A conservation genetic study of threatened, endemic southern African seabirds

By

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Declaration

I hereby declare that all the work presented in this thesis ("A conservation genetic study of threatened, endemic southern African seabirds") is my own, except where otherwise stated in the text. This thesis has not been submitted in whole or in part for a degree at any other university.

Signed in Cape Town on the 17 February 2014

Signed by candidate

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ABSTRACT

Molecular techniques have a broad, and growing, application in the field of wildlife conservation, ranging from the systematic identification and classification of taxa, through studying genetic connectivity between populations, to parentage and individual barcoding. While they are applied to a wide range of spatial- and temporal-scales, molecular approaches complement traditional methods used to classify, investigate and understand the natural world. This study uses multiple lines of evidence, at various scales, to investigate how seabird biology influences population-level responses to changing environments. The focal area is the Agulhas-Benguela Ecosystem (ABE) along the south-western coast of Africa. Globally, biodiversity loss due to environmental change in marine ecosystems is significantly affecting the phenology, distribution, dispersal patterns, and demographic rates of organisms across trophic-levels. Broad-scale changes are occurring that have consequences for both commercial fisheries and threatened marine top-predators. Seabirds are valuable indicators of the state of marine ecosystems, and changes in their distribution and dispersal patterns may reflect those of species in lower trophic-levels. This is the case in the ABE, where some endemic seabird species are better at responding to changes in their environment than others. Twentieth century shifts in the distribution of key pelagic prey species in the ABE have had serious consequences for endemic seabirds. The African Penguin *Spheniscus demersus*, Cape Gannet *Morus capensis* and Cape Cormorant *Phalacrocorax capensis* rely on these pelagic fish, and all three species are threatened and in decline. In this study population genetic and phylogeographic methods are used to: (i) quantify levels of genetic diversity, and determine regional-scale structure within all three focal species; (ii) explore fine-scale population structure in African Penguin; and (iii) compare wild and captive populations of African Penguins.

The conservation of genetic diversity is essential for the long-term persistence of species. Population genetics can help us to understand the evolutionary processes that have shaped patterns of genetic diversity in the focal species, and predict how they might respond to further environmental changes. Comparative phylogeography, combined with capture-mark-recapture models based on ringing data and annual census counts, provide the most complete picture of the micro-evolutionary forces at play in this unique ecosystem, and highlight seabird life-history characteristics may facilitate adaptation and survival under novel conditions. This is the first conservation genetic study of endemic seabirds in the ABE. Although the three focal species differ in a number of aspects of their breeding and foraging

ecology, and in some life-history characteristics, they have evolved under similar selective pressures across their shared range, and represent natural replicates that allow us to determine the dominant drivers of population genetic change. Flexibility in foraging behaviour and the degree of breeding site fidelity exhibited by each of the three focal species affect the rate and effectiveness of their demographic responses to changes in their environment. Understanding connectivity among seabird populations is crucial for their long-term conservation, and has been investigated in numerous studies of seabird species from around the world. Similar to many of these, this study found very low levels of genetic structure among populations of all three focal species based on DNA sequence data, suggesting long-term gene-flow among them, despite potential physical and non-physical barriers. Overall, the patterns observed suggest that high connectivity characterises their breeding regions, and most breeding colonies, buffering the respective populations against environmental variability. These results were supported by fine-scale analyses of the African Penguin using microsatellite markers that also suggested high levels of gene-flow, which may have masked genetic signatures of the regional- and colony-level bottlenecks experienced by this species. Microsatellite-based genetic diversity and fine-scale structure were also compared among wild and captive populations of African Penguins to assess the genetic consequences of a planned conservation breeding program. The genetic composition of birds in captivity largely reflects that found in wild populations. The success of reintroduction in terms of decreasing extinction risk in the wild is uncertain, and should be implemented as part of a broader management plan that addresses the primary threats to wild populations. Further research is required to improve our understanding of many aspects of endemic southern African marine avifauna and better inform our ability to ensure their continued persistence in this system.

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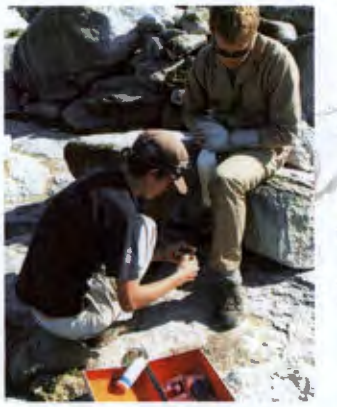

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here is the deepest secret nobody knows
(here is the root of the root and the bud of the bud
and the sky of the sky of a tree called life; which grows
higher than soul can hope or mind can hide)
and this is the wonder that's keeping the stars apart

i carry your heart(i carry it in my heart)

e.e. cummings

TABLE OF CONTENTS

LIST OF ACRONYMS

ABE:	Agulhas Benguela Ecosystem.
AMOVA:	Analysis of molecular variance.
ATPase-6:	adenosine triphosphate synthase subunit 6 (mitochondrial gene name).
BFIB or BFIB-I7:	beta-fibrinogen intron 7 (mitochondrial gene name).
COI:	cytochrome oxidase 1 (mitochondrial gene name).
cyt b:	cytochrome b (mitochondrial gene name).
DEA:	Department of Environmental Affairs, South Africa.
D_{EST}:	Jost's (2008) pure estimate of differentiation.
EBUS:	eastern boundary upwelling system.
EC:	Eastern Cape Province of South Africa.
ESU:	evolutionarily significant unit
F'_{ST}:	Meirman's (2006) standardised FST (corrected for F_{STmax})
FCA:	factorial correspondence analysis.
F_{IS}:	one of Wright's (1943) three hierarchical fixation indices, also known as the inbreeding coefficient ($F_{Individual - Subpopulation}$).
F_{IT}:	one of Wright's (1943) three hierarchical fixation indices ($F_{Individual - Total}$).
F_{ST}:	one of Wright's (1943) three hierarchical fixation indices ($F_{Subpopulation - Total}$).
F_{STmax}:	maximum possible theoretical value of F_{ST}, given the data set (used in standardisation).
G'_{STH}:	Hedrick's (2005) standardised GST.
G'_{STN}:	Nei's (1987) standardised GST.
G''_{ST}:	Hedrick's (2011) further standardised GST.
GAPDH:	glyceraldehyde 3-phosphate dehydrogenase (mitochondrial gene name).
G_{ST}:	Nei (1987) derivative of multi-allelic FST.
G_{STmax}:	maximum possible theoretical value of GST, given the data set (used in standardisation).
H_E:	expected heterozygosity.
HWE:	Hardy-Wynberg Equilibrium.
H_O:	observed heterozygosity.
IAM:	infinite allele model (of microsatellite mutation).
IBD:	Isolation-By-Distance.
LD:	linkage disequilibrium.
MCMC:	Markov-chain Monte Carlo method (applied in Bayesian phylogenetic analyses).
MHC:	Major Histocompatibility Complex.
MJ:	median joining.
MK:	mean kinship
ML:	maximum likelihood.
NADH2 or ND2:	nicotinamide adenine dinucleotide dehydrogenase subunit 2 (mitochondrial gene name).
NADH3:	nicotinamide adenine dinucleotide dehydrogenase subunit 3 (mitochondrial gene name).
N_{Ae}:	number of effective alleles.
NAM:	Namibia.
Ne:	effective population size.
NGS:	Next-generation sequencing

NZG:	National Zoological Gardens of South Africa.
PAZAAB:	African Association of Zoos and Aquaria.
PCoA:	principal coordinate analysis.
PD:	power of discrimination
PE:	probability of exclusion
RAD:	restriction-site associated DNA
RM:	reduced median.
SANCCOB:	Southern African Foundation for the Conservation of Coastal Birds.
SMM:	Stepwise Mutation Model.
SNP:	single nucleotide polymorphism.
TOA:	Two Oceans Aquarium
TPM:	two-phase mutation model.
uH_E:	unbiased expected heterozygosity.
WAZA:	World Associations of Zoos and Aquariums.
WC:	Western Cape Province of South Africa.

CHAPTER 1: Conservation genetics and seabirds

"The one process now going on that will take millions of years to correct is the loss of genetic and species diversity by the destruction of natural habitats. This is the folly our descendants are least likely to forgive us." E. O. Wilson (1984, p. 12)

INTRODUCTION

We are living in the Anthropocene ('the human epoch') and our collective impact on the environment is considered a global geophysical force (Crutzen 2002; Steffen et al. 2007). Globally, biodiversity loss is driven by the unsustainable harvesting of natural resources to fulfil the increasing demand created by unprecedented human consumption (Ehrlich & Wilson 1991; Pimm et al. 1995; Rands et al. 2010; Ehrlich & Ehrlich 2013). Worldwide, marine ecosystems are increasingly under pressure due to the combined effects of over-exploitation, pollution, invasive species, environmental degradation and climate change (Jackson 2008; Baum & Worm 2009; González-Solís & Shaffer 2009; Worm et al. 2010), and the rate of global marine biodiversity loss is increasing (Butchart et al. 2010). The rates of projected climatic change exceed the rates at which species have adapted to past changes (Quintero & Wiens 2013), and current species extinctions are rising globally. Nevertheless, the evolutionary and ecological consequences of this Anthropocene 'mass extinction' are largely unknown (Loreau et al. 2001; Crutzen 2002; Jackson 2008).

High levels of biodiversity within marine ecosystems are linked to the ability of these systems to withstand environmental perturbations (Worm et al. 2006; Palumbi et al. 2009; Taylor et al. 2011b), and the loss of diversity disrupts ecosystem functioning with implications for the long-term resilience of natural systems in the face of global change (Palumbi et al. 2008; Doney et al. 2012). Anthropogenic activities affect the diversity and abundance of marine organisms, especially top-predators (Lotze & Worm 2009; Baum & Worm 2009; Worm et al. 2010). Although many marine species and populations have evolved developmental, genetic and demographic adaptations to deal with naturally variable environments, they may lack the capacity to survive rapid, severe anthropogenic environmental transformation (Parmesan 2006; Chevin et al. 2010; Sears & Angilletta 2011). The current – and predicted future – rate of change, resulting from the synergistic effects of multiple simultaneous stressors on ecosystems, represents a formidable barrier to persistence for many species (Jackson 2008) and is significantly affecting the phenology, distribution, dispersal patterns, and demographic rates of marine organisms (Wolf et al. 2010; Weimerskirch et al. 2012; Doney et al. 2012). Predicting the consequences of environmental

change for marine ecosystems, and their constituent species, is complicated, but it is nonetheless critical for the development of effective long-term marine conservation strategies (Jiguet et al. 2007; Wolf et al. 2010).

Seabirds as indicators of ecosystem change

Evolutionary and ecological studies of seabirds have provided insight into the effects of marine habitat degradation and environmental change, and the associated destabilization of complex marine food webs worldwide (Croxall et al. 2012). Seabirds are informative in this context due to their trophic status, and the relative ease with which we can monitor their distribution, abundance and reproductive success.

Long-term datasets exist for numerous seabird species worldwide, allowing researchers to explore how species have responded to past changes in their environment and to develop predictions as to how they might be affected by on-going human-induced environmental change. Seabirds are collectively one of the most threatened groups of birds worldwide (Butchart et al. 2004; Croxall et al. 2012) and have become a conservation priority in recent years, partly due to the information they can provide regarding the state of coastal and oceanic systems (Oatley et al. 1992; Crawford 2007b; Piatt & Sydeman 2007; Croxall et al. 2012). Marine ecosystems, especially upwelling ecosystems, are complex and difficult to monitor directly, so indicators that integrate changes in biotic and abiotic factors are highly valuable (Piatt & Sydeman 2007; Saraux et al. 2011). Changes in the distribution and abundance of top predators in marine ecosystems often reflect changes in the biological or oceanographic processes that characterise those ecosystems (Crawford & Altwegg 2008; Einoder 2009; Distiller et al. 2012). For example, seabird population sizes and reproductive performance are determined largely by the availability of prey and are expected to reflect environmentally-induced fluctuations in prey resources (Durant et al. 2009). Pelagic seabirds are 'samplers' of pelagic prey that is mobile, patchily distributed and difficult to survey (Cherel & Weimerskirch 1995), and can be used as indicators of the spatial distribution and changes in abundance of marine resources (Boersma 2008; Waller 2011).

The 'seabird syndrome' and a role for DNA-based methods in marine conservation

Using seabird-derived indices to approximate the status and distribution of commercially important fish stocks necessitates a thorough understanding of the nature and reliability of the links between seabird population biology and behaviour, and prey stock size (Hunt et al. 1996). Seabirds are entirely dependent on the marine environment for at least part of their

life-cycle (Schreiber & Burger 2001; Friesen 2007; Taylor & Friesen 2012) and often are highly mobile, making them good candidates as indicators of broad-scale ecosystem change (Boersma 2008; Durant et al. 2009). A number of adaptive life-history characteristics buffer seabirds against short-term fluctuations in their food supply (Crawford 1999; Crawford & Altwegg 2008); e.g. some seabirds are specialist predators, whereas others have a more flexible diet, switching prey type more readily when conditions change (Durant et al. 2009); some seabird species show exceptionally high breeding site fidelity, whereas other species disperse to breed (Crawford et al. 1994; Schreiber & Burger 2001); survival, longevity, and age at first breeding are elevated in offshore compared to inshore foragers (Hunt & Furness 1996; Croxall & Davis 1999); some species are more mobile than others, and juveniles may visit, and potentially disperse to, non-natal colonies more frequently in such species (Reed et al. 1999; Morris-Pocock 2012). Differences in these life-history and behavioural characteristics result in varying responses to ecosystem change among species (Ricklefs 1990). Phenotypic plasticity in life-history traits enables some species to respond rapidly to changes in their habitat or food availability, while others struggle under novel conditions (Chevin et al. 2010; Devictor et al. 2012). Plasticity is a natural character under some genetic control and is thought to increase with genetic variation at the individual and population levels (Reed et al. 2006). When environmental changes alter an ecological regime, selection acts on this genetic variation and a population level response is elicited (Reed et al. 2006). Several species-specific attributes determine a species' ability to track optimal environmental conditions, including diet (foraging mode), natal dispersal, habitat preference, ecological specialization and breeding strategy (Jiguet et al. 2007).

Seabirds are generally long-lived species with delayed sexual maturation - thought to be linked to the time required to gain sufficient foraging experience and/or local knowledge of the spatiotemporal availability of food that is necessary to forage efficiently enough to survive and meet the demands of breeding (Ricklefs 1990). Seabirds often breed colonially and only attempt to breed annually or biennially (Schreiber & Burger 2001), resulting in large numbers of immature birds at breeding colonies (Votier et al. 2010). Many seabird species exhibit life-history characteristics that have evolved due to the constraints associated with breeding on land and foraging at sea e.g. strong mate fidelity (social monogamy) and natal site fidelity (Croxall & Davis 1999; Schreiber & Burger 2001). Seabird clutch sizes are generally small, especially in offshore foragers, due to the additional constraint of central-place foraging, which is necessary during clutch incubation and chick-rearing (Schreiber &

Burger 2001). This means that optimal mate and breeding site choice are important to maximise reproductive output, as are short distances between breeding and foraging grounds - to reduce the cost of rearing chicks (Schreiber & Burger 2001). Together, these highly conserved life-history traits have been termed the "Seabird Syndrome" because they restrict the population's growth rate, and hence its ability to recover from declines (Gaston 2004).

Several potential benefits exist to applying conservation genetic methods in the study of seabird biology; e.g. using molecular markers to study their evolution and ecology (Friesen 2007; Taylor & Friesen 2012). Seabirds are practical study organisms to investigate micro-evolutionary and ecological processes because data (e.g. chick growth rate) and samples (e.g. genetic samples or diet samples) can be collected with relative ease at colonies during the breeding season; sufficient genetic sample sizes can be accrued to robustly test hypotheses regarding physical versus non-physical barriers to gene-flow (e.g. isolation by distance versus natal site fidelity; Friesen 2007). It is also possible to individually mark chicks with durable metal or silicone bands or transponder chips to track their movements and breeding behaviour over their entire lives, providing useful estimates of dispersal and mate fidelity for comparison with genetic data (Young 2010). Unfortunately, the collection of such data usually requires recapture or re-sighting of birds and this 'recovery effort' is usually not equally distributed across the range of seabirds with a low percentage recovery of rings deployed (Votier et al. 2010), resulting in data that is often biased and statistically challenging to analyse in a way that yields robust conclusions. Other constraints restrict the use of transmitter or logger technologies to track the long-term movements of individuals (Hazen et al. 2012), such as the cost of devices, memory and battery life limitations, as well as ethical concerns about the impacts of devices on individual birds (especially if deployed in large enough numbers to detect rare dispersal events). Natural tracers (e.g. stable isotopes, trace elements) have also been used to investigate seabird movements during the non-breeding season i.e. where their primary foraging grounds are at a broad scale (Cherel & Hobson 2007; Tierney et al. 2008; González-Solís & Shaffer 2009; Lorrain et al. 2009; Wiley et al. 2012). These methods, however, are seldom useful for investigating dispersal and population connectivity among colonies of breeding seabirds because they cannot provide spatial data at a fine scale resolution relative to appropriate genetic markers (Avise et al. 2000; Lowe & Allendorf 2010; Taylor & Friesen 2012; Moseley et al. 2012). Evolutionary genetic theory as applied to threatened species provides a powerful complementary approach to these more traditional methods when studying seabird population responses to changes in

the environment. Improving our understanding of the interactions between threatened top predators and their environments, both at the individual and population levels, will improve our ability to predict the potential demographic consequences of environmental change, protect threatened species more effectively and improve their utility as indicator species (Palumbi 2003; Distiller et al. 2012; Ramírez et al. 2013).

Global threats to seabirds, and a role for conservation genetics

The main threats facing seabirds globally include competition with various fisheries (e.g. long-line and trawl fisheries), marine pollution (e.g. plastic, oil), disease (e.g. avian cholera), disturbance at breeding sites, and predation by introduced predators (e.g. cats, rats or pigs; Croxall et al. 2012). Added to these threats are the risks incurred by species as their population sizes decrease (Gilpin & Soule 1986; Spielman et al. 2004; Allendorf & Luikart 2007) e.g. localised extinction resulting from demographic or environmental stochasticity (Brook et al. 2002; Allendorf & Luikart 2007; Goldsworthy & Page 2007; Boersma & Rebstock 2014) or the deleterious effects of inbreeding (Frankham 2005; Jamieson 2011). In the face of these diverse threats, there is a clear role for conservation genetic studies of threatened seabirds.

The field of conservation genetics aims to establish the evolutionary processes that have shaped the diversity found in modern populations, and provide conservation scientists and managers with tools to ensure their persistence in threatened taxa (Frankham 2010; Avise 2010). The field of population genetics has a long theoretical and empirical history that focuses on the evolutionary forces that generate and maintain natural population genetic diversity, and how these processes result in some populations with higher levels of diversity than others (Wright 1931; Hedrick 2004; Allendorf et al. 2010; Lowe & Allendorf 2010; Leffler et al. 2012). The application of modern molecular biology tools to the study of ecology and evolution has provided a powerful approach to complement existing methods for defining, identifying and prioritizing threatened populations and for finding the most effective strategy to conserve them (Carty et al. 2009). Understanding how environmental degradation and/or change affect natural processes, such as dispersal, in threatened seabirds is crucial to identify effective conservation actions. Molecular tools are increasingly being employed in conservation to investigate genetic variation, demographic history, gene-flow and genetic sources and sinks (Allendorf & Phelps 1981; Lacy 1987; Beaumont 1999; Broquet & Petit 2009; Hellberg 2009; Taylor & Friesen 2012) in a variety of threatened species, especially those with fragmented ranges (Avise 1995; Milot et al. 2008; Lukoschek

et al. 2008). They have also been used in combination with other types of data to infer the colonization history of seabirds (Young 2010). The geographic distribution of genetic diversity can provide insights into various aspects of seabird ecology that are important for their conservation, such as barriers to dispersal (Steeves et al. 2003; Morris-Pocock et al. 2010a), metapopulation dynamics (Bouzat et al. 2009) and foraging ecology (Wiley et al. 2012). Data of this type can help conservationists identify priority populations and better understand connectivity between them (Crooks & Sanjayan 2006; Carty et al. 2009; Blomqvist et al. 2010). Dispersal, environmental tolerances and biotic interactions shape the geographic range of a species (Bohonak 1999; Hui et al. 2012). The degree of genetic structure within the range of a species can reflect these interactions and provide insights into the processes that generate and maintain genetic diversity (Wright 1965; Slatkin 1993; Grosberg & Cunningham 2001; Gaggiotti et al. 2009; Blamey et al. 2012). Multi-species comparative studies are powerful in this regard, as they allow for the discovery of common factors responsible for shaping patterns of genetic diversity (Taylor & Friesen 2012; Barbosa et al. 2012).

Genetic diversity and adaptive potential

There are several possible ways that populations may respond to changes in their environment. They can adapt *in situ* to changes (through natural selection), move to where conditions are more suitable (dispersal) or adjust their phenotype (depending on the reaction norm of the species) to better cope with the changes. Populations characterised by high levels of genetic variation are predicted to have greater fitness (Reed & Frankham 2003) at the individual and population levels, and higher adaptive potential (sometimes called 'evolvability'; Willi et al. 2006; Bouzat 2010; Frankham 2010). These populations are considered better able to adapt to changes in their environments (Willi et al. 2006; Bouzat 2010) because of how populations evolve – by changes in the frequencies of alleles as a result of mutation, genetic drift, gene-flow and selection (Hedgecock et al. 2007). A species' capacity to adapt to changing conditions, either by dispersing to where conditions are more favourable, or adjusting its phenotype to suit new conditions, determines whether populations will persist or go extinct (Hoelzel 2010; Pichegru et al. 2010b; Buckley & Kingsolver 2012). If a population lacks the appropriate (additive) genetic variation in some direction in phenotype space (i.e. variation in a single trait or set of traits that improve fitness under novel selective pressures), that population's evolutionary potential is constrained (Gomulkiewicz & Houle 2009). Quantitative genetic constraints occur when this additive variation is limited,

but the constraints are considered surmountable if the population has enough time to respond to a selective regime and adapt before demographic extinction occurs (Gomulkiewicz & Houle 2009). Plasticity in traits involved in ecological and physiological tolerances (e.g. thermal and hydric limits), phenology, dispersal potential and fitness all influence the way in which a species responds to environmental change (Somero 2010, 2012; Buckley & Kingsolver 2012). On this basis, many conservation genetic studies are initiated to quantify and characterise the genetic diversity of threatened populations (Frankham 2010). Importantly, Willi et al. (2006) emphasise that factors other than levels of quantitative genetic diversity within populations can influence their evolutionary potential. For example, if only suboptimal conditions remain across the range of a species, e.g. due to habitat loss or environmental change, that species' response to selection will be impeded irrespective of levels of variation (unless there is potential for adaptation that allows species to adapt to the "new" habitat). 'Environmental Stress' (Willi et al. 2006) is often higher under suboptimal conditions in fragmented or degraded habitats and has been shown to increase the intensity and direction of selection. Optimal, pristine habitat no longer exists for many species, and the ecological and evolutionary consequences of deteriorating environmental conditions are likely to be devastating and irreversible for many of them. An ecosystem approach to conservation that aims to preserve biological diversity and natural processes is thought to be the best way of ensuring the long-term persistence of threatened species in marine ecosystems under pressure from fishing, climate change, pollution and other human-induced threats (Gray 1997).

The study of pattern and process: The rise of evolutionary genetic methods

Phylogeography is the study of the processes that control the geographic distribution of genetic lineages within and among species (Avise et al. 1987; Avise 2000; Knowles 2009). Studies primarily consider the spatial distribution of alleles or haplotypes (Avise 2000) seeking explanations for observed spatial patterns of intraspecific genetic diversity among the evolutionary forces that influence closely related lineages, such as mutation, selection, genetic drift and gene-flow (Avise et al. 1987; Knowles 2004; Beheregaray 2008; Nielsen & Beaumont 2009). Population genetic methods are often used to assess population connectivity and estimate gene-flow, as dispersal can be difficult to measure directly (Koenig et al. 1996; Whitlock & McCauley 1999; Hedgecock et al. 2007; Lowe & Allendorf 2010). This type of phylogeographic inference is based on the observation that patterns of neutral genetic variation among individuals of a species contain signatures of that species' demographic

history (Amos & Harwood 1998; Knowles 2009; Welch et al. 2012b). A common goal of phylogeographic research is the identification of barriers to gene-flow within species (Avise 2000). Barriers to gene-flow reduce connectivity among seabird populations, and can be physical barriers (e.g. the geographic distance between colonies or regions) or oceanographic features (e.g. currents, gyres or upwelling cells, which could influence where the birds forage; Raymond et al. 2010; Weimerskirch et al. 2010; Morris-Pocock 2012). Non-physical barriers to gene-flow may also influence the genetic structure of species and populations. Such factors include strong philopatry and/or mate fidelity, local adaptation that prevents immigrants from establishing, and habitat preference e.g. pelagic species may encounter and disperse to non-natal colonies more often than inshore foragers (Burg & Croxall 2001; Morris-Pocock 2012). It has also been suggested that gene-flow is elevated in seabirds that inhabit spatially and temporally variable cold-water upwelling systems (Taylor et al. 2011a).

Barriers to gene-flow can differ markedly among species, but comparative phylogeographic studies of broadly sympatric species or those with common ecological traits allow for more general conclusions about gene-flow (Avise 2000; Morris-Pocock et al. 2010a; Calderón et al. 2014). Similarly, studies comparing species that differ in particular ecological traits, or represent a spectrum of such traits, but are otherwise similar, can be used to test if particular traits influence gene-flow (Morris-Pocock 2012). Understanding population connectivity is fundamental to population ecology and crucial when managing populations for conservation (Paetkau et al. 2004). However, the nature of the link between genetic connectivity and demographic connectivity is complex. Genetic methods can provide insights regarding dispersal rates, but several authors have advised that such results be interpreted with caution (Waples 1998; Whitlock and McCcauley 1999) and, whenever possible, in combination with data on demographic rates, movement behaviour and/or estimates of reproductive success of immigrants and residents (Hedgecock et al. 2007; Lowe & Allendorf 2010). Results from studies estimating dispersal rates from genetic data are often ambiguous due to low resolution of molecular markers (i.e. the retention of shared ancestral polymorphisms, and low precision of model-based estimates; Bossart and Prowell 1998). Additionally, because even limited gene-flow (one to ten migrants per generation) is sufficient to homogenise allele frequencies between populations (Palumbi 2003), and inferences about gene-flow are made on evolutionary time-scales, understanding the ecological and management ramifications of low genetic structure is difficult, due to the much reduced time frame of interest (Bossart & Prowell 1998; Waples 1998; Palumbi 2003). Also important here is that, for various reasons,

one study that uses particular genetic markers may find little significant population genetic structure, where another that utilises a different suite of loci and/or samples may detect significant structure (e.g. selectively neutral versus adaptive genetic markers).

Statistical methods for the inference of population connectivity

The main reason for caution when interpreting data on genetic connectivity is that indirect methods for measuring gene-flow from genetic data alone are derived from equilibrium-based estimates of population structure (i.e. models assume that the populations of interest have reached equilibrium between migration and genetic drift; Bossart and Prowell 1998; Hedgecock et al. 2007; Burton 2009). The dominant models employed when investigating population genetic structure are Wright's (1931) Island Model, the stepping-stone model (Kimura & Weiss 1964) and various metapopulation models (Hanski 1991, 1994) – all of which are defined by the pattern of gene flow and determine the probability of populations sharing genes. Population genetic theory predicts that the genetic composition of a population reaches a state of equilibrium as a result of opposing evolutionary forces acting over sufficiently long periods in the absence of any environmental change. For example, genetic drift and diversifying selection result in divergence, whereas dispersal homogenises gene pools (Hedgecock et al. 2007). It is unlikely that any natural populations fulfil such an assumption, especially those of seabirds inhabiting inherently variable marine ecosystems (Bossart and Prowell 1998; Whitlock and McCauley 1999), and given the context of global habitat destruction and degradation, pollution, exploitation and climate change.

Indirect estimates of gene-flow also assume that genetic differences between populations accumulate due to genetic drift and mutation, and that individuals who disperse to new populations to breed cause the homogenization of the respective gene pools (Burton 2009). The observed level of population divergence is therefore assumed to reflect gene-flow between two such populations at equilibrium. This divergence is typically expressed as a fixation index (φ_{ST} for haplotypic mitochondrial sequence data and F_{ST} for co-dominant microsatellite data) or as differences in standardised allele frequencies among populations (Wright 1931; Excoffier et al. 1992; Hudson et al. 1992; Balloux & Lugon-Moulin 2002). Some of the various conceptually related standardised fixation indices, differentiation indices and other alternatives that are increasingly employed include F'_{ST}, G_{ST}, G'_{ST}, G''_{ST}, AMOVA, private alleles and Jost's D (Hedrick 2005; Jost 2008, 2009; Heller & Siegismund 2009; Ryman & Leimar 2009; Gerlach et al. 2010; Bird et al. 2011). Wright's F_{ST} is based on diploid genes, ranges from 0 (identical allele frequencies) to 1 (no shared alleles between

populations) and was originally developed as part of a set of three hierarchical parameters (F_{ST}, F_{IS}, F_{IT}) to investigate how genetic variation in natural populations is partitioned among populations and individuals (Wright 1931, 1943; Bird et al. 2011). Under Wright's Island Model, gene-flow is expressed as the number of migrants (N_em) exchanged between populations at equilibrium, and is related to F_{ST} through the estimate $N_em = \frac{1-F_{ST}}{4F_{ST}}$, where N_e is the effective population size of the local population and m is the proportion of new individuals (migrants) entering the population in each generation (Hedgecock et al. 2007; Burton 2009). For maternally inherited, haploid mitochondrial DNA, population differentiation is defined based only on the female population size and female migration rates and, therefore, the degree of differentiation tends to be higher that that estimated from nuclear markers (Whitlock and McCauley 1999). Also, there are essentially half as many allele copies compared to the diploid genome, resulting in stronger genetic drift and increased expected differentiation among populations; here gene-flow is estimated as $N_{e♀}m_{♀} = \frac{1-\phi_{ST}}{2\phi_{ST}}$, where $N_{e♀}$ is the effective population size of females and $m_{♀}$ is their migration rate under the Island Model (Whitlock and McCauley 1999).

All natural populations violate the assumptions of the Island Model (Whitlock and McCauley 1999), which include that (1) there is an infinite number of populations, (2) all populations are composed of the same number of individuals (N), (3) migration rates between populations are equal, (4) there is no selection or mutation, and (5) each population persists until it reaches migration-drift equilibrium. The last assumption is important because of the likely retention of a historical signal of gene-flow in contemporary non-equilibrium populations, such that a large estimated number of migrants (N_em or $N_{e♀}.m_{♀}$) – corresponding to low F_{ST} estimates – can represent a low level of recent or current genetic exchange, or, alternatively, a high level of historical gene-flow between large populations, followed by isolation and subsequent drift (Burton 2009). This ambiguity means that conservation management decisions based purely on low levels of differentiation (low F_{ST} values) can be erroneous (Burton 2009), as low pairwise population estimates of F_{ST} can be interpreted as reflecting contemporary gene-flow or recent common ancestry of currently isolated populations. Inferring the absence of connectivity between two populations when F_{ST} or φ_{ST} approaches 1 (and $N_em \sim 0$) is more straight-forward than judging the degree of connectivity when $F_{ST} \approx 0$ (Hedgecock et al. 2007). In the latter case, it may be tempting to conclude that gene-flow is high, but there is no way of proving that the populations have reached equilibrium

(Hedgecock et al. 2007; but see Birky et al 1989). In fact, indirect methods widely used to estimate gene-flow do not distinguish between an exchange of 1000 individuals over 100 generations from 10 migrants every generation ($N_em = 10$ in both cases), and this lack of temporal resolution makes the assessment of population dynamics from genetic data less useful for conservation management (Hedgecock et al. 2007). Additionally, because F_{ST} estimates are reciprocally related to the number of migrants, small errors in estimating low F_{ST} values make moderate levels of gene-flow indistinguishable from panmixia (Palumbi 2003; Hedgecock et al. 2007). That is to say, as levels of gene-flow increase, estimates of F_{ST} and its analogues become small in relation to their confidence intervals, making it difficult to assess subtle population genetic subdivision without requiring unattainably large sample sizes to obtain adequate statistical power (Palumbi 2003; Kelly et al. 2010). Subtle deviations from panmixia, however, can have critical demographic and evolutionary implications (Kelly et al. 2010). Such populations may be demographically isolated e.g. exhibit different survival rates or breeding success, but not genetically disconnected (Taylor & Friesen 2012). Where gene-flow is sufficient to prevent population genetic divergence and local adaptation, but not sufficient to allow populations to be treated as a single demographic unit, populations are often referred to as management units (MUs, Moritz 1994a; Wallace et al. 2010). Taylor & Friesen (2012) suggest that management units can be identified by differences in allele frequencies at molecular markers (see also Moritz 1994s). Bossart & Prowell (1998) list some of the above, among other potential sources of "error and ambiguity surrounding estimates of genetic structure and gene-flow" (see Box 3, p. 204).

The occurrence of an allele in more than one population or colony is considered evidence of gene-flow – assuming that identical alleles are identical by descent – however, if the resolution of the chosen molecular marker is low, this pattern could represent retained shared ancestral polymorphisms (a recent split; i.e. incomplete lineage sorting) and not contemporary gene-flow (Wright 1965; Bulgin et al. 2003; Kelly et al. 2010). Theoretically, the higher mutation rate of more polymorphic markers should provide finer temporal resolution with respect to gene-flow, but the conceptual difficulties with established metrics to describe genetic connectivity mentioned above, in combination with the realization that fixation indices decrease as the diversity of the chosen marker increases (Hedrick 1999), has resulted in a number of alternative metrics being developed, with emphasis on standardised (expressed relative to their maximum possible value) fixation indices (G'_{ST}, F'_{ST}, φ'_{ST}) and pure indices of genetic differentiation (D, D_{est}, Jost 2008; Bird et al. 2011). As molecular

methods advanced in the 1980s and 1990s, and new, more variable genetic markers were discovered, methods to analyse these novel datasets also evolved (Meirmans & Hedrick 2011). Weir and Cockerham (1984) and Excoffier et al. (1992) developed the Analysis of Molecular Variance (AMOVA) approach for haplotypic mitochondrial sequence data, and Slatkin (1995) developed R_{ST}, based on a stepwise mutation model, for microsatellite data (Weir & Cockerham 1984; Slatkin 1995). AMOVA performs permutations to test for significant deviations of F_{ST} or φ_{ST} from the null expectation i.e. random distribution of alleles (F_{ST}) or haplotypes (φ_{ST}) among populations (Excoffier et al. 1992; Bird et al. 2011). R_{ST} is not affected by the amount of within-population variation and provides unbiased estimates of the number of migrants that are more accurate than those based on F_{ST} (Balloux et al. 2000), provided the microsatellite markers follow a stepwise mutation model (Meirmans & Hedrick 2011). Wright's F_{ST} (Wright 1943) remains a useful tool for comparative analyses of gene-flow (Neigel 2002), but is no longer used to estimate the number of migrants. F_{ST} and its analogues are useful for studying intraspecific population genetic structure, but they have been criticised as a way to investigate the processes that have generated such structure (Meirmans & Hedrick 2011).

The relatively new field of statistical phylogeography combines coalescent theory (Kingman 1982), and Bayesian or maximum likelihood statistical inference (Kuhner et al. 1995; Knowles 2004, 2009) to infer evolutionary process under posterior probability distributions. Methods for estimating connectivity in this framework include direct methods, such as assignment tests and clustering methods. Assignment tests calculate genotype likelihoods or probabilities that an individual originated from a particular population based on the expected frequency of that individual's multi-locus genotype (Hedgecock et al. 2007), while minimising deviations from HWE and LD. Unlike direct estimates, indirect estimates of gene-flow reflect dispersal rates over evolutionary time-scales, and are unlikely to reflect contemporary changes in movement patterns across specific landscapes, which biologists require for short-term management decisions (Paetkau et al. 2004).

Direct estimation of population genetic connectivity is a relatively new application of assignment tests and assumes the all putative source populations are sampled randomly, can be genetically defined *a priori* and are in Hardy Weinberg equilibrium, and that the markers are not linked (Manel et al. 2003; Hedgecock et al. 2007). Alternative assignment tests include the partial Bayesian assignment test (Rannala & Mountain 1997; Wilson & Rannala 2003) and full Bayesian inference (Pritchard et al. 2000). The former estimates allele

frequencies and the latter generates posterior probabilities for each potential source population, which can be interpreted as the probability that an individual belongs to a particular source population (Avise 2004; Knowles 2004, 2009). Direct methods generally perform better when estimating connectivity over a few generations, but still may fail to detect the genetic effects of stochastic, or recurrent (e.g. decadal-scale regime shifts), events that influence contemporary connectivity and demography (Paetkau et al. 2004; Hedgecock et al. 2007). Although indirect and direct methods of estimating gene-flow are currently limited in their ability to elucidate the temporal scale and magnitude of connectivity in natural systems (Hedgecock et al. 2007), they are widely used in conservation genetic assessments, as they do provide valuable insight into the genetic structure of populations and can be powerful in a multi-species, comparative context, especially when combined with tagging or tracking data.

The challenges involved in accurately estimating contemporary population connectivity based on population genetic models alone have limited the direct application of molecular data in many conservation contexts (Neigel 1997; Bossart & Prowell 1998; Waples 1998; Palumbi 2003). Molecular methods alone cannot currently fully resolve management questions about contemporary population connectivity, largely because (i) the level of migration necessary to erase most of the genetic signal (anything above a few individuals per generation) is difficult to estimate accurately over the time-scales of interest to conservation managers, and (ii) migration rate may be too low to be of consequence when trying to rebuild populations of threatened species (Waples 1998). Indeed, Waples (1998) emphasises that the biology and life history of a species of interest should be used to guide the design of the sampling regime so that additional information can be incorporated into genetic estimates of migration rates.

STUDY SYSTEM: THE AGULHAS-BENGUELA ECOSYSTEM

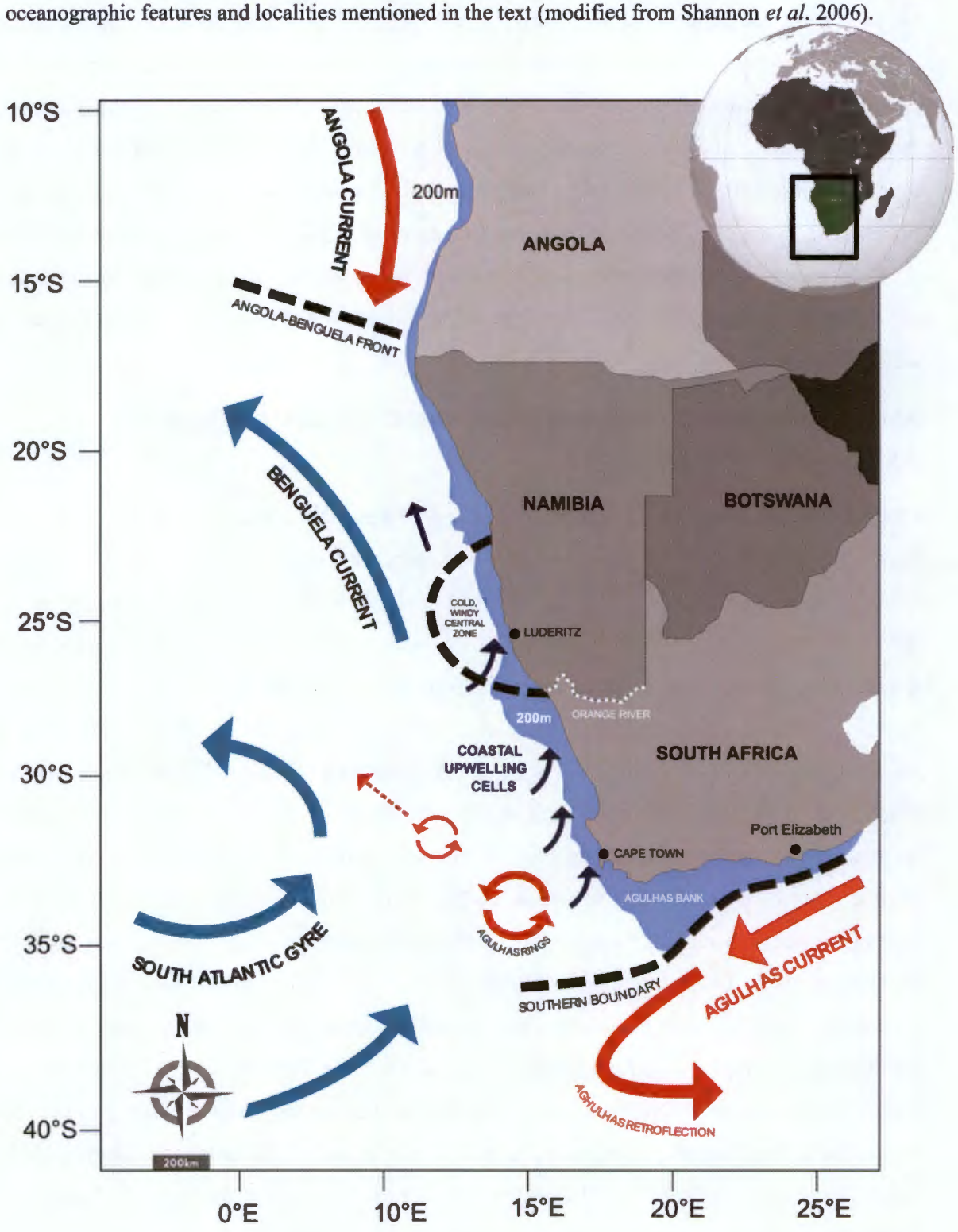
The Agulhas-Benguela Ecosystem (ABE) extends from southern Angola at around 5°S 12°E to Port Elizabeth in the Eastern Cape Province of South Africa (34°S, 26°E; Figure 1.1) and it is one of the four major eastern boundary upwelling systems globally (Shannon et al. 1992; Fennel 1999; Hutchings et al. 2009). These upwelling systems occur when local atmospheric circulation drives surface waters offshore forcing the upwelling of nutrient-rich subsurface waters (Shannon et al. 2006; Hutchings et al. 2009). The Humboldt Ecosystem off the west coast of South America is the only comparable ecosystem in the southern hemisphere, and due to their overall similarity, a number of comparative studies have been carried out

between the two systems (Moloney et al. 2005; Crawford et al. 2006c). The ABE is flanked by two warm temperate boundary currents: the Angola Current in the north-west and the Agulhas Current in the south-east. Like all upwelling systems, the ABE is a highly productive ecosystem that enriches, concentrates and retains sufficient nutrients for the establishment of large populations of pelagic fish (Hutchings et al. 2009). It is also a dynamic ecosystem system, characterised by spatially and temporally variable oceanographic features and phenomena (Moloney et al. 2005; van der Lingen et al. 2006b; Hutchings et al. 2009). The ABE is subdivided into northern and southern sub-systems, separated by a persistent upwelling cell around Lüderitz in south-central Namibia (26°S, Figure 1.1; Van der Lingen et al. 2006a).

Shifting distributions: environmental variability and fishing in the Agulhas-Benguela Ecosystem

Many top predators in the ABE feed on shoaling pelagic fish, primarily sardines (*Sardinops sagax*) and anchovies (*Engraulis encrasicolus*), which have been exploited by commercial fisheries in the region since the 1940s (Griffiths et al. 2004; Crawford 2007a; Crawford et al. 2007c; Coetzee et al. 2008). These pelagic fish species represent a critical mid-trophic-level that regulates energy transfer from lower levels to apex predators in what has been termed a "wasp-waist" ecosystem structure (Cury et al. 2000; Shannon et al. 2004). Sardines and anchovies are two of only a few key species that control the lower and upper trophic-levels (Cury et al. 2000), such that when their distribution and/or abundance is affected by climatic fluctuations or other perturbations (e.g. intense or localised fishing pressure), the whole trophic network is impacted (Cury et al. 2003). Worldwide stocks of sardine and anchovy fluctuate based on a suite of complex biotic and abiotic conditions and interactions (Crawford & Shelton 1978; Lluch-Belda et al. 1992; Hunt et al. 1996; Schwartzlose et al. 1999); accordingly, population dynamics in their seabird predators are also highly sensitive to the resulting fluctuations in food availability (Cury et al. 2000; Perry et al. 2010). Decadal- or longer-scale changes in food availability characterise marine ecosystems where sardine and anchovy are the principal prey species, independent of human exploitation (Shackleton 1987; Lluch-Belda et al. 1989, 1992). During the last century, however, technological advances in industrial fisheries worldwide, combined with increasing demand by the growing global human population, have resulted in increased catches, which affect top-predator populations through direct competition for prey (Frederiksen et al. 2004).

Figure 1.1 The Agulhas-Benguela Ecosystem (ABE) off the coast of southern Africa showing oceanographic features and localities mentioned in the text (modified from Shannon *et al.* 2006).



In the ABE, the abundance, diet and breeding success of endemic seabirds is strongly affected by fluctuations in prey availability (Boyer & Hampton 2001; Kirkman 2007; Crawford 2007b; Figures 1.2 and 1.3). Competition with purse-seine fisheries and mortality as a result of entanglement in fishing gear (Ryan et al. 2002; Grantham et al. 2008; Petersen et al. 2009) also have a negative effect on seabird populations e.g. prior to 2008 an estimated 2500 Cape Gannets *Morus capensis* were killed per year in the hake-trawl fishery alone (Watkins et al. 2008).

Exploitation of fish stocks and a possible link to regional seabird declines and range shifts

The pelagic fish stocks in the northern Benguela off Namibia are thought to be separate from those in the southern Benguela and western Agulhas systems off South Africa (Coetzee et al. 2008). In the South African system, there are at least two "functionally distinct units" of sardines, one on the west coast and one on the Agulhas Bank, each with separate spawning grounds (Coetzee et al. 2008). A recent molecular study of the Namibian and South African sardine stocks showed that sardine mitochondrial and microsatellite markers exhibited high genetic diversity, but that no clear spatial patterning was evident (Hampton 2013). Over the past few decades, major changes in sardine and anchovy distribution and abundance have been reported in both Namibia and South Africa (Shannon et al. 1992; van der Lingen et al. 2006a). In Namibia, the collapse of pelagic fish stocks in the 1970s has been attributed to extensive overfishing (Crawford et al. 1983, 2007a; Lewis et al. 2006; Kirkman 2007; Hutchings et al. 2009; Moseley et al. 2012), with serious consequences for seabirds breeding in that region (Figure 1.2 and 1.3a). The Namibian African Penguin *Spheniscus demersus* was the first seabird population that responded to the depletion of forage fish in the northern Benguela, and the number of breeding pairs decreased by ~90%. The response of the Cape Gannet population lagged behind that of the African Penguin, but the long-term regional population decline was greater, decreasing by ~95% (Figure 1.2). The Namibian Cape Cormorant *Phalacrocorax capensis* population responded last, and was the least affected of the three species, decreasing by ~75% (Crawford 2007b).

More recently (1990s-2007), in the southern Benguela off the coast of South Africa, a south-eastward shift of approximately 400km was recorded in sardine and anchovy distributions; breeding Cape Gannets, African Penguins and Cape Cormorants (Figures 1.2 and 1.3b) tracked this change to varying degrees (Fairweather et al. 2006; Crawford et al. 2007a; Kirkman 2007). The reason for the shift in the core distribution of sardine and anchovy in

South Africa has been the subject of a number of recent studies (van der Lingen et al. 2006; Roy et al. 2007; Coetzee et al. 2008; Crawford et al. 2008b). Climate change (Roy et al. 2007) and overfishing on the west coast (Coetzee et al. 2008) are thought to be the two major factors driving the observed shifts (Sabarros et al. 2012). But it is difficult to disentangle the effects of fishing from those of environmental change (Tasker 2000; Hsieh et al. 2006). Although fishing pressure is thought to play a role in the shift in pelagic fish, similar eastward distributional shifts have been observed in other species, including West Coast Rock Lobsters *Jasus lalandii* (Cockcroft et al. 2008; Blamey et al. 2012), the invasive Mediterranean Mussel *Mytilus galloprovincialis* (Viladomiu 2004 thesis), Crowned Cormorants *Phalacrocorax coronatus* (Crawford 2007a; Crawford & Ryan 2011), Hartlaub's Gulls *Chroicocephalus hartlaubii* (Zietsman 2011) and Bank Cormorants *Phalacrocorax neglectus* (Crawford et al. 2008a). As many of these species are not dependent on commercially exploited resources, global environmental change effects seem to be the most plausible explanation (Zietsman 2011). A number of studies report trends in oceanographic parameters that could explain the shifting distributions of species and in so doing implicate a role for contemporary climate change (Roy et al. 2007; Rouault et al. 2009, 2010), but the inherent high variability of the ABE introduces noise into these analyses that could mask the signal of long-term climate change. Irrespective of the ultimate cause, the shifting distributions of pelagic fish have significant implications for valuable commercial pelagic fisheries in South Africa and for seabirds breeding and foraging in the ABE (Crawford 1998; Crawford et al. 2008b, 2008c; Grémillet & Boulinier 2009; Okes et al. 2009; Pichegru et al. 2010a; Moseley et al. 2012; Distiller et al. 2012). The effect on avian top-predators in parts of the southern Benguela region is exacerbated by heavy fishing pressure that continues in areas with low fish abundance because the land-based fisheries processing plants were established nearby decades ago (Pichegru et al. 2009). A better understanding of connectivity among breeding regions may play a crucial role in determining the fate of these threatened top-predator species, but estimating parameters of connectivity can be a complex task and often requires the integration of information from multiple sources.

Figure 1.2 Changes in the abundance of (a) Cape Gannets and (b) African Penguins in the northern and southern parts of the Benguela Upwelling Ecosystem (updated from van der Lingen et al. (2006))

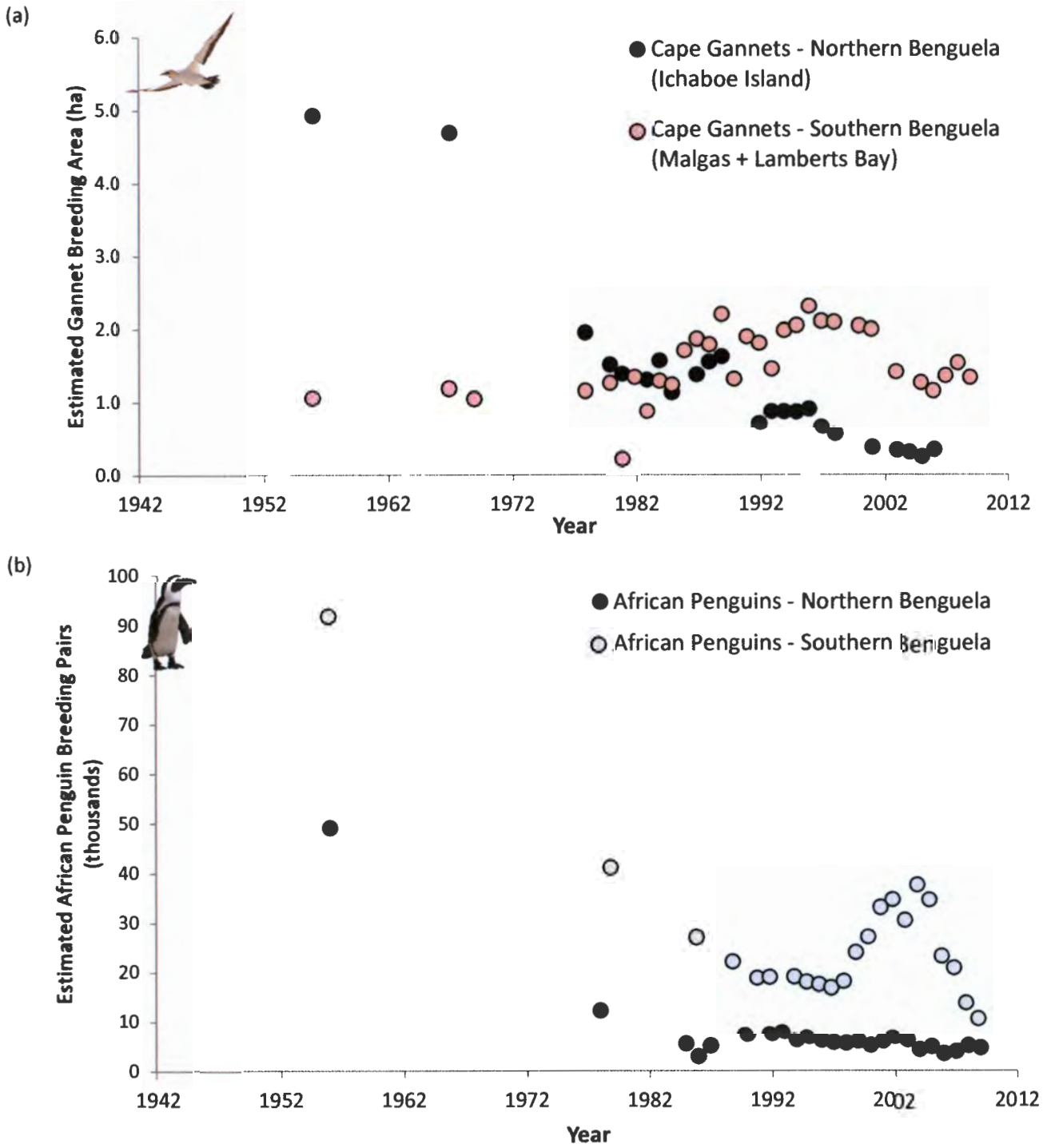
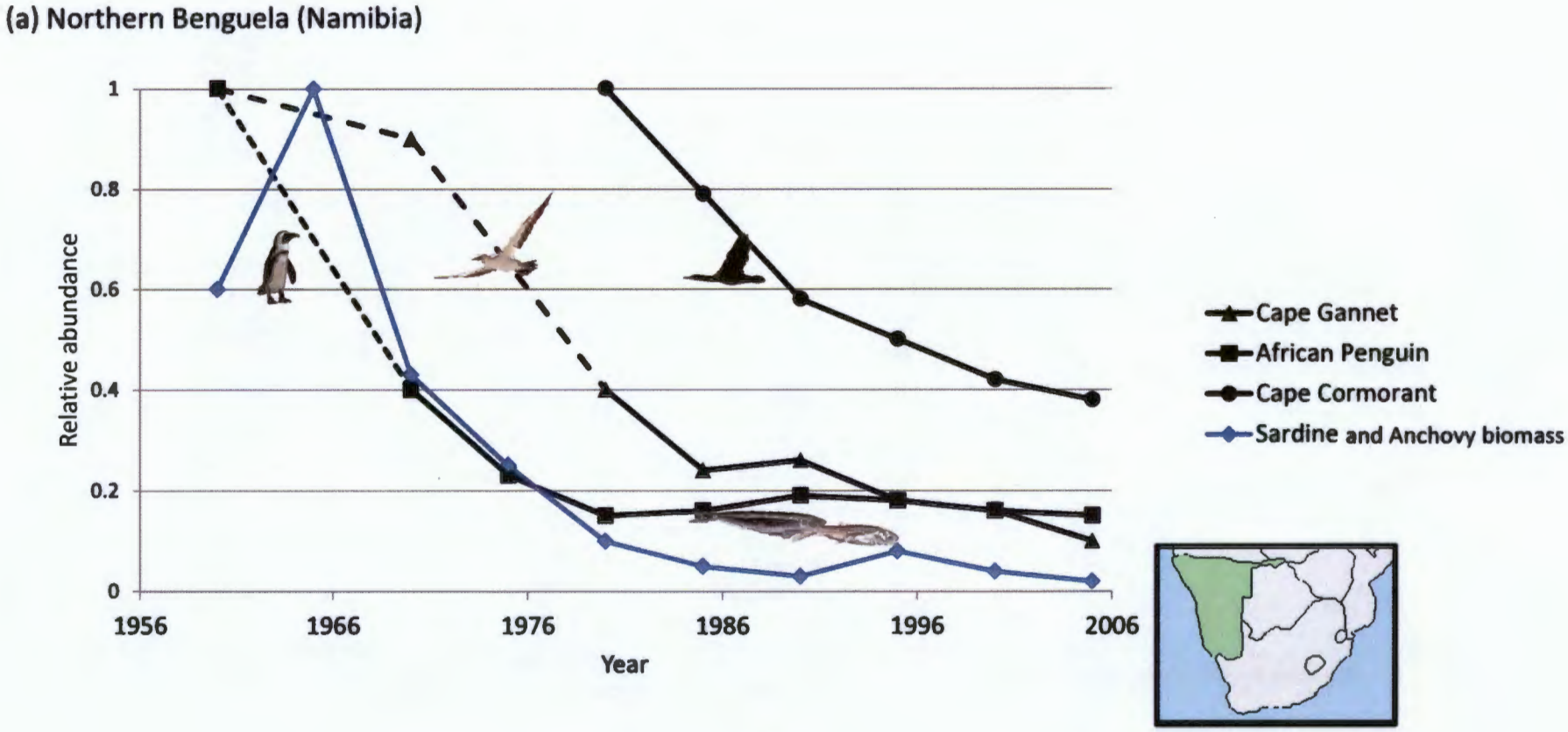
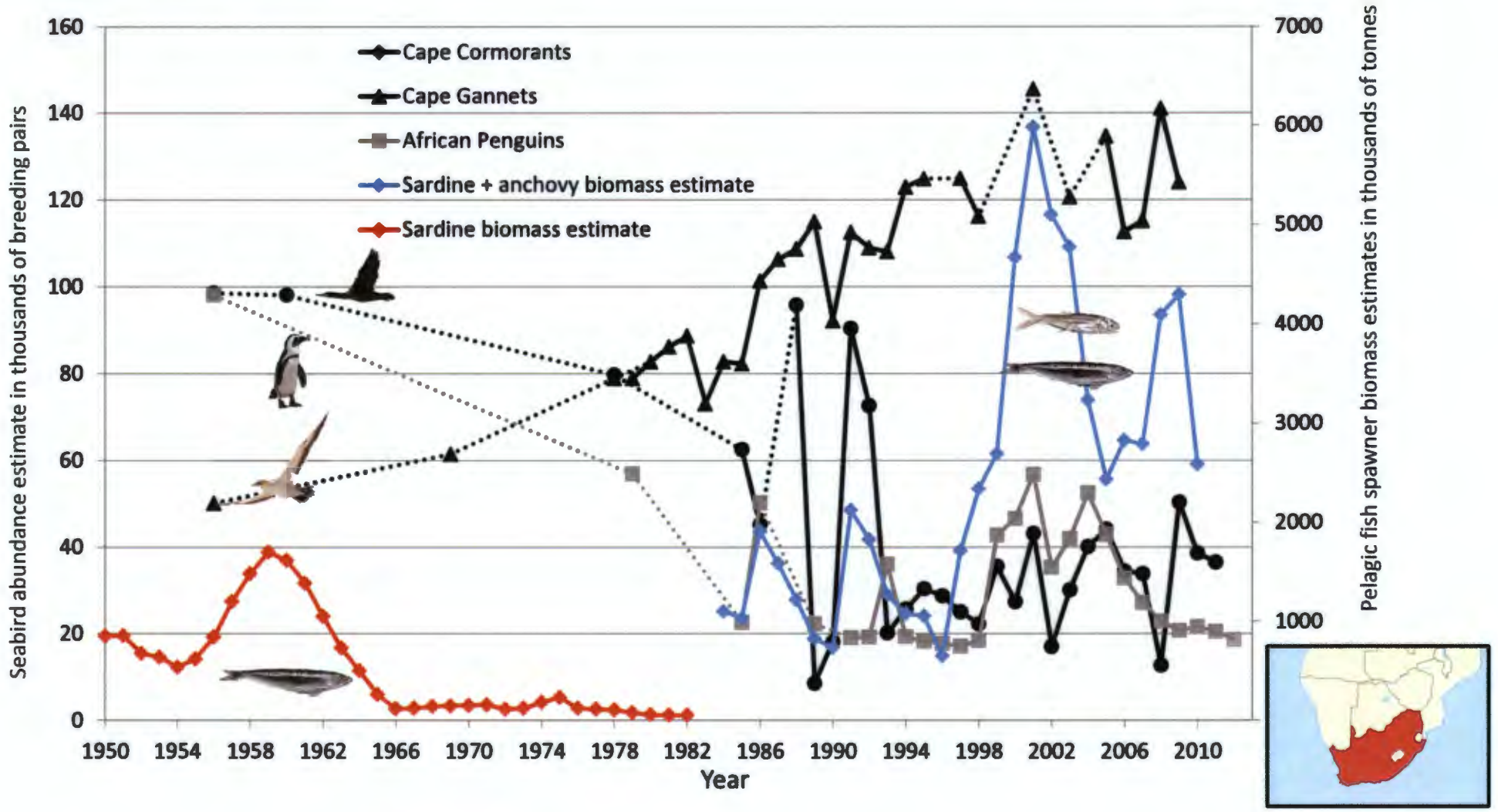


Figure 1.3 (a) The relative abundance of Cape Gannets, African Penguins and Cape Cormorants in Namibia (northern Benguela), showing the simultaneous collapse of the pelagic fish (sardine and anchovy biomass combined) at 4 year intervals between 1950 and 2012 (adapted from Crawford 2007). (b) Number of breeding pairs of Cape Gannets, African Penguins and Cape Cormorants in South Africa (southern Benguela), and the annual sardine and anchovy biomass estimates. Dashed lines represent gaps in the annual census data. Pelagic fish abundance data from 1950–1982 are VPA-derived (Butterworth 1983) and those for the period 1984–2005 are derived from hydro acoustic surveys (Fairweather et al 2006).



(b) Southern Benguela (South Africa)



Seabird dynamics as a function of long-term variability in the ABE

Long-term fluctuations in sea-level, climate and productivity are likely to have played a significant role in shaping the present ABE seabird community (Roberts et al. 2011). Based on fossil evidence, there has been a complete change in the ABE seabird community, except for the cormorants, since the mid-Pliocene (Olson 1985). Olson (1983, 1985) concluded that, because there was a colder, more sub-Antarctic nutrient-rich marine environment in the region in the late Tertiary than at present, seabird taxa more typical of cold-water systems moved north from the southerly latitudes near and around Antarctica, and subsequently went extinct due to a combination of oceanographic changes and sea-level fall (Schreiber & Burger 2001). Sea-level fluctuation causes both inundation and emergence of islands; i.e. low sea levels expose previously submerged land and high sea levels flood low-lying areas, thereby isolating high lands, and when the reverse processes occur, suitable island habitats are lost (Schreiber & Burger 2001).

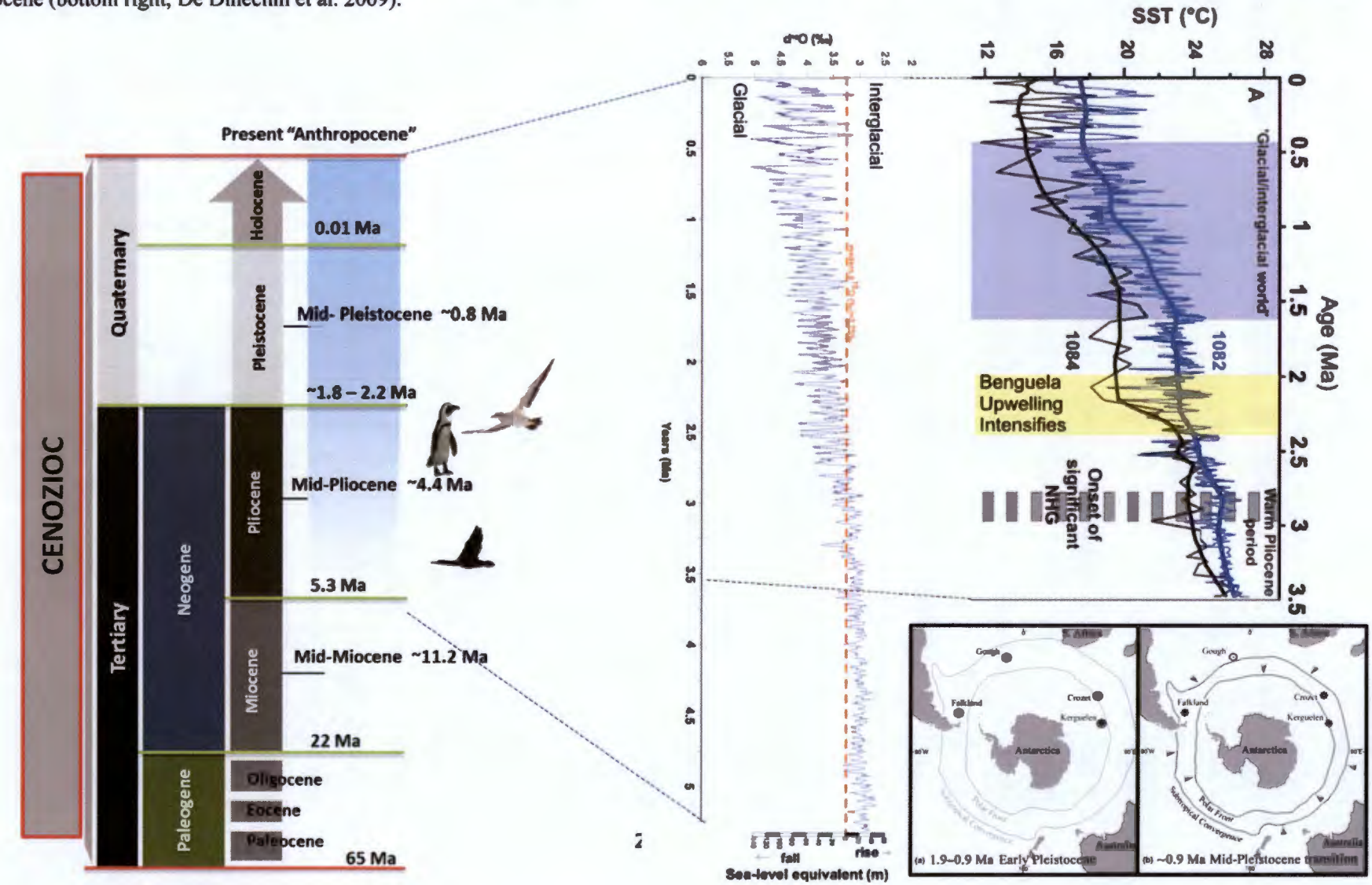
Ksepka and Thomas (2012) point out that an Early Pliocene ~90 meter high-stand, which coincided with a putative peak in penguin diversity in the Benguela, created island habitat and possibly increased the number of suitable nesting sites (Roberts et al. 2011; Ksepka & Thomas 2012); e.g. the contemporary land-surface area (120km^2) and length of coastline (200km) in the Saldanha Bay region of the Western Cape were affected: the former reduced to 20km^2 and the latter increased to 270km (Thomas & Ksepka 2013). Globally, average sea-level has declined since the Pliocene (Zachos et al. 2001; Lisiecki 2005), and the amplitude of sea-level rise and fall has increased dramatically, causing relatively rapid shifts in the coastal environment for penguins and drastically reducing breeding habitat for penguins and other seabirds (Ksepka & Thomas 2012).

Paleoceanographic studies also suggest that the Benguela and Agulhas Currents were differentially influenced during major transitions between glacial and interglacial conditions in the Middle to Late Pleistocene, with the former weakening and/or shifting towards the north, and the latter strengthening and intruding into the Atlantic (Flores et al. 1999). Sediment cores collected from the Benguela Current (Giraudeau et al. 2001; Chen et al. 2002) and from the Agulhas Current Retroflection (Flores et al. 1999; Berger et al. 2002; Krammer et al. 2006) suggest that de-glaciation during the Pleistocene resulted in warmer less productive water pulsing around the southern tip of Africa, which would have undoubtedly affected the seabirds breeding there (Figure 1.4). Recent paleoclimatic data indicate that the increased amplitude of climatic variation over the last five million years is more prominent

than any change in a single climatic factor i.e. the common signal amongst $\delta^{18}0$ oxygen isotopes (Lisiecki 2005; Miller et al. 2005), sea-level, bottom-water temperature, sea surface temperature (Marlow et al. 2000; Etourneau et al. 2009; Filippelli & Flores 2009), productivity (Jahn et al. 2003), upwelling intensities (Grant & Bowen 1998) and dust records (DeMenocal 2004) is that their variation has increased dramatically (Potts 1996, 1998). This variability could also explain the elimination of taxa with cold water affinities from the Benguela Ecosystem, despite the continued presence, and likely intensification, of the cold upwelling current since the Miocene. The alternating northward (expanding) and southward (contracting) migration of the Subtropical Convergence over the last ~2 million years (de Dinechin et al. 2009; McKay et al. 2012) is another source of large-scale variability that would have profound effects for temperate seabirds and those inhabiting sub-Antarctic Islands (Chen et al. 2002).

Modern seabirds are known to forage along this oceanic front, which is associated with increased levels of productivity (Jouventin et al. 2006; Bard & Rickaby 2009). Yet another source of environmental variability involves fluctuations in prey availability at ecological and evolutionary time-scales. Grant & Bowen (1998) investigated global genetic diversity and structure among populations of sardines and anchovies and found low genetic diversity, which they attributed to founder events and sequential bottlenecks resulting from long-term climatic variation and extinction-recolonisation events. Interestingly, sardines and anchovies from the ABE share haplotypes with fish in Australia and Europe, respectively – pointing to a scenario of long-distance dispersal in which anchovies dispersed from the Pacific to the Atlantic Ocean - almost certainly via the ABE - as recently as the Pleistocene, and subsequently, very recently from Europe to the ABE (Grant & Bowen 1998). Similarly, the estimated age of *Sardinops* is 15-24 million years, but genetic divergence among contemporary global sardine populations reveals a shallow history that coalesces in less than half a million years (Grant & Bowen 1998). In the context of long-term, high-amplitude climatic variation at various scales (e.g. Figure 1.4), Potts (1996) introduced the term "variability selection" that describes the effects of repeated, dramatic environmental shifts. Variability selection links adaptive change to large-scale environmental variability and predicts that versatile, plastic behaviours will evolve in highly variable environments (Potts 1998; Berger et al. 2002; DeMenocal 2004; Grove 2011). Seabirds exhibit a number of life-history and ecological characteristics that buffer populations against changes in their environment, and likely evolved in response to the inherent variability of the ABE.

Figure 1.4 Geological history from the Paleocene epoch until the present (spanning the Cenozoic Era), and the periods and epochs mentioned in the text (left). Fluctuations in sea-level based on $\delta^{18}O$ (centre; red dashed line is present sea-level) and as sea-surface temperature (indicating upwelling in the Benguela, right; NHG=Northern hemisphere glaciation) are also shown (Lisiecki 2005; Etourneau et al. 2009). Subtropical Convergence during the Early Pleistocene (bottom right, De Dinechin et al. 2009).



GENERAL RESEARCH AIMS AND THESIS STRUCTURE

The overarching aim of this research is to address knowledge gaps in our understanding of the conservation genetic status of threatened seabirds in the Agulhas-Benguela Ecosystem (ABE). Using data from molecular markers, and supported by available ringing records and census data, this thesis explores the population genetic structure and phylogeographic history of three species of endemic, coastal-breeding southern African seabirds: the Cape Gannet *Morus capensis*, African Penguin *Spheniscus demersus* and Cape Cormorant *Phalacrocorax capensis*. By comparing and contrasting the observed patterns for each of these three largely sympatric, threatened species this thesis explores the influence of their ecological and life-history traits on regional genetic connectivity and the probabilities of their persistence into a future of broad-scale environmental and ecological change. The first part of this study (Chapters 2 and 3) explores the comparative phylogeography and evolutionary history of the three species endemic to southern Africa and assesses their relative vulnerabilities and responses to the observed shift in their shared prey resource in the ABE. Valuable information is also gleaned from comparative studies of closely related species that occupy analogous niches in other upwelling ecosystems around the world. The second part of this thesis (Chapters 4 and 5) focuses on the African Penguin and explores the value of fine-scale molecular data (microsatellites) to its conservation.

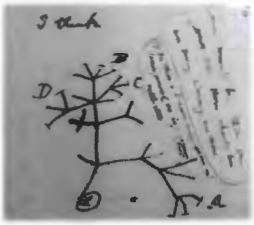
This thesis is structured into four data chapters that provide both a broad- and fine-scale investigation of seabird conservation genetics in the ABE. Chapter 1 provides a general introduction to the study system and the threats facing seabirds worldwide. It contextualises the research presented in this thesis within the broad arena of Conservation Genetics, and describes some aspects of the methodologies employed. Chapter 2 describes the history of the ABE and the evolution of the associated seabird fauna. It details what is known about the deep origins of the study species and their historical biogeography in the dynamic ecosystem in which they evolved, and presents new phylogenetic relationships among cormorants and shags, to provide a taxonomic framework for the comparison of Cape Cormorants to closely related species. Chapter 3 details a comparative genetic study of the three focal species, specifically investigating the utility of sequence-based markers to detect population structure. The focal species are broadly sympatric and rely mainly on the same prey base, but they differ in a number of ecological traits i.e. foraging ranges, flexibility in prey and foraging habitat preferences, clutch sizes and breeding phenology. These differences allow an examination of the extent to which ecological traits affect genetic structure among seabird populations in the ABE. Top-predators, such as seabirds, are likely to have adapted their

foraging strategies and movements to the distribution of their shared prey across scales (Reed et al. 1999; Fauchald & Tveraa 2006; Erikstad et al. 2009).

In Chapters 4 and 5, biparentally inherited microsatellite markers are used to explore the fine scale spatial distribution of genetic variation within one of the study species, the African Penguin. This species has the highest threat status of the three focal species and, based on its life-history and behavioural characteristics, is under the greatest pressure to respond to environmental change. Chapter 4 describes the population genetic structure and tests for signatures of recent population declines among free-living populations of the species. Chapter 5 explores *ex-situ* genetic variation in captive populations of African Penguins (from South African zoos and aquaria) and assesses a role for these populations as a potential source for supplementing wild populations.

Chapter 6 is a General Discussion of the significance of the results in the context of environmental change, marine biodiversity conservation and conservation breeding programmes.

CHAPTER 2: The dynamic evolutionary history of the Agulhas-Benguela region, and deep origins of the study species



*"**Seen in the light of evolution**, biology is, perhaps, intellectually the most satisfying and inspiring science. Without that light it becomes a pile of sundry facts - some of **them interesting or curious but making no meaningful picture as a whole.**"*

T. Dobzhansky (1973, p. 129)

Summary

Globally, population genetic and phylogeographic structure in seabirds have proven difficult to predict based on species' taxonomy, distribution, vagility or life-history characteristics. Little or no population differentiation has been detected in some highly philopatric species over scales of up to thousands of kilometres, while significant structure has been detected among colonies on small islands or archipelagos. Understanding connectivity among seabird populations remains challenging and is important to ensure that appropriate conservation strategies are implemented. Molecular methods have been employed in studies of numerous seabird taxa that differ in their environmental tolerances, foraging modes, developmental rates and patterns, choice of nesting sites, colony densities, and phylogenetic origins. As the literature grows, general patterns are emerging regarding the relative importance of physical and non-physical barriers to gene-flow, and the roles of historical and contemporary processes, in shaping seabird populations. Approaches that combine independent sources of evidence e.g. banding data and molecular data are proving the most powerful for studying population connectivity.

Published reviews largely neglect the focal groups of this study (Suliformes and Sphenisciformes). Given the close interspecific relatedness within the genera *Spheniscus* and *Morus*, comparisons with congeneric species will help elucidate the historical and contemporary drivers of population genetic and phylogeographic patterns within African Penguins *S. demersus* and Cape Gannets *M. capensis*. Comparisons with other cormorants may also shed some light on the patterns observed among Cape Cormorant *Phalacrocorax capensis* populations. Differences in life-history and behavioural characteristics will affect the way in which population-level responses to environmental changes manifest, so a thorough understanding of these differences is necessary to properly interpret comparative phylogeographic results.

INTRODUCTION

This chapter describes the focal study species in detail in order to contextualize them in terms of their foraging ecology, breeding biology and movement patterns. Additionally, this chapter has taxonomic elements insofar as it describes the evolutionary patterns that have been found in seabirds that are closely related to the focal species or those that share life-history and/or behavioural characteristics with the focal species. Reviewing similar studies of seabirds informs later interpretation of evolutionary patterns, and helps to identify potentially important drivers of the observed patterns. Phylogeographic studies aim to elucidate the fundamental links between population processes and regional patterns of genetic diversity (Avise et al. 1987; Bermingham & Moritz 1998; Avise 2000; Beheregaray 2008), and the analysis of genetic data in a comparative framework, comparative phylogeography, seeks to identify the shared processes that shape the distribution of genetic diversity within co-distributed taxa (Bermingham & Moritz 1998; Arbogast & Kenagy 2001; Rossetto et al. 2009). These taxa can be seen as "natural replicates" that may show phylogeographic concordance because they have been subjected to the same environmental history (Arbogast & Kenagy 2001). Ideally, the species being compared should be of a similar evolutionary age and be sufficiently similar in biology and distribution that they are likely to share responses to changes in the environment (Zink 2002; Lawson 2010). The interpretation of empirical data in such studies should, whenever possible, incorporate independent information about climate, geology and paleobiology of the landscape, and the biology, taxonomy and demographic history of the species under consideration (Bermingham & Moritz 1998; Zink 2002).

Seabirds and the Agulhas-Benguela Ecosystem

Based on archaeological records the Agulhas-Benguela Ecosystem (ABE) off the coast of southern Africa has supported seabirds for millions of years (Olson 1983, 1985; Klein et al. 2004; Olson & Hearty 2009; Ksepka & Thomas 2012; Thomas & Ksepka 2013). Evidence from palaeocoeanographic studies of the ABE indicates that upwelling off south-western Africa initiated approximately 8-10 million years ago (Mya), with current patterns and intensity emerging 2.0-3.2 Mya, during the Pliocene-Pleistocene cooling transition (Marlow et al. 2000; Etourneau et al. 2009). Recent studies using a number of proxies of palaeo-productivity derived from sediment cores (Ocean Drilling Program, 1985-2003) report varying results with respect to the age of the system (Kastanja et al. 2006; Etourneau et al. 2009), but all suggest that shifts toward higher productivity are associated with cooling and

increased wind-driven upwelling (Marlow et al. 2000; Berger et al. 2002; Jahn et al. 2003; Lazarus et al. 2006; Etourneau et al. 2009).

The ABE supports a wide variety of seabird species from phylogenetically diverse lineages, some of which visit the coast to forage in the productive ABE waters, and 16 of which breed in the region (Crawford et al. 2006c). Most of these species are not each other's closest extant relatives, and often their putative sister taxa inhabit the coastlines of other continents (Schreiber & Burger 2001; Hockey et al. 2005; Baker et al. 2006; Patterson et al. 2011). Closely related seabird species inhabiting other eastern boundary upwelling systems (EBUS) around the world are considered ecologically analogous to ABE endemics (Crawford et al. 2006c). They depend on forage fish in the same way and are subject to similar anthropogenic threats. Although seabirds were highly likely to have been present in the ABE prior to the initiation of upwelling, information regarding species assemblages in the region, and their relative abundance is difficult to ascertain from available fossil material. The contemporary lineages that characterise the seabird community of the Benguela Ecosystem are likely to have colonised the region after the initiation of upwelling, because the whole ABE relies on the productivity resulting from that process (Olson 1983, 1985; Schreiber & Burger 2001). Estimates of species divergence times support this proposal; e.g. the Cape Gannet *Morus capensis* diverged from the Australasian Gannet *M. serrator* between 0.5 and 4 Mya (Patterson et al. 2011), and African Penguins *Spheniscus demersus* diverged from Magellanic Penguins *S. magellanicus* approximately 3.5 Mya (Baker et al. 2006; Ksepka & Thomas 2012). Distantly related sympatric seabirds in the ABE that rely on similar resources and face similar natural threats have had to adapt to the same environmental changes and, although they may differ in their ecological traits, comparisons between them may provide insight into the adaptive importance of plasticity in terms of the species' ability to respond to a changing environment.

A number of ABE breeding endemics are similar to each other in that they rely on the same prey resource – shoaling pelagic fish species, also known as forage fish (Crawford et al. 1992b, 2001). Conservation genetic studies of these related species will provide an additional comparative context within which to interpret the results presented in this thesis. Many studies have compared the survival, breeding success and other characteristics of the eight endemic breeding species in the ABE, taking advantage of their shared life-history and behavioural characteristics (e.g. natal site fidelity, longevity, small clutch size, mobility; Crawford et al. 2006b, 2008d, 2009), and shared threats. Seabirds endemic to the ABE generally have a poor conservation status, with nine out of 16 classified as Near Threatened, Vulnerable, Endangered or Critically Endangered (Crawford et al. 2012; Table 2.1). This

thesis focuses on two Endangered and one Vulnerable ABE endemic that depend on pelagic fish (sardines *Sardinops sagax* and anchovies *Engraulis capensis*) for their survival.

Table 2.1 Seabirds that breed in the Agulhas-Benguela Ecosystem (ABE), their endemism to the region and IUCN red list status. Taxon names in bold are the focal species of this thesis.

Common Name	Scientific Name	Breeding endemic	IUCN Status (2012)
African Penguin	***Spheniscus demersus***	**Y**	**EN**
Cape Gannet	***Morus capensis***	**Y**	**VU**
Cape Cormorant	***Phalacrocorax capensis***	**Y**	**EN**
Bank Cormorant	*Phalacrocorax neglectus*	Y	EN
Crowned Cormorant	*Phalacrocorax coronatus*	Y	LC
African Black Oystercatcher	*Haematopus moquini*	Y	LC
Hartlaub's Gull	*Larus hartlaubii*	Y	LC
Damara Tern	*Sterna balaenarum*	Y	NT
Kelp Gull	*Larus dominicanus vetula*	Y	LC*
Swift Tern	*Sterna bergii bergii*	Y	LC*
White-breasted Cormorant	*Phalacrocorax lucidus*	N	LC
Great White Pelican	*Pelecanus onocrotalus*	N	LC (NT*)
Leach's Storm Petrel	*Oceanodroma leucorhoa*	N	LC (CR*)
Grey-headed Gull	*Larus cirrocephalus*	N	LC
Caspian Tern	*Sterna caspia*	N	LC (NT*)
Roseate Tern	*Sterna dougalli*	N	VU

* In southern Africa (not elsewhere in the breeding range)

Historical exploitation of seabirds in the ABE

Historical data on the abundance and distribution of endemic, coastal breeding seabirds in the ABE are relatively well recorded, because the eggs and guano of some species were very lucrative natural resources and extensively harvested as far back as the 1840s (Griffiths et al. 2004), and possibly earlier. The peak in initial disturbance for seabirds breeding in Namibia was the 1843–1845 "white-gold rush", when approximately 300 000 tonnes of guano was removed from the "Penguin Islands", primarily Ichaboe Island (which supported the largest gannetry at the time). A layer approximately 20 meters deep of "high quality" guano was removed from Ichaboe Island, possibly causing some species to start breeding at Hollamsbird, Halifax and Possession Islands (Crawford et al. 1983). Guano collecting ships then turned their attention to the inferior, but still profitable deposits at other breeding colonies. Subsequently, formalised harvesting concessions were granted by the British

Crown (Cape Colony, 1845–1890), South African government (1890–1994) and Namibian government (1994–present). Due to the availability of cheaper artificial fertilisers, the worldwide demand for guano dropped after World War II (Crawford et al. 1983). The removal of guano has had devastating and enduring consequences for breeding seabirds on the islands affected, due to the associated human disturbance, flooding and scarcity of suitable nesting sites (Zietsman 2011).

The disturbance and habitat destruction associated with early (mid-19th to mid-20th century) exploitation certainly had an impact on the survival, distribution and breeding success of seabirds in the region (Crawford et al. 1983), but the extent of this impact is not known because the first comprehensive seabird counts were only carried out in the mid-1950s - more than 100 years after harvesting began (Rand 1959, 1963a,b). Since the 1950s, more regular monitoring by conservation authorities has been undertaken on most species (Crawford et al. 2012). The regional population dynamics of each focal species are discussed in detail in Chapter 3, but overall, the differences in their foraging ecology, breeding biology and dispersal capabilities appear to have influenced their population demographic responses to an altered foraging environment.

STUDY SPECIES

The focal species of this study are the Cape Gannet *Morus capensis*, the African Penguin *Spheniscus demersus* and the Cape Cormorant *Phalacrocorax capensis*. All three species are breeding endemics in the temperate Agulhas-Benguela Ecosystem (ABE), and were historically the most abundant seabirds in the region (Adams et al. 1992).

Figure 2.1 The three focal species of this study: From left to right, the African Penguin, Cape Gannet (Photographs: L. Nupen) and Cape Cormorant (Photograph: V. Barquete). The sexes are alike in all three species (Hockey et al. 2005).

The focal species are largely sympatric across their breeding ranges and often breed on the same coastal islands (Hockey et al. 2005). Breeding colonies can be broadly divided into three geographic regions (Figure 2.2): colonies in the northern Benguela, along the Namibian coastline; those in the southern Benguela, along the coast of the Western Cape Province of South Africa; and colonies along the south east coast of South Africa, off the Eastern Cape Province (Hockey et al. 2005). Cape Gannets breed at six localities in the ABE, African Penguins at 28 and Cape Cormorants at 70, however only 23 of the latter colonies are regularly monitored, as they represent more than 90% of the population (Crawford et al. 2007b). In Namibia, Cape Gannets breed on three offshore islands (from north to south): Mercury Island, Ichaboe Island and Possession Island. African Penguins and Cape Cormorants also breed on these three coastal islands, but penguins breed at an additional seven localities in Namibia (populations are known to be extinct on Pomona Island and North Reef) and the breeding range of the Cape Cormorant extends north to Ilha dos Tigres in southern Angola (Hockey et al. 2005; Crawford et al. 2007b). Although Cape Cormorants breed at numerous localities in this region, the vast majority breed on the three islands mentioned above, the artificial platforms constructed at Cape Cross (north and central platforms, 1950s; Figure2.2), Swakopmund and Walvis Bay (Bird Rock, 1930), and Penguin Island, Seal Island and three smaller islands towards the south (Crawford 2007a).

In South Africa, the Cape Gannet breeds at three colonies: Malgas Island and Lambert's Bay in the Western Cape, and Bird Island in the Eastern Cape, where the largest gannetry exists today. The African Penguin currently breeds at 11 colonies in the Western Cape, having ceased breeding in the northernmost colony at Lambert's Bay in 2006. About 40% (~44 000 pairs) of the Cape Cormorant population breeds in the Western Cape, more than half of which breed at Dyer Island. African Penguins breed at two colonies in the Eastern Cape, one of which is, at present, the largest penguin colony (St Croix Island, estimated 8500 pairs).

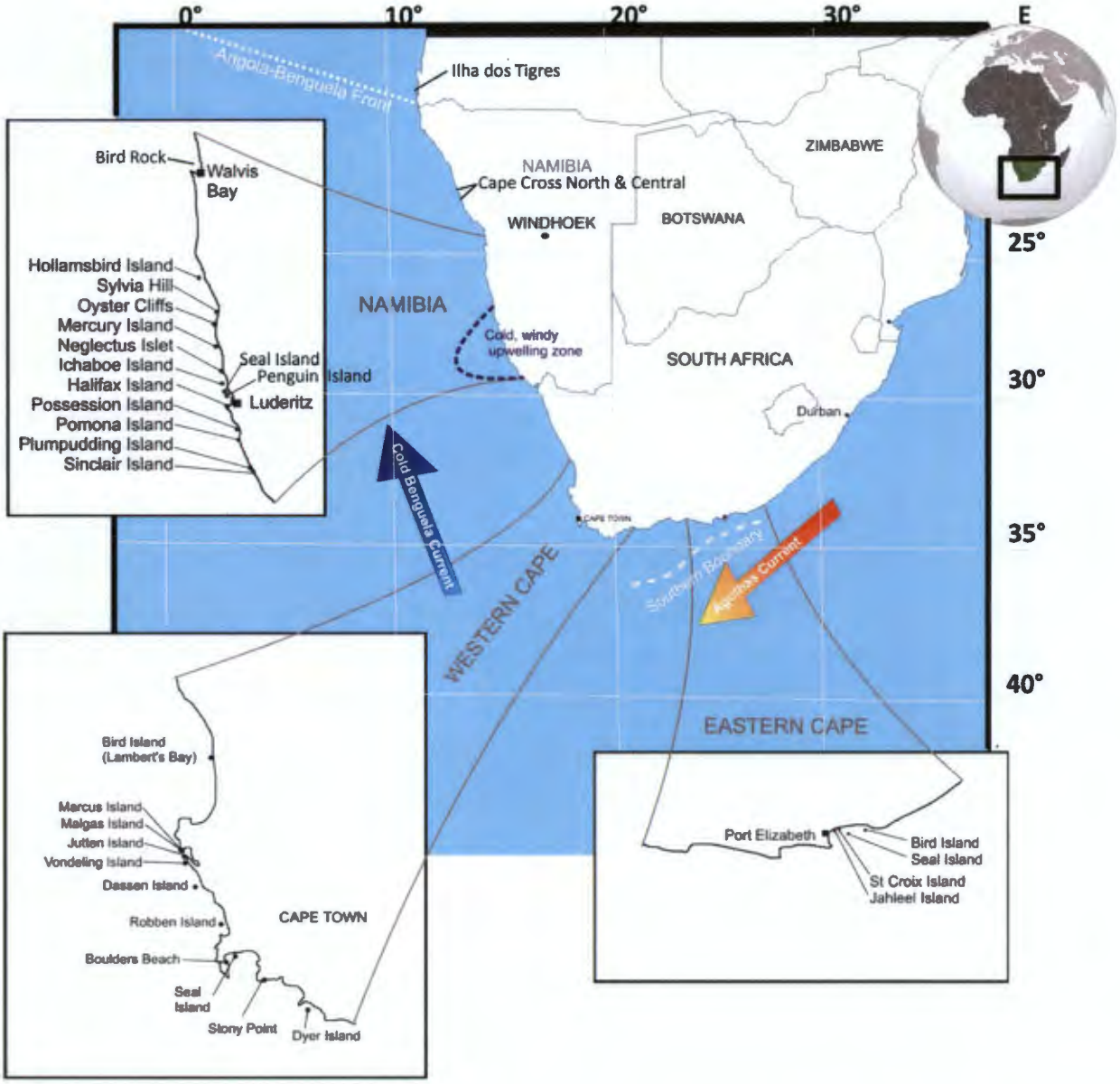
Evolutionary emergence of the study species in the Benguela System

Although this study is the first population genetic study on each of the focal taxa, they have each been included in family-level molecular phylogenies: the Sulidae (Friesen & Anderson 1997; Patterson et al. 2011), Phalacrocoracidae (Kennedy et al. 2009) and Spheniscidae (Baker et al. 2006) have undergone recent molecular phylogenetic treatments (Kennedy et al. 2001; Friesen et al. 2002; Banks et al. 2006; de Dinechin et al. 2009).

No fossil seabirds have been recovered from southern African deposits that pre-date the development of the Benguela upwelling system i.e. the first known South African fossil seabird fauna appeared at approximately the same time as the development of the ABE

(Schreiber & Burger 2001). The late Mid-Miocene global cooling (Monterey Excursion ~10Mya) coincided with the initiation of cold upwelling in the ABE (Roberts et al. 2011).

Figure 2.2 The Agulhas-Benguela Ecosystem off the coast of southern Africa showing oceanographic features and breeding localities of the three focal species of this study in Namibia, the Western Cape and the Eastern Cape.



Global seabird diversity was higher in the late Miocene, but declined during the Pleistocene, possibly due to the diversification and spread of large marine mammals (Olson & Hasegawa 1979) and a reduction in suitable breeding habitat due to changes in sea-level (Roberts et al. 2011; Ksepka & Thomas 2012; Thomas & Ksepka 2013). Early Pliocene (~5Mya) fossil deposits in the Western Cape indicate that the Benguela seabird community was more

diverse than at present, with evidence of breeding pelagic birds represented by an albatross (Family Diomedeidae), a storm petrel (Family Hydrobatidae), three prions (Family Procellariidae), and a diving petrel (Family Pelecanoididae) – in addition to the penguins and cormorants mentioned above (Olson 1983, 1985; Klein et al. 1999; Cruz-Uribe et al. 2003; Ksepka & Thomas 2012). Warheit (2001) emphasises the importance of the seabird fossil record and asserts that processes measured in both ecological time (e.g. dispersal, competition) and in geological or evolutionary time (e.g. plate tectonics, development of ocean currents, sea-level fluctuations) shape the structure of contemporary seabird communities and populations (Schreiber & Burger 2001). The Benguela Ecosystem is considered a prime example of how historical environmental change (sea-level fluctuations and changes in climate, productivity and oceanography) have shaped the composition of seabird communities (Schreiber & Burger 2001).

The Cape Gannet is one of three species of gannets worldwide, the others being the Australasian Gannet *M. serrator*, and the Northern or North Atlantic Gannet *M. bassanus* (Friesen & Anderson 1997; Patterson et al. 2011), which are all confined to temperate environments. These species, along with seven typically more tropical booby species (Friesen & Anderson 1997; Friesen et al. 2002; Patterson et al. 2011), comprise the family *Sulidae*, which belongs to the Order Phalacrocoraciformes (previously Pelecaniformes). Cape Gannets are distinguishable from the Australasian Gannet based on the length of their gular stripe (which is approximately 35% longer in *M. capensis*) and by differences in tail feather colouration (*M. serrator* has white outer tail feathers). Australasian Gannets have been recorded at South African colonies numerous times (Cassidy 1983; Olson 1985; Berruti 1988; Dyer 1990; Dyer et al. 2001) and Cape Gannets have been observed breeding at Australian colonies (Crawford et al. 1983; Dyer et al. 2001; Robertson & Stephenson 2005). At least ten Cape Gannets have also been reported as vagrants in Spain, Argentina, Brazil and Peru (García-Godos 2002; Rebstock et al. 2010). Neither of the other two species is considered threatened and both populations are increasing (www.iucnredlist.org). For Northern Gannets this increase has been attributed to long-term (century-scale) warming of surface waters, resulting in increased mackerel *Scomber scombrus* availability (Montevecchi & Myers 1997; Barrett 2008). The increase in Australasian Gannet numbers over the past century is likely due to the long-term warming trend in sea-surface temperature (SST) in south-eastern Australia (Schwartzlose et al. 1999), which is associated with an elevated abundance of Victorian pilchard *Sardinops sagax* (Bunce et al. 2002). The Cape Gannet is the only member of the Sulidae that breeds in Africa, although a small *Sula* (booby) species has been described from the Langebaanweg and Duinefontein fossil deposits in the Western

Cape (Olson 1985; Schreiber & Burger 2001). The split between the Australasian and Cape Gannet has been estimated (from genetic data) at 2-4.1 Mya (Patterson et al. 2011), although there is some debate as to whether they should be considered sub-species or full species (Robertson & Stephenson 2005). Several extinct gannet species had a more equatorial distribution, similar to that of extant boobies, and Olson (1985, p.7) postulated that "*Morus* probably did not disperse to the Southern Hemisphere until after the early Pliocene", as no gannet fossils are known from any site in the Western Cape.

The African Penguin is the only extant penguin species breeding in Africa, although at least four contemporaneous fossil taxa have been described from the Western Cape (Olson 1983, 1985; Baker et al. 2006; Ksepka & Thomas 2012). The other extant "banded penguins" (Genus *Spheniscus*) are the temperate adapted Magellanic Penguin (*S. magellanicus,* sister to the African Penguin), Humboldt Penguin (*S. humboldti)* and Galápagos Penguins (*S. mendiculus*) of the west and south coasts of South America and the Galápagos Islands respectively (Baker et al. 2006). All four of the extant *Spheniscus* penguins are threatened, with IUCN categories ranging from Near Threatened (Magellanic Penguin) to Endangered (Galápagos Penguin). The African Penguin and Magellanic Penguin diverged 2.3-4.5 Mya based on molecular data (Baker et al. 2006). The remaining 14 species of penguin – 12 of which appear on the IUCN Red List (Waller 2011) – are confined to Antarctica, sub-Antarctic Islands in the Southern Ocean, New Zealand and southern Australia.

Spheniscus likely has a South American origin. The oldest *Spheniscus* fossil is known from late Miocene (11–13 Ma) deposits in Peru (Göhlich 2007), where other *Spheniscus* fossils have also been discovered (*S. urbinai, S. chilensis* and *S. megaramphus*). The oldest known penguin fossil from Africa is a single Miocene bone from the Western Cape (Olson 1983; Thomas & Ksepka 2013), and Thomas & Ksepka (2013) describe a diverse group of Middle to Late Miocene (10 to 12 Mya) penguins ("Sphenisciformes *A" to "D")* from southern Africa that approximate the size range of modern penguins. The relationship between these fossil taxa and those described from southern African Pliocene deposits (5.3 to 2.6 Mya) are however uncertain (Thomas & Ksepka 2013). *Inguza predemersus* (previously *Spheniscus predemersus*), *Nucleornis insolitus, 'P.' huxleyorum* and *D. hendeyi* are represented in Pliocene fossil deposits from the Western Cape, but appear to be only distantly related (not ancestral) to the extant *Spheniscus* species (Ksepka et al. 2006; Ksepka & Thomas 2012) and all were extinct prior to the Mid-Pleistocene. Ksepka and Thomas (2012) posit that these Pliocene fossil taxa from southern Africa represent two separate Cenozoic colonization events, and that the ancestor of *S. demersus* was the third and most recent arrival. A Bayesian reconstruction of ancestral areas strongly suggests a South American origin for the

African Penguin, sometime between the early Pliocene and Mid-Pleistocene, but whether it occurred contemporaneously with the other penguin taxa remains uncertain (Ksepka & Thomas 2012). In fact, the oldest fossil evidence of African Penguins in the Western Cape dates to only 270 – 400 thousand years ago (Klein et al. 1999; Cruz-Uribe et al. 2003; Thomas & Ksepka 2013). If African and Magellanic Penguins shared a common ancestor 2.3-4.5 Mya, as indicated by molecular data, then African Penguins must have evolved *in situ* from an established Late Miocene / Early Pliocene ancestor that colonised Africa from South America.

The Cape Cormorant is one of five cormorant species that breed in southern Africa, the others being the Bank Cormorant *Phalacrocorax neglectus*, Crowned Cormorant *P. coronatus*, White-breasted Cormorant *P. (carbo) lucidus* (see Table 2.1) and Reed Cormorant *P. africanus*. The species-level phylogeny for the Phalacrocoracidae is incomplete, but places the Cape Cormorant as sister to a group of macro-cormorants (Kennedy et al. 2000), represented in the phylogeny by the Japanese Cormorant *P. capillatus* and Great Cormorant *P. carbo*. The White-breasted Cormorant, which is widely considered a regional variant of the Great Cormorant, is therefore the closest sympatric relative of the Cape Cormorant, but based on morphology, the Japanese Cormorant is likely more closely related. Some 40 species of cormorants and shags worldwide make up the family Phalacrocoracidae and a comprehensive molecular phylogeny that incorporates all extant species is yet to be published. This makes estimating the time of emergence of the Cape Cormorant difficult, although Olson (1985) describes two fossil cormorants from the Western Cape. Klein et al. (1999) found evidence indicating that Cape Cormorants have bred in that region for at least as long as the African Penguin, but found no evidence of Cape Gannets.

Ecological Comparison of study species

The African Penguin, Cape Gannet and Cape Cormorant share many life-history and ecological characteristics (Hockey et al. 2005) and accordingly face similar anthropogenic threats (Griffiths et al. 2004). As is the case with many seabirds, these endemic species are monogamous, long-lived, colonial breeders with small clutch sizes (Hockey et al. 2005), and exhibit varying degrees of mate and breeding site fidelity (Brown et al. 1982; Hockey et al. 2005). All three species are central-place foragers while breeding (Hockey et al. 2005; Sabarros et al. 2012). The different population-level responses of the three study species to changes in their environment observed over the last few decades provide information about the behavioural flexibility of each species in terms of dispersal, foraging ecology and breeding biology. Controlling for relatedness and differences in life-history characteristics,

these species essentially represent independent replicates to study the adaptive response of top-predators to changes in the environment.

Breeding Biology

Cape Gannets and African Penguins are monogamous and exhibit strong breeding site fidelity once they have settled to breed (Crawford et al. 1994; Whittington 2002; Hockey et al. 2005). Breeding adults, therefore, show less flexibility than juvenile birds when responding to environmentally induced changes in prey availability. Cape Cormorants exhibit more flexibility in choosing their mate and breeding locality (Crawford et al. 1994), as is evidenced by their readiness to colonise artificial breeding habitats, and the large number (>70, Table 2.2) of localities at which they have been recorded breeding (Cooper et al. 1982; Hockey et al. 2005). The relative plasticity of these life-history characteristics may buffer Cape Cormorant populations from changes in their environment. Estimates of Cape Cormorant adult survival are lower than for African Penguins (Randall 1983) or Cape Gannets (La Cock et al. 1987; Crawford et al. 1992a, 2008b; Crawford & Dyer 1995) and the lifespan of adults is shorter; time to accrue mating experience is therefore reduced for this species, possibly impacting breeding success. However, the ability of Cape Cormorants to move between breeding localities provides a different kind of advantage by allowing birds to exploit regional fluctuations in prey availability.

Cape Gannets and Cape Cormorants are naturally surface nesters (Table 2.2), whereas African Penguins preferentially nest in burrows (Kemper et al. 2007), and therefore require appropriate substrate for burrowing. Following the removal of guano (into which penguins naturally scrape their burrows) at most breeding localities, African Penguins nest in the open, making them vulnerable to heat stress, disturbance and predation (Kemper et al. 2007). Predation by Cape Fur Seals *Arctocephalus pusillus pusillus* affects African Penguins and Cape Gannets significantly, especially in the southern Benguela, where it is implicated in causing the local extinction of the Lambert's Bay African Penguin colony in 2006 (Makhado et al. 2006; Crawford et al. 2008c). Also in the Western Cape, predation by Great White Pelicans affects Cape Gannets and Cape Cormorants (Table 2.2), often causing massive breeding failures (Crawford et al. 2008c; de Ponte Machado 2009; Mwema et al. 2010). Differences in breeding phenology may reduce interspecific foraging competition and enable better resource sharing (Sabarros et al. 2012). Breeding phenology differs among the study species, and sometimes among regions within each study species' range (Crawford et al. 1995; Hockey et al. 2005). In South Africa and Namibia, the peak in breeding for Cape Gannets and Cape Cormorants falls between September and March (du Toit 2002; Crawford et al. 2008b). African Penguins breed all year round, but peak between October and

December at most Namibian breeding localities (Kemper et al. 2007; Kirkman 2007). In the Western Cape, the peak breeding season for African Penguins is between March and July, about one month later than in the Eastern Cape (Crawford et al. 1995; Hockey et al. 2005). The regional differences in breeding phenology in African Penguins may make adult breeding dispersal between regions less likely. The study species also differ in their respective average clutch sizes, with Cape Cormorants and African Penguins laying on average more eggs than Cape Gannets (Hockey et al. 2005). Cape Cormorants start breeding at a younger age (~2 years) than African Penguins and Cape Gannets (~4 years, Table 2.2). Cape Cormorant populations can, therefore, be expected to better take advantage of favourable conditions in terms of increased breeding success compared to African Penguins and Cape Gannets, which have a slower population-level response due to evolutionary constraints on breeding (Crawford 1999).

Foraging ecology

The focal species differ markedly in their respective foraging ranges, habitat preferences and levels of behavioural flexibility, which will affect their responses to changes in their environment, such as displacement of their shared, preferred prey. All three species have undergone distributional shifts in their core breeding ranges and are decreasing in numbers overall. Different foraging ranges, food preferences, prey-switching ability and propensity to colonise new breeding localities (Hockey et al. 2005) reflect their respective evolutionary adaptations to the ABE. All three study species forage near their breeding colonies and near the coast (Furness & Cooper 1982). This suggests the existence of cultural foraging grounds, which have been implicated as an impediment to breeding dispersal (Schreiber & Burger 2001; Friesen et al. 2007). Recent tracking studies of adult Cape Gannets and African Penguins strongly support this suggestion (Adams & Navarro 2005; Petersen et al. 2006; Cook et al. 2012), even outside their breeding seasons (Grémillet et al. 2008b; Harding 2013). These species are all reliant on sardine and anchovy for their survival, but each species has a different foraging mode (Adams et al. 1992). The non-breeding distributions of these species are not yet well characterised.

The foraging range of African Penguins during their breeding season is 20 to 60km from their colonies (Petersen et al. 2006; Ludynia et al. 2012), similar to that of Cape Cormorants (40km), but significantly shorter than that of Cape Gannets (100 – 250km; Hockey et al. 2005). African Penguins are obligate swimmers, and can pursue prey to depths of over 100m (Whittington 2002; Hockey et al. 2005; Sabarros et al. 2012). Cape Gannets are considered more pelagic (Grémillet et al. 2004) and, although their foraging range varies across

breeding regions (Moseley et al. 2012) they are the most mobile of the three study species (Table 2.2). Cape Cormorants are capable of long-distance movements, but tend to forage inshore. Cape Gannets are plunge-divers that detect prey from the air and dive ~10m in pursuit of prey, sometimes pursuing prey underwater (Ropert-Coudert et al. 2004). They forage on fisheries discards when available (Grémillet et al. 2008b). Cape Cormorants are pelagic- and benthic-feeding pursuit foragers (Wilson & Wilson 1988; Cook et al. 2012) that dive for benthic prey in shallow (10-30m) water near the shore, but also feed on pelagic prey (up to 100m), often in association with Cape Gannets (Hockey et al. 2005; Cook et al. 2012). All three species preferentially feed on sardine and anchovy (Crawford 1999), but proportions of prey species vary spatially and temporally across their ranges (Hockey et al. 2005), and all three species have the ability to switch to alternative prey.

Differences in foraging mode appear to have influenced how each of the focal species has responded to reduced prey availability in the past. Before the collapse of the sardine stock off Namibia, all three species fed primarily on this resource (all >90%), but afterwards they had to rely on alternative prey species (Crawford 1999; Hockey et al. 2005). All three species increased their consumption of pelagic goby *Sufflogobius bibarbatus* in Namibia (Crawford et al. 1991), but African Penguins relied on cephalopods, where Cape Gannets and Cape Cormorants switched to anchovy (Crawford et al. 1991). As anchovy became less available in the Northern Benguela, alternative prey species were (1) too deep in the water column for gannets, which increased their consumption of saury *Scomberesox saurlis scombroides,* and (2) beyond the foraging range of breeding African Penguins (Crawford et al. 1985), leading to massive population declines. The situation off South Africa was different for all three species: Cape Gannet colonies grew or remained stable (Crawford 1999), reflecting their ability to switch effectively between sardine and anchovy (Crawford et al. 2007b).

Table 2.2 Summary of the breeding biology and foraging ecology of the three study species.

Species	Breeding Biology						Foraging and predation			
	No. colonies	Longevity (ad. survival)	Mate fidelity / Breeding site fidelity	Ave. Clutch size (age at 1st br.)	Peak breeding	Nesting	Foraging range (br. ad.)	% obs. <50km of coast / breeding colony	Pelican/gull predation	Seal predation
African Penguin	28	High (0.88-0.96)	strong (86.2%) / strong (ad. 60%)	2 (~4yr)	Oct-Dec (NAM) Mar-Jul (SA)	Burrows	20-60km	85/75 (inshore)	low	high
Cape Gannet	6	High (0.93)	strong/strong (ad.)	1 (~4yr)	Sep-Mar	Surface	100-250km	70/75 (~pelagic)	high	high
Cape Cormorant	>70	Moderate (0.84)	Weak/moderate	2.4 (~2yr)	Sep-Mar	Surface	~40km	95/80 (inshore)	high	low

The proportion of Cape Cormorants breeding in South Africa increased until the 1980s, subsisting largely on anchovy (Crawford et al. 1991), but as the sardine recovered and anchovy became scarce, this species exhibited poor breeding success (Crawford 1999). As sardine recovered in the Western Cape, three new African Penguin colonies were established there (Stony Point (1982), Robben Island (1983) and Boulders (1985)). Breeding Cape Cormorants perform poorly in a sardine-dominated ecosystem and likely disperse to areas where anchovy is abundant (Crawford 1999). As sardines shifted eastwards, their availability to breeding seabirds in the westernmost colonies of the Western Cape decreased, but increased for seabirds in the Eastern Cape, especially Cape Gannets, which have large foraging ranges (Crawford et al. 2008b). Numbers of Cape Gannets breeding in the Western Cape and the Eastern Cape were significantly correlated with sardine distribution, decreasing in the west and increasing in the east. By 2005, the core distribution of sardine was out of reach for breeding gannets in the Western Cape (Lewis et al. 2006), but was increasingly available to those in the Eastern Cape (Crawford et al. 2008b, 2009). Due to their shorter foraging range, African Penguins breeding in the Eastern Cape did not benefit from the eastward displacement of prey (Crawford et al. 2008b), and by 2005, sardines were out of reach for penguins breeding in both the Western and Eastern Cape (Crawford et al. 2008b).

The reduction in food availability has resulted in Cape Gannets foraging further off-shore and feeding on nutritionally suboptimal prey items (Mullers et al. 2009), such as hake *Merluccius* spp. offal from demersal trawlers (Grémillet et al. 2008b; Moseley et al. 2012). The lower energy content of this diet, combined with increased foraging effort, likely reduced the breeding success of Cape Gannets in the Western Cape (Moseley et al. 2012). In South Africa, the eastward shift of breeding gannets (Figure 3.3) tracked shifts in the distribution of their prey (sardine and anchovy) over the past two decades (Fairweather et al. 2006; Crawford et al. 2007a; Kirkman 2007). The numbers of Cape Gannets breeding at the westernmost colonies in South Africa decreased by ~35% between 1987 and 2007, while increasing by over 100% at Bird Island in the Eastern Cape (Crawford et al. 2008b), however there was no detectable decrease in survival in the Western Cape (Distiller et al. 2012). The primary mechanism driving this shift in the breeding distribution of Cape Gannets is uncertain i.e. the relative roles of adult or juvenile movement versus differential breeding success at colonies in different regions, are unknown. Crawford et al. (1983) reasons that the growth of the South African colonies must be attributed partly to the movement of juvenile birds, as numbers have grown too fast to be accounted for by increases in local breeding success and survivorship alone. Based on ringing data, census counts and the relative

proportions of each species breeding in the northern and southern Benguela, it has been suggested that African Penguins and Cape Gannets are buffered against decadal-scale ecosystem changes by the emigration of first-time breeders from natal colonies to colonies or regions where foraging conditions are more suitable (Crawford 1999; Crawford et al. 2008c; Pichegru et al. 2010b).

Life-history theory predicts that seabirds will respond to deteriorating environmental conditions e.g. reduced food availability, increased predation and extreme weather events, by reducing their current reproductive effort in favour of survival, so that lifetime reproductive success can be maximised (Oro & Furness 2002). Minor reductions in food availability will, therefore, result in modified activity budgets and/or prey switching in adults, but not affect their survival; decreased breeding success and chick growth rate are expected following moderate/longer term food shortages, but decreases in adult survival rates will only be observed following severe food shortages, by which time the effects on breeding success would be catastrophic (Oro & Furness 2002). For philopatric seabirds, juvenile dispersal to where conditions are better may be a life-history trade-off that allows a population to track changes in food availability, but during times of severe food shortage, when recruitment is low, the pool of potential dispersers is small, which will constrain the population's ability to respond.

Regional patterns of demographic change suggest that Cape Gannets have dispersed *en mass* to breed in regions where conditions are more favourable, which contradicts the observed natal site and breeding site fidelity (Crawford et al. 1994; Distiller et al. 2012). Demographic changes in African Penguin populations i.e. the proportion of birds breeding in each broad geographic region, suggests that they too have attempted to track changes in prey distribution. The trends observed among Cape Cormorant populations appear to be following the same trajectory, but are lagging behind the other two study species. Various authors have suggested that the major effect of the changes in pelagic fish distribution is exerted on juvenile recruitment rather than dispersal or survival i.e. pre-breeders that prospect at breeding colonies other than their natal colony may settle there to breed, while adults that have previously bred (or attempted to breed) at a particular colony will remain there (Crawford 1999; Distiller et al. 2012). These studies raise the possibility that natural or historical dispersal patterns (levels of gene-flow) have been disrupted or elevated by human- or environment-induced changes in food availability over the past few decades. Foraging and breeding behaviour of the three seabird species considered in the present study suggest that their three breeding regions represent demographically separate populations; however no molecular studies have been carried out to investigate levels of gene-flow between these

regions. If there is congruence in biogeographic patterns among these groups of distantly related taxa, a simultaneous, historical cause could be inferred as the primary determinant (Hoberg 1992).

There is increasing evidence that individual plasticity (the ability of an individual with a given genotype to alter its phenotype in response to environmental changes) is an important mechanism for populations to overcome changes in their environment (Nussey et al. 2005; Ellegren & Sheldon 2008), including climate change (Parmesan 2006; Fretwell et al. 2014). Genetic variation represents the pool of possible genotypes – and phenotypes – in a population and selection sifts out the fittest individuals or those best able to survive and reproduce in the prevailing environmental conditions, necessitating a thorough understanding of the factors that influence the generation and maintenance of genetic diversity over the long-term (Taylor et al. 2011b). Levels of genetic diversity have not previously been quantified across the ranges of any of the focal species of this study. The similarities between these three sympatric species present an excellent opportunity to investigate how gene-flow and population genetic differentiation in seabird communities are affected by changes in the marine environment (Morris-Pocock 2012).

Movement patterns inferred from ringing data

The demographic and evolutionary trajectories of populations can be profoundly influenced by dispersal and the degree of genetically effective dispersal is thought to be positively correlated with dispersal capacity across taxa (Dearborn et al. 2003). Movement capabilities may not reflect true dispersal patterns within seabirds, which are generally reluctant to disperse, despite being highly mobile, due to the benefits of philopatry and coloniality (Milot et al. 2008). Breeding site fidelity and mate fidelity improve lifetime reproductive output in long-lived birds, which is especially important for seabirds given their generally poor juvenile survival rates (Schreiber & Burger 2001). Little is known with confidence about the relative propensity of the three study species to disperse to new colonies or regions to breed, but for seabirds in the ABE more generally, 'nomadism' or dispersal to non-natal colonies seems to be constrained by an attachment to existing breeding colonies (Crawford et al. 1994). However, historical disturbance by humans and competition with Cape Fur Seals *Arctocephalus pusillus pusillus* have resulted in changes in breeding localities of even those species thought to be the least nomadic (Crawford et al. 1994; Roux et al. 2003). African Penguins and Cape Gannets show strong fidelity to specific localities (Table 2.2), whereas Cape Cormorants are thought to exhibit more flexibility in this regard (Crawford et al. 1994). Schreiber & Burger (2001) report the site and mate fidelity respectively as 60% and 86% for

African Penguins (similar to Magellanic and Galápagos Penguins, Table 2.2), and 90% and 84% for Northern Gannets (no data for Cape Gannets). Breeding site and mate fidelity have not been quantified for Cape Cormorants, but the site fidelity of cormorants is generally low, ranging from 49% to 62% (Schreiber & Burger 2001), and mate fidelity is even lower (40 - 69%, Schreiber & Burger 2001).

The propensity of seabirds to disperse is thought to evolve under diverse, sometimes opposing forces e.g. intraspecific competition, inbreeding avoidance, spatial and temporal heterogeneity of resources and the fitness costs associated with dispersing – it may be advantageous to offspring to return and breed at their natal colony where their parents' genotype was successful (Lequette et al. 1995; Milot et al. 2008). Movement between breeding colonies only influences gene-flow if immigrant birds settle and breed successfully (Dearborn et al. 2003). It is also important to consider whether birds were re-sighted during the breeding season. All else being equal, readily nomadic species are likely to respond more rapidly to changes in their environment by moving to localities where the cost of reproduction is lower and are, therefore, better candidates for indicators of ecosystem health (Crawford et al. 1994).

Cape Gannet ringing data

Various authors have investigated Cape Gannet movement patterns and survival using ringing records (Broekhuysen et al. 1961; Oatley 1988; Oatley et al. 1992; Klages 1994; Distiller et al. 2012) from the South African Bird Ringing Unit (SAFRING, Animal Demography Unit (ADU), University of Cape Town; Oschadleus & Underhill 1999). Cape Gannets are the third most-ringed birds in southern Africa, with over 140 000 ringed since the 1950s (Oschadleus & Brooks 2006). The SAFRING database currently contains over 106 566 records, with 2.5% confirmed deaths and 29% re-traps. This database provides the opportunity to study Cape Gannet movement patterns over multiple generations (Oschadleus & Underhill 1999). Spatial and temporal ringing effort and recapture rates are uneven across breeding colonies (Klages 1994), with the vast majority of individuals ringed at the two biggest colonies: Ichaboe Island (Namibia) between 1956 and 1981 (Kemper & Crawford 2007), and Bird Island (Eastern Cape) since 1982.

In 1988, 77 400 Cape Gannets had been ringed and only 849 (1.1%) were re-trapped at colonies other than their ringing site (Oatley 1988), indicating strong site fidelity (Crawford et al. 1983; Oatley 1988). Klages (1994) later assessed site fidelity and non-breeding dispersal of juvenile and adult birds at Bird Island (Algoa Bay, Eastern Cape), and established that all ringed survivors of post-fledging dispersal returned to their natal-colony

to breed. That study found little evidence of inter-colony exchange - although seven individuals from Western Cape colonies were observed on Bird Island, none bred (Klages 1994). Klages (1994) reported low levels of breeding dispersal between Bird Island and other gannet colonies. Interestingly, on the west coast, between 1978 and 1997, 59 Cape Gannets that were ringed as chicks were reported breeding at colonies other than their natal colony (Crawford 1999). Another 27 were repeatedly observed at non-natal islands over long periods (>6yrs) and possibly also transferred colonies. Sixteen of these 86 known or supposed breeding immigrants were ringed as chicks at Namibian colonies and moved to Lamberts Bay or Malgas Island (Western Cape colonies), and 70 birds were exchanged between Malgas Island and Lamberts Bay (Crawford 1999). Overall, the majority of birds were faithful to the breeding colony where they initiated breeding (Crawford 1999). More recently, Oschadleus & Brooks (2006) found that adult Cape Gannets usually remain within 540km of their breeding site, and that while adult birds moved a maximum of 3 300km from their breeding colony, juveniles (under two years) migrated up to 6 800km (Oschadleus & Brooks 2006). Most inter-colony movement occurred between the closest colonies (Malgas and Lambert's Bay), but some movement occurred between South African and Namibian colonies. The most isolated colony was Bird Island, Algoa Bay (Oschadleus & Brooks 2006). Between 1975 and 2006 only two gannets ringed in Namibia were retrapped in the Eastern Cape, compared to 116 birds that moved between Namibia and the Western Cape and 98 between the Western Cape and Eastern Cape. Distiller et al. (2012) investigated movement between the three South African colonies (no Namibian colonies included), and found their results suggest that Cape Gannets are highly philopatric, and that connectivity is stronger between geographically proximate colonies (Distiller et al. 2012).

African Penguin movement data

Adult and juvenile African Penguins are known to visit colonies other than those at which they breed or at which they were born, but they do not migrate to breed at new colonies regularly (Randall et al. 1987; Whittington 2002; Whittington et al. 2005a). Juveniles have been known to travel considerable distances after fledging and adults sometimes forage a large distance away from their breeding colony (Whittington et al. 2005a). Breeding adults show strong fidelity to partners and consequently return to the same breeding colonies, even though conditions at those colonies may have changed (Hockey et al. 2005; Crawford et al. 2007c). SAFRING and the South African Department of Environmental Affairs Oceans and Coasts division curate flipper-banding databases, which provide information regarding the movement and settlement of African Penguins. An early study by Randall et al (1987)

analysed the 184 recaptures (out of a total of 14 500 African Penguins ringed between 1952 and 1984).

Whittington et al (2005a) investigated immigration and emigration rates using flipper-banding data (birds banded as chicks) and estimated that 2% of first time breeders settle to breed at non-natal colonies. Approximately 23 400 African Penguin chicks were flipper banded at breeding colonies between 1970 and 1998 and these included birds from Namibia and the Eastern and Western Cape of South Africa (Whittington et al. 2005b). In that study, a banded chick was assumed to have emigrated if it was observed incubating or nest-guarding at a colony other than its natal colony. Whittington et al (2005a) also investigated which years had the highest emigration estimates. The banding data suggest that the majority of emigrants settle at breeding colonies within the same region (Namibia, Western Cape or Eastern Cape) as their natal colony (n = 4004 re-sighted after banding, 598 seen breeding, n = 514 bred at natal colonies, n = 84 emigrated, n = 13 / 84 emigrants settled in a different region).

Cape Cormorant movement data

Berry (1977) conducted a study on Cape Cormorants at the Swakopmund guano platform (n=25 colour-ringed adults), and found evidence for breeding site fidelity (four of the 25 birds were re-sighted breeding < 10 m of their original ringing site). Crawford et al. (1994) compared the degree of "nomadism" (i.e. how often breeding birds change breeding locality in successive years) of 13 seabird species that breed in southern Africa, and proposed that the degree and pattern of variation in the number of Cape Cormorants breeding at six localities in the Western Cape (WC) suggests "considerable movement of Cape Cormorants between their breeding localities" (pp233). This conclusion contradicts that of Berry (1977) and, unfortunately, the existing ring-recovery data provides no specific evidence of breeding birds moving between islands, nor any examples of birds breeding at any colony other than their natal colony (Underhill et al. 1999). The majority (>50%) of the 553 Cape Cormorant ring recoveries (out of 15 426, data from 1999) represented nestling mortality at the ringing site (Underhill et al. 1999). Over half of the birds that fledged were recovered further than 100km from their ringing site (n=139 >100km, n=32 > 1000km) and most of these movements were along the western coast of southern Africa (Underhill et al. 1999). Although there is no record of a Cape Cormorant ringed as a chick breeding at a location other than its natal colony, the SAFRING ringing data demonstrate the movement potential of this species, with at least four Namibian birds re-sighted in the WC (average distance 1398km), four WC birds recovered in Namibia (average distance = 1511 km) and one Cape Cormorant covering

2102km (ringed in the Eastern Cape and recovered in Namibia). It is evident that the Cape Cormorant exhibits more flexibility in its dispersal and breeding behaviour than the Cape Gannet and African Penguin. Dispersal is a critical process in that it allows populations to cope with environmental changes (Kokko & López-Sepulcre 2006), and the flexibility in Cape Cormorant breeding behaviour may be key to its long-term survival in a changing ecosystem, as it allows individuals to respond to environmental cues and breed more successfully (Crawford et al. 1994). Crawford et al. (1994), on the other hand, states that the uncertainty that arises as a result of nomadic species changing their breeding localities at a higher frequency than their non-nomadic counterparts, makes nomadic species more difficult to protect.

EVOLUTIONARY GENETIC PATTERNS IN SEABIRDS

Population genetic structure in seabirds is commonly thought to be caused by two primary factors: philopatry to breeding colonies (non-physical) and physical barriers to dispersal (historical and contemporary) e.g. wide stretches of unsuitable ocean habitat or land, glaciers and long-term oceanographic changes (e.g. Moum & Arnason 2001, Morris-Pocock et al. 2008). The importance of these barriers to gene-flow, as well as the role of specific life-history characteristics, in shaping population genetic structure in seabird species, remains uncertain. Most seabird species exhibit some degree of philopatry to natal and breeding colonies, and many have naturally fragmented distributions because suitable nesting habitat, often on islands, may be distributed over hundreds or even thousands of kilometres (Schreiber & Burger 2001; Milot et al. 2008). The relative importance of evolutionary histories, differences in foraging and breeding behaviours, and mobility in determining population genetic structure among the three focal species is not currently known, however, insight into potential explanations can be found among studies of other seabird species. Various classes of molecular markers have been applied to seabird populations globally, and have made it possible to detect otherwise cryptic genetic structure resulting from different aspects of the species' biology or environment (Goostrey et al. 1998a; Winney et al. 2001; Steeves et al. 2005b; Friesen 2007; Morris-Pocock et al. 2011; Bicknell et al. 2012; Calderón et al. 2014).

Mark-recapture data have been collected for numerous seabird taxa, but is rarely taken into consideration when interpreting population genetic or phylogeographic studies (Pearce & Talbot 2006; Pearce et al. 2008; Taylor & Friesen 2012). One exception is a study of inter-island movements and population differentiation in the Great Frigatebird *Fregata minor* (see Appendix 2.1), which combined re-sighting data with amplified fragment length

polymorphism (AFLP) data (Dearborn et al. 2003). Although inter-island movements were regularly observed, the study found significant genetic structure among island populations. This result is surprising given that an estimated four migrants per generation would be sufficient to homogenise neutral alleles across populations at equilibrium (Hartl 2000; Dearborn et al. 2003). The apparent paradox is plausible because over half of the re-sightings occurred during the non-breeding season, and morphological differences exist between populations of Great Frigatebirds, suggesting that selection plays a role in maintaining genetic differentiation (Dearborn et al. 2003). Interestingly, a more recent study found no evidence of population genetic structure among Great Frigatebirds on the Galápagos archipelago (Appendix 2.1; Levin & Parker 2012). Another study that combined genetic data with ring returns established that populations of Wandering Albatross *Diomedea exulans* were demographically isolated (one migrant per generation), but not genetically structured (Milot et al. 2008). Similarly, Young (2010), found that rare dispersal events have prevented differentiation among populations of Laysan Albatross *Phoebastria immutabilis* (Appendix 2.1), and have enabled this otherwise highly philopatric species to colonise new breeding sites (Young 2009, 2010; Hunt & Wilson 2012; Taylor & Friesen 2012).

Population genetic and phylogeographic structuring in highly mobile seabirds are difficult to predict from the biology of a species (Dearborn et al. 2003; Friesen et al. 2007; Taylor & Friesen 2012). For example, differentiation among Atlantic Puffin *Fratercula arctica* populations is low (Appendix 2.1), despite marked variation in body size across their range (Moen 1991). A comparative mitochondrial phylogeographic study of sympatric razorbills *Alca torda* and Common Murres *Uria aalge* in the Atlantic Ocean, found some evidence for weak differentiation among populations of the former species (Appendix 2.1), but no phylogeographic structure in the latter (Moum & Arnason 2001). The authors suggest that the observed differences in genetic structure between these two species result from the specialised feeding preferences of Common Murres (almost exclusively shoaling fish), which cause fluctuations in their population sizes (repeated bottlenecks) and leads to unstable population structure (Moum & Arnason 2001). The lack of population structure among Atlantic populations of Common Murres is congruent with the results of a similar study employing microsatellites (Riffaut et al. 2005). A more recent study of Common Murres that included mitochondrial sequence data, and expanded the number of samples and sampling range found some evidence for genetic structure among Atlantic colonies, but not Pacific colonies (Appendix 2.1, Morris-Pocock et al. 2008), probably due to the differential effects of Pleistocene glaciation across oceanic basins that restricted gene-flow between the east and west Atlantic colonies (Morris-Pocock et al. 2008). Banding data indicate that there is

contemporary natal dispersal of juveniles within the eastern Atlantic, but that adult Common Murres are philopatric (Morris-Pocock et al. 2008).

Some seabird species whose ranges encompass two or more oceanic basins show different genetic patterns across their range e.g. Black-legged Kittiwakes *Rissa tridactyla*, Leach's Storm Petrels *Oceanodroma leucorhoa* and Common Murres exhibit different patterns in the Atlantic and Pacific portions of their ranges (Walsh et al. 2005; Morris-Pocock et al. 2008), suggesting a possible role for physical barriers to gene-flow. Highly mobile species with little or no genetic structure include (Appendix 2.1) Thick-billed Murres *Uria lomvia* (Birt-Friesen et al. 1992), Short-tailed Shearwaters *Puffinus tenuirostris* (Austin et al. 1994), Sooty Terns *Sterna fuscata* (although shallow structure exists between Atlantic and Indo-Pacific rookeries due to sea-level fluctuations and rare pulses of gene-flow driven by stochastic events across the meta-population, Avise et al. 2000), Fairy Prions *Pachyptila turtur* (Ovenden et al. 1991), Ancient Murrelets *Synthliboramphus antiquus* (Pearce et al. 2002), Waved Albatrosses *Phoebastria irrorata* (Appendix 2.1; the authors suggest that genetic homogenization is due to post-fledging dispersal, Huyvaert & Parker 2006), Grey-headed Albatrosses *Thalassarche chrysostoma* (Appendix 2.1; the authors posit that genetic panmixia despite breeding philopatry is due to the pelagic foraging mode of this species and mixing between juveniles at foraging grounds, Burg & Croxall 2001), Cory's Shearwaters *Calonectris diomedea* (Randi et al. 1989; da Silva & Granadeiro 1999), Black Guillemots *Cepphus grylle* (Kidd & Friesen 1998a, 1998b), Pigeon Guillemots *Cepphus columba* (Kidd & Friesen 1998a, 1998b) and Buller's Albatrosses *Thalassarche bulleri* (despite banding data indicating strong philopatry; Van Bekkum et al. 2006). Crested Auklets *Aethia cristatella* and the abundant Least Auklets *A. pusilla* both appear to be panmictic within the North Pacific (Walsh et al. 2005). No genetic differentiation was detected among White-capped Albatrosses *Thalassarche steadi*, however, in the closely related Shy Albatross *T. cauta*, there was evidence of genetic structure. Longer distances among populations of the latter species were proposed to explain the different patterns (Abbott & Double 2003).

Significant differentiation between populations of Black-browed Albatrosses *Thalassarche melanophris* was explained by spatial differences in foraging grounds leading to demographic isolation (Burg and Croxall 2001). Similar reasoning was employed by Hailer et al. (2011) to explain the unique genetic signature of the Galápagos population of Magnificent Frigatebirds *Fregata magnificens* compared to the homogeneity of the other populations sampled - although the authors speculated that philopatry, selection and non-breeding ranges may have also played a role in this case. Significant population structure has been detected in a number of seabird species (Appendix 2.1), including: Marbled Murrelets

Brachyramphus marmoratus (Congdon et al. 2000), Cory's Shearwaters *Calonectris diomedea* (Rabouam et al. 2000), Razorbills *Alca torda* (Moum & Arnason 2001), Hawaiian Petrels *Pterodroma sandwichensis* (Wiley et al. 2012; Welch et al. 2012a) and Band-rumped Storm Petrels *Oceanodroma castro* (Smith et al. 2007).

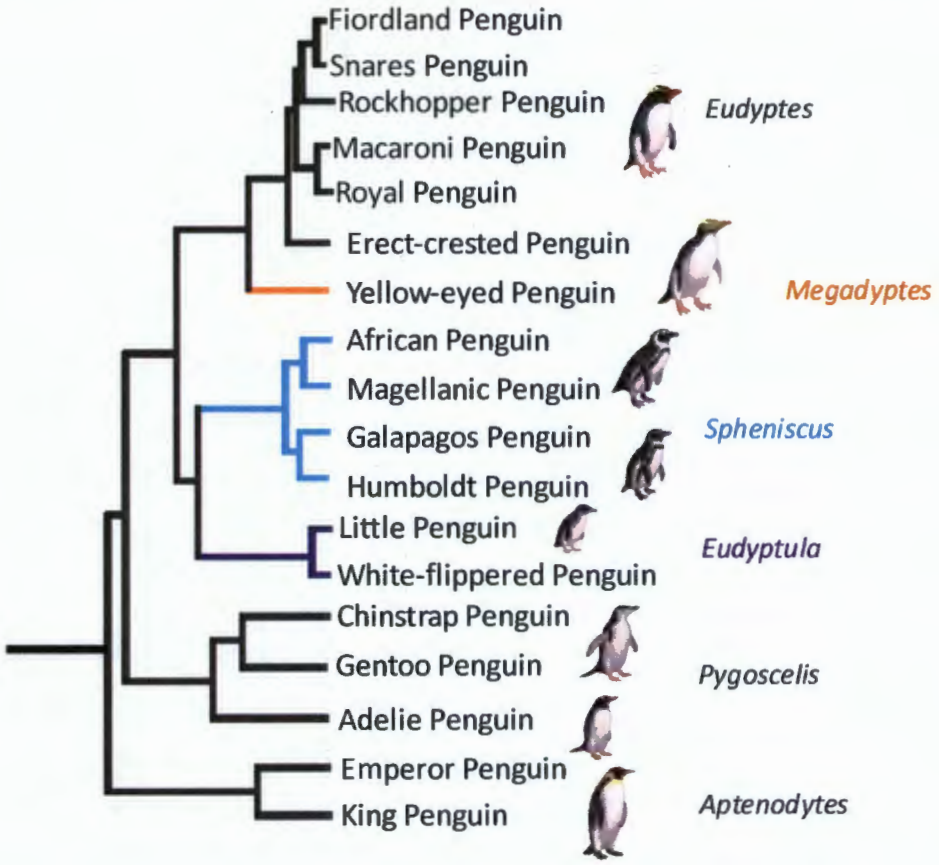
Comparisons among genetic studies of seabird populations should be made carefully, because of – among other factors – differences in the analytical methods and genetic markers used (e.g. allozymes, restriction fragment length polymorphisms (RFLPs), sequence data, microsatellites), the extent and intensity of sampling, the geographic scale of the research, the life-histories and effective population sizes of species, and their demographic and evolutionary histories (Dearborn et al. 2003; Friesen et al. 2007). Pearce et al. (2008) contemporary site fidelity should not be used to infer population structure and demographic independence because theirs, and many other studies, have found inconsistent relationships between population differentiation and dispersal (Pearce et al. 2008).

Overall, the evolutionary genetic pattern exhibited by seabird species is often not what is expected based on their mobility, distribution, philopatric tendencies, foraging mode or other aspects of their biology. Some of the studies mentioned above are included in a review of the mechanisms driving population differentiation in seabirds (Friesen et al. 2007). However, that review only included studies that employed mitochondrial sequence data and only considered one penguin, one cormorant and four booby species among 53 seabird species (Friesen et al. 2007). The results of a number of studies that are relevant to the present investigation, and some that been published recently, are outlined below.

Evolutionary genetic studies of penguins

The most recent molecular phylogenetic treatments of penguins (Tsuda et al. 2001; Baker et al. 2006) suggested that the Spheniscidae arose 50 to 70 Mya, and classify penguin species into six extant genera (Figure 2.4). Phylogenies based on morphology include numerous fossil taxa and largely agree with the molecular data regarding the age of the family (Giannini & Bertelli 2004; Bertelli & Giannini 2005; Slack et al. 2006; Ksepka et al. 2006, 2010; Göhlich 2007; Clarke et al. 2007, 2010; Jadwiszczak & Mörs 2011; Fordyce & Thomas 2011; Thomas et al. 2011; Ksepka & Thomas 2012). The taxon most closely related to penguins is currently a subject of debate (Mayr 2005; Watanabe et al. 2006), but they are thought to be allied to the Procellariiformes (Hackett et al. 2008).

Figure 2.4 Phylogenetic tree of extant penguin species (Baker et al. 2006; Ksepka & Thomas 2012)



The evolutionary history and phylogeography of Antarctica's **Adélie Penguins** *Pygoscelis adeliae* have been studied intensively (Ritchie 2001; Roeder et al. 2001, 2002; Lambert et al. 2002; Ritchie et al. 2004; Shepherd & Lambert 2005; Hinke et al. 2007; Millar et al. 2008; Banks et al. 2009; Subramanian et al. 2009; Trivelpiece et al. 2011). Roeder et al. (2001) found no evidence of genetic differentiation in Adélie Penguins across their range (Table 2.3; Roeder et al. 2001a). They attributed the high levels of gene-flow to episodic dispersal in response to variable environmental conditions and weak genetic drift due to the very large effective population sizes of Adélie Penguin populations (Roeder et al. 2001). Ritchie et al. (2004) identified two monophyletic mtDNA lineages among contemporary and sub-fossil Adélie Penguin samples (Table 2.3) and also did not detect present-day phylogeographic structure i.e. one lineage was present at all sampled colonies, and although the second was largely restricted to the Ross Sea, no significant genetic structure was found. The authors posit that the observed pattern is due to Antarctic glaciation separating the historically connected Adélie Penguin populations into refugia during the last glacial maximum, and subsequent admixture i.e. secondary contact in a warming environment (Ritchie et al. 2004).

A different aspect of paleo-environmental change, the shifting position of the Polar Front and Subtropical Convergence, is implicated in driving the Pleistocene speciation among island populations of another Southern Ocean species, the Rockhopper Penguin *Eudyptes chrysocome* (de Dinechin et al. 2009). During the Early Pleistocene, the Subtropical Convergence migrated as far north as southern Africa and may have played a role in the evolutionary history of many temperate and sub-Antarctic seabird species (de Dinechin et al. 2009). Banks *et al.* (2006) sampled individuals representing all putative Rockhopper Penguin subspecies: the Southern *E. c. chrysocome*, Eastern *E. c. filholi* and Northern Rockhopper *E. c. moseleyi,* and found strong evidence for population differentiation (global φ_{ST}=0.9, Table 2.3) between populations from the Falklands, Crozet and Kerguelen Islands, and Amsterdam (Banks et al. 2006). In a similar study, Jouventin et al. (2006) found evidence of mtDNA population structure among putative Rockhopper Penguin subspecies (Table 2.3). These results, and a comparison of pairwise genetic distances between penguin sister-taxa (Banks et al. 2006), provided motivation for taxonomic revision of Rockhopper Penguins.

Korczak-Abshire et al. (2011) investigated genetic structure among two breeding colonies of the rapidly declining Chinstrap Penguin *Pygoscelis antarctica* in the South Shetland Islands, and found no evidence of population differentiation (Table 2.3; Korczak-Abshire et al. 2012). The authors hypothesize that this pattern is due to considerable gene-flow between populations, and that the violation of natal philopatry may be a response to population declines caused by decreasing availability of their main prey, Antarctic krill *Euphausia superba* (Hinke et al. 2007; Trivelpiece et al. 2011). A recent study of another polar species, the King Penguin *Aptenodytes patagonicus* compared historical radiocarbon-dated samples to modern samples, and found considerable genetic diversity (h~1) among the latter, despite severe population declines (Heupink et al. 2012).

Table 2.3 A summary of population genetic studies conducted on penguins, including their estimated global population size (breeding pairs, bp), mitochondrial (mtDNA) target region, and estimates of genetic diversity (haplotype diversity, *h;* nucleotide diversity, π) and differentiation (global or pairwise range* φ_{ST}), and microsatellite sample sizes (N_{micro}), number of loci and results (Heterozygosity (H_O), global and/or pairwise range of F_{ST}) and the threat status based on the IUCN Redlist of threatened species (2013; Lc=least concern, Nt=near threatened, Vu=vulnerable, EN=endangered). Results are colour coded according to study.

Species	Scientific name	Census population size (bp)	IUCN	mtDNA region*	mt diversity (h |π)	φ_{ST}*	N_{micro}	H_O	F_{ST}	Study
King Penguin	*Aptenodytes patagonicus*	>500 000	Lc	CR	~1|0.0308 (n=17)	-	-	-	-	Heupink et al. (2012)
Rockhopper Penguin (Southern & Northern)	*Eudyptes chrysocome*	Southern Rockhopper >1.2mil Northern >250 000	South Vu; North EN	CR, NADH2; cytb, COI, 12S	0.93|0.013 (n=45 | n=20)	0.4 to 0.79; 0.922	–	–	–	Jouventin et al.(2006); De Dinechin et al. (2009); Banks et al. (2006)
Macaroni Penguin	*Eudyptes chrysolophus*	>9 mil	Vu	cytb, COI, 12S	0.94|0.015 (n=19)		–	–	–	Jouventin et al. (2006)
Adelie Penguin	*Pygoscelis adeliae*	>2 mil	Nt	CR	0.997|0.038 (n=381)	0.029	442 (7 loci)|557 live, 96 ancient	0.25 to 0.9	0.0007	Roeder et al. (2001); Ritchie et al. (2001, 2004)
Little Penguin	*Eudyptula minor*	> 100 000	Lc	CR	(n=222)	AUS=0.11 NZ=0.774	350 (5 loci)	0.077 to 0.685	0.018*	Meredith & Sin (1988); Banks et al. (2002, 2008); Overeem et al. (2008); Peucker et al. (2009)

Yellow-eyed Penguin	*Megadyptes antipodes*	> 3500 indiv.	EN	CR; CR	0.834|0.009 (extinct, n=43)), 0.547|0.004 (extant, n=100); South Island 0.31|0.0012; Sub Antarctic 0.69|0.0028 (n=100)	0.144 (between South and Sub-ant)	350 (12 loci); 27	South Island 0.31; Subantarctic 0.44; Historic 0.33	0.108	Boessenkool et al (2009a, 2009b, 2010);
Galápagos Penguin	*Spheniscus mendiculus*	~1000 indiv.	EN	–	–	–	46|116 (both 5 loci)	3%|0.4	- 0.0025 to 0.0405	Akst et al (2002); Nims et al (2008)
Humboldt Penguin	*Spheniscus humboldti*	>2 500	Vu	–	–	–	336 (12 loci)	0.71	- 0.0010 to 0.0104	Schlosser et al. (2008)
Chinstrap Penguin	*Pygoscelis antarcticus*	> 2 mil	Lc	–	Φ_{PT}=0.077 (n=122)	–	–		–	Korczak-Abshire et al. (2011)
Magellanic Penguin	*Spheniscus magellanicus*	>1 mil	Nt	COI	0.812|0.003 (n=87)	0.057	46 (5 loci); 231 (4 loci)	46%; 0.592	0.011*	Akst et al. (2002); Bouzat et al. (2009)

*CR = control region, COI = cytochrome oxidase I, cytb = cytochrome b, NADH2 = NADH dehydrogenase subunit 2, 12S = mitochondrial 12S ribosomal RNA

The evolutionary history and conservation genetic status of the endangered Yellow-eyed Penguin *Megadyptes antipodes* has recently been thoroughly investigated (Boessenkool et al. 2009a, 2009b, 2010; Lopes & Boessenkool 2009). Boessenkool *et al.* (2009a) investigated temporal changes in Yellow-eyed Penguin mtDNA genetic diversity using prehistoric, historic and modern samples, and established that modern birds have undergone a rapid range expansion as a result of the human-induced extinction of the closely related, previously undescribed and morphologically distinct Waitaha Penguin *M. waitaha* (Table 2.3). In a more comprehensive study of modern Yellow-eyed Penguins, Boessenkool et al. (2009b) detected significant phylogeographic structure between sub-Antarctic breeding colonies and those on New Zealand's South Island (Table 2.3), despite some haplotypes being shared among these regions. No significant structure was detected within the two regions. The authors attribute this pattern to a founder event and limited gene-flow across the water mass that separates the two regions, which includes the subtropical convergence (Boessenkool et al. 2009b). Boessenkool et al. (2010) again expanded the Yellow-eyed Penguin dataset to include microsatellite data from historical samples and estimated the effective population size of the South Island population at 6 to 30% of the census population size (~2200 individuals, Table 2.3), raising concerns about the long-term viability of this population.

Morphological and molecular data place the Little Penguin *Eudyptula minor* as sister to *Spheniscus* (Bertelli & Giannini 2005; Baker et al. 2006), and the population genetics and phylogeography of *E. minor* has been the subject of a number of studies (Meredith & Sin 1988; Banks et al. 2002, 2008; Slack et al. 2003; Billing et al. 2006; Overeem et al. 2008; Peucker et al. 2009). Meredith & Sin (1988) detected a latitudinal cline in allele frequencies among four populations of Little Penguins representing three morphologically distinct subspecies (White-flippered Penguin *E. m. albosignata*, Northern Blue Penguin *E. m. iredalei* and Cook Strait Blue Penguin *E. m. variabilis*). Banks et al. (2002) investigated the putative subspecies relationships within Little Penguins more comprehensively (including the remaining three subspecies: *E. m. chathamensis, E. m. minor* and *E. m.novaehollandiae*) and found evidence for only two clades. More recently, Overeem et al. (2008) found no evidence of phylogeographic structure among Little Penguins from southeast Australia, corroborating the findings of Banks et al. (2002, Table 2.3). The genetic homogeneity among Little Penguin colonies has been attributed to contemporary juvenile recruitment and historical population expansion due to increased availability of breeding habitat as a result of post-Pleistocene sea-level rise (Overeem et al. 2008). A more recent, range-wide mtDNA

phylogeographic study of Little Penguins (Peucker et al. 2009) found little structure among Australian colonies (Table 2.3), and confirmed their close relatedness to a subset of colonies in New Zealand, but found significant structure among the remaining, morphologically variable New Zealand localities (Table 2.3). The observed structure is not, however, congruent with the proposed subspecies classification. The existence of two major lineages that appear to have undergone secondary contact has repeatedly been observed (Banks et al. 2008; Overeem et al. 2008), leading to calls for *E. minor* to be split into two distinct species (Figure 2.4).

African Penguins are very closely related to Magellanic *Spheniscus magellanicus* (~1% divergence) and Humboldt Penguins *S. humboldti* (2% divergence; Banks et al. 2008) and some researchers have speculated about on-going gene-flow among *Spheniscus* species. For example, Thumser & Karron (1994) found Nei's genetic distance to be extremely low (~0.002) among Humboldt, African and Magellanic Penguins. In a similar study, Grant et al. (1994, n=45 African Penguins from Stony Point) found Nei's D=0.017 between African and Magellanic Penguins, and proposed a late Pleistocene divergence between this sister species pair (Grant et al. 1994).

Bouzat et al. (2009) found very limited evidence of regional genetic structure across the range of Magellanic Penguins (Table 2.3), with some evidence for weak isolation-by-distance effects exhibited by colonies known to be associated with different foraging grounds, but no evidence of differentiation among colonies within breeding regions (Bouzat et al. 2009). Magellanic Penguins are known to have a far greater foraging distance during the breeding season (60 – 450km) than African Penguins (<60km, Table 2.2), and have been observed ranging further than 700km during the non-breeding season (Boersma & Rebstock 2009a, 2009b). Bouzat et al. (2009) propose that their results reflect a recent northward expansion of Magellanic Penguins (two large colonies have been established since the 1920s), and that the species conforms to a metapopulation model, with juvenile dispersal and large N_e reducing genetic drift and resulting in high genetic connectivity among breeding regions (Bouzat et al. 2009).

Akst et al. (2002) compared genetic diversity in Magellanic Penguins to the endangered, range-restricted Galápagos Penguin *S. mendiculus,* and found extremely low levels of genetic diversity in the latter species (Table 2.3). The authors hypothesise that serial genetic bottlenecks caused by El Nino–Southern Oscillation (ENSO) events since the Late Pleistocene are the primary driver of low diversity in Galápagos Penguin. Akst et al. (2002)

also estimated that species in the genus *Spheniscus* segregated some 0.5 to 0.8 Mya (Akst et al. 2002). A subsequent, more comprehensive study of genetic diversity and population structure in the Galápagos Penguin (Nims et al. 2008) found no evidence of differentiation among populations (Table 2.3). This study used a different suite of microsatellite markers, and reported higher levels of heterozygosity (Table 2.3). However, the Galápagos Penguin exhibited low genetic diversity based on the average number of alleles per locus when compared to other *Spheniscus* penguins (Nims et al. 2008). The Galápagos Penguin has a short foraging range during the breeding season (<25km, Steinfurth et al. 2008) and the historical population size is likely to be smaller than any of the other *Spheniscus* penguins due to the low carrying capacity of the Galápagos archipelago.

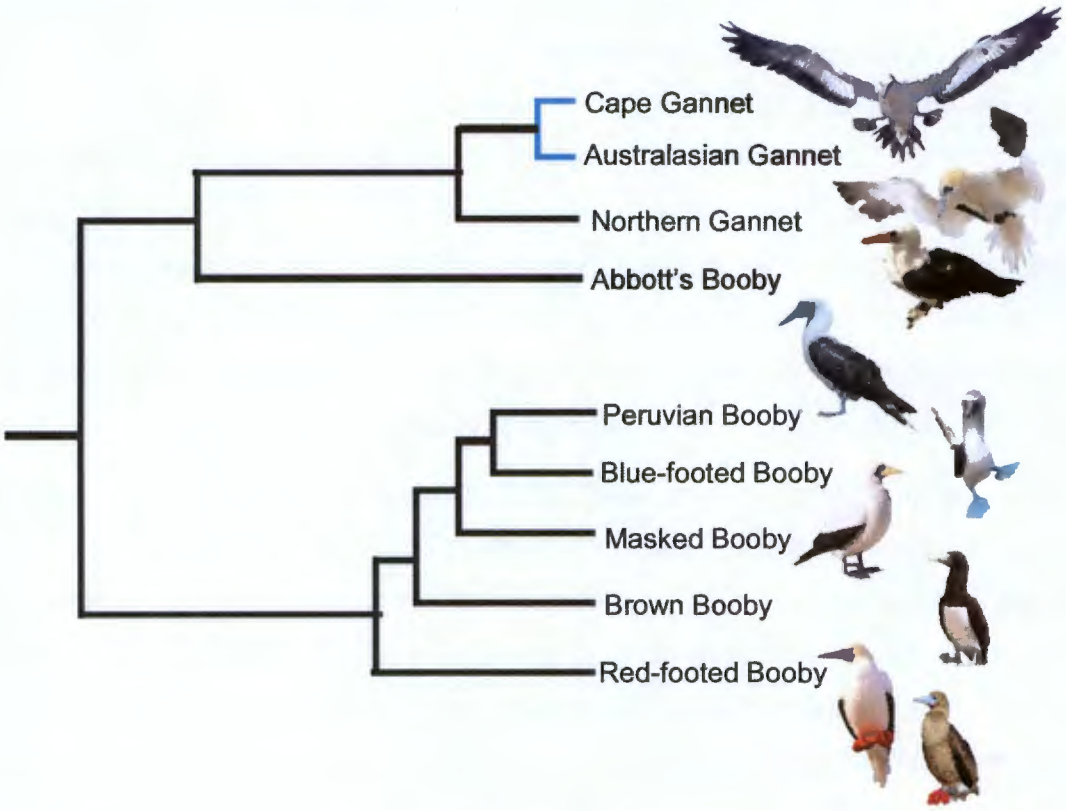
Gene-flow and population structure have been investigated in the vulnerable Humboldt Penguin *S. humboldti* (Schlosser et al. 2003, 2008). Schlosser *et al.* (2008) found that long-term gene-flow has occurred between colonies across the range of this species (some data included in Chapter 3; Table 2.3), and did not detect evidence of bottlenecks or founder events. A number of studies investigating adaptive major histocompatibility complex (MHC) diversity in *Spheniscus* penguins have corroborated the patterns observed for neutral markers (Kikkawa et al. 2005, 2009; Bollmer et al. 2007; Knafler et al. 2012).

Based on our knowledge of the biology and movement patterns of closely related penguin species, and the results of the above population genetic studies, gene-flow among African Penguin colonies can be expected to be high enough to overcome any divergence brought about by physical or non-physical barriers.

Phylogenetic and phylogeographic studies of sulids

A multi-locus phylogeny of the Sulidae showed that gannets and boobies are each monophyletic and diverged ~17 Mya (Patterson et al. 2011). The three extant gannet (*Morus*) species are closely related and are estimated to have arisen ~2.5 Mya (Patterson et al. 2011). Interestingly, although numbers of Cape Gannets *Morus capensis* decreased during the 20th century, the numbers of its two congeners, the North Atlantic *M. bassanus* and Australasian Gannets *M. serrator*, both increased steadily (Montevecchi & Myers 1997) to 343 000 pairs and 66 000 pairs respectively (Crawford et al. 2007a).

Figure 2.9 Phylogeny of extant boobies and gannets (Friesen & Anderson 1997).



Although Northern Gannets exhibit strong nest site and mate fidelity (Schreiber & Burger 2001), range shifts, local extinctions and the establishment of new colonies have been reported in North Atlantic Gannets (Barrett 2008; Fort et al. 2012). No population genetic studies have been carried out on any gannet species, but all species have been included in phylogenetic treatments of the Sulidae (Friesen & Anderson 1997; Friesen et al. 2002; Patterson et al. 2011). There have, however, been a number of studies on closely related booby species.

One relatively early study investigated the comparative phylogeography of the morphologically variable Masked (*S. dactylatra*), Red-footed (*S. sula*), and Brown (*S. leucogaster*) Boobies (Steeves et al. 2003), and found different patterns for each species (Table 2.4). Each of these species exhibit morphological variation across their range and differ in their foraging and breeding ecology. The study found strong, well-supported genetic differentiation among regional samples in all species (Table 2.4), and identified possible barriers to gene-flow as the Isthmus of Panama (which emerged 3 Mya, barrier for Red-footed and Brown Boobies) and the Eastern Pacific Basin (barrier for Brown Boobies). The authors explain the different patterns of divergence in the three largely sympatric species by highlighting differences in their foraging mode (near-shore versus offshore foragers),

breeding behaviour (ringing data suggest that Brown Boobies have a higher natal-site fidelity) and mobility (Steeves et al. 2003). Further work on the Masked Booby *S. dactylatra* (Steeves et al. 2005a, 2005b), expanded the geographic range of the study (Table 2.4), and found strong genetic differentiation between Indo-Pacific and Atlantic Ocean populations, and low levels of gene-flow among populations within ocean basins. Steeves et al. (2005a, 2005b) posited that the observed pattern is a product of isolation-by-distance across oceans, limited gene-flow across the Isthmus of Panama (physical barrier) and limited natal dispersal, local adaptation and genetic drift (non-physical barriers) within ocean basins.

The evolutionary history and phylogeography of Brown and Red-footed Boobies have also been further investigated (Morris-Pocock et al. 2010a; Morris-Pocock 2012). A comparative study between these two pantropical species revealed strong global population genetic structure in both species (Table2.4). Regional population divergence largely reflected subspecies classification based on morphology (Morris-Pocock et al. 2010a). The authors proposed that physical barriers, including the Isthmus of Panama and the Benguela Current, affected the evolution of both booby species, and that differentiation between populations inhabiting different ocean basins dates back to the Pleistocene (~0.3 to 1.8 Mya), with some colonies showing signs of recent secondary contact (Morris-Pocock et al. 2010a).

Divergence within ocean basins is lower among Red-footed Booby populations than among those of Brown Boobies, which the authors posit is due to long term gene-flow as a result of the marked differences in marine habitat preferences of these two species: Red-footed Boobies forage in pelagic waters (typically ~240km from their breeding colonies – identical to that of breeding Cape Gannets, and similar to that of Masked Boobies), whereas Brown Boobies forage very close to the shore (Schreiber & Burger 2001). This 'ecological barrier' decreases the probability that Brown Boobies will encounter and disperse to non-natal colonies. Morris-Pocock et al (2011) conducted a range-wide study of Brown Boobies and found strong evidence for population differentiation (Table 2.4) that closely reflects the recognised subspecies classification (Morris-Pocock et al. 2011). The authors propose that the divergence observed among colonies is due to historical genetic isolation (~1 million years without gene-flow) and limited contemporary gene-flow between distant breeding regions.

Table 2.4 A summary of population genetic studies conducted on sulids, including their estimated global population size, allozyme estimates of population differentiation, mitochondrial target region (mtDNA), sample sizes (N_{mt}) and estimates of genetic diversity (haplotype diversity, *h;* nucleotide diversity, π) and differentiation (global or pairwise range of φ_{ST}), and microsatellite sample sizes, number of loci (N_{loci}) and results (Heterozygosity (H_O), global and/or pairwise range of F_{ST}) and the threat status based on the IUCN Redlist of threatened species (2013; Lc=least concern, EN=endangered).

Species	Scientific name	IUCN	N_{pops}	N_{mt}	mtDNA*	mt diversity (h |π)	φ_{ST}*	N_{loci}	H_O	F_{ST}	Reference
Masked Booby	*Sula dactylatra*	Lc	4|14	64|120|292	cyt b|CR |CR	π= 0.0061 to 0.0196	0.62	–	–	–	Steeves et al (2003, 2005a, 2005b)
Peruvian Booby	*Sula variegata*	Lc	5	153	CR	0.98|0.019 to 0.021	0.005	7 (n=153)	–	0.003; 0.007 to 0.017	Taylor et al. (2011)
Brown Booby	*Sula leucogaster*	Lc	11|5	78|242|29	cyt b|CR|nDNA B-FIB, α-enolase, O-decarboxylae | Christmas Island cytb n=29	0.872)|0.005 to 0.02 (average 0.0106) 0.636|0.0011 CR 0.988 |0.022	0.94| nDNA 0.45 to 0.74	8 (n=215)	0.43 to 0.62 (average 0.53)	0.23; 0.03 to 0.43	Morris-Pocock et al. (2010, 2011, 2012) **Steeves et al. (2003)**
Blue-footed Booby	*Sula nebouxii*	Lc	9	173	CR	0.95 to 1 | 0.012 to 0.018	0.05	7 (n=173)	Δ	0.05	Taylor et al. (2011)
Nazca Booby	*Sula granti*	Lc	5	50	cyt b, ND2, COI (2145bp)	0.886 ± 0.028 | 0.001 ± 0.0001	0.127	8 (n=133)	0.58	0.07; -0.0003 to 0.164	Levin & Parker (2012)
Red-footed Booby	*Sula sula*	Lc	3|9|1	89|271	cyt b | CR | dloop on Christmas Island alone (n=19)	0.71 to 0.99 (average 0.912) | 0.007 to 0.014 (average 0.0109) h=0.980 | 0.0122	0.99| 0.8	10 (n=282)	average 0.531	0.24	Morris-Pocock et al. (2010, 2012a,b) **Steeves et al. (2003)**
Abbott's Booby	*Papasula abbotti*	En	1	41	cyt b | CR	cyt b h=0.095|0.0002 CR 0.789|0.0034	-	-	-	-	Morris-Pocock et al. (2012)

***CR = control region, COI = cytochrome oxidase I, cytb = cytochrome b, NADH2 = NADH dehydrogenase subunit 2, 12S = mitochondrial 12S ribosomal RNA**

Δ Appendix S5 of that paper gives microsatellite loci summary statistics, including sample sizes, allele frequencies, and observed and expected heterozygosities

Population genetic studies have also been conducted on the closely related Blue-footed *Sula nebouxii* and Peruvian Boobies *S. variegata* (Morris-Pocock et al. 2010b; Taylor et al. 2010a, 2011a, 2011b), which are known to hybridize in northern Peru (Ayala 2006; Figueroa & Stucchi 2008; Taylor et al. 2010b). Taylor et al. (2011b) found strong evidence for panmixia and higher than expected genetic diversity among five colonies of Peruvian Boobies distributed across their range on the Pacific coast of South America (Table 2.4), although the authors detected a significant relationship between genetic differentiation and geographic distance. Taylor et al (2011b) explained the observed pattern as a result of this species' specialization to the cold-water upwelling system of the Humboldt Current, which may elevate dispersal and reduce colony fidelity. The authors also cited evidence of long distance dispersal during times of severe environmental fluctuations (e.g. El Nino events), when breeding and survival depends on the species ability to track environmental change via dispersal to non-natal colonies (Taylor et al. 2011b). Also, suitable breeding sites for Peruvian Boobies are readily available, and could facilitate gene-flow across their range (Taylor et al. 2011b). Natural environmental fluctuations may be compounded by recent human-induced perturbations in that system, which may have resulted in higher gene-flow among colonies over recent generations of boobies (Taylor et al. 2011b).

The Blue-footed Booby exhibits weak range-wide population genetic structure (Table 2.4; Taylor et al. 2011a), purportedly due to high dispersal rates and low colony fidelity resulting from the selective pressures imposed by an unpredictable, variable foraging environment (Taylor et al. 2011a). Suitable breeding sites for Blue-footed Boobies are not distributed regularly within the Humboldt System as they are for the Peruvian Booby, but the general pattern of high gene-flow still exists. Morris-Pocock et al. (2010) showed that a large region of the mitochondrial genome underwent duplication in Brown, Red-footed and Blue-footed Boobies sometime before 3 Mya. The duplicated portion includes those genes used in the population genetic analyses discussed above and exists in at least four sulid species, including the Northern Gannet (Morris-Pocock et al. 2010b). However, Morris-Pocock et al. (2010) showed that the two copies of the control region are largely evolving in concert and should therefore not infringe on our confidence in these findings.

The Nazca Booby *Sula granti* was previously considered a subspecies of the Masked Booby, but has been elevated to species status based on morphology and genetic distinctiveness from *S. d. dactylatra, S. d. personata* and *S. d. californica* (Table 2.4; Friesen et al. 2002). A recent population genetic study (Levin & Parker 2012) revealed weak, but significant differentiation

(Table 2.4) among Nazca Booby populations breeding on the Galápagos archipelago (Levin & Parker 2012).

Based on our knowledge of closely related booby species, which show evidence of population structure only at large geographic scales i.e. ocean basins, or when populations are sparated by large physical bariers e.g. the Isthmus of Panama, it seems unlikely that Cape Gannets will show strong regional population structure.

Evolutionary genetic studies of cormorants

Cormorants belong to the family Phalacrocoracidae, which was classified under the order Pelecaniformes based on morphological and behavioural characters. However, since the advent of molecular systematics, the higher order systematics of the Pelecaniformes (now Phalacrocoraciformes; Siegel-causey 1997a; Kennedy et al. 2000; Kennedy & Spencer 2004; Smith 2010) has intrigued taxonomists and has been called "perhaps the most troublesome taxon in birds" (Siegel-Causey 1997, p.159), due to the incongruence of the molecular and morphologically-based phylogenies (Kennedy et al. 1996, 2000, 2005; Holland et al. 2010).

An early comprehensive study of the relationships within the Pelecaniformes based on skeletal and behavioural characters concluded that the group was monophyletic (Cracraft 1985). However, subsequent molecular systematic studies contradicted this finding (Sibley et al. 1988; Fain & Houde 2004; Hackett et al. 2008), and the Pelecaniformes were moved into the Ciconiiformes (Sibley et al. 1988). The paraphyly of the Pelecaniformes has since been widely accepted, with the six original families distributed among three clades within the Ciconiiformes. The "core Pelicaniformes" (anhingas, cormorants and sulids) are, however, consistently monophyletic, with Phalacrocoracidae sister to the Anhingidae, and frigatebirds sister to this cormorant-darter-gannet cluster (Christidis & Boles 2008). Christidis & Boles (2008) stated that the minimum taxonomic alteration to incorporate new, well-supported changes in relationships between these seabird taxa is to move the pelicans from Pelecaniformes to Ciconiiformes. This change necessitates a change in the ordinal name, and given that the cormorants are the most speciose of the remaining "Pelecaniform" taxa, Christidis & Boles (2008) suggested the order be renamed Phalacrocoraciformes, which has been commonly adopted.

A similar situation arose within the Phalacrocoracidae (cormorants and shags), in that different sources of evidence supported different taxonomic arrangements within the group (van Tets 1965; Kennedy et al. 2000). van Tets' (1976) phylogeny was based on behavioural, ecological and morphological characters, and separated shags (*Leucocarbo*) from cormorants (*Phalacrocorax*). Siegel-Causey (1988) separated the family into two sub-families,

Leucocarboninae and Phalacrocoracinae, which roughly corresponded with van Tets' (1976) genera. The main differences between these two phylogenies are a) rank i.e. the use of families, sub-families, genera and sub-genera, b) Siegel-Causey (1988) split van Tets' (1976) *Leucocarbo* into five genera and c) moved the marine shags (*Compsohalieus*) into Phalacrocoracinae (cormorants). Kennedy et al. (2000) attempted to resolve the phylogenetic relationships within Phalacrocoracidae using mitochondrial sequence data (12S, ATPase-6 and -8; Figure 2.4). The phylogeny differed substantially from those based on morphological and behavioural traits (van Tets 1965; Cracraft 1985; Hedges & Sibley 1994; Kennedy et al. 1996; Siegel-Causey 1997a). Kennedy et al. (2000) found that neither the traditional shags nor cormorants were monophyletic, but acknowledged sampling gaps in their data. The most basal taxon in their phylogeny is the Little Pied Cormorant *Microcarbo melanoleucos*, estimated to have diverged from the other shags and cormorants 12 Mya. Kennedy et al. (2000) hypothesised that the micro-cormorants (*Microcarbo*) are monophyletic, although no other samples were incorporated in their phylogeny. Two species of micro-cormorant occur in southern Africa: Reed *Microcarbo africanus* and Crowned Cormorants *M. coronatus*. The Cape Cormorant *Phalacrocorax capensis* was included in Kennedy et al.'s (2000) phylogeny, and was sister to a clade containing the Great Cormorant *P. carbo* and the Japanese Cormorant *P. capillatus* (Figure 2.4; Kennedy et al. 2000, 2001). This placement remained unchanged in Kennedy et al. (2009), although the authors cautioned that several important species had yet to be included in the tree, and acknowledge that this is required to gain better resolution, especially with respect to older branches in the phylogeny (Kennedy et al. 2009). The taxonomic uncertainty brought about by the incongruence of the molecular and morphology-based phylogenies (Figure 2.4), and the incomplete species sampling, motivated an expansion as part of the present study, which adds five putative species of *Phalacrocorax*, and presents additional phylogenies based on new mitochondrial and nuclear gene regions. A number of population-level evolutionary studies have been carried out for cormorant and shag species, but without a well-resolved phylogeny, it is difficult to ascertain whether the observed patterns are phylogenetically independent i.e. to what degree shared ancestry determines intraspecific population genetic or phylogeographic structure. The identity of the sister species of Cape Cormorants is currently disputed (although see Kennedy & Spencer, *in press*), thereby impeding comparisons with closely related species and, in turn, the interpretation of population-level molecular results presented in Chapter 3. In an attempt to clarify this, the sample sizes for Cape Cormorants and Bank Cormorants were increased, and two *Microcarbo* species and two subspecies of Blue-eyed Shags were added to the

Phalacrocorax sequence data available on GenBank, to produce an expanded phylogeny of the group.

Evolutionary affinities of the Cape Cormorant

Sample collection, DNA extraction and PCR amplification

The Cape Cormorant samples included in the ATPase-6 and COI phylogenies below are presented in Appendix 3.1c, and Appendix 2.2 lists the Crowned, Bank and Reed Cormorant samples, and the Crozet and Kerguelen Shags that were sequenced during this study. The molecular methods for mitochondrial DNA extraction, amplification and sequencing follow those described in Chapter 3. Blood samples were stored in Longmire's solution (100mM TRIS pH8, 100mM EDTA, 10mM NaCl, 0.5% SDS) and feather samples were stored in 99.9% ethanol. Whole genomic DNA was extracted using DNeasy© Blood & Tissue Kits (Qiagen, Valencia, CA), following the protocol on p. 25 of the handbook. Primer sequences and PCR conditions are reported in Appendices 3.3 and 3.4. There is some overlap in the Cape Cormorant results presented here and in Chapter 3 because the analyses were conducted at different scales (phylogenetic and population genetic scales, respectively). These results provide additional mitochondrial and nuclear sequence data to what has already been published, and expand the phylogenetic tree of cormorants and shags.

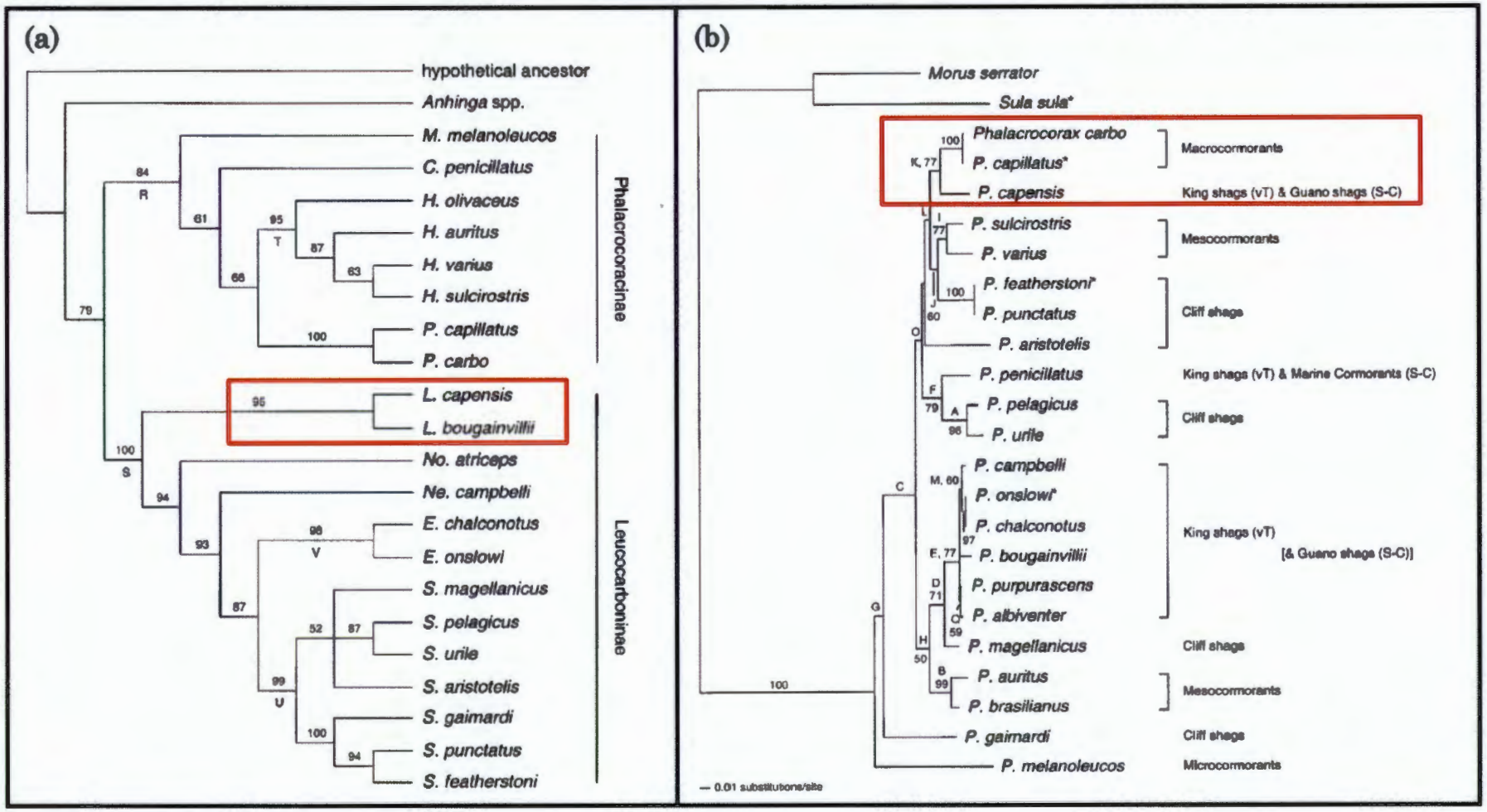
Phylogeny estimation

Maximum likelihood (ML) and Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analyses were conducted for each gene region in Mega 5 (Tamura et al. 2011) and MrBayes Version 3.1.2 (Ronquist & Huelsenbeck 2003) respectively. Statistical selection of the model that best described each dataset was implemented in jModeltest (Posada 2008), and MrModeltest 2.3 (Nylander 2008). Branch support values of ML trees were evaluated using bootstrap-resampling (1000 replicates). Bayesian analyses (six independent MCMC chains) were run for one million generations and trees were sampled every 100 generations. Nodal support for Bayesian estimates of phylogeny are in the form of Bayesian posterior probabilities.

Mitochondrial DNA (ATPase-6) phylogeny

Numerous outgroup taxa for the ATPase-6 phylogenetic analyses are available on GenBank, as this is one of the target regions used to reconstruct the molecular phylogeny of the Phalacrocoracidae (Kennedy et al. 2000, 2001, 2009). The full ATPase-6 alignment (n=113) was truncated to 567bp to include all available outgroup taxa (Figures 2.6; outgroup taxa are listed in Appendix 2.3).

Figure 2.4 (a) Morphology-based (generated using Siegel-Causey's (1988) data) and (b) mitochondrial sequence-based (12S and ATPase-6 and -8) phylogenetic trees adapted from Kennedy et al. (2000). Generic names follow Siegel-Causey (1988): shags and cormorants in separate subfamilies, Leucocarboninae and Phalacrocoracinae, respectively. Note the placement of the Cape Cormorant (boxed). Note also that *No. atriceps* (a) is equivalent to *P. albiventer* and *P. purpurascens* (b). Abbreviations: vT = van Tets, S-C = Siegel-Causey.



A number of additional sequences were generated during this study and are also in the ATPase-6 ML and Bayesian phylogenetic trees (Figure 2.6 and 2.7): Reed *Microcarbo africanus* (n=1), Bank *Phalacrocorax neglectus* (n=16) and Crowned Cormorants *P. coronatus* (n=3) from southern Africa, and Imperial Shags *P. atriceps* from Marion Island (n=5), Kerguelen Island (*P. (a.) verrocosus*, n=5) and Crozet Island (n=5), *P. (a.) melanogenis*). The two Japanese Cormorant sequences published on GenBank fall into very different clades in the phylogenetic trees (Figure 2.6): one (AB233986, from Okumura *et al.* (2005) is sister to the Pelagic Cormorant *P. pelagicus* (which breeds in the North Pacific, Japan, Siberia, the Aleutians, Alaska and western North America), and is closely related to *P. urile* (also from Alaska, the Aleutians and Japan). The other (AY009355, from Kennedy et al. (2000), no collection locality reported) is sister to the Great Cormorant. It seems that one of these samples has been misidentified. The ML and Bayesian phylogenetic trees for the ATPase-6 gene region were very similar and the clade containing the Bank and Cape Cormorants is not fully resolved. The Crowned and Reed Cormorants are very closely related, and fall into the currently monophyletic *Microcarbo* clade, as predicted by Kennedy et al. (2000). Mitochondrial 12S rRNA sequences for these two closely related *Microcarbo* species form an unresolved polytomy (data not shown, 92% bootstrap support, n=6), indicating recent divergence. Although the sample sizes are small, the Bank Cormorants form two well-supported clades that correspond with their collection localities in Namibia and South Africa. An analysis of the Beta-fibrinogen intron 7 gene (BFIB-I7) placed the Cape Cormorant (n=25, 91% bootstrap support) sister to the Great Cormorant (DQ881980), with the Bank Cormorant sister to that combined clade (92% bootstrap support).

The Imperial Shag is a colonially-breeding, marine species that forages in inshore waters around islands in the sub-Antartic and along Antarctic coastlines (Casaux & Barrera-Oro 2006). Its diet varies geographically, but includes fish, crustaceans, squid and benthic invertebrates (Gosztonyi & Kuba 1998; Casaux & Barrera-Oro 2006; Cook et al. 2007). The taxonomy of the Imperial Shag complex has proven to be challenging, due to incongruencies between morphological and molecular data. The current molecular phylogenies (Kennedy et al. 2000, 2001, 2005, 2009; Holland et al. 2010) have included a number of relevant putative species and subspecies: the South Georgian Shag *P. (a.) georgianus* (from South Georgia, the South Orkney Islands, South Sandwich Islands and Shag Rocks in the Scotia Sea); *P. (a.) purpurascens* (Macquarie Island); and the King Cormorant or White-bellied Shag (Falkland Blue-eyed Shag) *P. (a.) albiventer*, which is found on the southern tip of South America,

from central Chile round to central Argentina, and on the Falkland Islands. The Antarctic Shag *P. (a.) bransfieldensis* (from the Antarctic Peninsula and South Shetland Islands) was excluded from Holland et al.'s (2010) analysis due to missing data. Samples for the Heard Island Shag *P. (a.) nivalis* and the Imperial Shag *P. (a.) atriceps* (coastal southern Chile and Argentina) could not be obtained for the present study, but samples from Kerguelen Island representing *P. (a.) verrucosus*, and *P. (a.) melanogenis* from Crozet Island and Marion Island were added to the current molecular phylogeny. As expected, the Imperial Shags collected on Crozet (Crozet Shag), Kerguelen (Kerguelen Shag) and Marion Island (Crozet Shag *P. (a.) melanogenis*) fall into a well-supported clade containing other Imperial Shag subspecies (Macquarie Shag *P. (a.) purpurascens*, South Georgia Shag *P. (a.) georgianus* and Falkland Blue-eyed Shag *P. (a) albiventer*). Interestingly, birds from Marion Island and Kerguelen, representing different subspecies, seem to be more closely related than birds from Crozet. Overall, the level of divergence within the Imperial Shag complex is low and not well-resolved based on ATPase-6 or 12S rRNA (data not presented, Kerguelen and Crozet 12S rRNA samples form an unresolved polytomy, which includes the Macquarie Shag *P. (a.) purpurascens*). The taxonomic uncertainty in this complex is impeding conservation, as the IUCN recognises the Imperial Shag complex as a single species (classified as Least Concern), but acknowledges that "any change in taxonomy would result in the recognition of additional threatened species" (BirdLife International 2012).

Mitochondrial DNA (Cytochrome Oxidase I (COI)) phylogeny

Although COI was largely invariable among the Cape Cormorants sampled, numerous comparable sequences are available from the BOLD (Barcoding of Life Database) and GenBank (see Appendix 2.5). A number of COI sequences were also generated during the present study and are incorporated in the ML and Bayesian analyses of this expanded Cormorant COI dataset (n=71, 925bp). Based on COI, the Cape Cormorant is sister to the Bank Cormorant (Figure 2.8), and the Great Cormorant is sister to those two species. The Bank Cormorant is also sister to the Cape Cormorant in the cyt b gene tree (Chapter 3, Figure 3.26). Interestingly, this pattern of relatedness is reversed in the nuclear Beta-fibrinogen Intron 7 gene tree i.e. the Cape Cormorant is sister to the Great Cormorant (Appendix 2.5), as in published studies that include the Bank Cormorant (Kennedy et al. 2005; Holland et al. 2010). The concatenated phylogeny (2736 bp: 12S 189bp, Beta-Fib I7 513bp, COI 693bp, ATPase-6 567bp and cyt b 774bp) had large alignment gaps that caused a loss of resolution and more data are required to increase nodal support (data not shown).

Figure 2.6 Phylogenetic gene tree of all available *Phalacrocorax* ATPase-6 sequences (n=113, 567 bp). Numbers at the nodes are ML bootstrap values (below) and Bayesian posterior probabilities (above).

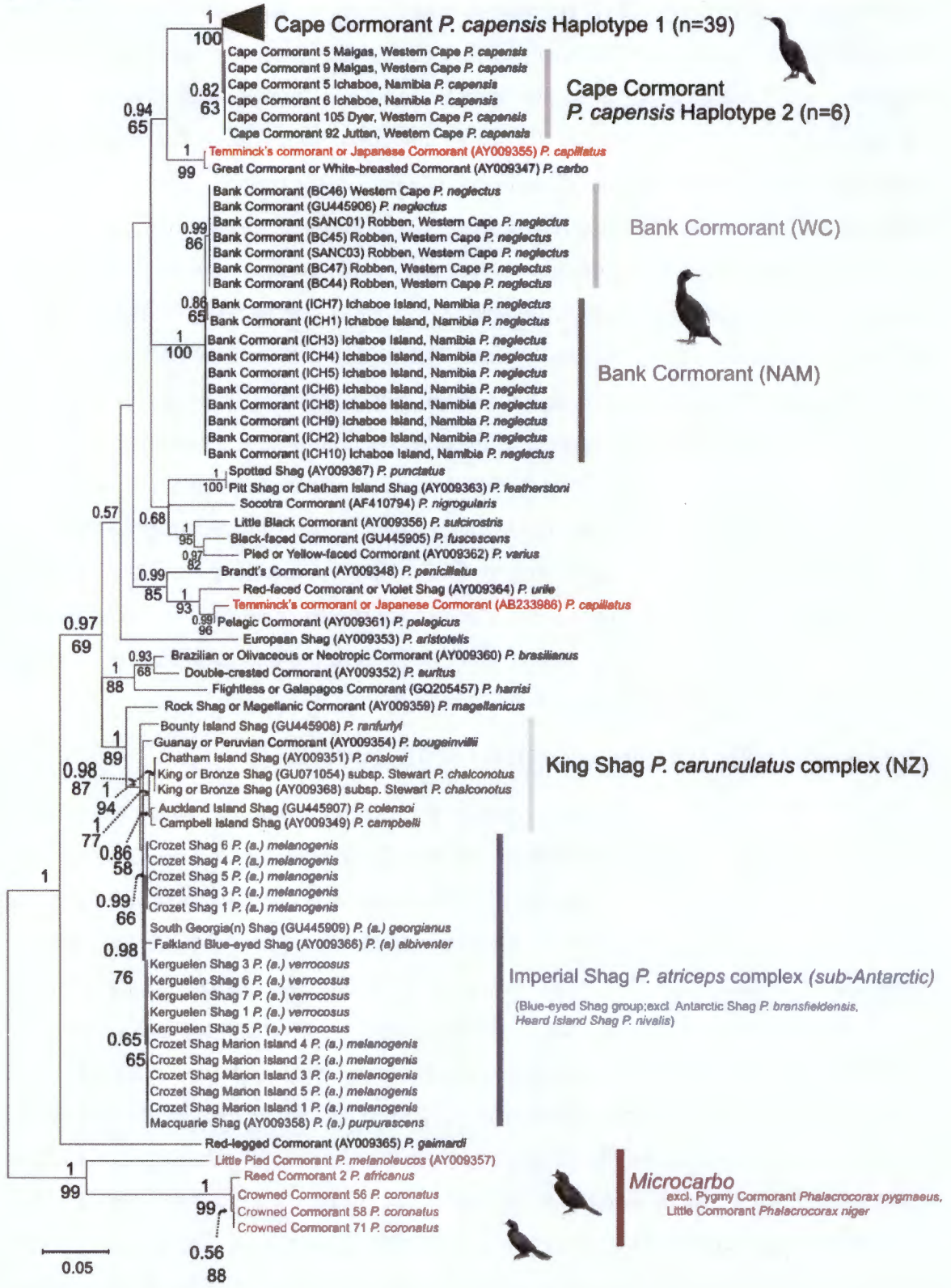
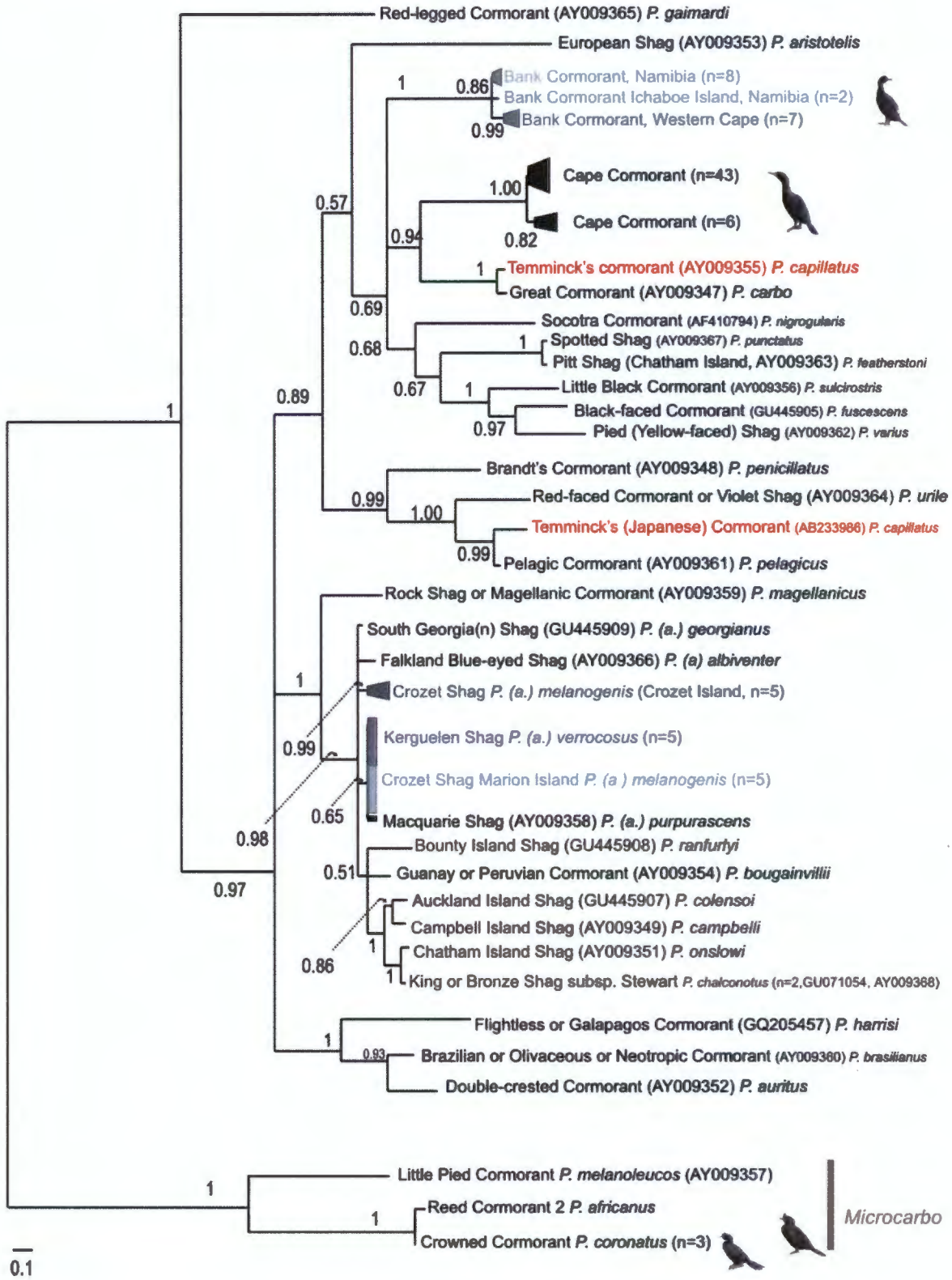


Figure 2.7 Bayesian phylogenetic tree based on the ATPase-6 sequence data generated during this study, and including sequences published on GenBank.

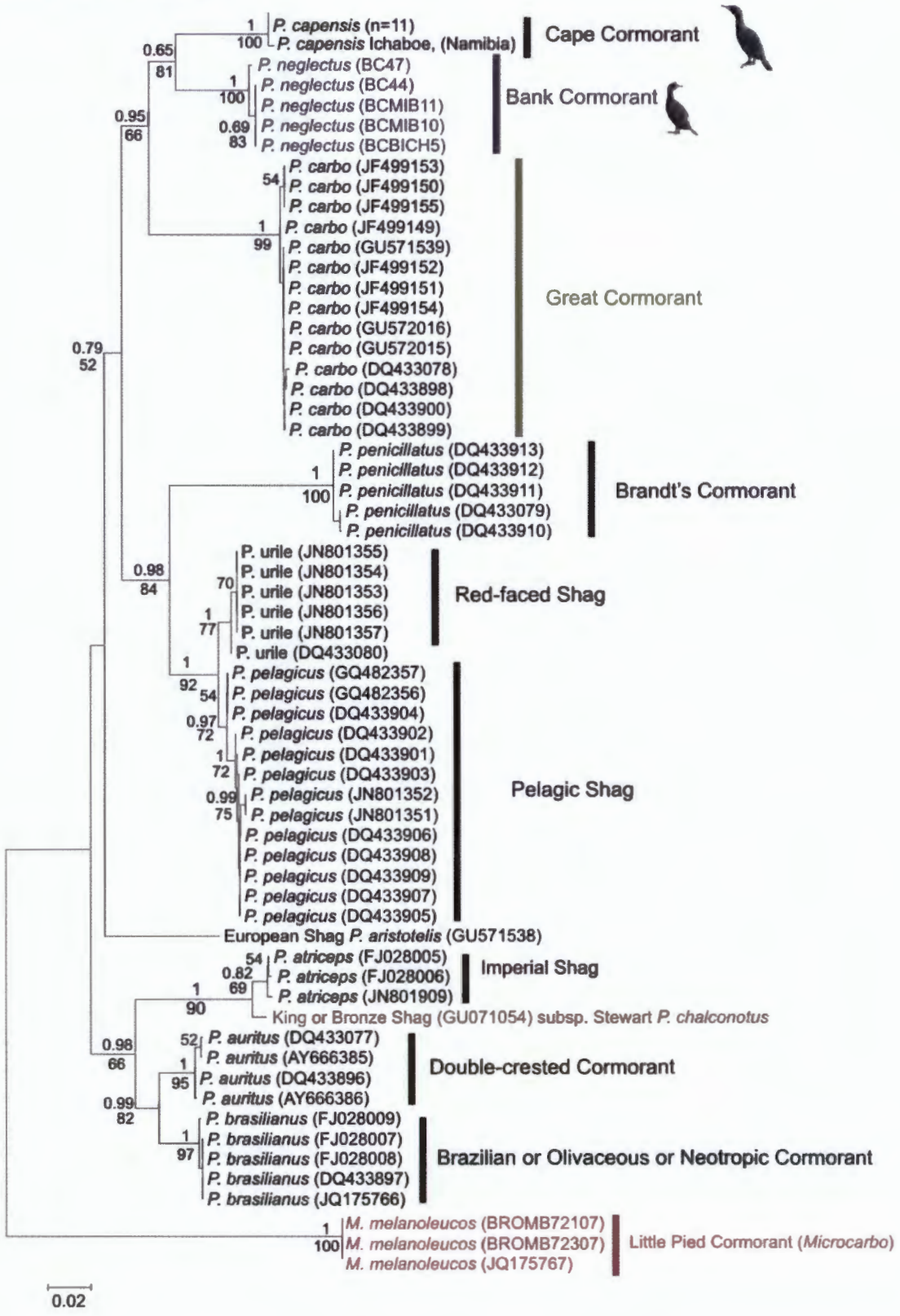


Population genetic and phylogeographic studies of cormorants and shags

There have been only a limited number of studies of genetic structure within cormorant or shag species, especially those restricted to marine environments (Table 2.5). Duffie et al. (2009) assessed colony- and island-level genetic differentiation among flightless, endangered Galápagos Cormorant *Phalacrocorax harrisi* populations. They found evidence for weak genetic structure both within and between populations on the only two islands where the species breeds (Table 2.5). The authors explained that this pattern was most likely due to the low vagility and strong colony philopatry exhibited by this species, and found evidence for an isolation-by-distance effect across the 5km gap that separates the two islands (Duffie et al. 2008, 2009). The Galápagos Cormorant is only distantly related to the southern African cormorants, and Kennedy et al. (2009) concluded that it is a recent offshoot of the adjacent mainland forms, the Double-crested *P. auritus* and Neotropic Cormorants *P. brasilianus,* and has subsequently evolved flightlessness.

Siegel-Causey (1997b) conducted an allozyme study of Rock Shag *Phalacrocorax magellanicus* populations and found evidence of significant genetic structure (Table 2.5; Siegel-Causey 1997b), and asymmetrical gene-flow along the coastline of South America. The author proposed that major glaciation events during the Pleistocene (35 to 15 Kya) and at the Pliocene-Pleistocene boundary (1.2 to 1.0 Mya) rendered the coastlines of southern Chile, Tierra del Fuego and southern Argentina uninhabitable for coastal breeding seabirds, and that encroaching ice and changing sea levels drove Rock Shag populations north into Pacific and Atlantic refugia, where population divergence occurred (Siegel-Causey 1997b). Conversely, another allozyme study of cormorants in South America by Rasmussen (1994) found little evidence for population differentiation among Atlantic and Pacific populations of the morphologically variable Imperial Shag *Phalacrocorax* (*Leucocarbo*) *atriceps* (Table 2.5), or among freshwater and coastal forms. The author concluded that Pleistocene glaciation had little impact on the evolutionary history of Imperial Shags, due to their life-history traits (colonial breeding, vagility, longevity and generalist foraging mode) and tolerance for cold conditions (Rasmussen 1994). The above results were corroborated in a recent comparative study of *P. magellanicus* and *P. atriceps* (Calderón et al. 2014), and the authors stress the importance of non-physical barriers in shaping the distribution of genetic diversity in these two species with contrasting life-histories.

Figure 2.8 Maximum likelihood (ML) phylogenetic tree based on all available *Phalacrocorax* COI sequences (n=71, 693bp). Numbers at the nodes are Bayesian posterior probabilities (above branches; based on 1 million generations, 6 independent chains, 2 runs) and ML bootstrap values (below braches; 1000 replicates).



A number of subspecies of Imperial Shag have been described from sub-Antarctic Islands, and are included in the most recent molecular phylogeny, including the South Georgia(n) Shag, the Falkland Blue-eyed Shag *P. a. albiventer,* and the Macquarie Shag *P. a. purparascens.* The Imperial Shags that breed on Marion Island, Crozet Island *P. a. melanogenis* and Kerguelen Island *P. a. verrocosus* were previously not included in any cormorant phylogeny, and appropriate gene regions were sequenced for several individuals from each population in the present study to confirm their position in the phylogeny of Kennedy et al. (2000, 2009).

In a study of continental (marine and freshwater) cormorants, Waits et al. (2003) found no evidence of population structure among three breeding areas of Double-crested Cormorants *P. auritus*, nor between two recognised subspecies (North Atlantic Double-crested Cormorant *P. a. auritus* and Southeastern Double-crested Cormorants *P. a. floridanus* that exhibit morphological differences and differences in migratory patterns (Waits et al. 2003). Mercer (2008) expanded on this study, incorporating more samples from across the range of this species (Table 2.5) and the other two recognised subspecies (Alaskan Double-crested cormorants *P. a. cincinnatus* and Pacific Double-crested Cormorants *P. a. albociliatus*). Mercer (2008) found evidence for population structure that reflected the subspecies classification e.g. the Alaskan subspecies was divergent from other subspecies (Table 2.5).

Goostrey et al. (1998), Winney et al. (2001) and Marion & Le Gentil (2006) studied another widespread, morphologically variable, inland-nesting (continental) cormorant, the Great Cormorant *Phalacrocorax carbo,* at 21 European colonies representing two of the six recognised subspecies (*P. c. carbo* and *P. c. sinensis;* Newson et al. 2004). The authors found some evidence for the genetic distinctiveness of these two subspecies (Table 2.5; Goostrey et al. 1998), including well supported reciprocal monophyly (Goostrey et al. 1998b; Winney et al. 2001; Marion & Le Gentil 2006). Sex differences in breeding site fidelity (Schjorring et al. 2000) and prospecting by first-time breeders (Schjorring et al. 1999) have been reported in *P. carbo*, and may enhance colony connectivity.

A recent study of European Shags *Phalacrocorax aristotelis* (Barlow et al. 2011) found evidence of only weak genetic structure (Table 2.5) among breeding populations, and even among recognised subspecies (*P. a. aristotelis, P. a. desmarestii* and *P. a. riggenbachi)*, despite high observed levels of philopatry and wide stretches of ocean separating colonies (Barlow et al. 2011). The authors hypothesised that the observed pattern of genetic admixture

is primarily due to juvenile dispersal, reinforced by rare, recent long-distance, cross-sea movements and a post-Pleistocene range expansion (Barlow et al. 2011).

Based on the available evidence regarding phylogeographic structure among populations of freshwater and marine cormorants (and shags), and given that very little divergence has been detected among even morphologically distinctive populations and recognised subspecies, it seems likely that the Cape Cormorant will not exhibit population genetic structure across its range. The drivers of population divergence among cormorants and shags appear to be primarily environmental among the few studies that have been carried out in this group i.e. historical or contemporary physical barriers seem to play a more significant role than non-physical barriers, although some evidence exists that sedentary species exhibit stronger genetic structure than dispersive species (Duffie et al. 2009; Barlow et al. 2011; Calderón et al. 2014).

CONCLUSIONS

Having reviewed a large proportion of the literature available on evolutionary genetic studies of seabirds it is possible to make some generalizations and predictions about what patterns to expect among populations of Cape Gannets, African Penguins and Cape Cormorants in the Agulhas-Benguela Ecosystem (ABE). Many seabird species show morphological variation across their ranges, but the focal species of this study, and all other seabird species endemic to the ABE, are morphologically conserved throughout their ranges. This general pattern likely reflects the evolutionary forces that have shaped seabird species that inhabit this variable ecosystem: Pleistocene glaciation did not affect the southern African coast to the extent that it impacted the southern coasts of South America and the South Pacific sub-region, and therefore probably had little influence on the evolutionary history of seabirds breeding in the ABE; the shifting position of the Subtropical Convergence during the Early Pleistocene, however, may have altered the marine environment for temperate southern African seabirds, but has not promoted divergence among their populations. The Subtropical Convergence is thought to have migrated as far north as the southern coast of Africa at a similar time to the emergence of at least two of the study species in the ABE.

Table 2.5 A summary of population genetic studies conducted on cormorants and shags, including their estimated global population size, mitochondrial target region (mtDNA), sample sizes (N_{mt}) and estimates of genetic diversity (haplotype diversity, *h;* nucleotide diversity, π) and differentiation (global or pairwise range of φ_{ST}), and microsatellite sample sizes, number of loci (N_{loci}) and results (Heterozygosity (H_O), global and/or pairwise range of F_{ST}) and the threat status based on the IUCN Redlist of threatened species (2013; Least Concern (Lc) and Vulnerable (Vu)).

Species	IUCN	Scientific name	N_{pops}	N_{mt}	mtDNA***	mt diversity (h |π)	φ_{ST} *	N_{loci}	H_O	F_{ST} or allozyme F_{ST}	Reference
Double-crested Cormorant	Lc	*Phalacrocorax auritus*	3; 23	18 ; 234	12S , 16S, CO3-ND3, cyt b; CR	0.66 to 1|0.0067 to 0.055	0.476	8 (n=395)	0.132 to 0.918	0.029*; 0.476	Waits *et al.* (2003); Mercer (2008)
Great Cormorant	Lc	*Phalacrocorax carbo*	21; **22;** 20	**SSCP n=418; sequenced n=74;** 231	**CR;** CR	0.58|0.0068	**SSCP n=0.441; sequenced n=0.26;** 0 to 0.446 (ave 0.131)	7 (n=541)	0.64 to 0.95	R_{ST} range 0 to 0.2, mean 0.069	Goostrey *et al.* (1998), **Winney *et al.* (2001);** Marion & Le Gentil (2006)
Galápagos Cormorant	Vu	*Phalacrocorax harrisi*	9	–	–	–	–	5 (n=223)	0.51 to 0.66	0.097*	Duffie *et al.* (2008, 2009)
Rock Shag	Lc	*Phalacrocorax magellanicus*	7; 16	–	ATPase-6	0.54	–	Allozyme (14 loci, n=90)|7 (n=151)	0.02-0.06|0.54	0.02 to 0.5|0.1	Siegel-Causey (1997) Calderon *et al.* (2014)
Imperial Shag	Lc**	*Phalacrocorax atriceps*	7	–	ATPase-6	0.76	–	8 (n=173)	2.2% (1.4-2.9%) | 0.71	Allozyme (n=114, 9 loc), F_{ST} 0.062|0.05	Rasmussen (1994) Calderon *et al.* (2014)
European Shag	Lc	*Phalacrocorax aristotelis*	20	66	NADH2 (320bp)	0.38|0.0019	low	7 (n=447)	0.46 to 0.76	0.055 (0.006 to 0.169)	Barlow et al. (2011)

***Where more than one study has been conducted on a species, the results of the most comprehensive are shown; *** P. atriceps may consist of up to 7 species, Lc status pending molecular phylogenetic treatment: "...it is clear that any change in the taxonomy would result in the recognition of additional threatened species" (IUCN Redlist). ***CR = control region, COI = cytochrome oxidase I, cytb = cytochrome b, NADH2 = NADH dehydrogenase subunit 2, 12S or 16S = mitochondrial 12S/16S ribosomal RNA**

light on the observed patterns among Cape Cormorant populations. In terms of demography reduced food availability may depress clutch sizes, breeding success and survival of the sympatric focal species, by forcing them to forage further from their colonies and switch to lower quality prey items. The interactions between the phenotypes of the focal species and their shared environment could potentially lead to congruent population genetic signals, depending on how flexible their respective adaptive strategies are for coping with changes in the marine environment. Given the declining population sizes of these threatened endemic seabirds, comparative studies are of interest to conservation biologists. Understanding connectivity between seabird populations is important for many reasons, including identifying source-sink populations, prioritizing distinct colonies for conservation, ascertaining the impacts of natural or human-induced environmental change, and assessing a species' capacity to colonize or recolonize available breeding habitat (Crooks & Sanjayan 2006; Carty et al. 2009; Blomqvist et al. 2010). Also, the unavoidable elimination of genetic diversity concomitant with population declines limits the responses of species, because their phenotype space will be reduced and their potential to adjust their position in the adaptive landscape will be compromised (Lande 1988; Lande & Shannon 1996; Fraser 2008; Chevin et al. 2010). Projected climate change will require these unique seabirds to adapt faster than they have had to in the past to be able to tolerate novel conditions.

Isolation-by-distance and other physical barriers to gene-flow have seldom been shown to lead to strong genetic structure among temperate and tropical seabirds at similar spatial scales to those considered in this study, particularly when little phenotypic or morphological variation exists. Suitable breeding habitat is unevenly distributed across the ABE, with large geographical distances separating regional groups of Cape Gannet and African Penguin colonies. A similar pattern exists for the Cape Cormorant, although it breeds at many more colonies along the south-western coast of Africa. The geographic distribution of breeding colonies raises the possibility that breeding endemics, especially Cape Gannets and African Penguins, may experience restricted gene-flow between these regions. Among the phylogenetically diverse seabirds in which this type of hypothesis has been tested, the majority have not exhibited strong phylogeographic structure, possibly due to the violation of natal philopatry in response to population declines caused by decreasing prey availability. The selective pressures imposed by foraging on unpredictable pelagic prey (sardines and anchovy) may promote dispersal among breeding colonies and lead to weaker-than-presumed adult philopatry and juvenile natal site fidelity (Taylor et al. 2011a). Importantly, changes in sea-level have historically drastically altered the availability of breeding space, which could have selected for dispersal propensity among seabirds breeding in affected regions. Even a few effective migrants among breeding regions and colonies will lead to the homogenization of their respective gene pools (Whitlock and McCauley 1999). The data available for the focal study species suggest that sufficient movement among breeding colonies and breeding regions is possible for this to occur (Crawford et al. 1994; Whittington et al. 2005a; Oschadleus & Brooks 2006). It has also been suggested that large historical population sizes, and waves of evolutionarily recent dispersal in response to environmental variability, may mask any contemporary genetic signal of divergence due to physical or non-physical barriers to gene-flow (Taylor et al. 2011a). These natural processes may also be exacerbated by anthropogenic forces during the past 150 years, which may have promoted gene-flow among colonies (Taylor et al. 2011a; Jeyasingham et al. 2013).

Given the close generic relationships among the species within *Spheniscus* and *Morus*, comparisons with related species will help elucidate the historical and contemporary drivers of population genetic and phylogeographic patterns within African Penguins and Cape Gannets e.g. *Spheniscus* species share ancestral adaptations, such as flightlessness, which could affect their dispersal capabilities, although this has not led to population structuring in other *Spheniscus* penguins. Comparisons with other cormorant species may also shed some

CHAPTER 3: Comparative phylogeography and genetic structure of three threatened seabird species in the Agulhas-Benguela Ecosystem

"I never saw a wild thing sorry for itself. A small bird will drop frozen dead from a bough without ever having felt sorry for itself."

(Self-pity, D. H. Lawrence)

Summary

This study constitutes the first range-wide investigation of genetic diversity and conservation genetic status of any endemic, threatened seabird species in the Agulhas-Benguela Ecosystem (ABE) in southern Africa. The primary objective was to investigate genetic diversity and population structure between the breeding regions of three sympatric seabird species using DNA sequence data. Although a number of ecological characteristics buffer seabirds from local environmental change over the short-term, longer-term broad-scale change is a serious threat to their survival. Recently, changing environmental conditions, in combination with fishing pressure, in the ABE have caused collapses and shifts in the distributions of several species, including the pelagic fish that many top predators depend on (sardine *Sardinops sagax* and anchovy *Engraulis* spp.). The resulting spatial mismatch between some breeding colonies of the focal species (African Penguin *Spheniscus demersus*, Cape Gannet *Morus capensis* and Cape Cormorant *Phalacrocorax capensis*) and their pelagic prey has led to reduced food availability for breeding seabirds, impacting their survival, breeding success and breeding distributions. Spatial variation in foraging conditions across their shared range likely affects dispersal rates and may disrupt gene-flow between regions, which has conservation implications. Differences in their evolutionary histories, ecologies and life-histories, these species have exhibited different demographic responses to environmental changes in their shared habitat. These may reflect their responses to historical environmental changes in the ABE that would leave detectable genetic signatures within each species.

To investigate genetic diversity and structure, breeding birds were sampled at colonies across their ranges, and a number of mitochondrial and nuclear DNA regions were sequenced. Results revealed all regions and species are dominated by a few high frequency haplotypes, and that genetic divergence is low among breeding regions and breeding colonies. This suggests high, long-term, range-wide genetic connectivity among populations. Gene-flow among breeding populations of the focal species is currently, or has historically been, sufficient to homogenise genetic diversity across their ranges.

Studies based on ringing data e.g. a multistate capture-mark-recapture (MCMR) modelling, corroborate these findings, and have shown that although breeding adults of all three species are faithful to their breeding sites and foraging grounds year after year, juvenile recruitment to non-natal colonies occurs more frequently than previously thought. This suggests that the population-level response to deteriorating environmental conditions may be mediated by juvenile prospecting and dispersal to more favourable regions. The highly connected population structure observed in these species suggests metapopulation dynamics, and has implications for their conservation management and long-term persistence in a highly variable environment.

INTRODUCTION

Seabirds that have evolved in highly variable marine environments exhibit a number of ecological and life-history characteristics that buffer their populations against ecosystem changes over different time scales (Schreiber & Burger 2001; Crawford & Altwegg 2008). The effects of anthropogenic exploitation of marine species, however, promulgate through food webs and amplify natural levels of variability (Hsieh et al. 2006; Doney et al. 2012), especially for top-predators. Flexibility in organismal traits and individual performance (e.g. physiological tolerance, behavioural flexibility) under novel environmental conditions determine a species' population-level response to environmental change (Doney et al. 2012; Buckley & Kingsolver 2012), and ultimately whether that species will adapt and persist, or go extinct under a new set of conditions.

Long-term environmental change has been shown to affect, among other ecological attributes, phenology (Parmesan 2006; Yang & Rudolf 2010; Jenouvrier & Visser 2011), distribution (Buckley & Kingsolver 2012), breeding success (Boersma & Rebstock 2014) and abundance (Williams et al. 2007) of numerous species globally. Recently, in the Agulhas-Benguela Ecosystem (ABE) off the coast of southern Africa, changing environmental conditions (Roy et al. 2007; Rouault et al. 2009, 2010), in combination with increased fishing pressure (Crawford 2007b; Crawford et al. 2008c), have been implicated in population declines and distributional shifts in several marine species, including sardine *Sardinops sagax* and anchovy *Engraulis capensis* (van der Lingen et al. 2006b; Coetzee et al. 2008; Cockcroft et al. 2008; Crawford et al. 2008b; Blamey et al. 2012). These pelagic fish, which are also the target of a valuable commercial fishery in the region, constitute a critical mid-trophic-level in the ABE, upon which many top-predators depend for their survival (Cury et al. 2000; Shannon et al. 2004). Among these top-predators are a number of threatened, endemic seabird species, which show varying degrees of population decline and regional distributional shifts that largely reflect the altered spatial availability of their shared prey resource (Crawford et al. 2008c; Chapter 1 and 2). These endemic seabirds breed primarily on coastal islands and their breeding habitat is not continuous, so they cannot shift their ranges optimally in response to changes in their environment. To survive and breed under the new conditions, seabird species will have to be flexible in one, or a few, aspects of their foraging and breeding biology. Conservation efforts targeting colonies or regional clusters of colonies with high intraspecific variation may help maintain the viability of these seabird populations that will have to adapt *in situ* to new conditions (Thomassen et al. 2011). With regards to the

use of seabirds as indicators of environmental conditions, conservationists should bear in mind that, due to various aspects of their biology, seabirds will lag behind environmental changes to varying degrees. The genetic consequences of the differential population responses of three seabird species to changes in the ABE are the focus of this chapter, as the observed genetic signal may reflect differences in their life-history characteristics, and foraging and breeding biology (Crawford et al. 2008b; Sabarros et al. 2012). As is the case for many seabird species globally, ringing (banding) data for all three study species suggest that adults are philopatric, which might influence levels of population genetic connectivity across the ranges of these species (Crawford et al. 1994; Whittington et al. 2005a; Oschadleus & Brooks 2006). Conservation genetic tools provide insight into levels of connectivity among populations and improve our understanding of the mechanisms driving the demographic responses by seabirds (Hedgecock et al. 2007; Bicknell et al. 2012; Welch et al. 2012a; Ramírez et al. 2013). The accelerating loss of global biodiversity has stimulated much research aimed at identifying species characteristics associated with extinction vulnerability (Lande & Shannon 1996; Spielman et al. 2004; Colwell et al. 2011). This is particularly relevant to seabirds, which are one of the most threatened groups of birds worldwide (Brooke et al. 2008; Croxall et al. 2012) and are a conservation priority in the ABE (David et al. 2003; Crawford 2007b).

Comparative phylogeography

The significance of conserving genetic diversity, and the evolutionary processes that generate and maintain it are increasingly recognised (Smith et al. 1993; Avise 2000; Reed & Frankham 2003; Spielman et al. 2004; Blomqvist et al. 2010; Bouzat 2010; Cardinale et al. 2012) and are explicitly incorporated into several international conventions and policies (Ehrlich & Wilson 1991; Moritz & Faith 1998; Laikre 2010; Hendry et al. 2010). The field of conservation genetics is growing rapidly as genomic technology becomes more prevalent (Romanov et al. 2009; Joop Ouborg et al. 2009; Allendorf et al. 2010; Ouborg et al. 2010; Hendry et al. 2010; Avise 2010) and our understanding of the value of genetics in ecology only increases with these types of studies (Brito & Edwards 2009). Population genetic studies, when coupled with ecological data, can yield powerful insights pertinent to conservation management e.g. surveys of DNA sequence data for resolving management units e.g. evolutionarily significant units or ESUs (Moritz 1994a; Frankham 2003; Boessenkool et al. 2009b), and estimating population connectivity (Joseph et al. 1995; Bowen 1999; Lowe & Allendorf 2010; Leidner & Haddad 2011). Limited or reduced population

connectivity is of major concern in conservation biology, as it increases population extinction risk (Matthiopoulos et al. 2005; Crooks & Sanjayan 2006; Blomqvist et al. 2010; Lowe & Allendorf 2010).

As discussed in Chapter 1, estimating ecological and demographic parameters (e.g. dispersal and population size) from genetic data is statistically challenging, especially for fluctuating populations, and it is difficult to obtain accurate estimates that are of practical use over time-frames relevant to conservation (Ewing et al. 2004; Broquet et al. 2009; Meirmans & Hedrick 2011). Despite these challenges, comparative population genetic and phylogeographic studies of ecologically similar, sympatric species do provide information about (i) the processes underlying those species' responses to shared historical environmental change and (ii) population connectivity across their shared ranges (Moritz 1994b; Avise 1995). It has long been recognised that genetic data from multiple co-distributed species can contribute significantly to our understanding of geographic and environmental phenomena that have shaped present day distributions of biodiversity (Avise et al. 1987; Hickerson et al. 2010). Such studies that compare intraspecific phylogeographic patterns of several sympatric species are, however, rare (Taberlet et al. 1998). Whilst geographical concordance can be identified at various levels (e.g. within a gene or species), the term 'comparative phylogeography' describes investigating the geography of gene-trees across multiple sympatric species (Bermingham & Moritz 1998; Riddle 2005; Kidd & Ritchie 2006). Given the current rate of biodiversity loss, such phylogeographic studies are important for our understanding about how the history of existing lineages can potentially influence their dynamics in the future (Bermingham & Moritz 1998; Hendry et al. 2010).

The use of mitochondrial (mtDNA) genes has dominated phylogeographic research in animals and mtDNA is considered a "leading indicator" of intraspecific population genetic structure (Zink & Barrowclough 2008). Some of the advantages of employing mtDNA are its non-recombining mode of inheritance and the putative selective neutrality of mtDNA variation (Avise 2000; Ballard & Rand 2005; Zink & Barrowclough 2008; Dowling et al. 2008). Isolating and sequencing animal mtDNA genes is also relatively easy and inexpensive, as most cells have a high copy number of mtDNA, and universal polymerase chain reaction (PCR) primers are more readily available, especially for vertebrates (Sorenson et al. 1999; Hickerson et al. 2010). For these reasons, numerous studies continue to employ mtDNA sequence data to infer taxonomic boundaries, population demographic history, historical levels of gene-flow and population genetic structure (Ball & Avise 1992; Friesen et al. 2007;

Zink & Barrowclough 2008; Hickerson et al. 2010; Bicknell et al. 2012). There are, however, some drawbacks to using mtDNA in phylogeographic studies, and these have recently been emphasised by various authors (Zink & Barrowclough 2008; Brito & Edwards 2009). These include that the entire mitochondrial genome must be treated as a single marker, as all mitochondrial genes are tightly linked and are inherited as a single unit; mtDNA is maternally inherited and therefore reflects only the female lineage; nuclear copies of mitochondrial genes (pseudogenes or NUMTS) can be accidently and preferentially amplified during PCR (Bensasson et al. 2001; Funk & Omland 2003), leading to erroneous conclusions e.g. NADH2 in *Spheniscus* penguins (Simeone et al. 2009); similarly, amplification of mtDNA genes can be complicated by gene duplication events within the mitochondrial genome itself – this has been documented in a number of seabird species including a number of species of sulidae, *Thalassarche* albatrosses, *Pygoscelis* penguins and *Eudyptula* penguins (Mindell et al. 1998; Ritchie 2001; Slack et al. 2003, 2006; Abbott et al. 2005; Morris-Pocock et al. 2010b); mtDNA diversity may not reflect quantitative variation for adaptively important traits (Bekessy et al. 2003); and finally, some deeper analytical and methodological problems exist regarding gene tree heterogeneity in topology and branch lengths – studies have shown that mitochondrial gene trees sometimes differ substantially depending on the locus sampled (Brito & Edwards 2009). Gene trees contain distinct genetic signatures of a species' demographic history, but also contain stochasticity brought about by genetic drift in historical populations (Knowles 2009). Recent methodological advances, including the rise of the coalescent (Kingman 1982), and statistical phylogeographic approaches that incorporate coalescent and mutational variance (Knowles & Maddison 2002) have ameliorated some of these analytical challenges (Knowles 2009). Multi-locus approaches are therefore preferred over single mtDNA-gene phylogeographic studies and are based on the assumption that gene trees estimated from multiple independent loci will show similar patterns due to shared historical events (Brito & Edwards 2009). The multi-locus approach should ideally use rigorous model-based methods (Pritchard et al. 2000), gene concatenation (with partitioning) and the 'total evidence' approach (Kluge 1989) i.e. combining data from molecular and non-molecular sources.

Seabirds are an evolutionarily interesting study group, as they are highly vagile i.e. they have high inter-population dispersal potential and can, theoretically, maintain high levels of gene-flow (Burg & Croxall 2001; Van Bekkum et al. 2006; Bicknell et al. 2012), but many species have highly restricted ranges and exhibit a high degree of natal or breeding philopatry

(Greenwood & Harvey 1982), potentially leading to population genetic differentiation (Burg & Croxall 2001; Dearborn et al. 2003; Friesen et al. 2007). The unpredictability of the relationship between movement patterns and population structure has become known as the "seabird paradox" and has been investigated in species worldwide (Friesen et al. 2007; Milot et al. 2008). Understanding the relative importance of selection, genetic drift and gene-flow in shaping contemporary populations is important for conservation planning (Bicknell et al. 2012), as is understanding how the life-history characteristics typical of seabirds (the "seabird syndrome") restrict species' responses to changes in their environments (Priddel et al. 2006; Votier et al. 2008; Péron et al. 2012), food availability (Oro & Furness 2002; Oro et al. 2004; Lewis et al. 2006; Crawford et al. 2006a, 2008b; Cahill et al. 2013) and predation (Votier et al. 2005; Bicknell et al. 2012). The three focal seabird species of this study exhibit typical seabird characteristics to varying degrees and, represent a 'natural experiment' that makes it possible to test how flexibility in various life-history traits, habitat preferences and foraging modes affect their response – at the molecular and population demographic levels - to changes in their environment. This investigation represents what has been termed "co-structure analysis", defined as "the comparison of population demographic and/or genetic structures between two or more species, with the aim of elucidating factors that determine that structure in one or more of those species" (Criscione 2008; Barbosa et al. 2012, p.1).

Conservation status of the focal species

The Cape Gannet *Morus capensis* has been classified as Vulnerable since 2000, with the global population estimated at ~150 000 pairs in 2006 (Crawford et al. 2007a). It historically bred at nine or 10 localities off the coasts of Namibia and South Africa (Crawford et al. 1983, 2007a), but has bred at only six of these since 1956 (Hockey et al. 2005; Crawford et al. 2007a). The Cape Cormorant *Phalacrocorax capensis* has been classified as Endangered since 2013 and breeds at 69 localities between Ilha dos Tigres in southern Angola, and Stag Island in the Eastern Cape Province of South Africa. Less than 2% of the breeding population (~100 000 pairs) occurs to the east of Cape Agulhas (Barnes 2000). The African Penguin *Spheniscus demersus* was classified as Endangered in 2010 (Crawford et al. 2011). The current estimated 21 000 pairs represents less than 2% of the pre-1900s population size, which is estimated at over 1.5 million birds (Shannon & Crawford 1999) prior to the exploitation of seabird islands by humans.

Cape Gannets, African Penguins and Cape Cormorants have all shown notable population declines and breeding range shifts in response to historical exploitation (Crawford et al.

1983). The numbers of these birds breeding in the ABE have declined over the last 50 years, and populations in Namibia have plummeted due to overfishing and a human-induced, possibly irreversible, ecosystem shift (Crawford et al. 2006b; Lynam et al. 2006). Overfishing and other anthropogenic activities in the northern Benguela ecosystem off the Namibian coast ultimately induced a critical ecosystem "regime" shift, where the recovery of the pelagic fish stocks, and by extension the avian top-predators, has been curtailed, as the pelagic trophic-level has become dominated by zooplanktivorous fish, pelagic goby *Sufflogobius bibarbatus* and jellyfish (van der Lingen et al. 2006a; Lynam et al. 2006). The northern Benguela is also affected by low oxygen events and large-scale warm water events (e.g. Benguela Niños) that do not occur in the southern Benguela. It has been suggested that energy flows within the northern Benguela ecosystem have been permanently altered (van der Lingen et al. 2006a), affecting the carrying capacity for top-predators (Crawford et al. 2007c) and causing irreversible decreases in the abundance of the seabirds there (Cury & Shannon 2004).

Overall population trends

After the collapse of the pelagic fish stock in Namibia, African Penguin, Cape Gannet and Cape Cormorant numbers decreased significantly there (Figure 3.1), but numbers of Cape Cormorants and Cape Gannets in South Africa increased (Crawford et al. 2007a). The total population size of Cape Gannets averaged 250 000 breeding pairs between 1956 and 1969, but this number fell to 150 000 pairs between 1978 and 2006 (Crawford et al. 2007a). There has been a long-term shift to the south and east in the core breeding distribution of Cape Gannets, with the largest colony now present in the Eastern Cape of South Africa (Figure 3.1). In Namibia, the Cape Gannet population crash observed since the 1970s has been attributed to the collapse of pelagic fish stocks there due to overfishing (Crawford et al. 1983, 2007a; Lewis et al. 2006; Kirkman 2007; Hutchings et al. 2009; Moseley et al. 2012). The global population of Cape Cormorants was estimated at about 110 000 pairs in 1956 (Rand 1963a, 1963b), and increased to a peak of about 247 000 pairs during 1977-1981, before decreasing to 72 000 pairs in 1996, when 37% of the population bred in South Africa (Barnes 2000; Crawford et al. 2007b). The demographic response of Cape Cormorants in Namibia appears to be delayed compared to African Penguins and Cape Gannets. Numbers decreased in the 1990s, due to disease outbreaks and food shortages in the Western Cape (Crawford et al. 2007b), but appears to have stabilised at approximately 100 000 pairs in 2006, with 44% breeding in South Africa. Following a long-term decline, the African Penguin population in South Africa showed some signs of recovery during the late 1990s and early 2000s, but did

not nearly attain the population sizes observed prior to the late 1970s (Crawford et al. 1995, 2011).

In summary, since the 1950s, trends in the proportions of sardine and anchovy occurring across the northern and southern Benguela showed striking similarity with the trends in numbers of breeding Cape Gannets and Cape Cormorants, and to a lesser extent African Penguins (Crawford et al. 2008c). The large increase in the Eastern Cape gannet population is likely driven by the increased availability of sardine in that region, reflected in an increase in the contribution of sardines to their diet (Sabarros et al. 2012). The primary demographic mechanism driving the consistently anticlockwise shifts in seabird breeding distributions is uncertain i.e. the relative roles of the movement of adult or juvenile birds between colonies in response to lowered food availability versus differential breeding success at the respective colonies, are not known with certainty. By comparing the observed responses (e.g. overall population growth, breeding success, changes in breeding distribution) of these three endemic seabirds, contrasting the differences in their life-history strategies, and combining modelling and molecular evidence, the present study seeks to gain more clarity about the movement propensity and relative vulnerability of these species to long-term perturbations in their foraging environment.

Regional population trends

Namibia

Census counts of African Penguins and Cape Gannets were carried out in 1956, but regular counts only began years later (Crawford et al. 2009). In Namibia, Cape Gannet and African Penguin numbers fell by 90% and 95% respectively between 1956 and 2008 (Figures 3.1 and 3.2). Between 1956 and 2006, numbers of Cape Gannets at their three Namibian colonies decreased by 85 – 98% (Crawford et al. 2007a) following the collapse of the sardine (*Sardinops sagax*) stock there (van der Lingen et al. 2006a). The overall number of Cape Gannets breeding in the northern Benguela fell from 204 000 pairs in 1956 to only 10 000 in 2006 (Crawford et al. 2007a). Approximately 380 pairs were recorded breeding at Possession Island in 2011(Kemper & Crawford 2007). Numbers of Cape Cormorants (Figures 3.1 and 3.2) decreased by more than 75% after 1978 (Crawford et al. 2007b). The collapse of the northern Benguela sardine stock off the coast of Namibia started in the south and so affected colonies of seabirds there first (Crawford et al. 1987). The effect on African Penguin breeding numbers preceded the observed decline in breeding Cape Gannets (Hockey et al.

2005), possibly due to the shorter foraging range of penguins and limited suitable breeding habitat. The range of Cape Cormorants extends further north, which may have prolonged their access to the diminishing sardine resource relative to Cape Gannets and African Penguins in the region (Crawford 2007). Also, Cape Cormorants extended their breeding range further north in the 1990s in response to the food shortage in northern Namibia (Crawford et al. 2007b).

Western Cape, South Africa

The South African sardine stock collapsed in the 1960s as a result of overfishing, but began to recover significantly in the 1970s and early 1980s, after which sardine and anchovy were more abundant off the south western coast of South Africa than in Namibian waters. During this period, three new African Penguin colonies were established: the Stony Point (1982), Robben Island (1983) and Boulders Beach (1985) colonies, which subsequently grew due to the increased availability of epipelagic fish in the Western Cape region (Crawford 1998; Cury et al. 2000; Crawford et al. 2011).

In the late 1980s, the eastward displacement of sardine and anchovy around the South African coast (Fairweather et al. 2006; Roy et al. 2007) resulted in a spatial mismatch between seabirds in the Western Cape and their prey, causing substantial decreases in the numbers of breeding seabirds there (Crawford et al. 2008c), especially towards the west. The number of breeding pairs of African Penguins in the Western Cape decreased from about 23 000 in 1987 to 13 000 in 1993 (Crawford et al. 2008b). These conditions have persisted, resulting in the overall numbers of breeding African Penguins falling from an estimated 56 000 pairs in 2001 to 21 000 pairs in 2009, representing a loss of > 60% over eight years (Crawford et al. 2011). The survival rates of adult penguins decreased over the same period and African Penguins attempted breeding further east at De Hoop Nature reserve, between 2003 – 2006 reaching a maximum of 18 pairs before abandoning that colony after 2006 (Crawford et al. 2007c).

Cape Gannets recovered along with the sardine stock in the Western Cape during the 1970s and late 1980s, peaking at almost 25 000 pairs in 1990. Numbers of Cape Gannets then declined to about 10 000 pairs in 2008, following the eastward shift in sardine distribution. The number of breeding Cape Gannets fell approximately 38% between 2001 and 2005, a pattern reflected in a reduction of sardine in their diet from 40% (average during 1987-2003) to approximately 6% in 2006 (Crawford & Altwegg 2008). At the westernmost colony,

Malgas Island, there was a long-term population decline of ~35% between 1987 and 2007 (Crawford et al. 2008b), however there was no detectable decrease in survival in the Western Cape over that time (Distiller et al. 2012). In 2006 Cape Gannets abandoned breeding entirely at the Lambert's Bay colony (Crawford et al. 2007a) and breeding success at Malgas Island was very low (Grémillet et al. 2008b), reflecting the eastward displacement of sardine and anchovy (Fairweather et al. 2006; Roy et al. 2007). The reduction in food availability has resulted in Cape Gannets foraging further off-shore and feeding on nutritionally suboptimal prey items (Mullers et al. 2009), such as hake offal from demersal trawlers (Grémillet et al. 2008b; Moseley et al. 2012). The lower energy content of this diet, combined with increased foraging effort is implicated in reducing the breeding success of gannets in the Western Cape (Moseley et al. 2012). Moseley et al. (2012) suggested that, although behavioural flexibility maintained the body condition of adult gannets on the West Coast, there may be long-term costs associated with increased foraging effort and low prey quality, which may eventually affect adult survival.

Similar shifts in the proportion of birds breeding were observed for Cape Cormorants and Swift Terns (*Sterna bergii*) within the Western Cape over the same time period i.e. numbers breeding in the southern portion of the province increased as prey moved south and east (Crawford & Altwegg 2008). The number of breeding pairs of Cape Cormorants in the Western Cape decreased from 96 000 pairs in 1988 to 90 000 pairs in 1991 and again to an average of 30 000 pairs between 1993 and 2006 (Crawford et al. 2008b). The number of Cape Cormorants breeding in the Western Cape between 1985 and 1992 reflected the biomass of anchovy and sardine available to them (Crawford & Dyer 1995). The proportion of birds in the Western Cape that bred at Dyer Island increased from 0.24 in 1988 to 0.80 in 2006, suggesting considerable movement of Cape Cormorants among colonies within the Western Cape (Crawford et al. 1994). Over 90% of the Cape Cormorants breeding in South Africa usually nest at only six off- shore islands in the Western Cape: Bird Island (Lambert's Bay), Malgas, Jutten, Vondeling, Dassen and Dyer Island (Cooper et al. 1982).

Eastern Cape, South Africa

The islands in Algoa Bay in the Eastern Cape have considerable significance for seabirds, as they are the only islands between Cape Agulhas and Mozambique. In South Africa overall (Western Cape and Eastern Cape), the number of breeding pairs of Cape Gannets increased from 50 000 in 1956 to 135 000 in 2006. This increase reflects the large increase in the numbers of birds breeding on Bird Island (Algoa Bay, Eastern Cape), which increased from

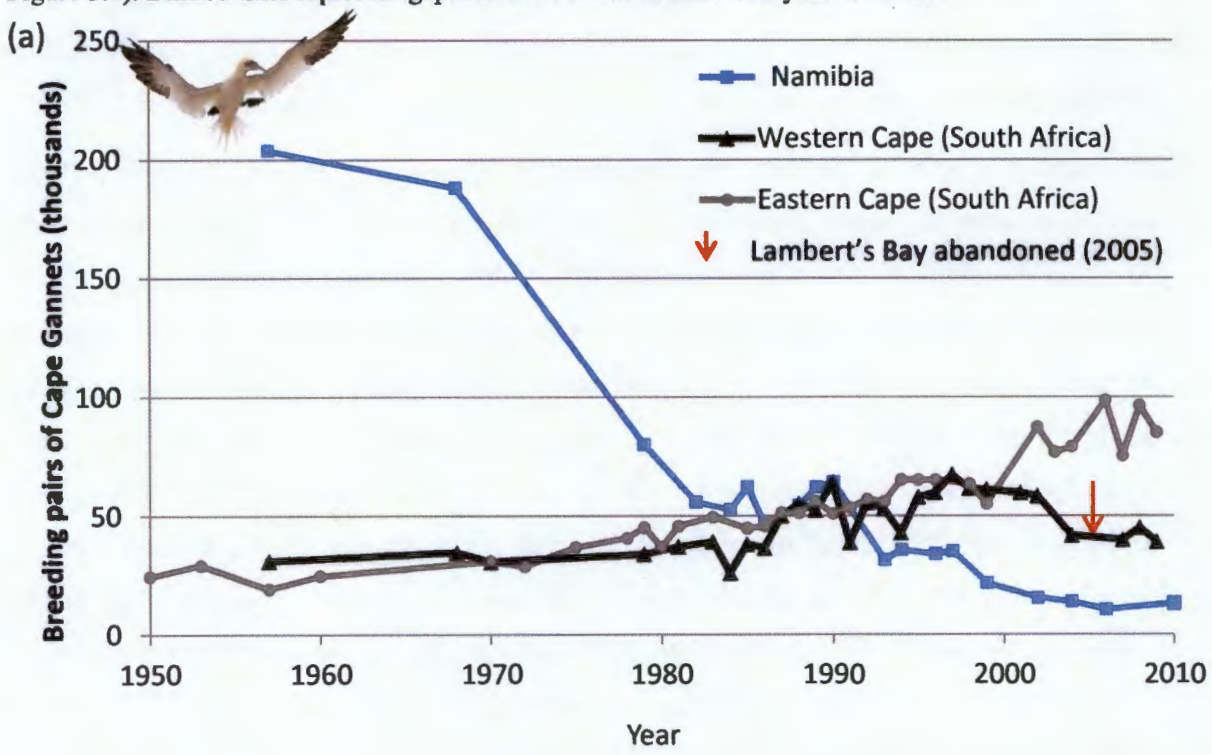
about 19 000 pairs in 1956 to over 50 000 pairs by 1986 and 98 000 pairs in 2006, but decreased to 80 000 in 2008 (Crawford et al. 2009). The Cape Gannet population trend in the Eastern Cape is markedly different from the rest of its breeding range, with the population more than doubling in size at Bird Island as prey became more available between 1980 and 2007 (Crawford et al. 2007a). Intraspecific competition is likely to be higher for gannets breeding in the Eastern Cape because of the size of the colony there (Moseley et al. 2012).

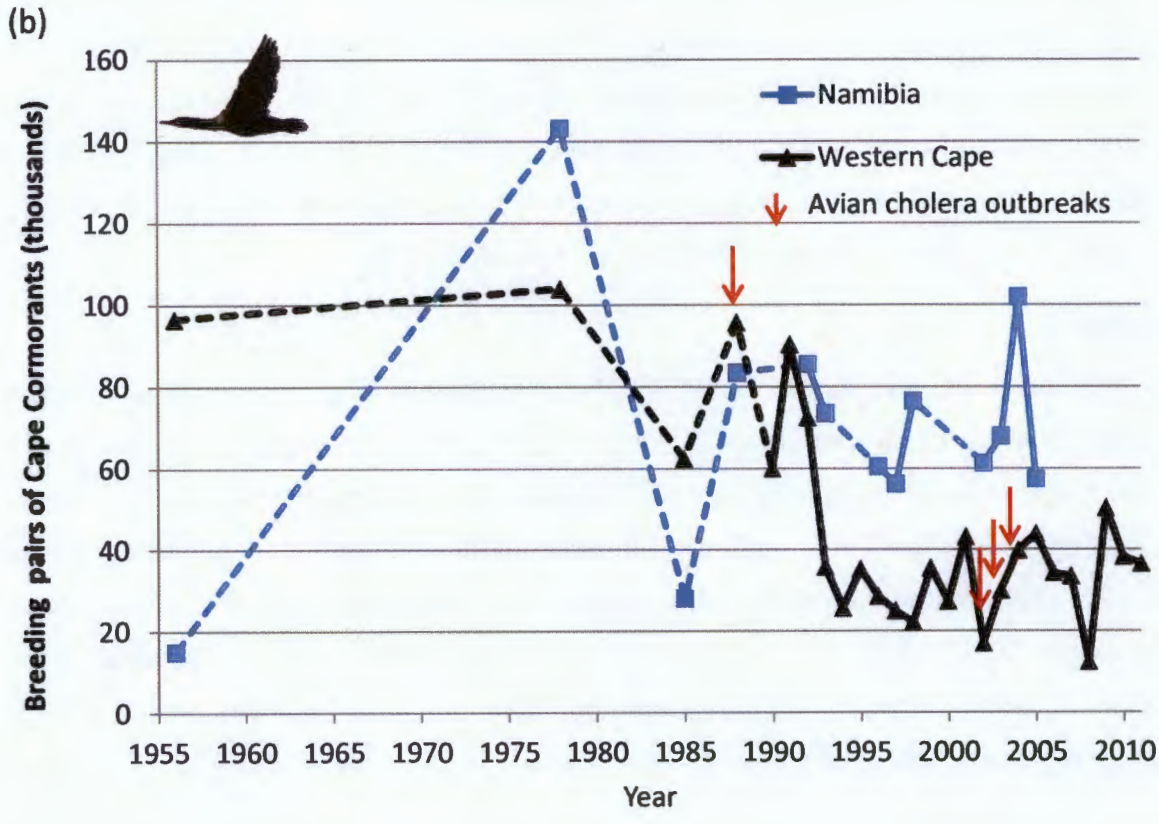
The number of African Penguins breeding in the Eastern Cape increased during the 1980s, but subsequently decreased dramatically, showing the worst decline between 2001 and 2003, when the population halved (Crawford et al. 2011). It has been suggested that although prey became increasingly available to gannets as it moved eastwards, it remained out of reach of penguins breeding in both the Western and Eastern Cape. Cape Cormorants breeding in the Western Cape have been affected by occasional, severe avian cholera *Pasteurella multocida* outbreaks since the 1940s (Crawford et al. 1992a; Waller & Underhill 2007). This disease affects other species of seabirds in the region, but has caused significant mortality only in Cape Cormorants, killing thousands of birds, mostly at the largest colony on Dyer Island (Waller & Underhill 2007). It has been suggested that the high mortality associated with avian cholera outbreaks has masked the consequences for this species of the eastward displacement of sardine and anchovy (Crawford et al. 2008b), but there is evidence that the proportion of Cape Cormorants breeding at the more southerly colonies in the Western Cape has increased as a result of the sardine displacement despite the loss of about 29 000 individuals to cholera between 2002 and 2006 (Waller & Underhill 2007). Only a small minority of the Cape Cormorant population breed in the Eastern Cape, but the numbers appear to be increasing (Figure 3.2) (Waller & Underhill 2007).

Aims

This chapter explores mitochondrial (mtDNA) and nuclear sequence variation to quantify population genetic structure across the breeding ranges of Cape Gannets, African Penguins and Cape Cormorants. It quantifies (i) nucleotide and haplotype variation at mtDNA and nuclear markers and (ii) population structure within and among the sampled regions. Evolutionary history is considered, together with ringing data and the life-history and ecological traits of these target species, to test hypotheses about the possible role of environmental change on dispersal and gene-flow in seabirds endemic to the Agulhas-Benguela Ecosystem (ABE).

Figure 3.1 Estimated number of breeding pairs of (a) Cape Gannets, (b) Cape Cormorants and (c) African Penguins in each of the broad geographic regions in which they breed. Data are shown only for those years in which geographically comprehensive counts were carried out. Eastern Cape colonies are not included for the Cape Cormorant; numbers in that region appear to be growing (see Figure 3.4). Dashed lines represent gaps between annual counts of 2 years or more.





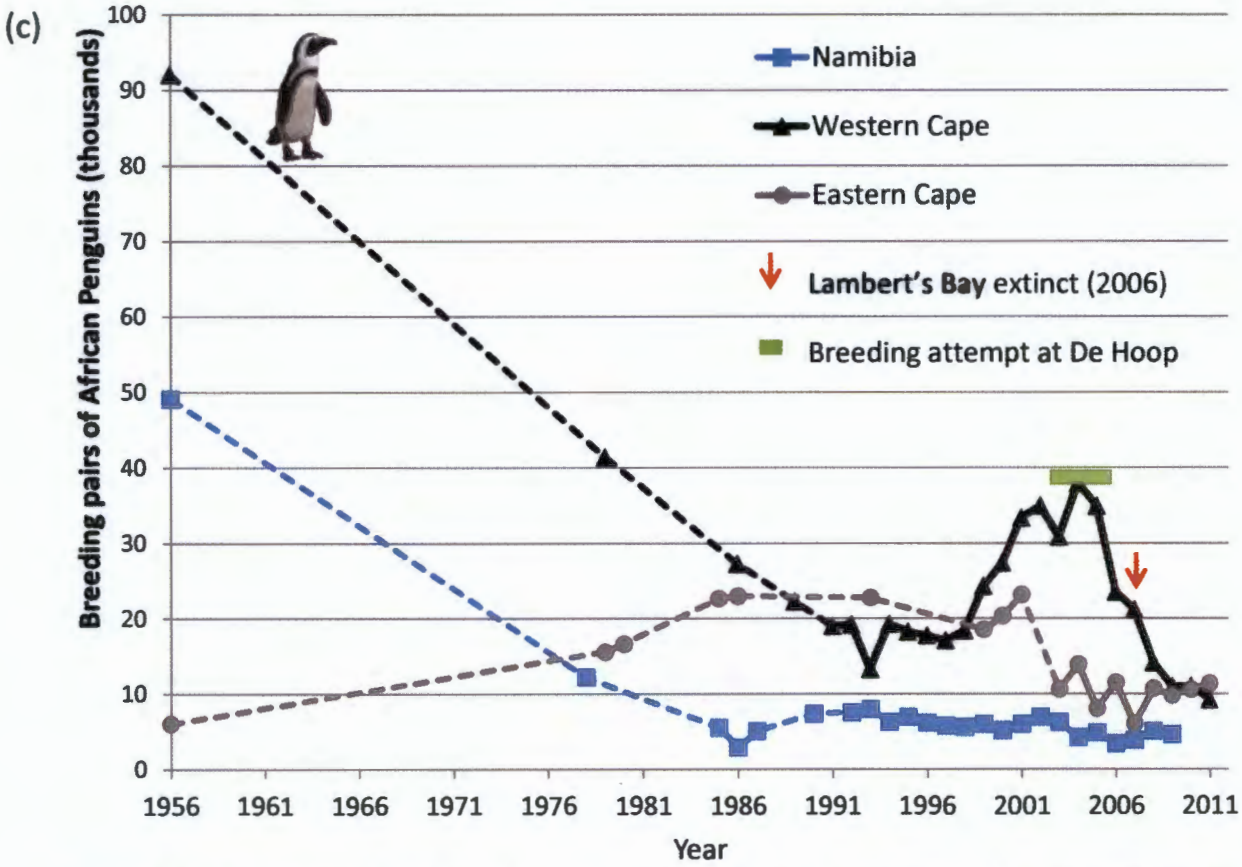
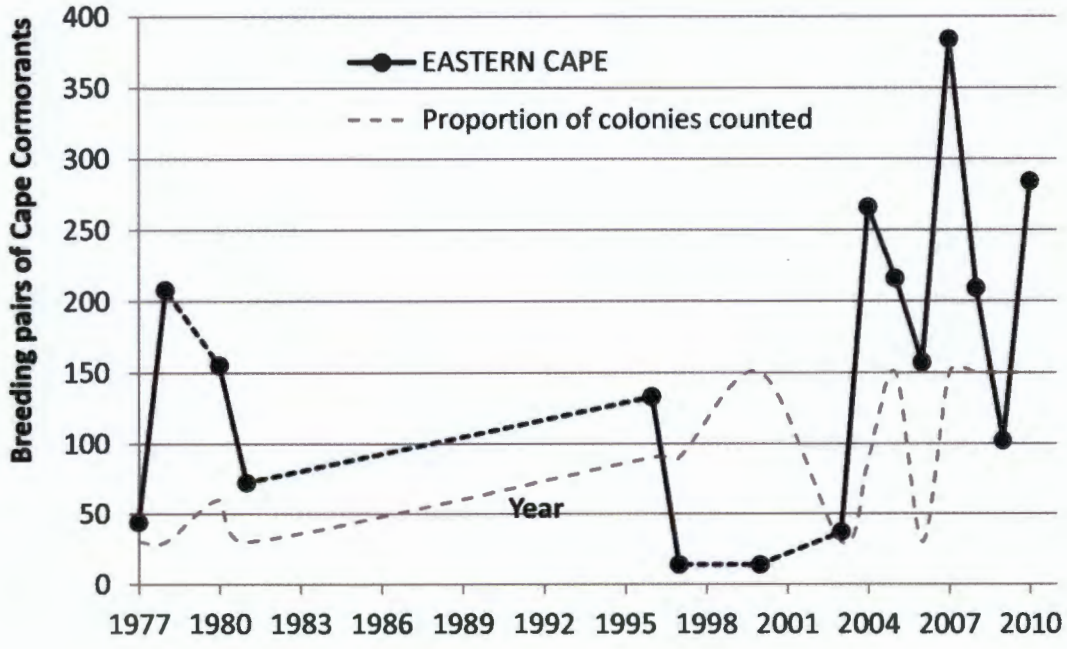


Figure 3.2 The estimated number of breeding pairs of Cape Cormorants at five colonies in the Eastern Cape Province (Jahleel Island, Brenton Rock, St Croix Island Seal Island and near Tsitsikamma Nature Reserve) between 1977 and 2010 – dashed line represents gaps in the annual census data of 2 years or more. The proportion of colonies counted in a given year is shown, as every locality was not visited every year.



Hypotheses regarding genetic diversity and structure were tested in a comparative framework i.e. comparing patterns of population structure among the focal species, and also comparing these results to closely related, congeneric species. The three focal species have all evolved over the same time-frame, under similar evolutionary constraints and environmental selective pressures. Mitochondrial DNA sequence analysis allows for inferences to be made about their respective histories in their shared environment. Given the long generation time of all three study species, any bottleneck signatures in mtDNA sequence data likely predate the more recent population declines, but inferences can be made regarding long-term genetic connectivity among populations.

Many phylogeographic studies report minimal structure in seabird species within ocean basins (Avise et al. 2000; Burg & Croxall 2001; Roeder et al. 2001; reviewed in: Friesen et al. 2007; Bouzat et al. 2009; Techow et al. 2009; Morris-Pocock et al. 2010a), but the focal species of this study are interesting because they occur at the boundary of the Atlantic and Indian Oceans. Also, glaciation events during the Pliocene and Pleistocene are frequently invoked to explain patterns of population genetic diversity and structure in seabirds (Pearce et al. 2002; Morris-Pocock et al. 2008), but the relative roles of ice-encroachment and sea-level rise (and fall) are often unclear. Generally, ice-encroachment would have caused species to shift their breeding distributions drastically towards the tropics, whereas sea-level changes are likely to inundate some colonies and create habitat for new colonies i.e. they may not have necessitated drastic shifts or dispersal to colonise new breeding regions. The coast of southern Africa was not subject to Plio-Pleistocene glaciation and any genetic signal at that temporal scale in the focal study species is, therefore, more likely to be related to changes in sea-level and the associated changes in habitat availability (Etourneau et al. 2009; Roberts et al. 2011; Ksepka & Thomas 2012).

Both foraging and wintering ecology of seabirds are also often used to explain genetic differentiation and, as technology improves and methods are developed to aid our understanding of the at-sea distribution of the focal species, its role in structuring populations can be better evaluated. Stable isotope analysis and preliminary data from GPS and geo-locator studies indicate a degree of regional foraging segregation among African Penguins, Cape Gannets and Cape Cormorants (L. Pichegru, K. Ludinya, T. Cook, pers. Comm.). A paucity of migration among colonies should elevate conservation concern in a species that complies with a metapopulation model, because low dispersal rates may be insufficient to offset declines in local populations (Overeem et al. 2008).

Natal and breeding philopatry (respectively, individuals breeding close to their birthplace or their previous breeding territory), are also life-history traits that might be expected to generate population structure (Greenwood & Harvey 1982; Alcaide et al. 2009). Restricted gene-flow typically leads to genetic differentiation among populations via drift, so sufficient dispersal and effective gene-flow throughout a spatially structured population will result in a similar genetic signal to that observed in a single, large panmictic population. Panmixia among seabird populations in the ABE may be explained by historical processes i.e. natural stressors in a variable environment, and perhaps to a smaller extent by persistent human-induced stressors (e.g. habitat destruction, fishing pressure) over the last 150 years, that promote dispersal among colonies (Taylor et al. 2011a; Jeyasingham et al. 2013).

MATERIALS AND METHODS

All necessary research and ethics permits were approved for the collection of samples (University of Cape Town Science Faculty Animal Ethics clearance number: 2009/V21/LN; Oceans and Coasts branch of the South African Department of Environmental Affairs permit number: RES2010/66; CapeNature (Western Cape Nature Conservation Board) permit number: AAA-004-00520-0035; SANParks permits were approved, but no permit number is supplied). Permits were obtained from the Namibian Ministry of Environment, Natural Resources and Transport (Wildlife Enforcement and Permits Division), Wildlife Trade and Conservation Section (also no permit number supplied on the permit) to visit Ichaboe Island and Mercury Island to collect blood samples, but were only permitted to sample Namibian Cape Gannet and African Penguin chicks. We were not permitted to visit Possession Island, but samples were obtained from previous studies there.

Sample collection and storage

All samples are archived at the Percy FitzPatrick Institute at the University of Cape Town. Collection localities are shown in Figure 2.2.

Cape Gannets

A total of 146 blood samples were taken from breeding adult Cape Gannets, and some chicks, from across their range (although not all samples were used in the present study): South African colonies (n=79): Malgas Island (n=25; 33.05 S, 17.93 E; October 2008), Bird Island, Lambert's Bay (n=25; 32.09 S, 18.30 E; February 2009), Bird Island, Algoa Bay (n=29; 33.84 S, 26.29 E; January 2009); Namibian colonies (n=67): Ichaboe Island (n=26 blood

samples, n=6 feather samples; 26.29 S, 14.94 E; February 2010 and January 2003 respectively) and Mercury Island (n=27, 25.72 S, 14.83 E; February 2010). Eight feather samples from Possession Island were available from previous research activities (collected in January 2003). Seventy-one of the 79 gannets sampled in South Africa were ringed (SAFRING numbers in Appendix 3.1).

African Penguins

A total of 159 blood samples were taken from breeding adult African Penguins (52 additional birds were sampled that are included in the microsatellite study), and some chicks, from across their range (although not all gene regions were amplified for all samples). Forty-four birds are included from the Eastern Cape: Bird Island, Algoa Bay (n=19; 33.84 S, 26.29 E); St. Croix Island, Algoa Bay (n=35; 33.80 S, 25.77 E); 25 chicks were sampled at their natal colonies in Namibia: Halifax Island (n=6; 26.65 S, 15.08 E), Ichaboe (n=11; 26.29 S, 14.94 E), Mercury (n=18; 25.72 S, 14.83 E). An additional 25 adult birds were included from Namibia that were rehabilitated after an oil spill that affected their colonies in mid-April 2009 (Halifax n=14; Ichaboe n=2; Mercury n=4; Possession n=5, 27.01 S, 15.20 E). A total of 50 African Penguins from Namibian colonies are, therefore, included. Fifty-five African Penguins from the Western Cape are included: Boulders Beach (n=7; 34.20 S, 18.46 E), Dassen Island (n=13; 33.43 S, 18.09 E); Dyer Island (n=8; 34.68 S, 19.42E); Jutten Island (n=8; 33.08 S, 17.96 E); Robben Island (n=7; 33.81 S, 18.37 E); Stoney Point (n=12; 34.37 S, 18.90 E). These sample sizes are different to those in Chapter 3, as some additional samples are included in the microsatellite study. Forty-two of the African Penguins sampled had been fitted with flipper-bands. Appendix 3.1 summarises the numbers of African Penguins sampled at each locality.

Cape Cormorants

A total of 95 blood and feather samples were taken from breeding adult Cape Cormorants, from Namibia and the Western Cape (currently only 2% of the population breeds in the Eastern Cape). Not all target regions amplified successfully in all samples. Twenty-three samples were collected in Namibia: Ichaboe Island (n=18, 26.29 S, 14.94 E); Bird Rock guano platform, Walvis Bay (n=5, 22.88 S, 14.54 E). Seventy-two samples were collected in South Africa: Dyer Island (n=33; 34.68 S, 19.42 E); Jutten Island (n=13; 33.08 S, 17.96 E); Malgas Island (n=16; 33.05 S, 17.93 E) and Robben Island (n=10; 33.81 S, 18.37 E). None of

the Cape Cormorants sampled were fitted with rings. Appendix 3.1 summarises the numbers of Cape Cormorants sampled at each locality.

Sample storage, DNA extraction, PCR and sequencing

Blood samples were stored in Longmire's solution (100mM TRIS pH8, 100mM EDTA, 10mM NaCl, 0.5% SDS) and feather samples were stored in 99.9% ethanol. Whole genomic DNA was extracted using DNeasy© Blood & Tissue Kits (Qiagen, Valencia, CA), following the protocol on pg. 25 of the handbook as directed by the manufacturer. The manufacturer's genomic DNA extraction protocol was modified for feather samples in that the Proteinase K incubation step was extended to 24 hours.

Two nuclear genes (Beta-fibrinogen intron 7 and GAPDH) and five mitochondrial genes (NADH dehydrogenase subunit 3, cytochrome oxidase I, ATPase 6, cytochrome b and NADH dehydrogenase subunit 2) were amplified using standard PCR techniques. The primers for each target region are presented in Appendix 3.3. PCR conditions are summarised in Appendix 3.4. PCR reactions were carried out on Applied Biosystems 2720 and Veriti® 96-Well Thermal Cyclers in 25µl reactions. Cycle sequencing of PCR products was performed with BigDye Technology and capillary sequencing instruments (Applied Biosystems 3130 and 3730 Genetic Analysers). All gene regions were direct sequenced using forward and reverse primers. PCR profiles had an initial 3 minute denaturing step (TD=94°C) and a final extension step of 5 minutes (TE=72°C). All PCRs were cycled 35 times. Reagents supplied with Supertherm and Kapa PCR kits (www.kapabiosystems.com) were used for all PCR reactions. The optimal annealing temperature (TA) for each primer pair and the corresponding annealing time for each profile, is given in Appendix 3.4, along with the concentrations of reagents.

Alignment and vetting of sequence data

Forward sequences and the reverse-complement of reverse sequences were aligned using the ClustalW Multiple Alignment (Thompson et al. 1994) accessory application in Bioedit v7.0.9.0 (Hall 1999). Published sequences for closely related species from GenBank were included in alignments to confirm that the correct target region had been amplified and to assess the overall quality of the alignment. Alignments were analysed for each gene region separately and combined. Sequences were obtained for two or more of the seven gene regions for 69 Cape Gannets, 62 Cape Cormorants and 105 African Penguins. Sequences were obtained for three or more of the seven gene regions for 28 Cape Gannets, 21 African

Penguins and 27 Cape Cormorants. Nuclear copies of mitochondrial genes (Numts) and duplicated regions of mtDNA have been reported in a number of seabird taxa, and are suspected to have affected some of the results of this study, especially for African Penguins (NADH2 and control region) and Cape Gannets (control region). Such sequences were omitted whenever possible.

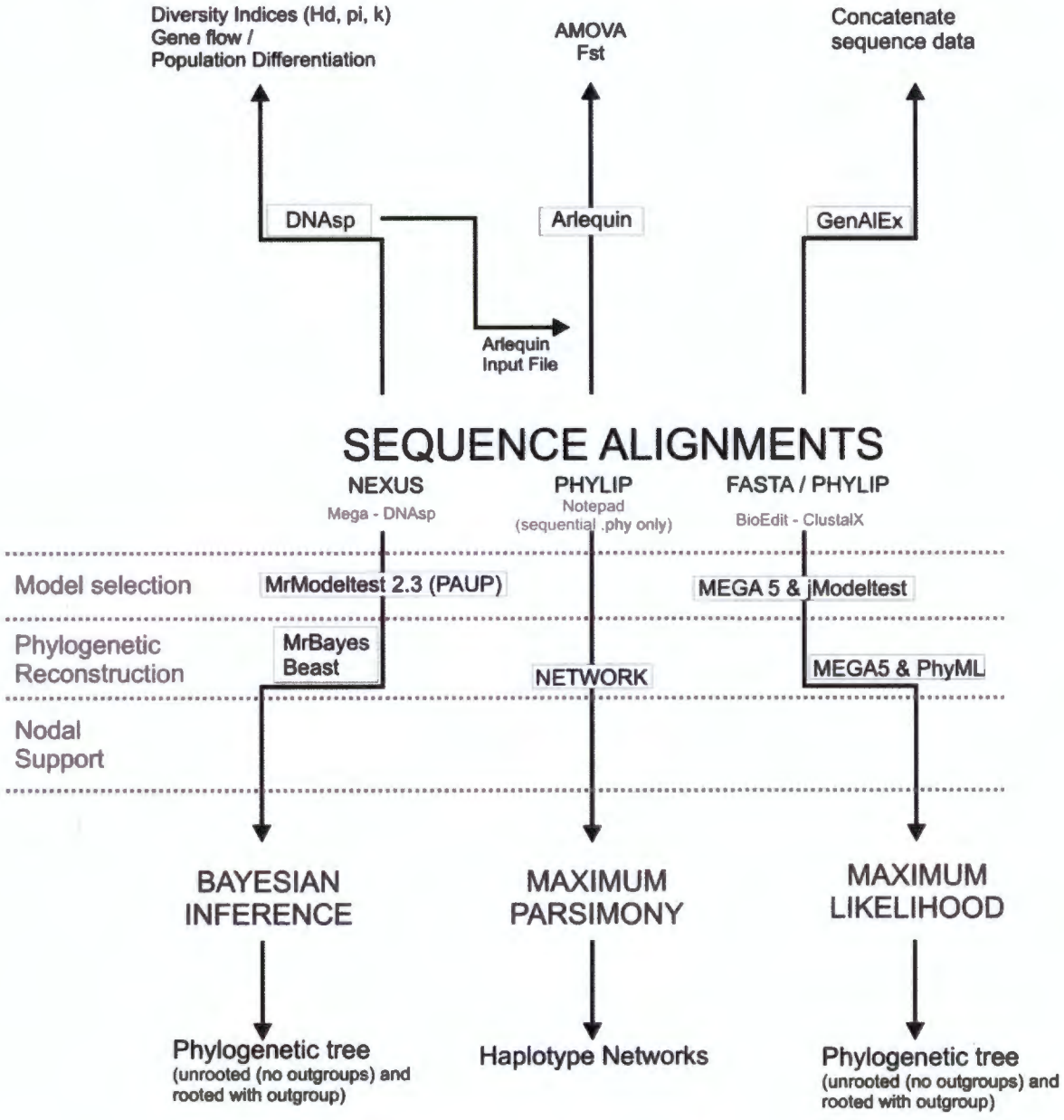
Genetic variation within populations (regional- and colony-level)

To compare genetic variation within each population (defined as breeding colonies (sampling locality), then grouped into geographical breeding regions), standard population genetic diversity indices were calculated based on each gene region (haplotype diversity, h and nucleotide diversity, π) using DNAsp Version 5.10.01 (Librado & Rozas 2009). Tajima's D was calculated for each gene region to test if it was in mutation-drift equilibrium and to investigate if there have been long-term changes in N_e (positive values of Tajima's D indicate historical N_e contraction (reviewed in: Peery et al. 2012)). If sequences were invariable across all individuals sampled, as were BFIBI7 and GAPDH in African Penguins, then no detectable genetic variation exists within or among populations. Overall genetic variation i.e. based on concatenated alignments is also reported for each species.

Population genetic and phylogeographic analyses

To test for population genetic structure, genetic differentiation between all pairs of colonies and all pairs of broad geographic regions was estimated (Figure 3.3) by calculating pairwise φ_{ST}, G_{ST}, D_{xy} and the average number of nucleotide differences (k) among them (significance ascertained by 1000 permutations) in DNAsp (Librado & Rozas 2009). The F_{ST} derivatives were used to determine whether the samples conform to panmictic or non-panmictic, population structure. This is the first (and sometimes the only) step of any analysis i.e. if populations are panmictic, then no significant spatial (or other) structure exists upon which many down-steam analyses are based (Kidd & Ritchie 2006). Hierarchical population genetic structure within each of the three study species was quantified using an analysis of molecular variance (AMOVA) to find φ_{ST} between each colony and each geographic region (Excoffier et al. 1992, 2009). AMOVA estimates indices of genetic structure based on haplotype frequencies and sequence differences between haplotypes. G_{ST} was calculated according to Nei's (1973) equation 9 in DNAsp (Nei 1973; Librado & Rozas 2009).

Figure 3.3 Diagram summarizing the software packages, methodologies and output from mitochondrial and nuclear sequence analyses.



Estimating evolutionary relationships among haplotypes

Phylogeographic methods explore the distribution of intraspecific lineages through time and space, and therefore have a strong phylogenetic component (Avise 2000). Algorithms for reconstructing phylogenies typically search among many possible trees (all possible trees if the number of operational taxonomic units OTUs is small enough), following some optimality criterion, for those trees that approximate the 'true tree' best (Avise 2004).

Phylogenetic methods are commonly used for determining evolutionary relationships among species i.e. species trees, but they can also be useful for determining the evolutionary relationships among lineages in well-defined populations within a species (Heled & Drummond 2010). The different methods used to recover these relationships among individuals, populations or higher taxa are associated with methodological strengths and weaknesses (Avise 2004; Steel 2005; Lemmon et al. 2009). Methods can be divided into: i) Distance-based approaches (UPGMA cluster analysis and the Neighbour-Joining method; phenetic, based on genetic distance matrices) and ii) Character-state approaches (Maximum Parsimony (MP), Maximum likelihood (ML) and Bayesian MCMC methods; performed directly on raw data). The latter three (character-state) methods are employed in this study. MP haplotype networks are sometimes better than bifurcating gene trees for visualising relationships between genes or haplotypes in closely related species, especially in cases involving reticulation. However, ML and Bayesian trees are both more useful than MP networks when describing relationships among populations and closely related species. Homoplasy, due to parallelism, convergence or evolutionary reversals of character states, can lead to unreliable, misleading phylogenies (Avise 2004). However, none of the species in this study are thought to be highly variable and homoplasy is not expected to significantly influence results of phylogenetic analyses.

Maximum Parsimony (MP), Maximum likelihood (ML) and Bayesian MCMC phylogenetic analyses were conducted for each gene region separately, and for combined datasets. MP haplotype networks (Polzin & Daneshmand 2003) were generated in Network v 4.6.1.0 (www.fluxus-engineering.com) using the default settings. MP analyses search for the most parsimonious tree(s) i.e. those that require the smallest number of character-state changes to explain the observed data (Avise 2004). Two different network building algorithms were employed in this study: (i) Reduced Median (RM) (Bandelt et al. 1995), which requires binary data i.e. one transition or transversion at each nucleotide position in the alignment and (ii) the Median-Joining (MJ) algorithm (Bandelt et al. 1999) that allows for ambiguities and multiple transitions or transversions at each nucleotide position in the alignment. These analyses were run using alignments for each gene region that included and excluded outgroup taxa.

Statistical model selection was carried out for each dataset in MEGA v 5.0 (Tamura et al. 2011), and also implemented in jModeltest (Posada 2008) and MrModeltest 2.3 (Nylander 2008). Model selection was based on Akaike Information Criterion (AIC) and Bayesian

Information Criterion (BIC) scores. Corrected Akaike Information Criterion (AICc) values, Maximum Likelihood value (-lnL) and the number of parameters (k) were also calculated (Appendix 3.2) (Posada 2008; Tamura et al. 2011). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites (Tamura et al. 2011) are given for each gene region (Appendix 3.2).

ML analyses were conducted in PhyML (Guindon & Gascuel 2003) and Mega 5.1 (Tamura et al. 2011). The best fit nucleotide substitution models for each dataset, determined by the various model selection programmes outlined above, were used in the Bayesian and ML phylogenetic analyses. ML methods use these data-informed models of evolutionary change as their optimality criterion i.e. they search for tree(s) that maximise the probability of observing the data, given a model that describes the expected behaviour of the substitution rate e.g. whether it is constant throughout the tree; whether it is the same between all nucleotide pairs (Avise 2004).

The reliability or significance of the inferred ML tree i.e. branch support values, is evaluated using bootstrap-resampling (1000 replicates). A bootstrap support value at a given node that exceeds 95% is generally considered to indicate that the topology at that branch is "correct" (Tamura et al. 2011). Bayesian phylogenetic inference is a variant of likelihood methods (Rannala & Yang 1996; Avise 2004) and also requires an evolutionary model of nucleotide substitution. As in ML, the parameters of the evolutionary model are estimated by maximizing the likelihood i.e. the probability of observing the data (a constrained multidimensional maximization is carried out to find the combined set of parameter values that maximise the likelihood function). However, Bayesian analysis differs from ML in a number of important ways: It specifies the prior distribution of tree topologies and the branch lengths of terminal taxa i.e. it treats tree topologies and branch lengths as random variables rather than as parameters (as in ML), and uses a Metropolis-coupled (a convergence acceleration technique) Monte Carlo Markov Chain (MCMC) to model nucleotide substitution (Rannala & Yang 1996); the use of priors (assumed distributions of the model parameters) means that independent information can be incorporated into analyses (Beaumont & Rannala 2004); when searching the likelihood landscape of possible trees, the MCMC process can leap valleys and avoid becoming trapped in local optima instead of the globally highest peak, as is possible in ML (Avise 2004). Where ML identifies a single tree (the one with the highest likelihood), Bayesian MCMC generates a probability based on the likelihoods of a best set of trees given the data and the evolutionary model specified i.e. the

posterior probability of each tree topology and the tree with the highest posterior probability is taken as the estimate of phylogeny (Ronquist & Huelsenbeck 2003).

Bayesian phylogenetic trees were estimated in MrBayes Version 3.1.2 (Ronquist & Huelsenbeck 2003) with six independent chains (each with different starting trees) that run simultaneously and occasionally exchange information to allow the MCMC process to jump across a valley in the likelihood landscape) i.e. if a chain is "trapped", it can escape when data is exchanged with another chain. Each Bayesian analysis was run for at least one million generations and trees were sampled every 100 generations. By monitoring the average standard deviation of split frequencies between two simultaneous runs, it is possible to ensure that the Bayesian MCMC process is converging.

RESULTS

Population genetic diversity indices based on all individuals sequenced for each gene region in each species are reported in Table 3.1. The population genetic diversity indices for each breeding region and each breeding colony sampled are given in the appendices (e.g. Appendices 3.5 and 3.6) for each species. A number of markers tested during this study were not variable enough to be informative for testing hypotheses about population connectivity. B-fibrinogen (nuclear DNA, B-fib intron 7) was invariable among 31 African Penguin samples from across their range, and only differed at one nucleotide position from a published sequence (GenBank accession number: EF552784). GAPDH was invariable among 15 African Penguin samples. Also for African Penguins, NADH2 (n=69) and control region (n=107) alignments showed consistent ambiguity at numerous nucleotides, indicating that either nuclear copies of the mitochondrial target regions (Numts) were being simultaneously amplified or a duplication within the mitochondrial genome has occurred in this species (Table 3.1). Both of these genetic phenomena have previously been reported in other penguin species (Ritchie 2001; Slack et al. 2003; Simeone et al. 2009). GAPDH was invariable in five Cape Cormorants from four different colonies in Namibia and South Africa and differed from *P. carbo* (AF339342) at six nucleotide positions. One Cape Cormorant ATPase-6 sequence was obtained from GenBank and incorporated into the alignment, but the published sequence was shorter than, and identical to, some of the samples collected for the present study and was excluded (AY009350). COI (925bp) was invariable among 11 Cape Cormorants from Namibia and South Africa, except for one nucleotide change in a bird from Ichaboe Island, Namibia. The nuclear B-fib alignment (567bp) for Cape Cormorants was invariable among 24 samples from six colonies, except for one nucleotide position, where the base was

consistently ambiguous on chromatograms (Table 3.1). It is likely that the two alleles were amplified simultaneously and, although these data could be used in population genetic analyses, there is too little variation at this marker to be informative. The B-fib sequences for Bank and Cape Cormorants were combined with *M. bassanus* (AY695213, EU739445, EF552786, EF881997), *M. serrator* JX683938, *S. dactylatra* AY695212, *Anhinga anhinga* (AY695210, EU739364, EF552751, DQ881941) and *Phalacrocorax* (*P. carbo*: DQ881980, *P. auritus*: AY695211, *P. melanoleucos*: JX683939) sequences from GenBank, and the Cape Gannet data, to produce a nDNA phylogeny (n=70, 514bp). Among the 12S (187bp) sequences, the five Bank Cormorant sequences generated during the present study (data presented in Chapter 2) were identical to the published sequence (GU445900), except for samples from Ichaboe Island, which shared a unique haplotype endemic to that region. Cape Cormorant cyt b aligns to site 13962 of the published *P. chalconatus* mt genome (GenBank accession number: GU071054). The Reed Cormorant differs from the Crowned Cormorant at 5 nucleotide positions in ATPase-6 target region (668) and three of these differences are shared with another *Microcarbo* species *P. melanoleucos*.

Molecular markers in Cape Gannets

Cape Gannet: NADH3 (400bp)

In the Cape Gannet NADH3 dataset (n=94, 400bp), five haplotypes existed among samples from all six breeding colonies (overall haplotype diversity h=0.143±0.049; nucleotide diversity, π=0.0004±0.0001; average number of nucleotide differences, k=0.147, Table 3.1). Haplotype diversity is a measure of the uniqueness of a haplotype within a population; a value of one indicates that all haplotypes within a colony are unique. Haplotype diversity was markedly higher at Namibian colonies, and in Namibia overall compared to colonies in the Western Cape and Namibia (Appendix 3.5 (a) and (b)) based on NADH3. Pairwise φ_{ST} estimates between breeding regions were very low and no significant population differentiation was detected between breeding regions or among the six colonies sampled (probabilities obtained by a permutation test with 1000 replicates, Table 3.2).

Table 3.1 Population genetic diversity indices based on each gene region for each of the three focal species: sample size (n), length of the alignment in base pairs (bp), the number of haplotypes identified (H), haplotype diversity (h±SD) and nucleotide diversity (π).

Species		NADH3	ATPase-6	COI	NADH2	GAPDH	B-FIBI7	Cyt b	Control Region
Cape Gannet	n	94	28	26	20	25	31	15	-
	bp	400	669	668	555	419	559	834	-
	H	5	4	10	4	5	2	2	-
	h	0.14±0.05	0.58±0.06	0.83±0.06	0.28±0.13	0.62	0.07	0.25	-
	π	0.0003	0.001	0.003	0.0005	0.002	0.0001	0.0003	-
African Penguin	n	124	130	38	69	15	31	-	107
	bp	358bp	672	668	527	369	575	-	520
	H	6	12	5	-	1	1	-	-
	h	0.18±0.05	0.244±0.05	0.33±0.09	suspected numts	invariable	invariable	-	suspected duplication
	π	0.0005	0.0004	0.0005	-	-	-	-	-
Cape Cormorant	n	71	47	11	-	5	24	41	-
	bp	393	682	925	-	380	567	864	-
	H	3	2	2	-	1	1	4	-
	h	0.52±0.02	0.23±0.07	largely invariable	-	invariable	invariable	0.615±0.04	-
	π	0.001	0.0003	-	-	-	-	0.0009	-

Table 3.2 Pairwise comparisons of genetic structure using φ_{ST}, G_{ST}, D_{xy} and the average number of nucleotide differences, k_{xy} at (a and b) the regional-scale and (c and d) colony-level based on the NADH3 dataset for Cape Gannets (400bp). φ_{ST} and k_{xy} above diagonals and G_{ST} and D_{xy} below.

(a) NADH3 (400bp)		φ_{ST}		
		NAM	**WC**	**EC**
G_{ST}	**Namibia (NAM)**		0.000	- 0.02
	Western Cape (WC)	0.006		0.000
	Eastern Cape (EC)	- 0.01	0.001	

(b)		k_{xy}		
		NAM	**WC**	**EC**
D_{xy}	**Namibia (NAM)**		0.134	0.203
	Western Cape (WC)	0.000		0.127
	Eastern Cape (EC)	0.001	0.000	

(c)			φ_{ST}				
		Ichaboe (NAM)	**Mercury (Namibia)**	**Possession (NAM)**	**Lamberts Bay (WC)**	**Malgas (WC)**	**Bird Island (EC)**
G_{ST}	**Ichaboe (NAM)**		-0.031	0	0	0	-0.03
	Mercury (NAM)	-0.022		0	-0.04	0	-0.027
	Possession (NAM)	0.033	0.035		0	0	0
	Lamberts Bay (WC)	-0.01	-0.021	0.027		0	0
	Malgas (WC)	0.022	0.016	1	0.001		0
	Bird Island (EC)	-0.02	-0.02	0.037	-0.011	0.014	

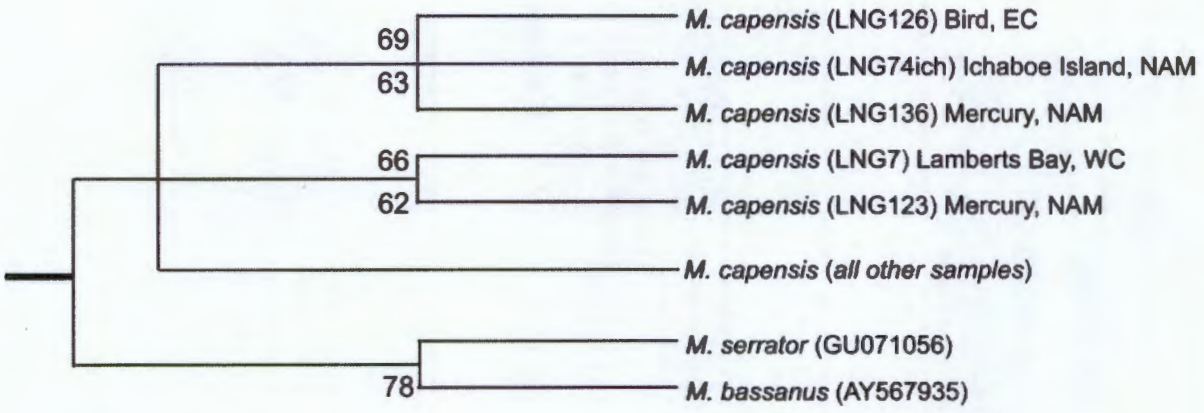
(d)			k_{xy}				
		Ichaboe (NAM)	**Mercury (Namibia)**	**Possession (NAM)**	**Lamberts Bay (WC)**	**Malgas (WC)**	**Bird Island (EC)**
D_{xy}	**Ichaboe (NAM)**		0.237	0.133	0.196	0.133	0.227
	Mercury (NAM)	0.0006		0.111	0.167	0.111	0.206
	Possession (NAM)	0.0003	0.0003		0.063	0	0.1
	Lamberts Bay (WC)	0.0005	0.0004	0.0002		0.063	0.163
	Malgas (WC)	0.0003	0.0003	0	0.0002		0.1
	Bird Island (EC)	0.0006	0.0005	0.0003	0.0004	0.0003	

Maximum Parsimony (MP), Maximum likelihood and Bayesian phylogenetic analyses

The median-joining (MJ) and reduced-median (RM) MP haplotype networks based on Cape Gannet NADH3 (400bp) included *M. bassanus* (GenBank accession number: AY567935; Treutlein & Wink, unpublished) and *M. serrator* individuals (GU071056; Gibb *et al.*, unpublished), and showed identical patterns. There is one dominant haplotype (Figure 3.4), which is exhibited by 87 of the 94 Cape Gannets sampled from all six breeding colonies. Unique, endemic haplotypes were found at Bird Island in the Eastern Cape and Ichaboe Island in Namibia (Figures 3.4 and 3.5). Other haplotypes were shared either between the Western Cape and Namibian colonies or between the Eastern Cape and Namibia. The dominant (most common) haplotype was shared by 92% of sampled birds representing all six colonies. The NADH3 haplotype representing the Northern Gannet is separated from all Cape Gannet samples by at 9 mutational steps, more than the Australasian Gannet (3 steps, Figure 3.5(a)).

The HKY model of nucleotide substitution was identified as the best at describing the NADH3 sequence alignments for Cape Gannets (Appendix Table A.4). The Bayesian and ML trees (Figure 3.4) based on NADH3 reflect the pattern observed in the haplotype network, and consist of a large polytomy (reflecting the dominant haplotype) of individuals sampled in all three breeding regions, with two weakly supported sub-clades: one containing individuals from the Western Cape and Namibia (65%, Bayesian posterior probability=0.66) and the other Namibia and the Eastern Cape (62%, Bayesian posterior probability=0.69).

Figure 3.4 Maximum likelihood (ML) and Bayesian phylogenetic tree (consensus tree, HKY model) based on the Cape Gannet NADH3 data set (numbers above nodes are Bayesian posterior probabilities; below the nodes ML bootstrap values).



Cape Gannet: ATPase-6 (669bp)

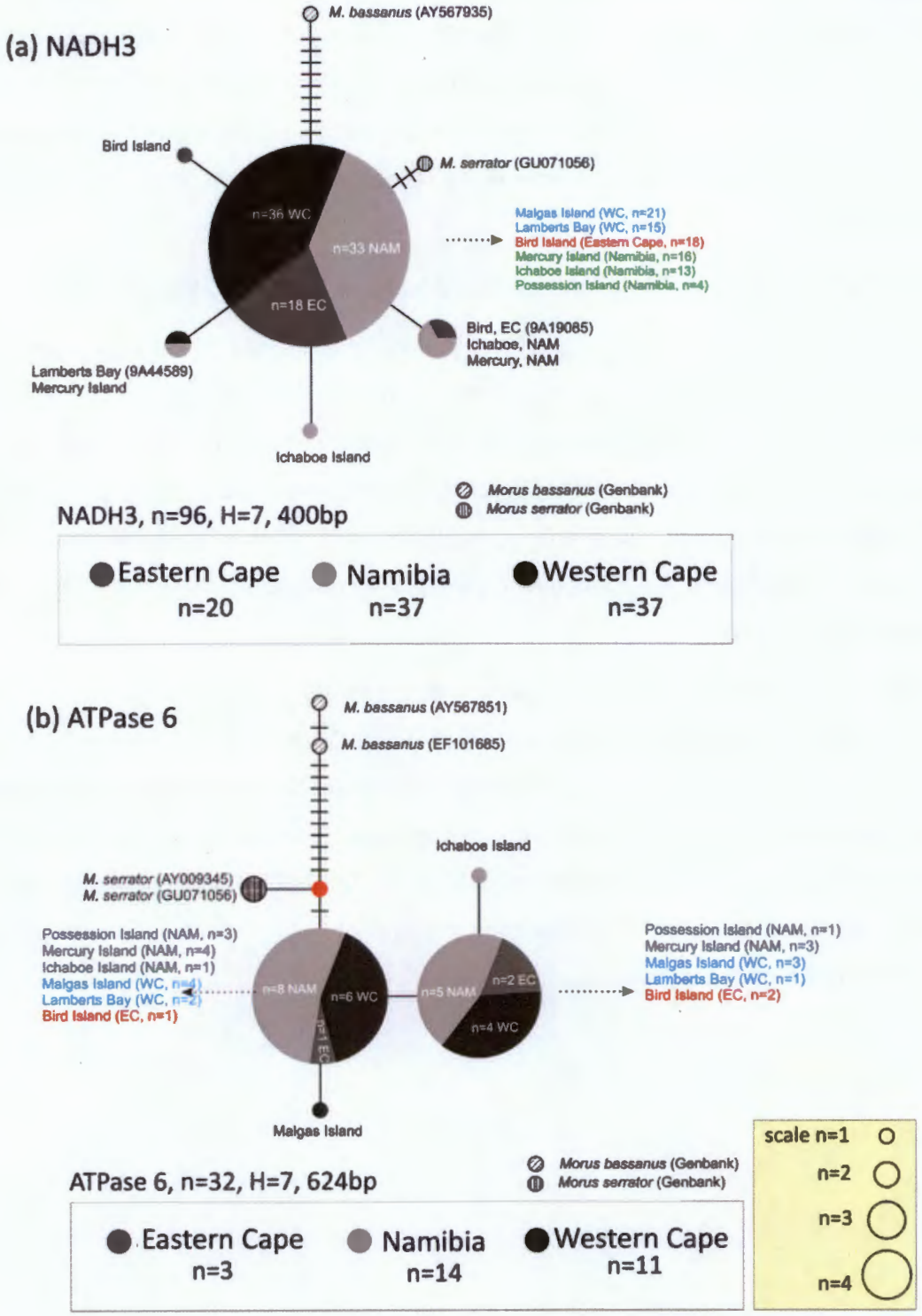
The ATPase-6 target region was amplified for 28 Cape Gannet samples (n=14 from Namibia, n=11 from the Western Cape and n=3 from the Eastern Cape). Four haplotypes were detected in this dataset (overall haplotype diversity h=0.577±0.055 and nucleotide diversity π=0.0012, Appendix 3.6 (a), Table 3.1). When populations are defined as breeding colonies, Possession Island in Namibia exhibits the lowest haplotype diversity, and Ichaboe Island the highest (0.5 and 1 respectively), although sample sizes for colony-level analyses are small (all n<9; Appendix 3.6 (b)). Pairwise φ_{ST} estimates between breeding regions were very low and no significant population differentiation was detected between breeding regions or among the six colonies sampled (probabilities obtained by a permutation test with 1000 replicates, Appendix 3.7).

Maximum Parsimony (MP), Maximum likelihood and Bayesian phylogenetic analyses

The MJ and RM MP analyses of the ATPase-6 data (both the full 669bp alignment and that truncated to 624bp) showed identical results (Appendix 3.8 and Figure 3.5(b)). The MJ network based on the full alignment showed a very similar pattern, but introduced a "missing haplotype" into the network. All MP analyses showed two dominant haplotypes (separated by an A-G transition at 550bp – Adenine is probably the ancestral state, as it is exhibited by both of the other gannet species). Endemic haplotypes were detected at Ichaboe and Malgas Islands (Figure 3.5 (b)).

The model of nucleotide substitution that best describes the ATPase-6 sequence alignments that include and exclude outgroup taxa was identified as the HKY model (Appendix 3.2). The ML tree based on the ATPase-6 alignment (669bp, Figure 3.6) mirrored the pattern exhibited in the haplotype networks in that there is one dominant clade representing the two dominant haplotypes (a second sub-clade is nested within the first). This clade and sub-clade both contain representatives from all six breeding colonies of Cape Gannets. There are only two other haplotypes among individuals.

Figure 3.5 (a) Cape Gannet haplotype network based on NADH3. Numbers in brackets are accession numbers for sequences extracted from the GenBank database or ring numbers (SAFRING) for ringed individuals sampled for the present study. **(b)** The relationships among the four haplotypes detected in Cape Gannets and four outgroup individuals based on the truncated ATPase-6 alignment (624bp). The size of the circles represents the number of individuals that possess a particular haplotype and the line between haplotypes represents a nucleotide change and tick marks along it indicate additional changes. Red circle represents **"missing haplotype"**.



Four gannet sequences were available on GenBank for comparison with the full ATPase-6 alignment (669bp): *M. serrator* AY009345 (Kennedy et al. 2000) and GU071056 (Gibb, unpublished); *M. bassanus* AY567851 (Treutlein & Wink, unpublished) and EF101685 (Hughes et al. 2007). These formed two well-supported clades in the Maximum likelihood (ML) tree (Figure 3.6). Other sulid species were also added to the analysis (Appendix 3.9): *Sula nebouxii* (Blue-footed Booby) EF101686 (Hughes et al. 2007), *Sula sula* (Red-footed Booby) AY009346 (Kennedy et al. 2000), *Sula dactylatra* (Masked Booby) AY941806 (Kennedy et al. 2005) and *Sula leucogaster* (Brown Booby) EF101687 (Hughes et al. 2007). The full sequences were not available for all species, so a truncated alignment of 624bp was used for this analysis. Cape Gannets form an unresolved polytomy that is sister to a clade containing the other two gannet species (Figure 3.7). The overall pattern observed in the Bayesian phylogenetic tree is very similar to that of the ML analyses above. Cape Gannets from all three breeding regions fall into two well-supported clades, with one sample from Malgas Island distinct from the rest (Appendix 3.9).

Cape Gannet: Cytochrome Oxidase I (COI)

In the Cape Gannet COI dataset (n=26, 668bp), there are nine polymorphic sites, six of which are parsimony informative. Ten haplotypes were identified (overall haplotype diversity, h=0.83±0.06; nucleotide diversity, π=0.0027±0.0005; average number of nucleotide differences, k=1.8, Appendix 3.10 (a)). At a regional scale, the Eastern Cape had the highest haplotype diversity, and the Western Cape exhibited the lowest (h=1 and 0.79 respectively). Although sample sizes for colony-level comparisons are low, Mercury Island in Namibia exhibited the lowest haplotype diversity (Appendix 3.10 (b)). Pairwise comparisons of genetic structure (φst) ranged from 0.11 (between the Western Cape and Namibia) to 0.36 (between the Western Cape and Eastern Cape, Appendix 3.11). G_{ST}, the average number of nucleotide differences between populations (k) and genetic distance (Dxy) are also reported (Appendix 3.11). The elevated estimates of population divergence (notably φ_{ST}) based on COI sequences may be an artefact of the small sample size, or it may be that this gene region has higher resolution (is more variable) for Cape Gannets compared to the ATPase-6 and NADH3 markers.

Figure 3.6 Maximum likelihood consensus phylogenetic tree based on the ATPase-6 sequence data (669bp). Numbers at each node indicate bootstrap support (1500 replicates) estimated in MEGA (below) and PhyML (above). Collection localities (colonies and regions) are indicated for each sample: Western Cape (WC, blue), Eastern Cape (EC, red) and Namibia (NAM, black).

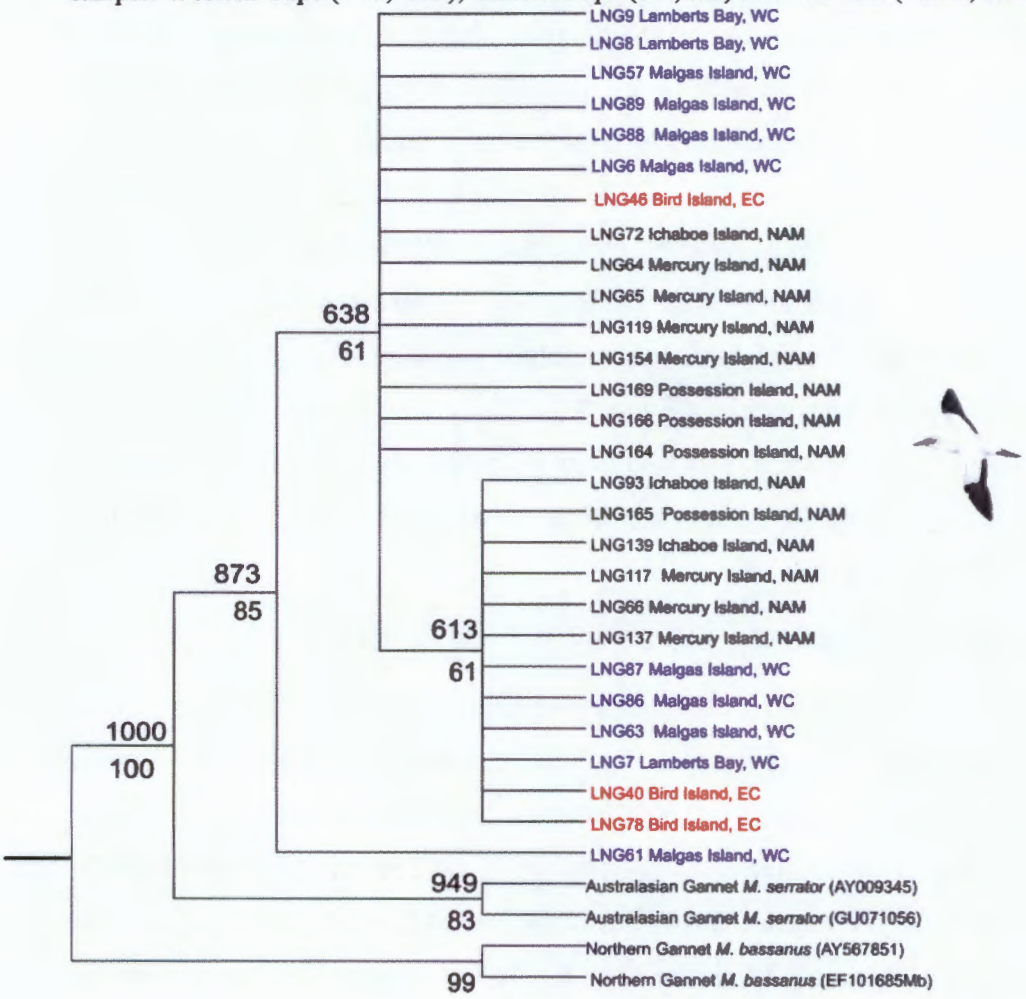
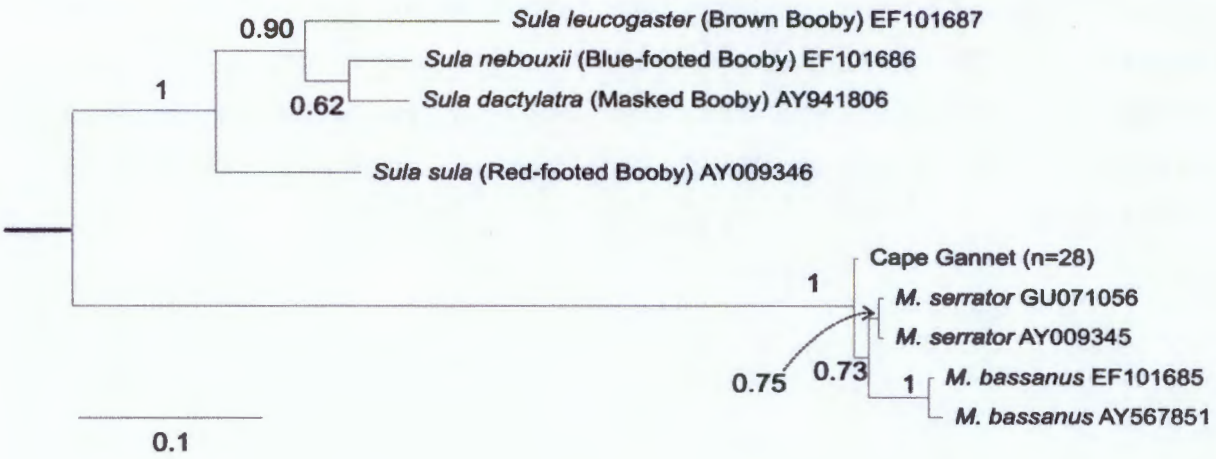


Figure 3.7 Bayesian Phylogenetic tree based on the ATPase-6 sequence data for Cape Gannets (669bp) and the outgroup taxa listed in the text. Numbers at the nodes are Bayesian posterior probabilities.



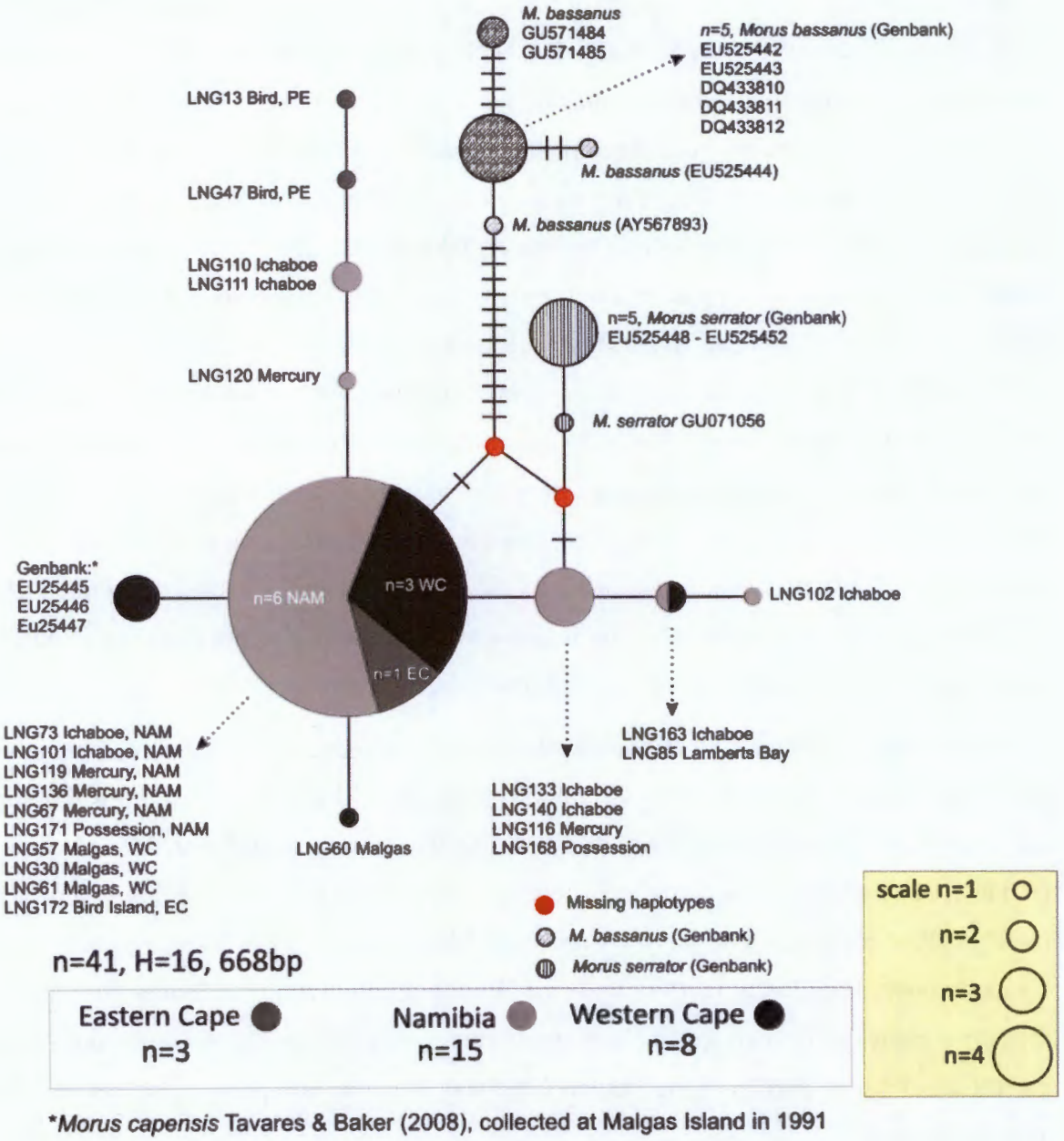
Maximum Parsimony (MP), Maximum likelihood and Bayesian phylogenetic analyses

Multiple COI sequences from other gannet species were available on GenBank and the Barcoding Life (BoL) databases: Australasian Gannet *M. serrator* GU071056 (Gibb et al., unpublished), EU525448 – EU525452 (Tavares & Baker 2008) and Northern Gannet *M. bassanus* AY567893 (Treutlein & Wink, unpublished), EU525442 - EU525444Mb (Tavares & Baker 2008), GU571485 and GU571484 (Johnsen et al. 2010) and DQ433810 - DQ433812 (Kerr et al. 2007). For the MP analyses of Cape Gannet COI (n=26, 668bp), the MJ and RM networks were identical (Appendix 3.12). Ten haplotypes were identified, with one dominant haplotype representing individuals from all three breeding regions. Endemic haplotypes existed at lower frequencies in all three regions: four in Namibia, two in the Western Cape and two in the Eastern Cape. One of the Western Cape haplotypes was found in three samples collected in 1991 from Malgas Island (Tavares & Baker 2008), which may be contributing to the elevated estimates of population divergence (compared to ATPase-6 and NADH3). One haplotype was shared between Namibia and the Western Cape. For the family–level network (n=41, 668bp, Figure 3.8), six Australasian Gannet and nine Northern Gannet COI sequences were included for comparison. One extra Australasian Gannet and three Northern Gannet individuals could be included by truncating the alignment to 600bp (n=45, EF101674 excluded due to multiple alignment gaps), however this caused a loss of resolution in the network and the fundamental relationships between haplotypes remained the same. RM analysis could not be conducted on the family-level analysis due to the presence of multi-state characters at numerous nucleotide positions in the alignment.

The model selected for the COI alignments was HKY (Appendix 3.2) in Mega (Tamura et al. 2011) and, although jModeltest (Posada 2008) selected the TPM2uf model using AICc, HKY was used for the Maximum likelihood analyses. The 50% bootstrap consensus ML tree based on the COI alignment excluding (n=26, 668bp, Appendix 3.13) and including outgroups (n=41, 668bp, Figure 3.9) strongly reflected the MP analyses above. The published Cape Gannet sequences collected in 1991 from the Western Cape (Tavares & Baker 2008) form their own clade, as do two groups of Cape Gannets collected for the present study: One comprised of individuals from the Eastern Cape and Namibia, and one of Namibia and the Western Cape.

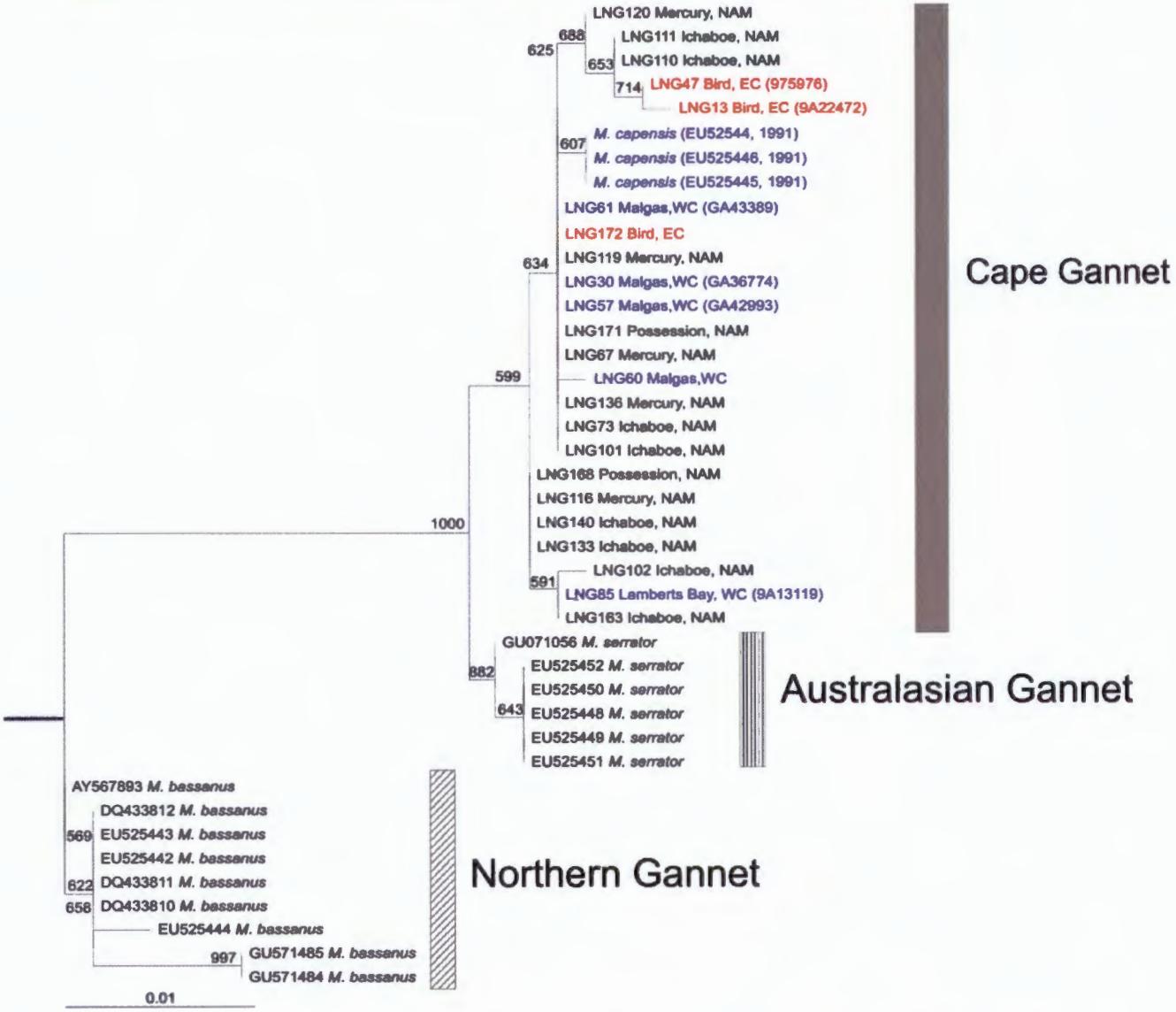
M. serrator and *M. bassanus* each form well-supported clades of their own, reflecting their phylogenetic relationship with *M. capensis* (Figure 3.9). The large polytomy represents individuals from all three breeding regions with a single shared haplotype.

Figure 3.8 The relationships between the 16 haplotypes identified in the Genus-level Cytochrome Oxidase I (COI) sequence dataset (n=41, 668bp), and their frequencies in each Cape Gannet breeding region (Eastern Cape, Namibia and Western Cape). Australasian Gannet (*Morus serraror*) and Northern Gannet (*M. bassanus*) COI sequences from GenBank are included. The size of the circles represents the number of individuals that possess a particular haplotype and the black line between haplotypes represents one nucleotide change.



**Morus capensis* Tavares & Baker (2008), collected at Malgas Island in 1991

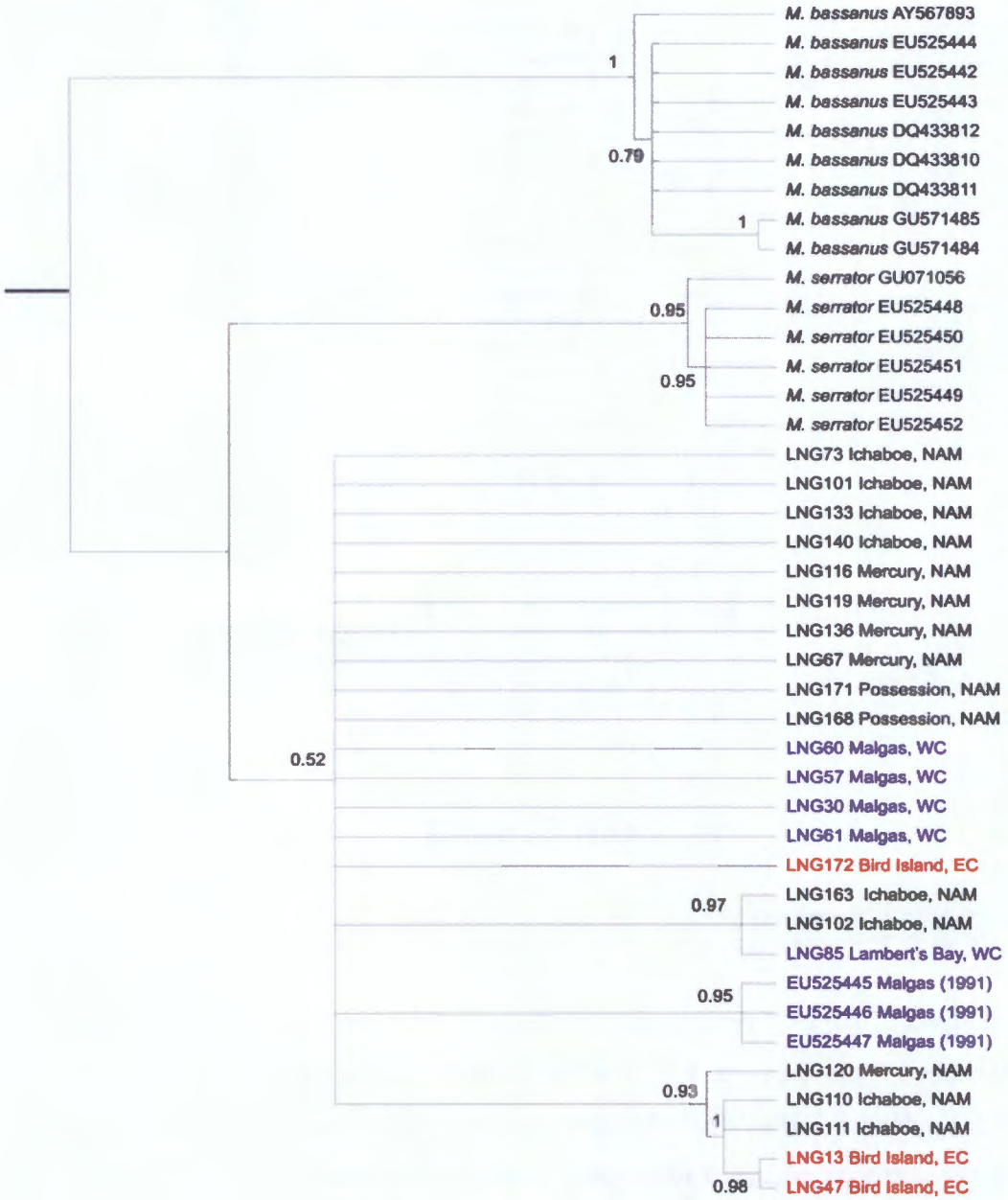
Figure 3.9 Maximum Likelihood neighbour joining tree generated by PhyML based on the HKY model and the Cape Gannet COI dataset, including outgroup taxa (n=41, 668bp). Branch support values are the result of a non-parametric bootstrap analysis (1000 replicates). Colours indicate regional collection locality (red = Eastern Cape, blue = Western Cape and black = Namibia).



The Bayesian analysis of the COI dataset was also based on the HKY model, identified by hierarchical Likelihood Ratio Tests (hLRTs) and the Akaike Information Criterion (AIC) in MrModeltest 2.3 (Nylander 2008). The COI Bayesian tree incorporating all three *Morus* species reflects the currently accepted phylogeny, with a well-supported clade containing Northern Gannets ancestral to the Australasian-Cape Gannet sister pair (Figure 3.10). Within the Cape Gannets, the well-supported structure largely reflects the pattern observed in the haplotype networks: one clade containing samples sourced from GenBank (suggesting an

alignment or sequencing artefact), a second clade containing birds from Namibia and the Eastern Cape, and a third from Namibia and the Western Cape.

Figure 3.10 Bayesian phylogenetic tree based on the COI gene region of three species of gannets (668bp, 41 samples, with HKY model selected by hLRT in MrModeltest). Values at nodes are Bayesian posterior probabilities. Colours indicate regional collection locality (red = Eastern Cape, blue = Western Cape and black = Namibia).



Cape Gannet: NADH2 (555bp)

Genetic diversity Indices and estimates of genetic divergence

The NADH2 target region was successfully amplified for 20 Cape Gannets (n=7 from the Eastern Cape, n=10 from Namibia and n=3 from the Western Cape). A total of four haplotypes were detected in the NADH2 dataset (haplotype diversity h= 0.284±0.128 and nucleotide diversity π=0.0005±0.0003, Appendix 3.14, Table 3.1). No significant population differentiation was detected between the three breeding regions (all φ_{ST}~0, Appendix 3.15). Sample sizes were too small to conduct analogous analyses at the level of breeding colonies.

Maximum Parsimony, Maximum likelihood and Bayesian phylogenetic analyses

The MJ and RM Network analyses of the NADH2 alignment (555bp) showed identical results. One Australasian Gannet *M. serrator* (GU071056, Gibb 2009) individual was included in the analysis (Figure 3.12 (a)). Although the sample size is small, there is again a large pool of shared haplotypes, reflecting a dominant lineage that is present in all regions. There are three haplotypes endemic to Namibia, but they only differ from the dominant haplotype at one nucleotide position each.

The model selected for the NADH2 alignment, including one *M. serrator* individual, was the HKY model. The 50% bootstrap majority-rule ML consensus tree contains only one clade (65%) containing Cape Gannet individuals from all regions. The Bayesian analysis showed a very similar pattern, with one well-supported clade (posterior probability=0.85, Appendix 3.16) containing all samples except one Cape Gannet (LNG103, Ichaboe Island).

Cape Gannet: Cytochrome b

The cyt b target region was amplified in 15 Cape Gannet samples (n=10 from Namibia, n=3 from the Western Cape and n=2 from the Eastern Cape). Among these sequences, two haplotypes were recovered (Table 3.1). The haplotype diversity was 0.25 and nucleotide diversity 0.0003 (Table 3.1).

Phylogenetic analyses

The MJ and RM Maximum Parsimony (MP) analyses of cyt b (834bp, Figure 3.12 (b)) showed identical results. One Australasian Gannet individual was included in the analysis. Although the sample size is small, two unique haplotypes are present. The dominant haplotype is shared among Cape Gannets from all three breeding regions, whereas the rarer haplotype is shared between the Western Cape and Namibia.

The rooted and unrooted Maximum likelihood (ML) trees based on the Cape Gannet cyt b alignment, with and without *M. serrator* (GU071056) as an outgroup (Appendix 3.17) reflect the pattern displayed in the haplotype network. The phylogeny is largely unresolved, with the dominant haplotype represented by a large polytomy containing gannets from all three breeding regions, and a clade comprised of the two individuals that possess the second, rare haplotype. A further eight related cyt b sequences and one Cape Gannet sequence from GenBank were included in a family-level ML and Bayesian phylogenetic analyses (Figure 3.11 and Appendix 3.18 respectively). The model selected was HKY+G (Appendix 3.2). The outgroup taxa included six Northern Gannet sequences AJ004229 – AJ004232 (Heidrich et al. 1998), AY567921 (Treutlein & Wink, unpublished) and U90001 (Friesen & Anderson 1997); two Australasian Gannet sequences GU071056 (Gibb, unpublished) and U90003 (Friesen & Anderson 1997); and one sequence from Abbott's Booby *Papasula abbotti* U90000 (Friesen & Anderson 1997). The single Cape Gannet cyt b sequence available, U90002 (Friesen & Anderson 1997) was collected at the Malgas Island gannet colony. All three gannet species form well-supported clades that reflect their evolutionary relationships and the pattern within the Cape Gannet clade remains identical to previous analyses. It is interesting to note that the "historical" (collected before 1997) Cape Gannet sequence falls outside the clade formed by Cape Gannets collected for the present study, although this pattern is not reflected in the Bayesian phylogenetic tree.

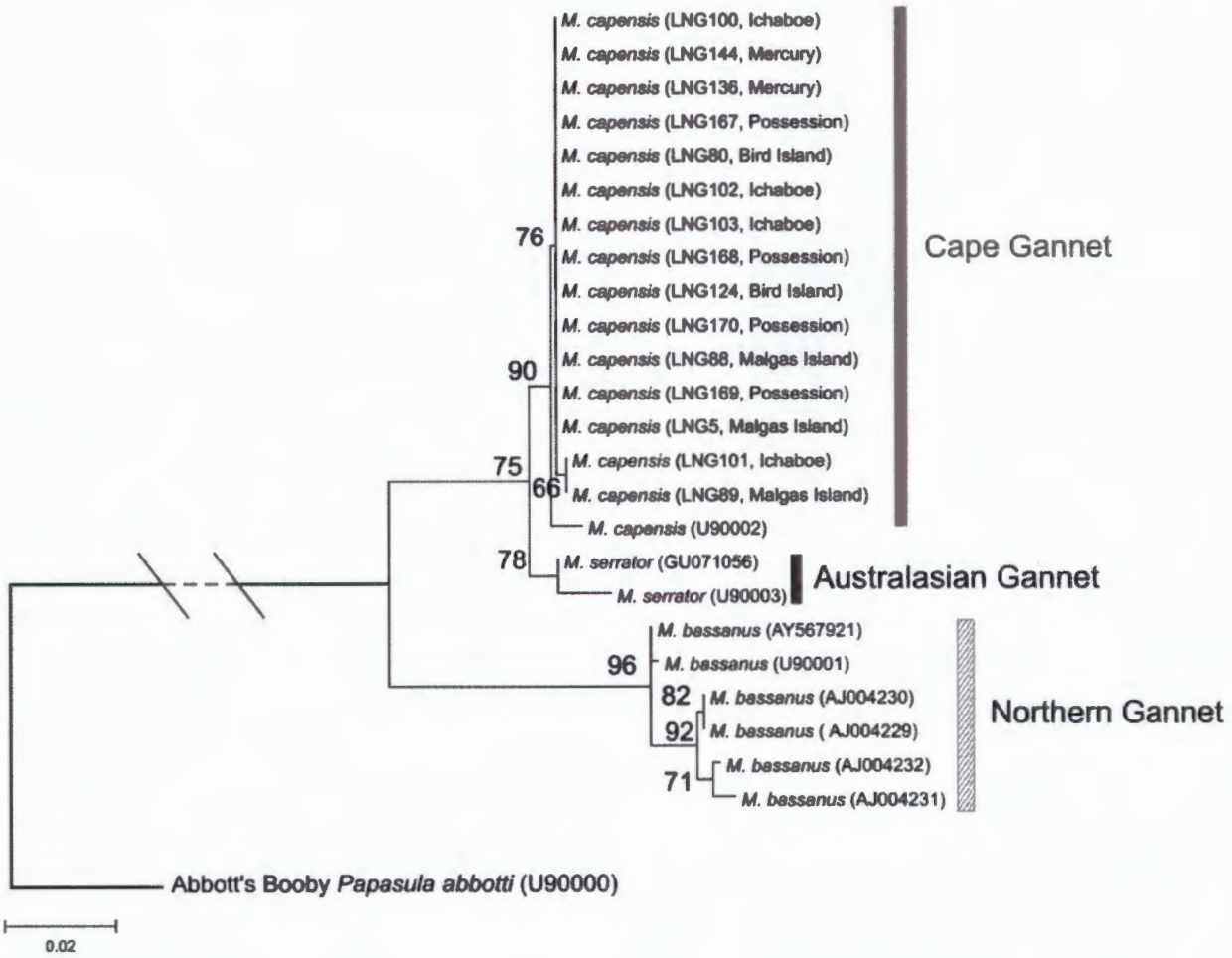
Cape Gannet: GAPDH

The nuclear GAPDH target region was successfully amplified for 25 of the Cape Gannets sampled (n=15 from Namibia, n=6 from the Western Cape and n=4 from the Eastern Cape). Five haplotypes or alleles were detected (h=0.62, π=0.0018, Table 3.1).

Maximum Parsimony, Maximum likelihood and Bayesian phylogenetic analyses

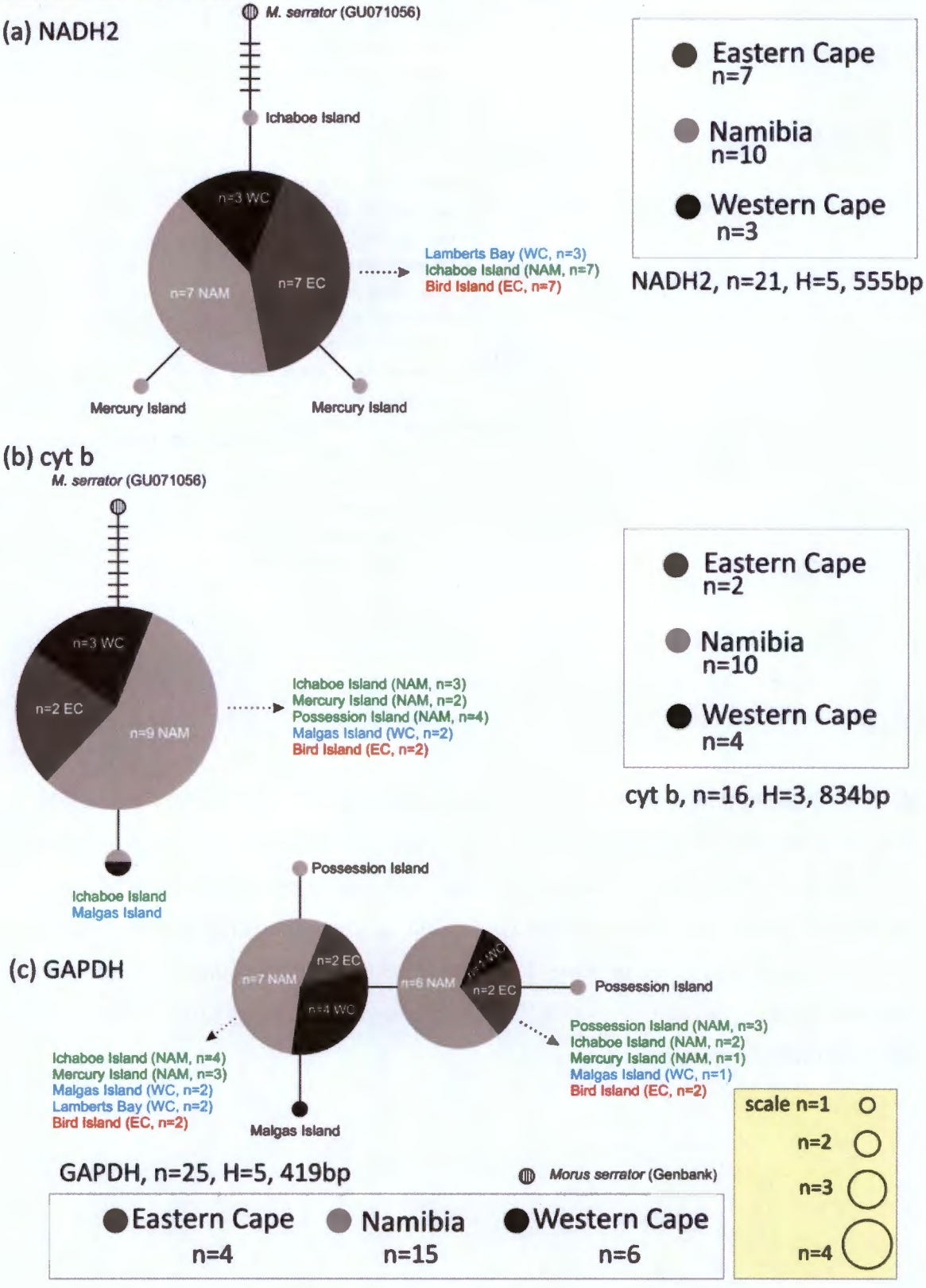
The only available comparable GAPDH sequences on GenBank were from *Fregata magnificens* (Magnificent Frigatebird, FR691314 and FR691315), which introduced multiple gaps into the Cape Gannet alignment. The RM and MJ networks, therefore, omitted these, and showed identical patterns for Cape Gannets (Figure 3.12 (c)). Although the sample size is small, the pattern observed in the GAPDH data is that there are two dominant haplotypes or alleles. Each of the dominant haplotypes contains representatives from all three breeding regions.

Figure 3.11 Maximum likelihood (ML) phylogenetic tree of *Morus* cyt b sequences (truncated to 794bp, HKY+G model) rooted on Abbott's Booby *Papasula abbotti*. Numbers in brackets are GenBank Accession numbers, and numbers at the nodes are ML bootstrap values).



ML and Bayesian phylogenetic analyses strongly reflected the MP analyses above, and the addition of the Magnificent Frigatebird as an outgroup species (truncated to 415bp, Appendix 3.19) did not affect the connections between haplotypes, represented by clades in the bootstrap consensus ML tree (branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed). The model chosen for this analysis was Kimura 2-parameter model – different to the HKY model chosen for the majority of the mitochondrial gene alignments (Appendix 3.2).

Figure 3.12 Cape Gannet Maximum Parsimony haplotype network based on **(a)** NADH2 (555bp) **(b)** cyt b (834bp), and **(c)** GAPDH (419bp). The size of the circles represents the number of individuals that possess a particular haplotype and the black line between haplotypes represents one nucleotide change with tick marks along the line indicating additional changes. Numbers in brackets are GenBank accession numbers.



Cape Gannet: Beta-fibrinogen Intron 7 (BFIB)

BFIB (559bp) was successfully amplified in 31 individuals from all three breeding regions (Namibia n=17, Western Cape n=9 and Eastern Cape n=5). Two haplotypes were recovered from this data: one dominant haplotype shared among individuals from all three breeding regions and the second only present in one individual from the Western Cape. The rare haplotype differed from the dominant haplotype by only one transversion (G-C at 104bp), and may represent a second allele.

Maximum likelihood and Bayesian phylogenetic analyses

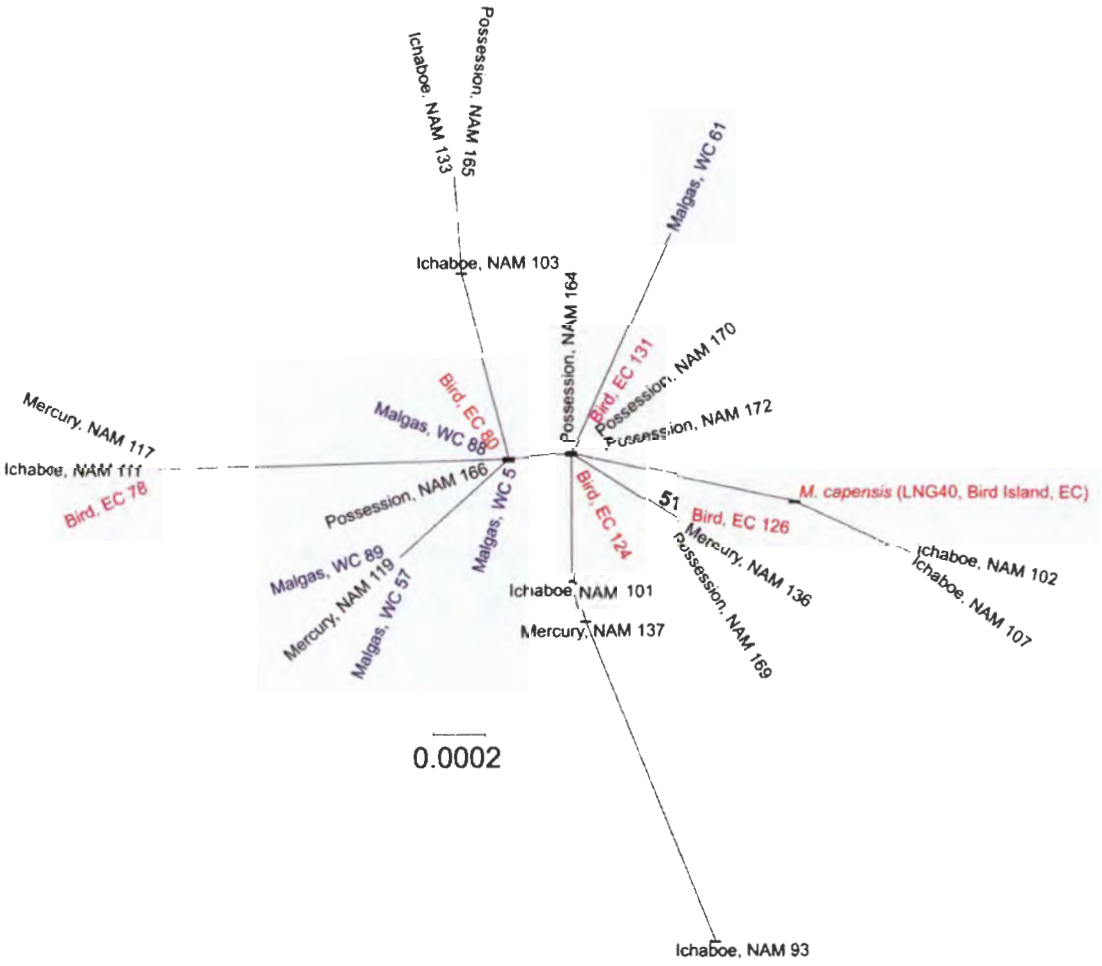
Although no BFIB sequences were available from other studies of Cape Gannets or their putative sister species, Australasian Gannets *M. serrator*, there were sequences for other sulids: Northern Gannets EU739445 (Hackett et al. 2008), AY695213 (Fain & Houde 2004), EF552786 (Paśko et al. 2011) and DQ881997 (Ericson et al. 2006); and *Sula dactylatra* AY695212 (Fain & Houde 2004). The model selected for this region was the Tamura-Nei model (Appendix 3.2). The ML tree with the highest log likelihood (-890.7823) is presented (Appendix 3.20), with bootstrap values (the percentage of 1000 trees in which the associated taxa clustered together is shown next to the branches) and Bayesian posterior probabilities. The model of nucleotide substitution selected for the alignment that included the darter *Anhinga anhinga* and Masked Booby *Sula dactylatra* sequences was the Hasegawa-Kishino-Yano (HKY) model. The tree with the highest log likelihood (-994.7732) is presented (Appendix 3.20).

Cape Gannet: Overall (concatenated, partitioned)

Two or more of the seven gene regions described above were successfully amplified in sixty-eight Cape Gannet samples (n=36 from Namibia, 21 from the Western Cape and 11 from the Eastern Cape), and 3 or more gene regions in 28 samples (n=17 from Namibia, n=6 from the Eastern Cape and n=5 from the Western Cape). The Maximum likelihood phylogenetic tree based on this data show the same general signal of little divergence among sequences, and the majority of lineages present in two or three regions (Figure 3.13). For the Bayesian analyses, the concatenated sequence datasets were partitioned by gene region and missing data substituted with question marks (Appendix 3.21). Each gene region was assigned the appropriate model of nucleotide evolution, all parameters were unlinked and rates were allowed to vary across partitions before conducting Bayesian MCMC analysis (2 million generations, 6 chains, potential scale reduction factor (PSRF) ~1 indicating convergence of

runs for all parameters). The Bayesian phylogenetic tree based on the combined, partitioned Cape Gannet sequence data has low resolution, with birds from all three breeding regions falling into two largely unresolved polytomies.

Figure 3.13 The unrooted, Maximum likelihood phylogenetic tree based on the combined (concatenated) Cape Gannet sequence dataset that includes individuals for which three or more of the 7 gene regions were successfully sequenced (n=28, 4104bp). Samples from Namibian colonies are shown in black, those from the Eastern Cape in red, and Western Cape in blue.



Molecular markers in African Penguins

African Penguin: NADH3 (358bp)

NADH3 was successfully amplified in 124 African Penguin samples (n=50 from Namibia, n=41 from the Western Cape and n=33 from the Eastern Cape). A total of six haplotypes were recovered, which differed by a maximum of two nucleotide substitutions (overall haplotype diversity, h=0.182±0.046 and nucleotide diversity π=0.0005, Appendix 3.22, Table

3.1). Pairwise comparisons of genetic structure (φ_{ST}) were very low (~0) and ranged from -0.007 (between the Eastern Cape and Namibia) to -0.014 (between the Western Cape and Namibia). G_{ST}, the average number of pairwise nucleotide differences between populations (k_{xy}) and genetic distance (D_{xy}) are also reported (Appendix 3.23). No significant differences were found between any of the colonies sampled, although Mercury Island in Namibia appears to be genetically distinctive among all breeding colonies in the three breeding regions. The Eastern Cape and Namibia appear to be the most genetically different among the three breeding regions based on this target region.

Maximum Parsimony, Maximum likelihood and Bayesian phylogenetic analyses

For the MP Network analyses of African Penguin NADH3 (n=124, 358bp), the MJ and RM networks were identical (Appendix 3.24). Six haplotypes were present among the sampled individuals, with two dominant haplotypes representing individuals from all three breeding regions. Endemic haplotypes existed at lower frequencies in all three regions: one in Namibia, two in the Western Cape and one in the Eastern Cape. These differed from the dominant haplotype by ony one nucleotide substitution each. Thirty-seven of the sampled individuals had flipper bands.

The model selected for the NADH3 alignments (358bp with and without outgroup taxa) was HKY (Appendix 3.2) in Mega (Tamura et al. 2011) and, although jModeltest (Posada 2008) selected the Jukes-Cantor and J80 models using AICc, HKY was used for the Maximum likelihood analyses. The 50% bootstrap consensus ML NADH3 tree rooted with *S. humboldti* (n=125, Appendix 3.25), and including *Pygoscelis adeliae* sequences (n=140, Figure 3.14) strongly reflected the pattern observed in the MP analysis above.

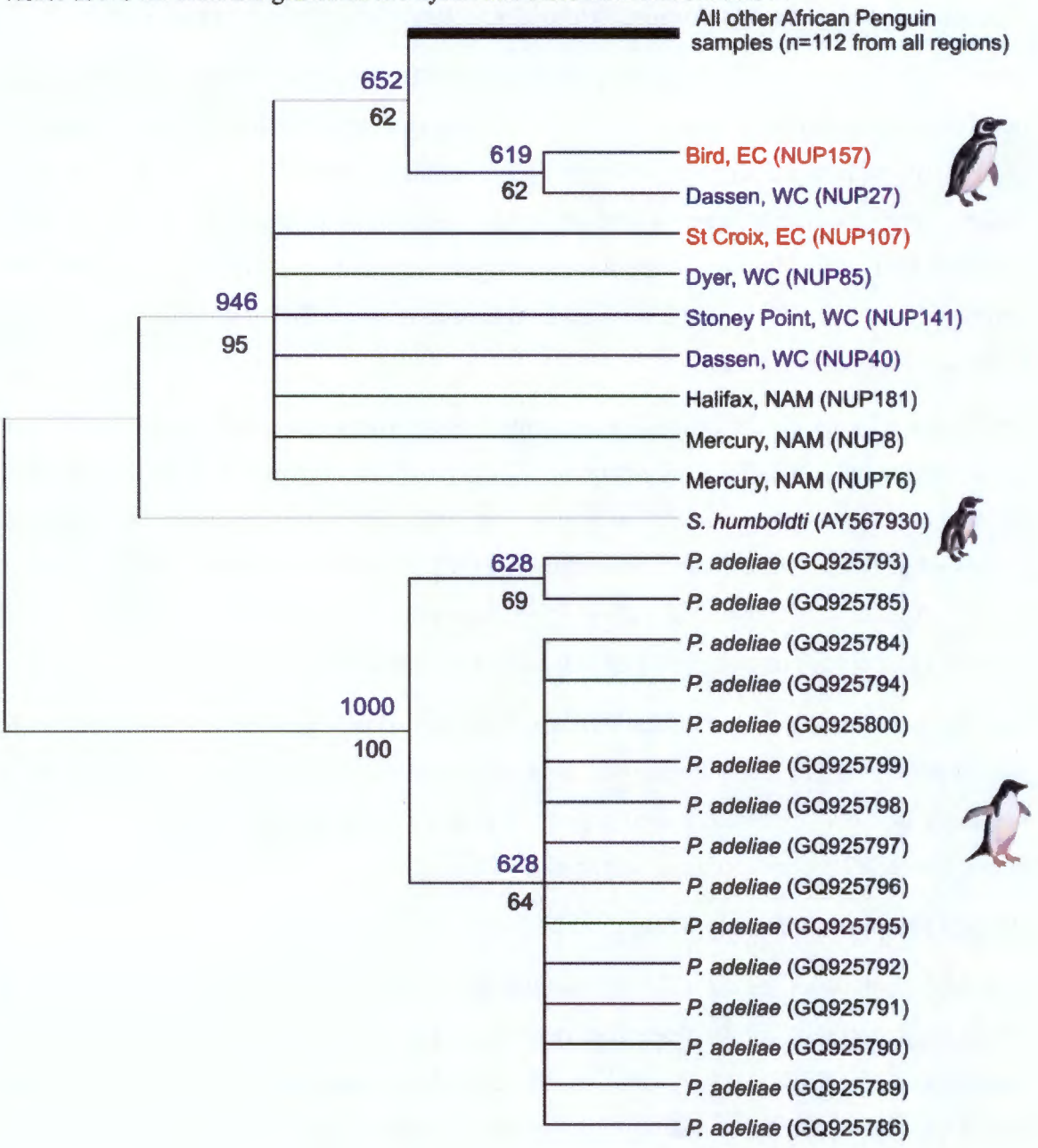
The Bayesian analysis based on the African Penguin NADH3 sequence data showed a very similar pattern to ML and MP analyses, with the majority of samples forming an unresolved polytomy and two moderately well-supported clades reflecting haplotypes that are shared among two or all breeding regions (Appendix 3.26).

African Penguin: ATPase-6 (672bp)

The ATPase-6 target region was successfully amplified in 130 African Penguin samples (n=50 from Namibia, n=49 from the Western Cape and n=31 from the Eastern Cape; Appendix 3.27, Table 3.1). A total of 12 haplotypes were detected (h=0.24±0.05 and π=0.0004; Appendix 3.27, Table 3.1). Pairwise comparisons of genetic structure (φ_{ST}) between breeding regions were low and ranged from 0.003 (between the Western Cape and

Namibia) to 0.026 (between the Eastern Cape and Namibia; Table 3.3). No significant population differentiation was detected between any of the colonies sampled i.e. when samples were assigned to colonies, and not broad geographic regions based on the sampling locality. It is notable, however that within all three breeding regions, pairwise **colony φ_{ST} values were ≤ 0 (Table 3.3).**

Figure 3.14 Maximum likelihood phylogenetic tree (50% bootstrap consensus tree) based on the NADH3 (358bp) sequence data, rooted with GenBank sequences from *P. adeliae* (n=140). Bootstrap values above the branches generated in PhyML and those from MEGA below.

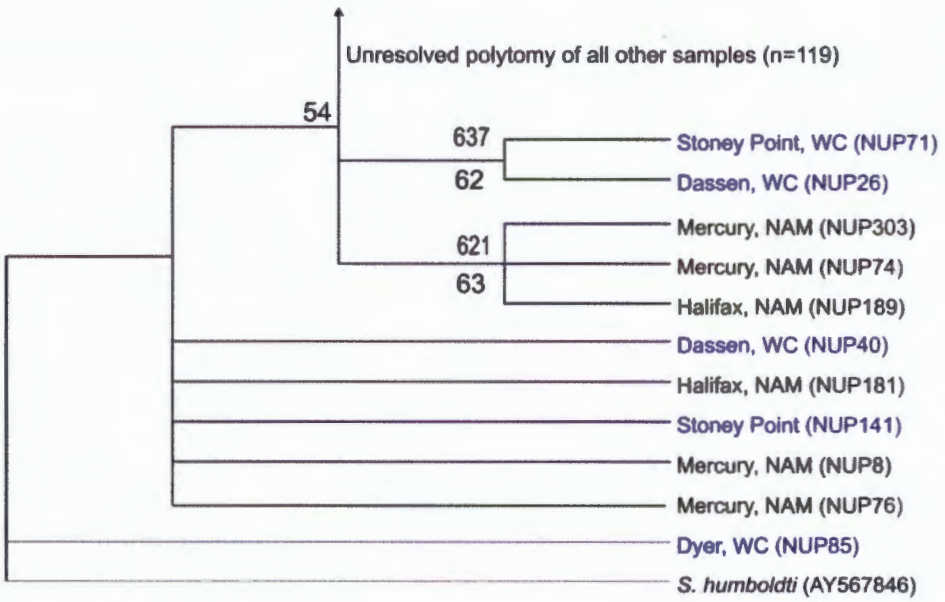


Maximum Parsimony, Maximum likelihood and Bayesian phylogenetic analyses

The RM and MJ haplotype networks based on the ATPase-6 sequence data are identical and show a dominant haplotype (exhibited by 88% of the African Penguins sampled) representing individuals from all three breeding regions and all of the twelve colonies sampled (Appendix 3.28). The second most common haplotype is shared by equal numbers of individuals from the Western Cape and Namibia. A number of endemic haplotypes occur in each breeding region (3 in Namibia, 6 in the Western Cape and 1 in the Eastern Cape).

The ML tree based on the African Penguin ATPase-6 sequence data (Figure 3.15) strongly reflects the pattern observed in the MP analysis, with the majority of samples forming a large, unresolved polytomy consisting of individuals from all three breeding regions and some of the rarer haplotypes falling into moderately well-supported clades. A lade containing only Western Cape individuals and another containing Namibian birds received the highest nodal support.

Figure 3.15 Maximum likelihood phylogenetic tree based on the African Penguin ATPase-6 dataset (n=130, 672bp). Numbers at the nodes are ML bootstrap values (1000 replicates) from MEGA (below nodes) and PhyML (above nodes). Sampling localities are indicated and individuals from the Western Cape are shown in blue.



Bayesian analysis of the African Penguin ATPase-6 data (GTR model), with the Humboldt Penguin (AY567846) included as an outgroup (n=132, 672bp), generated similar results to the ML analysis. The unrooted Bayesian gene tree (Appendix 3.29) shows similar

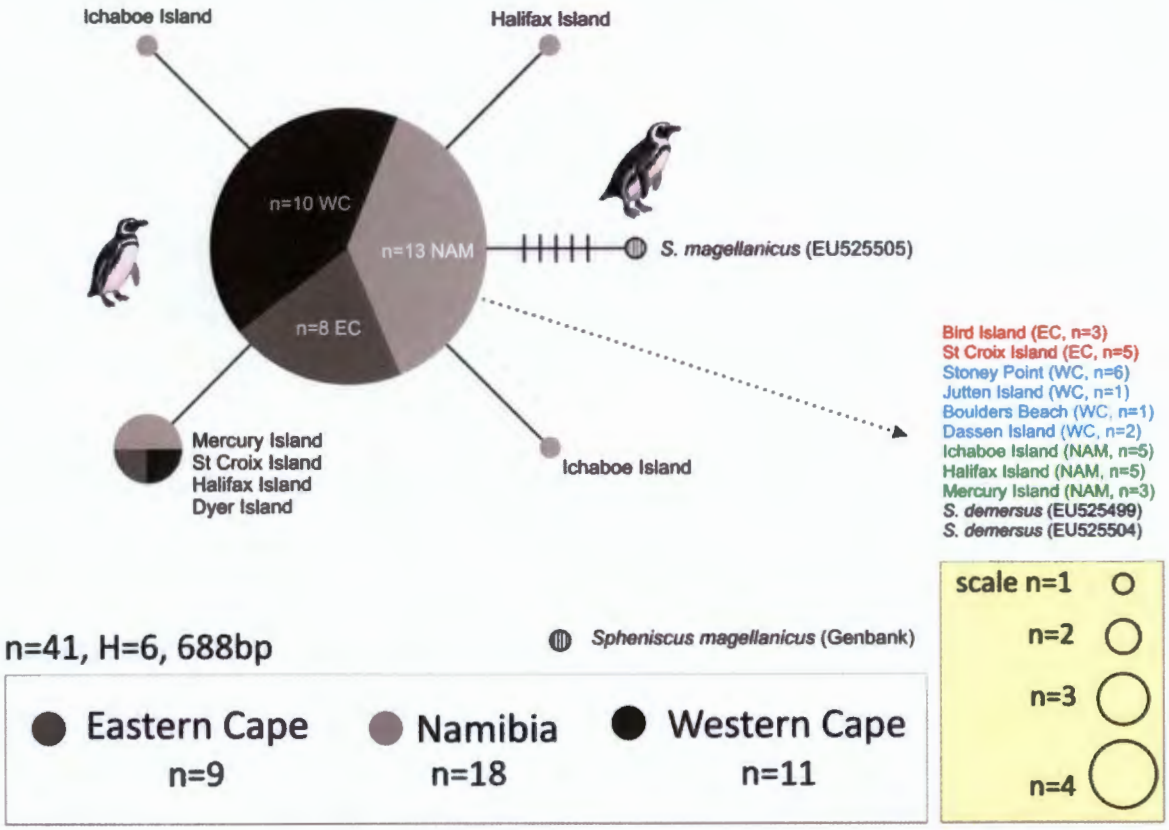
Table 3.3 Pairwise comparisons of genetic structure using φ_{ST}, G_{ST}, D_{xy} and the average number of nucleotide differences, k_{xy} at the regional (a and b) and colony (c) level based on ATPase-6 for African Penguins (672bp). Numbers in bold are significant; φ_{ST} and k_{xy} above diagonals and G_{ST} and D_{xy} below.

(a)		φ_{ST}		
		NAM	**WC**	**EC**
G_{ST}	**Namibia (NAM)**		**0.003**	**0.026**
	Western Cape (WC)	-0.002		**0.014**
	Eastern Cape (EC)	0.015	0.021	

(b)		k_{xy}		
		NAM	**WC**	**EC**
D_{xy}	**Namibia (NAM)**		0.357	0.192
	Western Cape (WC)	0.0005		0.236
	Eastern Cape (EC)	0.0003	0.0004	

(c)		φ_{ST}											
		Mercury (NAM)	**Ichaboe (NAM)**	**Possession (NAM)**	**Halifax (NAM)**	**Boulders Beach (WC)**	**Robben Island (WC)**	**Dassen Island (WC)**	**Jutten Island (WC)**	**Stony Point (WC)**	**Dyer Island (WC)**	**St Croix Island (EC)**	**Bird Island (EC)**
G_{ST}	**Mercury (NAM)**		**0.044**	**0.044**	-0.035	**0.044**	**0.029**	-0.012	**0.044**	-0.018	-0.024	**0.037**	**0.044**
	Ichaboe (NAM)	0.039		0	0	0	0	0	0	0	0	0	0
	Possession (NAM)	0.042	**1**		0	0	0	0	0	0	0	0	0
	Halifax (NAM)	-0.023	0.029	0.034		0	0	-0.024	0	-0.026	-0.029	0	0
	Boulders Beach (WC)	0.042	**1**	**1**	0.034		0	0	0	0	0	0	0
	Robben Island (WC)	-0.002	0.002	0.002	-0.01	0.002		0	0	0	0	0	0
	Dassen Island (WC)	-0.015	0.044	0.04	-0.021	0.04	-0.012		0	-0.065	-0.035	0	0
	Jutten Island (WC)	0.038	**1**	**1**	0.029	**1**	0	0.039		0	0	0	0
	Stoney Point (WC)	-0.012	0.06	0.048	-0.017	0.048	-0.009	-0.04	0.05		-0.039	0	0
	Dyer Island (WC)	-0.007	0.042	0.033	-0.014	0.033	-0.024	-0.021	0.035	-0.023		0	0
	St Croix (EC)	0.021	0.004	0.022	0.008	0.022	-0.007	0.022	0.01	0.036	0.018		0
	Bird Island (EC)	0.040	**1**	**1**	0.03	**1**	0.003	0.05	**1**	0.062	0.044	0.003	

Figure 3.16 The relationships among the five African Penguin COI haplotypes (n=38, 688bp), including two African Penguin sequences from GenBank and one Magellanic Penguin outgroup sequence (EU25505). The size of the circle represents the number of individuals with a particular haplotype and lines connecting each circle represent one nucleotide change (tick marks along connecting lines represent additional changes). Numbers in brackets are flipper-band numbers (SAFRING) or accession numbers for sequences extracted from GenBank.



African Penguin: Combined analyses

NADH3 and ATPase-6 were both amplified in 106 samples (n=41 from the Western Cape, n=47 from Namibia and n=18 from the Eastern Cape). An AMOVA based on this concatenated mitochondrial dataset (1030bp), subdivided into the three African Penguin breeding regions, showed that 5% of the variation was explained among populations and 95% within populations (defined as breeding regions).

Maximum Parsimony, Maximum likelihood and Bayesian phylogenetic analyses

The haplotype network based on the combined African Penguin dataset (Figure 3.17) is distinctively star-shaped, with the vast majority of samples sharing a single dominant haplotype, and a number of closely-related haplotypes (usually differing by only one base pair) found in all breeding regions. Individuals from the Western Cape exhibit five unique haplotypes and Namibian individuals exhibit four (Figure 3.17). Only two unique haplotypes

relationships among samples, although a third weakly-supported clade comprising individuals from Namibia and the Western Cape is more distinct.

African Penguin: COI (688bp)

The COI target region was successfully amplified in 38 African Penguin samples (n=18 in Namibia, n=11 in the Western Cape and n=9 in the Eastern Cape; Appendix 3.30). A total of five haplotypes were detected in the COI alignment (overall haplotype diversity, h=0.33±0.094 and π=0.00051; Appendix 3.30, Table 3.1). Pairwise comparisons of genetic structure (φst) between breeding regions were low and ranged from -0.04 (between the Western Cape and Namibia) and -0.11 (between the Eastern Cape and Western Cape). No significant population differentiation was detected between breeding regions based on the COI dataset. The COI sequence data could not be analysed at the colony-level due to small sample sizes, so some of the samples were pooled e.g. Stony Point and Dyer Island for a finer scale analysis.

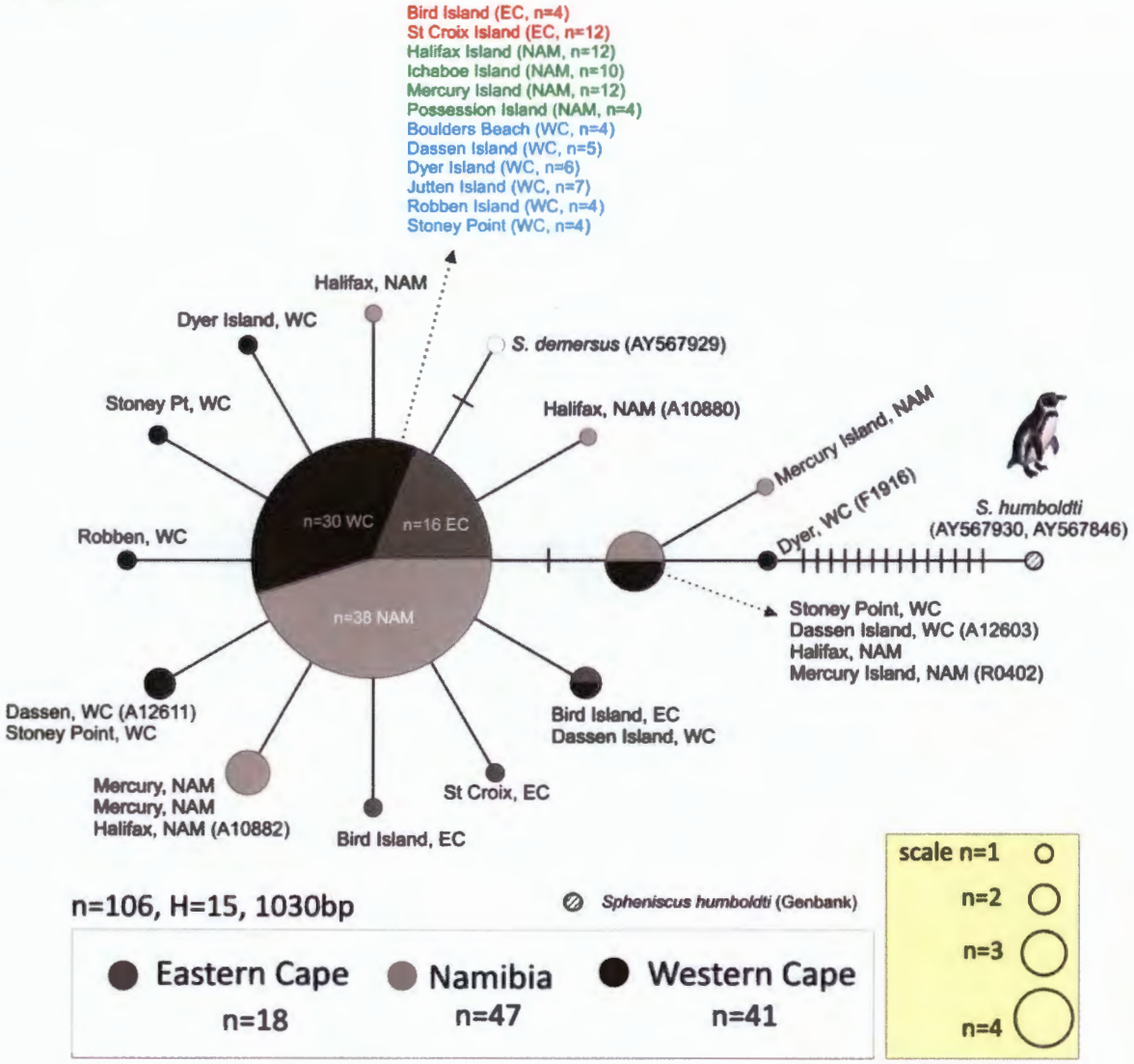
Maximum Parsimony, Maximum likelihood and Bayesian phylogenetic analyses

In the Maximum Parsimony haplotype network, the majority of sampled individuals (31 of 38 African Penguins from all three breeding regions) share a single COI haplotype (Figure 3.16). Three of the five unique African Penguin COI haplotypes represent single individuals from Ichaboe Island or Halifax Island in Namibia. The fifth haplotype is comprised of four samples representing breeding colonies in all three breeding regions of African Penguins. All intraspecific haplotypes differ by only one nucleotide change, compared to the 5 mutational steps separating the dominant haplotype from an outgroup sequence from a Magellanic Penguin (Figure 3.16).

The ML and Bayesian COI phylogenetic trees reflected the MP topology (Appendix 3.32), and strongly reflected the pattern observed in the MP haplotype network. The majority of individuals, representing nine colonies in all three breeding regions, exhibiting the dominant haplotype form a large unresolved polytomy. The rare haplotypes are distinct and one well-supported clade represents four birds from Namibia and South Africa that share a haplotype.

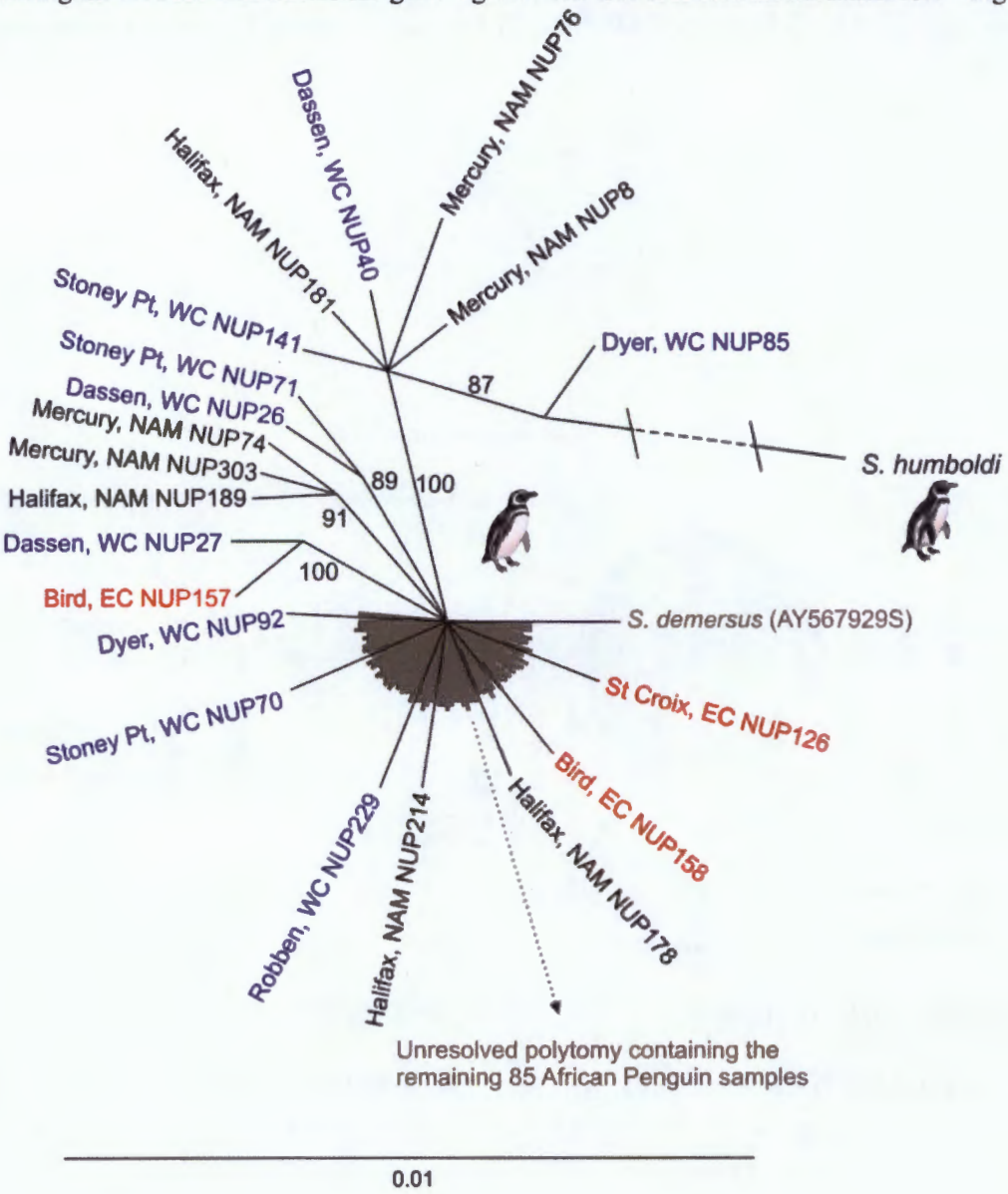
were evident among the 18 individuals from the Eastern Cape. ATPase-6 (672bp), COI (688) and NADH3 (358) were amplified in 21 of the same individuals, and a combined analysis (1718bp) might improve the resolution of the tree when more sequences are available. The general pattern observed across all gene regions analysed, however, is unlikely to change with the addition of more samples i.e. dominant genetic lineages are present in all three breeding regions of African Penguins.

Figure 3.17 The relationships among the 15 African Penguin haplotypes identified from the combined ATPase-6 (672) and NADH3 (358) sequences (n=104), including one Humboldt Penguin outgroup sequence and one African Penguin from GenBank. The size of the circle represents the number of individuals with a particular haplotype and lines connecting each circle represent one nucleotide change (tick marks along connecting lines represent additional changes). Numbers in brackets are flipper-band numbers (SAFRING) or accession numbers for sequences extracted from GenBank.



Bayesian analysis was conducted on a combined (concatenated), partitioned dataset, incorporating NADH3 and ATPase-6, with *S. humboldti* (NADH3: AY567930; ATPase-6: AY567846) as an outgroup taxon (n=106, 1030bp), and the appropriate models assigned to each gene region (Figure 3.18). The well-supported clades strongly reflect the pattern of relatedness among samples exhibited in the MP analysis above. The two dominant haplotypes form two distinct clusters, one containing the majority of samples (n=85), which form a large, unresolved polytomy (Figure 3.18). Two branches contain individuals from two or all three of the breeding regions, and two braches are restricted to single breeding regions.

Figure 3.18 Bayesian phylogenetic tree based on the combined, partitioned African Penguin dataset i.e. including the ATPase-6 and NADH3 gene regions. The tree is rooted on the Humboldt Penguin.



Molecular markers in Cape Cormorants

Cape Cormorant: NADH3 (393bp)

Genetic diversity indices and estimates of genetic differentiation

The Cape Cormorant NADH3 alignment (n=71) contained three haplotypes (overall haplotype diversity h=0.52±0.02 and nucleotide diversity π=0.001; Appendix 3.31, Table 3.1). Divergence between the combined Namibian colonies (Ichaboe Island and Bird Rock, Walvis Bay) and the combined South African colonies was low (φ_{ST}=-0.025; Table 3.4) and none of the measures of population differentiation were significant. No significant population differentiation was found when the data was analysed at the colony-level i.e. when individuals were assigned to source populations based on their collection locality (Table 3.4).

Maximum Parsimony, Maximum likelihood and Bayesian phylogenetic analyses

The NADH3 haplotype network for Cape Cormorants (n=71, 393bp) shows that the two dominant haplotypes identified above are present in similar proportions among Namibian and the Western Cape samples (Appendix 3.33). The network is rooted on the Stewart Island Shag *P. chalconotus* (GenBank accession number GU071054). One individual from Dyer Island represents a unique mitochondrial lineage.

The topologies of the ML and Bayesian phylogenetic trees based on the Cape Cormorant NADH3 dataset support the MP analysis and show two distinct clades that correspond to the two dominant haplotypes recovered among sampled individuals (Figure 3.19). Only one comparable *Phalacrocorax* NADH3 sequence (*P. chalconotus*, the Bronze Shag or Stewart Island Shag, mtDNA genome, Accession number: GU071054), and one partial sequence (Double-crested Cormorant *P. auritus*, 340bp NADH3; Accession number: AF373589) are currently available from GenBank and are included as outgroup taxa. Also, NADH3 sequences for two other cormorant species endemic to the region were generated during the present study: Bank Cormorants (n=14 from Namibian colonies) and Crowned Cormorants (n=2), and are included as outgroups in the ML and Bayesian analyses of an expanded Cormorant NADH3 dataset. All three endemic cormorant species form well-supported monophyletic clades, and the Bank Cormorant is sister to the Cape Cormorants (Figure 3.20). The Cape Cormorant clade is largely unresolved, but one well-supported sub-clade exists and represents the smaller of the two dominant haplotypes. There appears to be strong structure among the Bank Cormorants sampled, with one well supported intraspecific clade comprised of individuals from Namibia nested among samples from the WC and Namibia.

Table 3.4 Pairwise comparisons of genetic structure based on four commonly reported metrics calculated at the regional scale ((a) and (b)) and colony-level ((c) and (d)) based on the NADH3 dataset for Cape Cormorants (n=71, 393bp). φ_{ST} and k_{xy} above diagonals and G_{ST} and D_{xy} below.

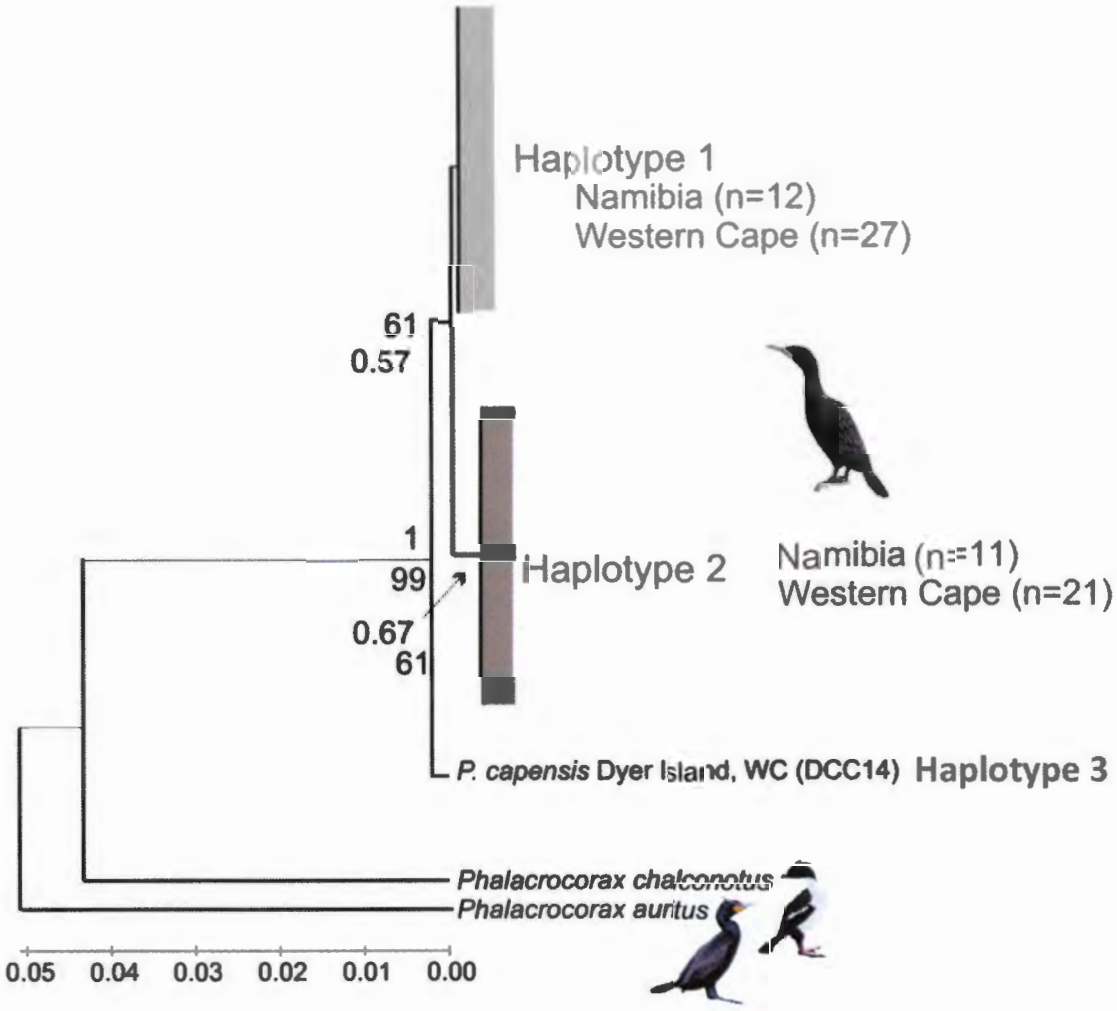
(a)		φ_{ST}	
		NAM	**WC**
G_{ST}	**Namibia (NAM)**		-0.025
	Western Cape (WC)	-0.01	

(b)		k_{xy}	
		NAM	**WC**
D_{xy}	**Namibia (NAM)**		0.517
	Western Cape (WC)	0.001	

(c)		φ_{ST}					
		Ichaboe Island (NAM)	**Bird Rock (NAM)**	**Malgas Island (WC)**	**Robben Island (WC)**	**Jutten Island (WC)**	**Dyer Island (WC)**
G_{ST}	**Ichaboe Island (NAM)**		-0.129	-0.068	-0.085	0.185	-0.04
	Bird Rock (NAM)	-0.02		-0.121	-0.182	-0.033	-0.136
	Malgas Island (WC)	-0.031	-0.032		-0.085	0.219	-0.037
	Robben Island (WC)	-0.031	-0.07	-0.038		0.086	-0.081
	Jutten Island (WC)	0.083	-0.015	0.108	0.044		0.088
	Dyer Island (WC)	-0.023	-0.019	-0.021	-0.03	0.054	

(d)		Average number of Nucleotide differences between populations (Kxy)					
		Ichaboe Island (NAM)	**Bird Rock (NAM)**	**Malgas Island (WC)**	**Robben Island (WC)**	**Jutten Island (WC)**	**Dyer Island (WC)**
D_{xy}	**Ichaboe Island (NAM)**		0.5	0.5	0.5	0.5	0.553
	Bird Rock (NAM)	0.001		0.5077	0.49	0.429	0.537
	Malgas Island (WC)	0.001	0.001		0.504	0.528	0.559
	Robben Island (WC)	0.001	0.001	0.001		0.46	0.544
	Jutten Island (WC)	0.001	0.001	0.001	0.001		0.496
	Dyer Island (WC)	0.001	0.001	0.001	0.001	0.001	

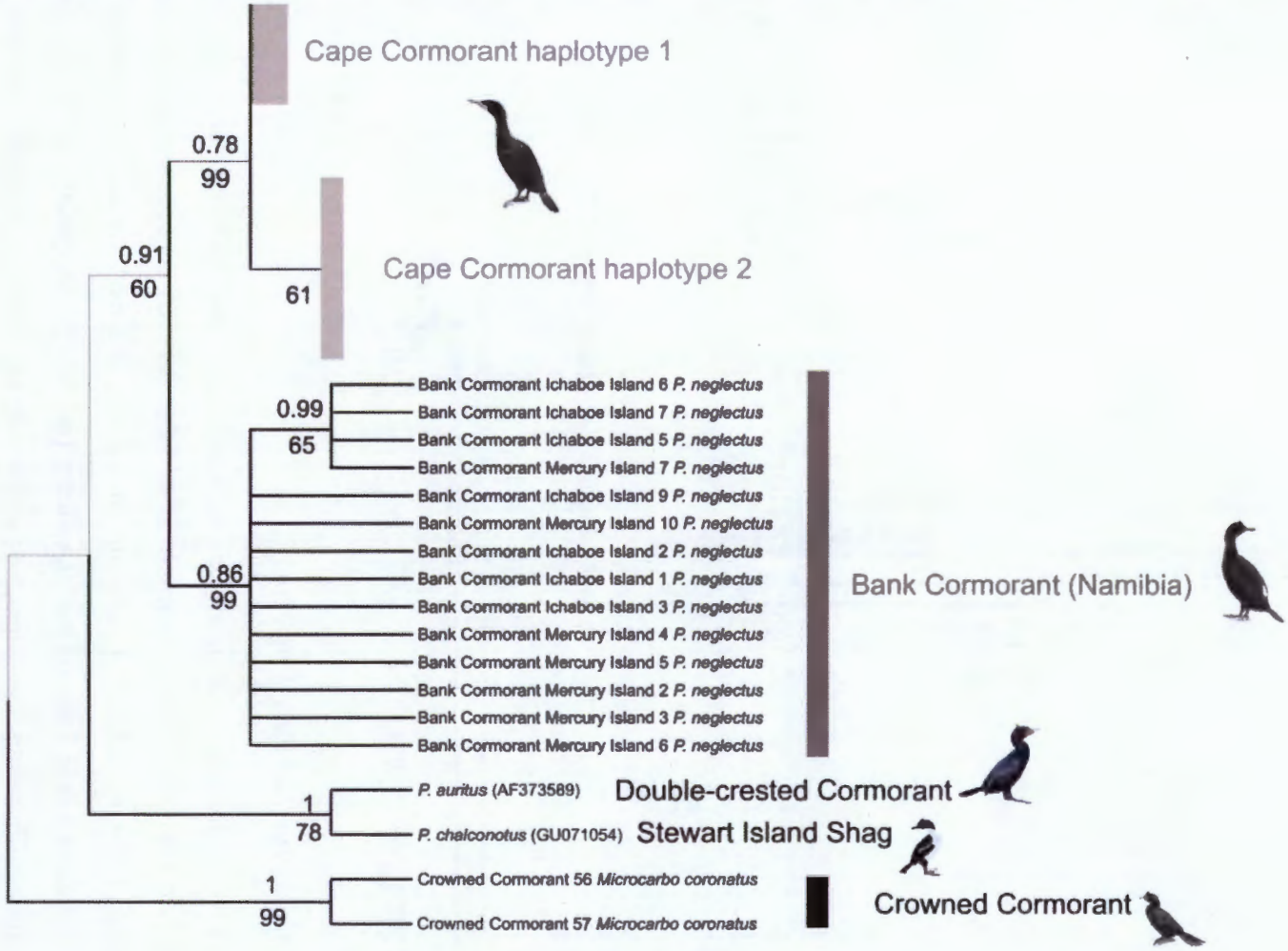
Figure 3.19 NADH3 gene tree for Cape Cormorants and two outgroup *Phalacrocorax* species (n=74, 394bp). Numbers at nodes are Bayesian posterior probabilities (above nodes) and Maximum likelihood bootstrap values (below nodes, 1000 bootstrap replicates).



Cape Cormorant: ATPase-6 (682bp)

Only two haplotypes were identified in analyses of the ATPase-6 alignment of Cape Cormorant sequences (n=47, Appendix 3.34, Table 3.1). Divergence between the combined Namibian colonies (Ichaboe Island and Bird Rock, Walvis Bay) and the combined South African colonies was low and not significant (Φ_{ST}=-0.052; Appendix 3.35). No significant population differentiation was detected between any two colonies sampled (Appendix 3.35).

Figure 3.20 Maximum likelihood 50% bootstrap consensus tree (based on the HKY+I model) generated from the *Phalacrocorax* NADH3 sequence data set (n=89, 389bp). Numbers at the nodes are Bayesian posterior probabilities (above) and ML bootstrap values (below branches, 1000 replicates).



Maximum Parsimony, Maximum likelihood and Bayesian phylogenetic analyses

The haplotype network based on the ATPase-6 target region for Cape Cormorants showed that the two haplotypes detected among sampled individuals occurred in similar proportions in Namibia and South Africa i.e. there was no spatial pattern observed in the distribution of haplotypes. No unique haplotypes were detected among all individuals sampled (Figure 3.21 (a)). The ML and Bayesian phylogenetic trees (Appendix 3.36) based on the Cape Cormorant ATPase-6 sequence data mirror the simple pattern observed in the MP analyses and the split between the two clades is well supported (ML bootstrap 65%, Bayesian posterior probability=0.95).

Cape Cormorant: Cytochrome b (864bp)

The cyt b target region was successfully amplified for 41 Cape Cormorant samples. Four haplotypes were identified (haplotype diversity $h=0.62\pm0.04$, nucleotide diversity $\pi=0.0009$; Appendix 3.37, Table 3.1). No significant differentiation was detected between Namibia and South Africa overall based this target region ($\varphi_{ST}=0.007$, $D_{xy}=0.001$, $G_{ST}=0.01$, $K_{xy}=0.72$) or when data was analysed at the colony-level.

Maximum Parsimony, Maximum likelihood and Bayesian phylogenetic analyses

The cyt b haplotype network for Cape Cormorants (Figure 3.21 (b)) showed a similar pattern to those based on NADH3 and ATPase-6 in that there are two dominant haplotypes comprised of Cape Cormorants from the Western Cape and Namibia. Two additional haplotypes were recovered among the cyt b sequences, one represented by an individual bird from Jutten Island (WC) and the other by four birds from Namibia and the Eastern Cape (Figure 3.21 (b)).

The Bayesian phylogenetic tree based on the Cape Cormorant cyt b sequence data (Appendix 3.38) reflects the haplotype network above. The two dominant haplotypes form well-supported clades, and contain individuals from Namibia and South Africa. Outgroup sequences were extracted from GenBank for the Maximum likelihood analyses of cyt b, and some were generated during the course of this study.

Figure 3.21 (a) The relationships among the Cape Cormorant ATPase-6 haplotypes (n=46, 682bp). **(b)** The relationships among the Cape Cormorant cyt b haplotypes (n=864bp). The size of the circle represents the number of individuals with a particular haplotype and connecting lines represent one nucleotide change (tick marks along connecting lines represent additional changes). Numbers in brackets are accession numbers for sequences extracted from GenBank. Numbers in bold along branches are Bayesian posterior probabilities and plain typeface are maximum likelihood bootstrap values (1000 replicates).

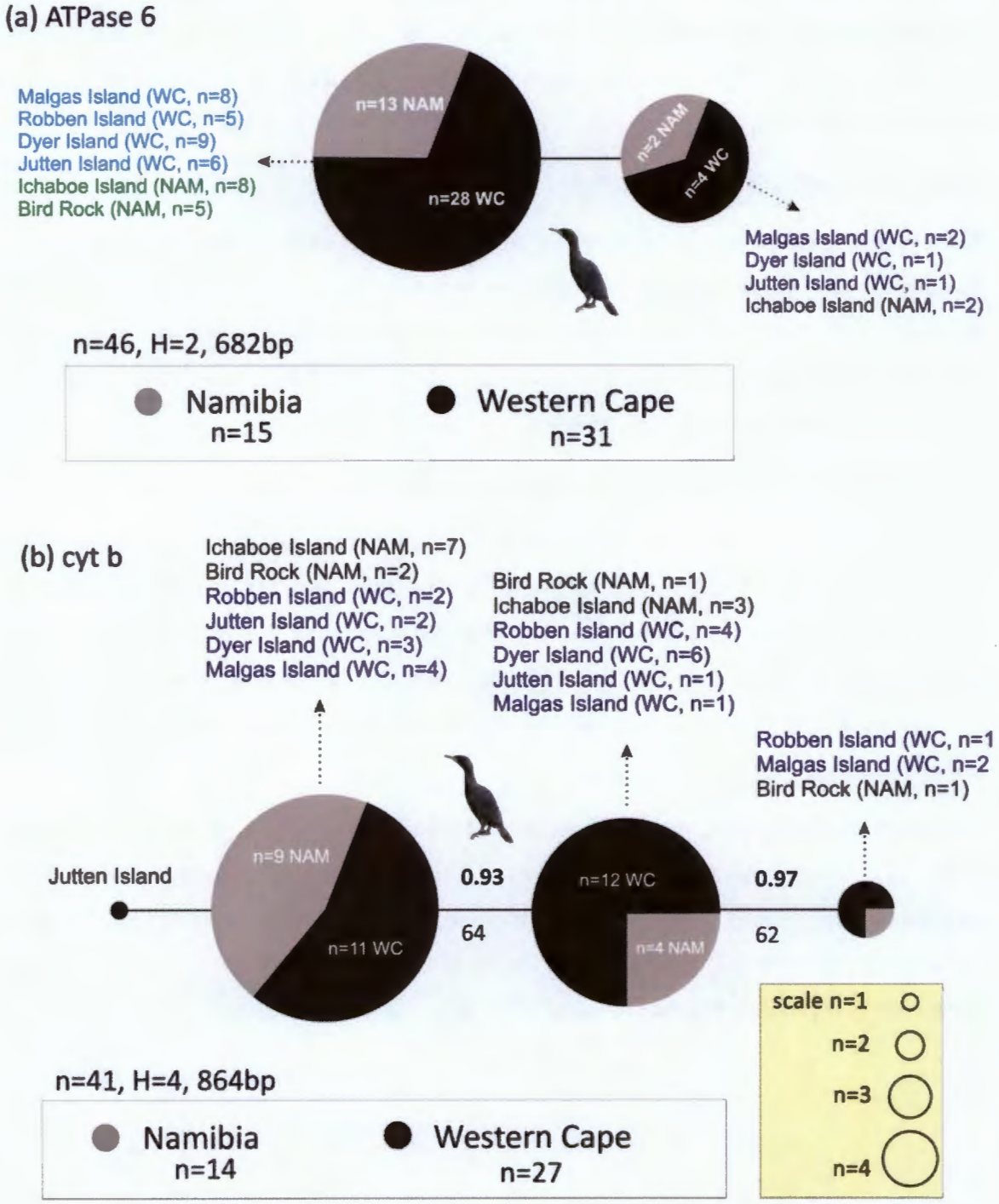
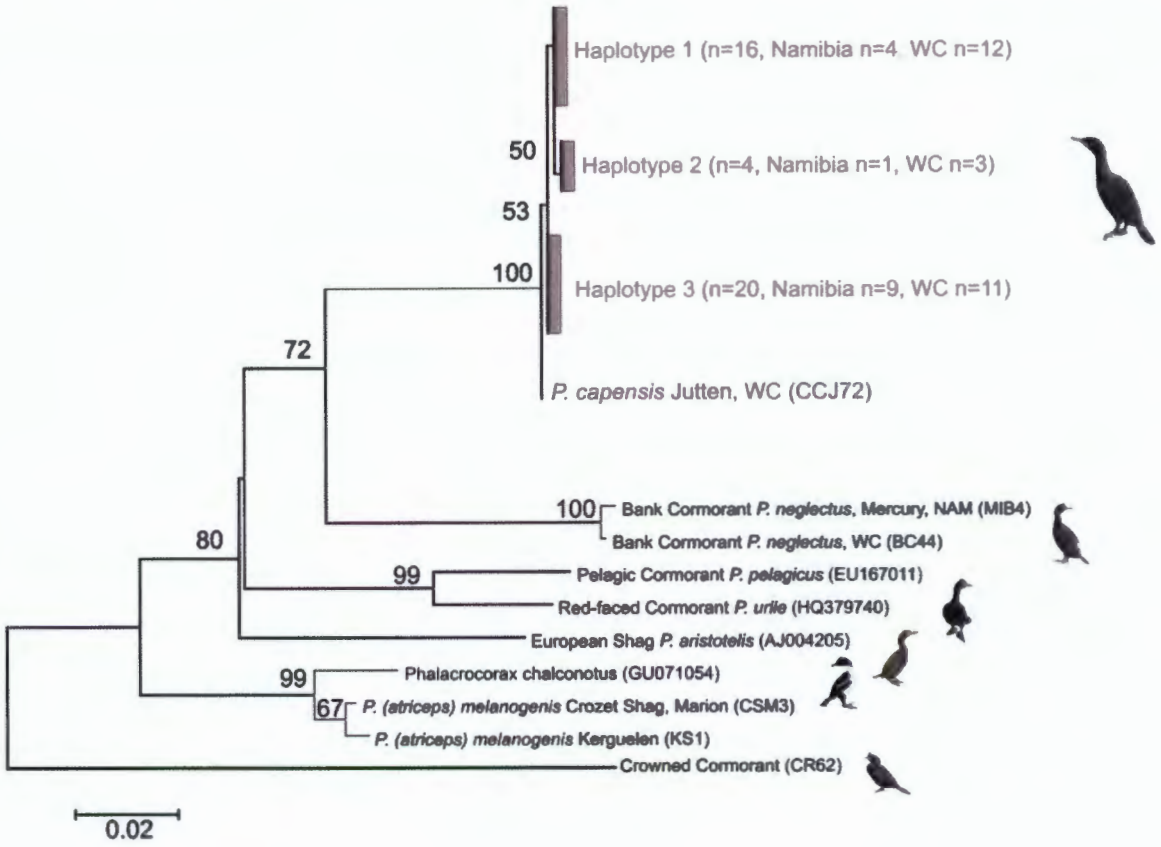


Figure 3.22 The cyt b (864bp) ML gene tree for Cape Cormorants (n=41) and related species, rooted on *P. coronatus*. Numbers at the nodes are bootstrap values (1000 replicates). Numbers in brackets are sample codes or GenBank accession numbers.



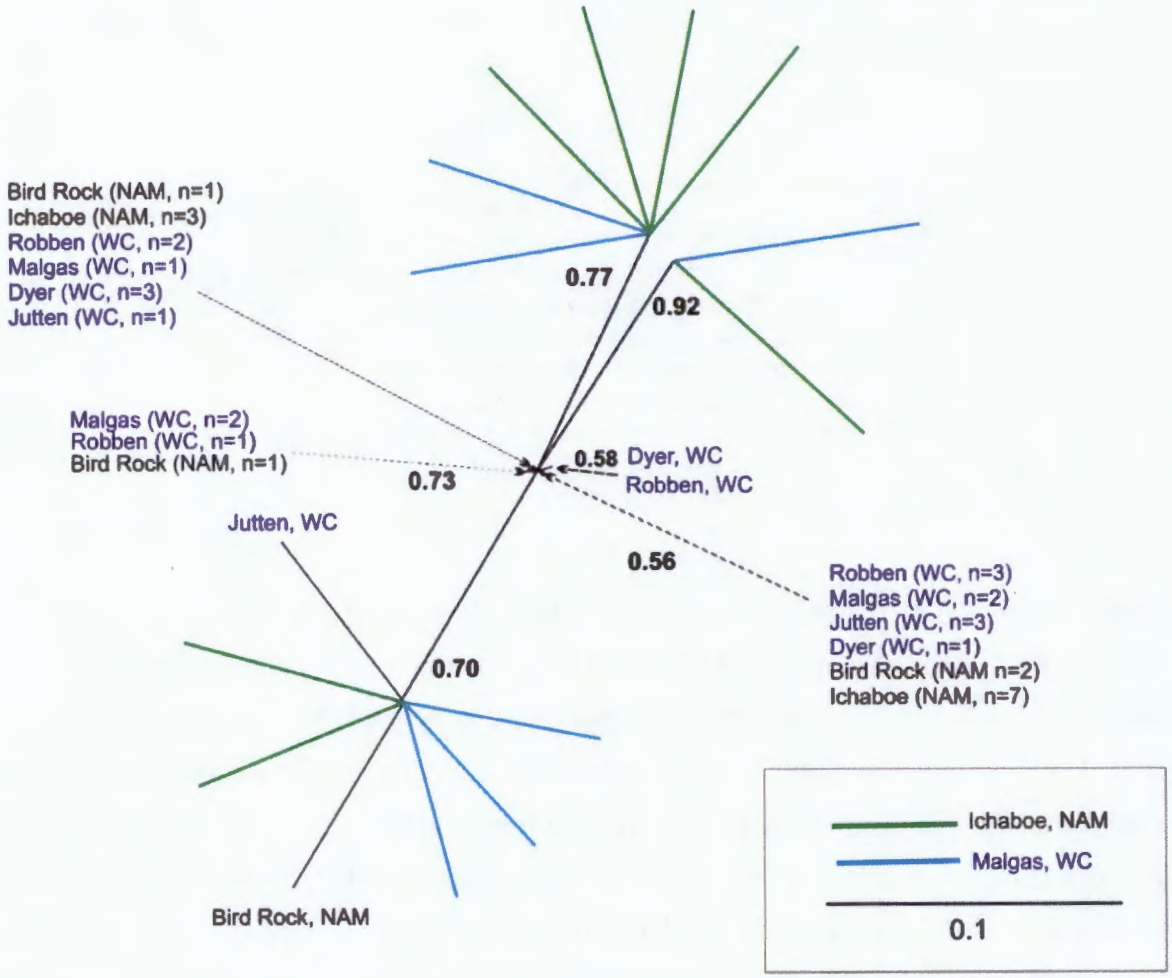
774bp of the cyt b region of the Stewart Island shag or Bronze Shag *Phalacrocorax chalconotus* (GU071054), Pelagic Cormorant *P. pelagicus* (EU167011), European Shag *P. aristotelis* (AJ004205) and Red-faced Cormorant *P. urile* (HQ379740) were included, as were two species of southern African cormorants, the Crowned Cormorant *Microcarbo coronatus*) and Bank Cormorant *P. neglectus,* and Imperial Shags *P. atriceps* or Crozet Shags *P. (atriceps) melanogenis* from Crozet and Kerguelen Islands (Figure 3.22). The ML phylogenetic tree is based on the HKY+G model and is rooted with *P. coronatus.* Cape Cormorants are monophyletic and the connections between sub-clades within the species reflect the same pattern observed in the MP analysis above.

Cape Cormorant: Overall

Both ATPase-6 and NADH3 were amplified in 31 samples (n=16 in the Western Cape and n=15 in Namibia). Both cyt b and NADH3 were amplified in 33 samples (n=19 in the Western Cape and n=14 in Namibia). Fifty individuals were represented by two or all three of

these genes and were included in a combined, partitioned Bayesian analysis (1939bp, Figure 3.23). Nineteen haplotypes were recovered among these 49 samples (haplotype diversity, h=0.92). The clades within the tree are largely unresolved, but some had high nodal support. All well-supported clades comprised individuals from both the Western Cape and Namibia (Figure 3.23).

Figure 3.23 Unrooted Bayesian phylogenetic tree based on a combined (concatenated), partitioned Cape Cormorant dataset including all individuals for which two, or all, of cyt b, NADH3 and ATPase-6 were successfully amplified (n=49, 1939bp). Numbers in bold type are Bayesian posterior probabilities. Individuals from the Western Cape are in blue and those from Namibia are in black.



DISCUSSION

Biodiversity, natural resources and species' ranges in the marine realm are largely influenced by inherent environmental variability (Parmesan & Yohe 2003; Parmesan 2006) together with anthropogenic activities (Tasker 2000; Höglund 2009; Worm et al. 2010). Co-distributed, ecologically similar species of similar evolutionary age are likely to have been

subject to the same selective pressures and threats over time, but may employ different strategies to cope with them. Species within the Agulhas-Benguela Ecosystem (ABE) have experienced long-term environmental variability over evolutionary scales, and more recent anthropogenic disturbance and exploitation over ecological time-scales. Notable changes in the abundance and distribution of African Penguins, Cape Gannets and Cape Cormorants have been recorded, and these may have population genetic consequences in terms of genetic diversity and population connectivity. Genetic sequence data provide a window into the history of these species, allowing us to better understand the microevolutionary forces that have shaped the distribution of genetic diversity among their populations.

Genetic Diversity

Relative to similar studies on closely related species, this study found low levels of genetic diversity overall and detected very few cases where levels of diversity differed among breeding populations of any of the study species. Within the general framework of marine ecosystem conservation, the role of marine conservation genetics is to merge evolutionary and ecological principles and techniques from population genetics with those from marine ecology, and apply them to marine biodiversity conservation (Ouborg 2010). Population genetic models, and numerous empirical studies, have shown that genetic drift and long-term reduced population size leave detectable genetic signatures in the form of depleted allelic and genotypic diversity within populations, and that the degree to which this occurs is a function of N_e (effective population size, defined as "the number of breeding individuals in an idealised population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration"; Wright, 1938, p430; (Wright 1931, 1938)). Although seabirds are generally long-lived, and only a small proportion of genetic variation may be lost per generation – mostly rare alleles or haplotypes – the massively reduced population sizes observed in populations of the focal species may have lead to a significant loss of genetic diversity and the associated potential to adapt to environmental changes (Willi et al. 2006; Gomulkiewicz & Houle 2009).

The focal species here face regional, seasonal, inter-annual and long-term changes in the availability of their shared prey resources and must cope with these changes in order to survive and breed (Shackleton 1987; Lluch-Belda et al. 1989; Crawford 1999). To ensure their long-term persistence, individuals must either adapt to novel local conditions or move to areas where conditions are more favourable. A number of life-history characteristics of

African Penguins, Cape Gannets and Cape Cormorants may act to buffer their populations from fluctuations in food supply (Hunt et al. 1996; Crawford 1999; Crawford & Altwegg 2008), and phenotypic plasticity in some of these traits undoubtedly improves the species' ability to track changes in food availability (Sabarros et al. 2012). Here, levels of neutral genetic diversity are used as a proxy for genome-wide diversity, which is especially important in threatened or endangered species, which characteristically exhibit low genetic diversity (sometimes half that of related non-endangered species (Frankham 2003)). Additionally, relative diversity among populations helps us to reconstruct the history of species, and provides a window into past population-level processes. Inbreeding and genetic drift in small, threatened populations act to increase their risk of extinction, and populations with low genetic variation cannot evolve and adapt, since evolution cannot proceed without genetic diversity.

Cape Gannet individuals sampled in Namibia exhibited the most unique haplotypes (11) across the seven gene regions surveyed, and those from the Eastern and Western Cape each exhibited three. This may reflect the historically much larger Namibian gannet population (Figure 3.1 (a)), and the larger number of breeding colonies. This overall pattern was different among African Penguin samples, where the Western Cape colonies exhibited the highest number of unique haplotypes (seven) across the gene regions surveyed, where Namibian birds exhibited four and those from the Eastern Cape, only two. Again, this likely reflects the historically much larger Western Cape penguin population (Figure 3.1 (c)), and the number of colonies comprising each breeding region. Cape Cormorant sample sizes were generally smaller for the six gene regions surveyed, and only three unique haplotypes were recovered: one in Namibia and two in the Western Cape. The NADH3 marker was amplified in all three focal species and overall genetic diversity was highest in Cape Cormorants (h=0.52, Table 3.1), intermediate in African Penguins (h=0.18, Table 3.1) and lowest in Cape Gannets (h=0.14, Table 3.1). Genetic diversity is difficult to compare directly among other gene regions due to differences in sample sizes and mutation rates between genes, but among variable mtDNA target regions (Table 3.1), Cape Gannets range from h=0.14 (NADH3) to 0.81 (COI), African Penguins from h=0.18 (NADH3) to 0.33 (COI) and Cape Cormorants from h=0.23 (ATPase-6) to 0.62 (cyt b). These results are comparable to a number of published studies (see Chapter 2), although many of these report diversity indices based on the mitochondrial control region (specifically the hyper variable D-Loop region), which are expected to be higher than other those based on more slowly evolving mitochondrial genes.

Among published studies of the Sulidae, the Cape Gannet exhibits lower haplotype and nucleotide diversity than the Nazca Booby (h=0.89, π=0.001; Table 2.4) at three comparable gene regions, with the exception of a slightly higher nucleotide diversity among COI sequences. The average diversity indices based on mitochondrial genes (h~0.4, π~0.001) are lower than those reported for all booby species, except the Endangered Abbott's Booby Papasula abbotti (Morris-Pocock et al. 2012), which occurs only on Christmas Island (cyt b, h=0.095, π=0.0002). This depressed genetic diversity may reflect the threat status (the Cape Gannet is listed as Vulnerable, whereas most booby species are classified as Least Concern), population and sample sizes, and the mutation rates of the gene regions chosen. Also, the range of Cape Gannets is restricted to only six colonies in southern Africa, whereas the Masked and Red-footed Booby are pantropical and exhibit higher levels of genetic diversity. The Peruvian Booby has a similar range to the Cape Gannet, but exhibits markedly higher levels of genetic diversity across its range. Genetic diversity across the range of Cape Gannets is at the lower end of the spectrum of genetic diversity indices reported for seabirds globally. Based on the most intensively surveyed gene region (NADH3), Cape Gannets from Namibia exhibited markedly higher levels of genetic diversity than those from the Western Cape or Eastern Cape. In terms of conservation management implications, Cape Gannets appear to have lower genetic diversity relative to other sulids, and Namibian colonies may, therefore, be considered conservation priorities.

Similarly, when compared to studies of other spheniscids, the African Penguin exhibits low haplotype and nucleotide diversity, for example, the haplotype diversity at the COI locus for the Magellanic Penguin (sister to the African Penguin) is more than double that detected in the African Penguin. The ATPase-6 and NADH3 gene regions were most intensively surveyed, and African Penguins from the Western Cape showed consistently higher levels of genetic diversity than those from Namibia and the Eastern Cape. This pattern, however, does not seem to hold at the colony-level i.e. within the Western Cape, Boulders Beach and Jutten Island colonies exhibited consistently low genetic diversity (h~0, n=12) compared to Dassen Island, Stony Point and Dyer Island colonies (h range 0.25 to 0.524).

Cape Cormorants showed similar levels of genetic diversity (h=0.25 - 0.52) among Namibian colonies and Western Cape colonies based on the three most intensively studied mitochondrial markers, and showed considerably higher levels of genetic diversity across their range at the NADH3 locus than either of the other two focal study species.

Genetic variation was generally higher among Cape Cormorants than among the less abundant and largely sympatric African Penguins and Cape Gannets. These results may reflect that, because of their more flexible foraging behaviour, larger clutch sizes and lower tendency to be philopatric, the Cape Cormorant has retained genetic diversity through their recent range-wide population decline. The levels of genetic diversity among Cape Cormorants are, however, lower than those reported in comparable studies of other Phalacrocoracidae (Waits et al. 2003; Marion & Le Gentil 2006): the Double-crested and Great Cormorants - although these species are expected to exhibit higher levels of genetic diversity, as they are both classified as Least Concern and their ranges and population sizes are much larger than those of the endangered Cape Cormorant, and incorporate marine and freshwater populations. The genetic variation in Cape Cormorants is more similar to that of the coastal, but wide-ranging European Shag *Phalacrocorax aristotelis* (Barlow et al. 2011), based on the NADH2 gene of 66 individuals from ten colonies, haplotype diversity was 0.38 (nucleotide diversity π=0.002), slightly lower than the NADH3 estimates from Cape Cormorants. Similar molecular markers were employed in a study of Magnificent Frigatebirds (Hailer et al. 2011), but levels of genetic diversity were much higher in that species (h=0.82, π=0.003). The molecular markers employed did not provide the necessary resolution to investigate the historical demography of Cape Cormorants, and it is unlikely that enough time has passed for any mtDNA marker to show signs of reduced genetic diversity due to the recent population decline.

Although some of the observed patterns of regional differences in genetic diversity are evident across gene regions, the various genetic markers employed sometimes seem to contradict each other, possibly due to differences in sample sizes and substitution processes (resolutions). Although mitochondrial genes are thought to be predominantly inherited as a single unit, some evolve faster than others and may, therefore, produce apparently divergent results (due to differences in resolution). Neutral genetic diversity among the African Penguins and Cape Gannets sampled is lower than that reported for non-threatened relatives, but does not seem to be as depleted as what has been described in some Endangered and Vulnerable species (see Tables 2.3 to 2.5).

Connectivity between breeding regions

This study found indirect evidence for strong, long-term connectivity among populations of all three study species. Gene-flow and dispersal occur at different spatial and temporal scales, however, dispersal is difficult to measure, especially for seabirds, in which rare, long-distance

movements are likely to connect populations (Lowe & Allendorf 2010). Metapopulations with strong interpopulation genetic connectivity will show weak population genetic structure i.e. an overall pattern of panmixia. This study investigated the degree to which genetic structure characterises the three focal species due to physical or non-physical barriers to gene-flow across their respective breeding ranges. The term 'population' is used to indicate all individuals collected at a defined geographic area (breeding region or breeding colony). A population was considered genetically structured if haplotype frequencies were significantly different between two populations ($P<0.05$). 'Phylogeographic structure' implies the existence of geographically-defined population-specific genealogical lineages (monophyletic populations), but no such patterns were observed in any of the species. Strong phylogeographic structure would be indicative of prolonged genetic isolation of populations (Friesen et al. 2007). The molecular markers used in this study provide insight into historical genetic connectivity, i.e. the degree to which gene-flow has affected evolutionary processes in subpopulations in each species (Lowe & Allendorf 2010), and suggests that significant historical connectivity across the distributions of these species has occurred. This suggests that contemporary sub-populations may be connected through gene-flow and, therefore, exhibit metapopulation dynamics that have likely buffered them against genetic diversity loss (Ramírez et al. 2013).

A recent review of the mechanisms involved in population differentiation (estimated from mtDNA data only) in seabirds (Friesen et al. 2007) highlighted the role of physical barriers to gene-flow, in that species separated by large land or ice barriers generally exhibited higher levels of population genetic and phylogeographic structuring than those species for which no such barrier existed. Oceanographic features (e.g. relative strengths of the Benguela and Agulhas currents, Benguela upwelling cells, various fronts and eddies, the Agulhas Bank) have been shown to play a role in seabird movements and in genetic processes for numerous marine taxa. Friesen et al. (2007) also found that seabirds resident at or near particular breeding colonies all year round exhibit increased levels of population genetic structure. Based on global positioning system (GPS) tracking data, this appears to be the case for adult African Penguins and Cape Gannets (Cape Cormorants have not been tracked in their non-breeding season to my knowledge). Importantly, not all factors affecting gene-flow and population genetic structure are contemporary; evolutionary forces such as historical fragmentation, population bottleneck events, range expansion, isolation by distance, retained ancestral variation and long range colonization also play a role (Friesen et al. 2007). Indeed,

studies of high latitude seabirds have found that observed population genetic structure can be accounted for by divergence in multiple glacial refugia during the Pleistocene (Kidd & Friesen 1998a; Moum & Arnason 2001).

Given that population differentiation can be strong in seabirds that are highly mobile, and therefore capable of overcoming most geographical barriers to dispersal, non-physical barriers must also influence gene-flow, population genetic structure and phylogeographic structure (Friesen et al. 2007). Examples in the literature include habitat preferences, mate choice, cultural foraging grounds, nonbreeding distribution and natal site fidelity (Friesen et al. 2007). Among Cape Gannets, regional φ_{ST} values based on NADH3 were low ($\leq$0.0003) and not significant, indicating high genetic connectivity among breeding regions. This pattern was unchanged when samples were analysed at the colony-level (individuals from all six breeding colonies were included in the analysis), with no significant genetic differentiation detected between any colony pair (all $\varphi_{ST} \leq 0$). Sample sizes were smaller for the other gene regions analysed, but estimates of regional genetic differentiation were also very low ($\varphi_{ST} \sim 0$) for ATPase-6, NADH2 and cyt b. Analyses of nuclear sequence data (B-fib I7 and GAPDH) yielded similar results (regional φ_{ST}<0.06). Based on COI, Cape Gannets from the Eastern Cape were more different from those in Namibia (average φ_{ST}=0.29) than from birds from the Western Cape (average φ_{ST}=0.17), indicating a possible role for isolation by distance, although sample sizes were small. Based on NADH3 (n=94), overall φ_{ST} was -0.008 and G_{ST} was -0.0023 for Cape Gannets. This low level of regional population genetic structure contradicts what was found for Masked (*S. dactylatra*), Red-footed (*S. sula*), and Brown (*S. leucogaster*) Boobies (Steeves et al. 2003) based on cyt b sequence data. Although they are close relatives of the gannets, these booby species exhibit morphological variation across their shared range, and vary in their foraging and breeding ecology. The genetic differentiation was high at a regional-level for all three species (φ_{ST}=0.62, 0.99 and 0.94 for Masked, Red-footed, and Brown Boobies, respectively). The observed structure was likely due to the Isthmus of Panama (a physical barrier which emerged 3 Mya) and other long-term physical barriers. Differences in foraging modes among these three boobies (near-shore versus offshore foragers), breeding behaviour (Brown Boobies are highly philopatric) and mobility (Steeves et al. 2003) may have also contributed to the incongruences observed among these three species i.e. intrinsic rather than environmental factors.

It has also been shown that Masked Boobies exhibit population structure across their pantropical range (Steeves et al. 2005a), with strong genetic differentiation between Indo-

Pacific and Atlantic Ocean populations, and low levels of gene-flow among populations within ocean basins (Steeves et al. 2005b). Although Cape Gannets technically inhabit both the Atlantic and Indian Oceans, the distances between regions are more comparable to "within ocean basin" investigations i.e. the isolation-by-distance effects between oceans are at a much larger spatial scale for masked boobies (>2000km). Also, there are no physical barriers of the scale of the Isthmus of Panama separating breeding regions for Cape Gannets. Divergence within ocean basins is lower among Red-footed Booby populations than among those of Brown Boobies, which may be due to long term gene-flow as a result of the marked differences in marine habitat preferences of these two species: Red-footed Boobies forage in pelagic waters (typically ~240km – interestingly, identical to the estimate for Cape Gannets and similar to that of Masked Boobies), whereas Brown Boobies forage very close to the shore, with similar foraging ranges to Cape Cormorants and African Penguins. This 'ecological barrier' decreases the probability that Brown Boobies will encounter and disperse to non-natal colonies. The ability of Cape Gannets to switch prey may also play a role in buffering their populations, to some extent, against environmental changes: it has been demonstrated that Cape gannets depend on fishery waste when their natural prey is scarce, but revert to feeding on natural resources whenever available, showing highly flexible foraging behaviour (Tew Kai et al. 2013). The combined effects of non-physical barriers, such as philopatry, fidelity to foraging grounds, and genetic drift in declining populations have not been sufficient to produce a mitochondrial genetic signal among Cape Gannet population, in the face of on-going gene-flow.

Little population structure was found among populations of Peruvian and Blue-footed Boobies, which inhabit the Humboldt Upwelling System on the Pacific Coast of South America, the most similar EBUS to the Benguela System (Taylor et al. 2011a). That pattern was explained as a result of these species' specialization to a cold-water upwelling system, which may elevate dispersal rates and reduce natal-site fidelity (Taylor et al. 2011b). Given that the Cape Gannet is also endemic to a cold-water upwelling system, there are strong parallels to these ecologically analogous South American Booby species. Long distance dispersal during times of severe disturbance (e.g. the historical exploitation by humans, the present shift in the distribution of prey resources, Benguela Nino events), when breeding and survival depends on the gannets' ability to track environmental change via dispersal to non-natal colonies, may have also played a role in producing the observed pattern of high genetic connectivity among regions and colonies (Taylor et al. 2011b).

Similar factors appear to be influencing genetic structure among African Penguin populations: regional φ_{ST} values based on NADH3, ATPase-6 and COI were small and not significant (<0.026). Based on ATPase-6, overall φST and G_{ST} were 0.01, but only one of the six measures of genetic differentiation performed by DNAsp was significant (P<0.05). Differentiation between the Eastern Cape and Namibia was highest (φ_{ST}=0.026), indicating a possible role of isolation by distance, as these two regions are furthest apart along the coast. The Eastern Cape and Western Cape were more differentiated (φ_{ST}=0.014) than the Western Cape and Namibia (φ_{ST}=0.0027), indicating tighter connectivity between the latter two regions. Tajima's D indicated significant deviation from neutrality at this gene region (D=-1.98, P<0.05), which may be as a result of the drastic population decline in African Penguins over the last century (approximately ten African Penguin generations), however, a mismatch distribution based on ATPase-6 showed that the data fit a constant population size model better than a declining population model (data not shown), suggesting that the demographic bottleneck has not yet resulted in a genetic signature of a bottleneck, based on the gene regions surveyed. Isolation by distance (IBD) analyses based on this data showed no correlation between geographic and genetic distance (data not shown).

The high connectivity inferred among populations of African Penguins is similar to what has been found for other members of their genus: High levels of gene-flow are reported for populations of Magellanic Penguins (Bouzat et al. 2009), Humboldt Penguins (Schlosser et al. 2003, 2008) and Galápagos Penguin (Nims et al. 2008) – the latter two are based on microsatellite data only (F_{ST} ~0.01 for all *Spheniscus* species, see Table 2.3) - despite differences in their range-sizes, threat status, foraging modes and mobility. This pattern is also generally present among populations of morphologically conserved penguin species; i.e. where no differences in morphology have been detected across their range e.g. Adelie (Roeder et al. 2001), Chinstrap (Korczak-Abshire et al. 2012) and Little (Overeem et al. 2008; Peucker et al. 2009) Penguins, although the former two species depend on krill, and the latter on squid, as their primary food resource, rather than fluctuating populations of pelagic shoaling fish.

In a comparative study of the life-history characteristics of seabirds in the Humboldt and Benguela Upwelling ecosystems, it was found that, in the Humboldt system, where adverse environmental conditions are more frequent, resident seabird taxa are able to recover more rapidly than their counterparts in the Benguela, where such perturbations occur less often (Crawford et al. 2006c). Based on the results of the present study, African Penguins appear to

be capable of shifting their distribution in response to environmental variability in the ABE i.e. to a similar degree to their Humboldt counterparts (Crawford & Jahncke 1999; Crawford et al. 2006c; Vargas et al. 2007), but that such changes still lead to a reduction in the breeding population.

Among Cape Cormorants, the NADH3 dataset indicated a lack of regional genetic differentiation between Western Cape and Namibian colonies (φ_{ST}=-0.025). Estimates of colony-level genetic differentiation based on NADH3 were also low (overall φST <0) and no significant differences were detected. The only positive φ_{ST} values based on NADH3 involved comparisons with Cape Cormorants from Jutten Island (4 of 5 comparisons > 0), although this pattern did not hold true for ATPase-6 and cyt b gene regions.

Based on molecular data (Figures 2.6 and 2.7), the closest extant relative of the Cape Cormorant is either the Bank, Japanese or Great Cormorant (Kennedy et al. 2009). Based on morphological data, the sister species is either the Guanay Shag or the Socotra Shag (Siegel-Causey 1988; Holland et al. 2010). Information about genetic structure is not available for most of these putatively close relatives, although the preliminary data presented in this study indicate strong regional phylogeographic structure in the Bank Cormorant (see Chapter 2, Figures 2.6 and 2.7). Interestingly, among the Bank Cormorants sampled for the present study, no birds from Namibia shared ATPase-6 haplotypes with those from the Western Cape (n=10 from Namibia, n=7 from the Western Cape; 588bp). Although the sample sizes are small, the Bank Cormorant appears to be highly differentiated at a regional-level (overall φ_{ST}=0.92, P<0.001; G_{ST}=0.66, P=0.0002). This cormorant breeds from November to April in Namibia and all year round in the Western Cape (although breeding peaks between May and October), and it does not rely heavily on sardine and anchovy stocks, as do the focal species of the present study (Hockey et al. 2005). Bank Cormorants forage closer inshore than Cape Cormorants (Ludynia et al. 2010, 2012) and although data on breeding dispersal are rare, they appear to be highly philopatric (Crawford et al. 1999, 2008a; Hockey et al. 2005). Bank Cormorants are endemic to the ABE and face many of the same threats as Cape Cormorants. Although shifting pelagic fish stocks (towards the east) are not expected to affect the breeding distribution of Bank Cormorants to the same degree as the Cape Cormorants, a similar shift in the distribution of one of their preferred prey species, the West Coast Rock Lobster *Jasus lalandii,* is thought to be driving regionally divergent demographic trends in the species (Crawford et al. 2008a). Mercury and Ichaboe Islands in Namibia support >70% of the global population (there are fewer than 500 pairs breeding in the Western Cape) and

those populations are in rapid decline, necessitating further research on regional connectivity. The contrasting patterns of genetic structure in Cape and Bank Cormorants imply a strong role for foraging mode (inshore versus offshore) as a mechanism driving population differentiation in the ABE.

By and large, strong genetic structure among cormorants is rare (see Chapter 2). Genetic structure has been investigated among European populations of the Great Cormorant (Goostrey et al. 1998b; Winney et al. 2001; Marion & Le Gentil 2006), which comprises two genetically distinct subspecies (Marion & Le Gentil 2006). Genetic structure among colonies within breeding regions i.e. within subspecies, was found to be weak (φ_{ST}=0.04). Sex differences in breeding site fidelity (Schjorring et al. 2000) and prospecting by first-time breeders (Schjorring et al. 1999) are thought to contribute to the high connectivity. Weak population genetic structure was also found among populations of Double-crested Cormorant, and between two recognized subspecies (Waits et al. 2003). A more recent investigation of genetic structure in this widespread species (Mercer 2008), found some evidence for population structure that partially reflected the subspecies classification (four subspecies; global φST=0.48). Despite strong philopatry, wide stretches of ocean separating colonies and the existence of recognised subspecies, population genetic structure was weak among breeding populations of European Shags (Barlow et al. 2011). This coastal-breeding marine benthic forager has some morphological and behavioural similarities with Cape Cormorants, which may explain, in part, their shared pattern of weak population genetic structure. Barlow et al. (2011) hypothesise that the pattern of genetic admixture among European Shags is primarily due to juvenile dispersal, reinforced by rare, recent long-distance, cross-sea movements and a post-Pleistocene range expansion (Barlow et al. 2011). An allozyme study of the morphologically variable Imperial Shag in South America (Rasmussen 1994) found little evidence for population differentiation among coastal Atlantic and Pacific populations. Imperial Shags inhabit freshwater and marine habitats (Rasmussen 1994), and that study included birds from continental South America and the Falklands Islands. The authors found little difference among inland freshwater and coastal forms (Rasmussen 1994). Conversely, another allozyme study of the Rock Shag found evidence of significant genetic structure along the coastline of South America (Siegel-Causey 1997a). Major glaciation events during the Pleistocene that rendered coastlines in the south uninhabitable for coastal breeding seabirds are thought to have shaped this pattern, with encroaching ice and changing sea levels

driving populations north into Pacific and Atlantic refugia where population divergence likely occurred (Siegel-Causey 1997b).

Overall, relative population differentiation estimates are highest among African Penguins, followed by Cape Cormorants, which exhibit only slightly higher levels than Cape Gannets. However, this comparison should be interpreted with caution due to differences in sample sizes and in the gene regions analysed. Relative to population genetic studies of other seabirds, however, none of the focal species exhibit strong population genetic or phylogeographic structure based on the gene regions surveyed i.e. neither breeding regions nor breeding colonies represent evolutionarily distinct units. This could be due to historical factors or ongoing contemporary genetic connectivity. Given the findings of this study, it is tempting to conclude that gene-flow is high, but low estimates of F_{ST} and φ_{ST} can reflect either ongoing gene-flow or recent common ancestry of currently isolated populations (Hedgecock et al. 2007; Knowles 2009). This is when additional, independent estimates of dispersal e.g. from ringing or banding studies, and historical species distribution accounts become important. The data emerging from tracking studies of Cape Gannets in the non-breeding season and of juvenile African Penguins also provide insight into the probability that birds move between breeding regions and colonies.

Congruency of molecular and movement data

Dispersal and gene-flow can occur at very different temporal scales, and this study infers connectivity based on both minimal estimates of genetic differentiation, together with ringing data (Lowe & Allendorf 2010). Although the movement data (based on banding) do not incorporate information about the breeding status of re-sighted birds, they do reveal that African Penguins and Cape Gannets readily move among breeding regions and breeding colonies. Given their mobility, and based on studies of other cormorants, it is likely that the distances between breeding regions do not represent a movement barrier to Cape Cormorants either. The homogenization of genetic diversity throughout the ranges of these three species in the ABE indicates that levels of gene-flow are likely to be sufficient to minimise any vicariant effects that philopatry or natal-site fidelity might have on the spatial distribution of genetic diversity. Despite the distances between breeding regions and a number of potential oceanographic 'barriers' to gene-flow, genetic connectivity among breeding regions appears to be high, allowing all three species to respond at the population level to changes in their environment.

The general pattern of genetic panmixia has been shown for a number of marine species in lower trophic-levels in the ABE (Matthee et al. 2005; Neethling et al. 2008), including the pelagic prey of the focal study species (Hampton, 2013). If differences in climate, habitat quality or other factors among breeding regions have resulted in different selective regimes, the degree of selection is not strong enough to overcome the homogenizing effect of gene-flow. This informs the third hypothesis tested in this study, in that populations (regions or colonies) do not represent distinct evolutionary units for any of the focal species. The results also indicate that, despite the drastic recent declines in their population sizes, genetic drift has not caused any regional or colony-level divergence among populations of the focal species, probably due to large historical effective population sizes and consistent gene-flow among populations. The 'dispersal corridors' between regions may represent an important factor for conservation strategies to consider, as the ability of these species to track changes in food availability may determine, to some degree, their long-term survival (Knowles 2009).

Ringing effort and rates of ring recoveries are much lower in Cape Cormorants than in the other two focal species, reflected by the fact that none of the birds captured for the present study (n~150) were ringed. However, based on the available SAFRING data (spanning 1950 to 2012), Cape Cormorants have been known to move between breeding regions: from the Western Cape to Namibia (e.g. Saldanha Bay or Lambert's Bay to Walvis Bay - ring numbers 20019, 22261, 22401, 22680), Western Cape to Bird Rock (ring number 20469), from the Western Cape eastwards (towards the Eastern Cape (e.g. Malgas to De Hoop (ring number 20136)), from Namibia to the Western Cape (e.g. Cape Cross to Noordhoek, ring number 24856). It is also evident from the ringing data that Cape Cormorants move around extensively within breeding regions (e.g. ring numbers 858416, 862017 and 862094) and sometimes venture inland (e.g. 21648), but little is known about their breeding status from ringing records, especially among earlier records. It is difficult to infer breeding status based on the breeding season (for any of the focal species) from the ringing data because the re-sighting date does not necessarily reflect when an individual dispersed.

In the early 1990s, African Penguin breeding adult site fidelity was estimated at 61–89%. Subsequently, individual breeding African Penguins have not been recorded breeding or settling at more than one island (Crawford et al. 1994). Fifty of the Cape Gannets in the NADH3 alignment were ringed (41 of them as chicks at their natal colonies, two ringed as adults) and have been re-sighted a variable number of times. Only two of the ringed individuals in the NADH3 dataset have been re-sighted at colonies other than those where

they were ringed and they were breeding at non-natal colonies when the samples were collected for the present study. One chick ringed at Ichaboe Island in Namibia in 2004 (LNG82, 9A39753) was sampled at the Lamberts Bay (Western Cape) colony and one chick ringed in Lamberts Bay in 1998 (LNG26, 9A13614) was sampled at Malgas Island. The vast majority of ringed Cape Gannets in the NADH3 dataset were sampled while breeding at colonies where they were ringed as chicks.

Cape Gannets are the second most abundant avian top-predators breeding in the ABE and have the largest foraging range of the breeding endemic seabirds, which theoretically affords them the greatest flexibility to respond to spatial changes in the distribution of their prey (Grémillet et al. 2008a). Interestingly, stable-isotope studies have shown that adults remain in the same oceanic habitats year round and do not undertake long migrations after reproducing (Jaquemet & McQuaid 2008). Also, there are clear differences between the stable-isotope signatures of Cape Gannets from Ichaboe (northern Benguela), Malgas (southern Benguela) and Bird Island (east coast, ABE), and very little difference between birds from the same colony, reinforcing the hypothesis that Cape Gannets show a high degree of foraging site fidelity (Pichegru et al. 2007; Grémillet et al. 2008a; Jaquemet & McQuaid 2008) Juveniles, however are known to migrate substantial distances post-fledging, which is what is likely to be driving distributional shifts of Cape Gannets in response to a shifts in their preferred prey species (Crawford et al. 2006b, 2008c). Fourteen of the Cape Gannets in the ATPase-6 dataset were ringed. One individual ringed in 1984 (LNG40, 953991) was 25 years old at the time of sampling. Ringing data confirms that all individuals were sampled at either their natal colony (ringed as chicks) or at the only colony they have been sighted at (ringed as adults or no primary ringing data).

The data described in the present study suggest that there has been historical long-term gene-flow between breeding regions i.e. that strong genetic connectivity has buffered the effects of environmental or human-induced changes in food availability. Given that adults of the focal species show a high degree of breeding site fidelity, gene-flow is most likely mediated via juvenile recruitment, and levels of recruitment have been sufficiently high to homogenise genetic diversity across their breeding ranges. Breeding adults are unlikely to move, but may do so when conditions deteriorate drastically: e.g. "The attachment of Cape Gannets to nest sites, and the low frequencies at which they visit other islands and form new colonies, indicate that the species is seldom nomadic as a breeder. Only under exceptional circumstances, such as during severe disturbance by man or displacement by seals, will Cape

Gannets change their locality of breeding" (Crawford et al. 1994, p.233). If physical (e.g. isolation by distance, the Luderitz upwelling cell or other oceanographic currents or features) or non-physical barriers to gene-flow (e.g. natal site fidelity) existed between breeding regions, we would expect to find phylogeographic structuring among populations.

The role of variability and behavioural inertia in shaping seabird 'metapopulations'

The structure of marine ecosystems, and the distribution and abundance of their constituent species, is driven primarily by environmental processes (Cury et al. 2001), and it has been shown that they respond drastically to inter-annual changes and inter-decadal climatic variations. Given the highly variable nature of the Agulhas-Benguela Ecosystem (ABE) in which Cape Gannets, African Penguins and Cape Cormorants have evolved over the past ~4 to 0.8 million years, environmental stochasticity in e.g. productivity or sea-level may have previously resulted in a spatial and/or temporal mismatch in the distribution of the seabirds and their prey (possibly local extinction and re-colonization of sardines and/or anchovies). Indeed, population genetic studies of sardines and anchovies in the ABE indicate that regional collapses have occurred without the additional pressure of fishing (Grant & Bowen 1998). Juvenile recruitment to non-natal colonies is one life-history strategy that has been shown to buffer seabird species against changes in their environments, and this has likely remained plastic in the focal study species, allowing them to adjust their breeding distribution in response to such changes.

It has been suggested that the degree of site fidelity exhibited by birds should be correlated with the stability of their habitat, such that a high degree of site fidelity is expected in stable habitats, and a lower degree of site fidelity is expected in unstable habitats (Ganter & Cooke 1998). To reproduce successfully in a spatially and temporally variable environment, iteroparous species must exhibit considerable behavioural flexibility over their lifetimes (Reed et al. 1999). To maximise lifetime reproductive output, seabirds have evolved life-history strategies to cope with environmental variability. Central-place foraging during breeding imposes time and energy constraints on adult seabirds, and if conditions deteriorate beyond certain thresholds, they will abandon breeding in favour of their own survival - which will manifest eventually at the population-level (Erikstad et al. 1998, 2009). Decreased natal-site fidelity and flexible foraging behaviour in response to variable food availability may represent crucial adaptations to the dynamic environment of the ABE, and allow populations of the focal species to survive, and reproduce, despite regionally adverse conditions – as has

been suggested for some Humboldt Upwelling System endemics (Taylor et al. 2011a). This plasticity has been proposed in seabirds (Pichegru et al. 2010b), and for Cape Fur seals (Matthee et al. 2005; Skern-Mauritzen et al. 2009).

In the face of environmental change, the strong genetic connectivity among breeding regions of the focal species is encouraging, because metapopulation dynamics likely increase their capacity to adapt to such change. However, the projected rate of climate change may surpass their ability to track changes in their environment. The unusual rate and extent of anthropogenic alterations of the environment may exceed the capacity of developmental, genetic, and demographic mechanisms that seabird populations have evolved to deal with environmental change i.e. combined with fishing pressure in the ABE, endemic seabirds may face stronger selection than their populations can cope with. Population responses to changes in the environment can be in the form of micro-evolutionary responses to selective pressure, such as adaptation or behavioural, morphological or physiological flexibility (Charmantier et al. 2008). Species that have evolved in a variable environment should have the ability to withstand changes in their environment and respond appropriately by adjusting aspects of their life-histories or their phenotypes. Understanding the limits of a species' adaptability under different environmental conditions is important for their conservation and for predicting the consequences of long-term environmental change e.g. climate change (Charmantier et al. 2008).

Adequate additive population genetic diversity provides insurance against changes in environmental conditions, and confers greater flexibility on populations for coping with anthropogenic or natural stress (Palumbi et al. 2008; Dawson et al. 2011). The present study focussed on only the larger populations of the focal species, and where populations have been small for a long time, genetic drift may have had a stronger influence (Willi et al. 2006). If the focal species are genetically compromised and show inertia in adapting to changing conditions, they may not be able to survive the ecological trap represented by the mismatch between the core breeding distributions of the focal species and the core distribution of their prey (Pichegru et al. 2010b). A number of species have reportedly shifted their distributions polewards (Weimerskirch et al. 2012; Péron et al. 2012) to accommodate changes in their environment, however there is no suitable breeding habitat further south for Cape Gannets, African Penguins and Cape Gannets, and the area suitable for breeding in regions of high prey availability is limited, or does not exist. Fluctuating environments favour behavioural plasticity (Svanbäck & Eklöv 2006), but seabirds also have conservative evolutionary

constraints on their life-history characteristics (Schreiber & Burger 2001). Few studies investigate individual variation in responses to fluctuating conditions, or how selection acts on these individual differences, despite this information being essential for understanding how populations will cope with future, rapid environmental change (Reed et al. 1999).

CONCLUSIONS

The influence of environmental change on seabirds has been especially noticeable at high latitudes (Crawford & Altwegg 2008), and its variability is higher in oceanic relative to terrestrial habitats (Hunt et al. 1999). The results presented here contribute - using molecular techniques – to our understanding of how seabirds in the ABE may be buffered against changing environmental conditions through strong genetic connectivity; this appears to be primarily mediated through juvenile recruitment to non-natal colonies. This knowledge is important for effective conservation of this species because, although adult survival is unlikely to be affected by variability of prey stocks (adults can shift to alternate prey or migrate to seek prey in other regions), breeding birds are tied to their colonies (Hunt et al. 1996), and local fluctuations in fish recruitment can have a dramatic effect on seabird reproductive success. Any reduction in breeding success will result in fewer juvenile recruits, which in turn restricts the population as a whole from responding to the change in food availability. Conservation efforts that encourage high breeding success must, therefore, continue to be prioritised.

CHAPTER 4: Evidence for high contemporary connectivity between breeding regions of African Penguins *Spheniscus demersus* throughout their range

"We must all hang together or, assuredly, we shall all hang separately" Benjamin Franklin

SUMMARY

Using published microsatellite markers for the genus *Spheniscus*, and novel species-specific primers for the African Penguin (*Spheniscus demersus*), we investigated population genetic and phylogeographic structure across the breeding range of this species. African Penguins representing 12 colonies distributed throughout the range in the Agulhas-Benguela Ecosystem off the coast of southern Africa were genotyped at 12 microsatellite loci. Genetic diversity and population structure were investigated at regional- and colony-levels, and the results compared to those of published studies of closely related species. Genetic diversity across the species range was reasonably high (average H_E~0.6), and overall allelic richness ranged from 1.9 to 13.3 alleles per locus.

Various fixation indices and pure indices of differentiation were employed to investigate hierarchical partitioning of diversity (among individuals, colonies and regions). Overall F_{ST} estimated from and analysis of molecular variance (AMOVA) at the regional and colony-levels were very low, suggesting high gene-flow among populations. Overall R_{ST} at the regional-level revealed slightly more evidence of regional population structure, but detected stronger structuring at the colony-level, indicating that colony-level processes are driving structure across the metapopulation. Although no significant evidence of population bottlenecks was detected, global heterozygote deficiency was significant at the regional- and colony-scales. Regionally, Namibia exhibited significant heterozygote deficiency, and at the colony-level Halifax Island, Mercury Island and Dassen Island showed this pattern.

Spatially explicit and spatially independent analyses at both the regional and colony-level corroborate the finding that, although there is little evidence for regional genetic structure across the range of African Penguins, and connectivity between most breeding colonies is sufficient to homogenise genetic diversity between them, some African Penguin colonies are evolutionarily distinctive. These results corroborate findings based on mitochondrial and nuclear sequence data for African Penguins. Regionally, connectivity between Namibia and the Eastern Cape is the highest, as might be expected based on shifts in the African Penguin's breeding distribution. The highest population differentiation was observed between colonies within the Western Cape. The roles of population demographic history, genetic drift and gene-flow in shaping the observed pattern are discussed.

INTRODUCTION

Many seabird species exhibit a high degree of breeding site fidelity, reflecting the evolutionary advantages of returning to the same breeding site. These include familiarity with the breeding or foraging ground, factors involving mate-fidelity and behavioural adaptation to local conditions (Schreiber & Burger 2001; Dearborn et al. 2003; Milot et al. 2008). Most seabirds will remain faithful to their breeding site once they have initiated breeding, reflecting the costs involved in dispersing to breed in a foreign colony. If conditions deteriorate over the long-term (decadal to century scale), the fidelity of adult seabirds remains strong i.e. they continue attempting to breed, often increasing their foraging effort and/or switching prey (Pichegru et al. 2007; Cury et al. 2011; Croxall et al. 2012; Moseley et al. 2012). Juvenile seabirds that have not yet found a mate or attempted to breed, however, are more nomadic, and may prospect at non-natal colonies during post-fledging dispersal (Reed et al. 1999; Schjorring et al. 1999). The number of young birds that disperse to breed at non-natal, unfamiliar colonies (where conditions may be better better), and the increased survival and breeding success at those colonies, will result in demographic changes; i.e. an increase in numbers at "good quality" colonies. Once juvenile or immature birds have settled at a non-natal colony to breed, they are unlikely to move again. This movement of juveniles, and the resulting genetic connectivity among populations, may represent a buffer against long-term changes in fish abundance. It means that seabirds with a high degree of natal site and breeding site fidelity may take a number of years to respond to changes in food availability and that breeding success is likely more strongly affected than adult survival.

The African Penguin *Spheniscus demersus* is endemic to southern Africa and it is the only penguin species that breeds in Africa. African Penguins have experienced long term decline since the 1800s, with numbers decreasing steeply in recent years (Griffiths et al. 2004; Crawford et al. 2011). Historical exploitation and current threats to the survival of this species have resulted in it being classified as Endangered (Crawford et al. 1995, 2011) and it has become an iconic conservation flagship species in the region. The distribution of breeding colonies in the Agulhas-Benguela Ecosystem (ABE) reflects the species' reliance on the pelagic fish that abound in this highly productive ecosystem (Shelton et al. 1984; Crawford & Altwegg 2008), and their survival is critically dependent on the continued availability of fish in the vicinity of their breeding colonies (Crawford 1998; Crawford et al. 2011). Pelagic prey species are generally patchily distributed in the ocean (Ryan et al. 2012), and their abundance and distribution are affected by environmental variability at a variety of scales (Shackleton

1987; Lluch-Belda et al. 1989; Crawford 1998; Crawford et al. 2001; van der Lingen et al. 2006b), which in turn influenc penguin populations.

A number of life-history characteristics of African Penguins buffer them against changes in prey availability over the short term (Crawford & Altwegg 2008). For example, African Penguins are central place foragers when breeding and need to find sufficient prey in close enough proximity to their breeding colony to survive and raise their young (Pichegru et al. 2010a) – but if there is a reduction in local food supply such that adults cannot cope with the additional costs associated with rearing their chicks, they will abandon breeding, allowing them to forage further afield to regain body condition and survive to breed the following season (Sabarros et al. 2013). Mark-recapture studies indicate that adult African Penguins show a high degree of breeding site- and mate-fidelity (Whittington et al. 2005a, 2005b), but juvenile recruitment to non-natal breeding colonies may be more common and has likely driven the eastward shift in the core breeding distribution of this species observed over recent decades (Crawford et al. 2008c; Pichegru et al. 2009), following similar shifts in their preferred prey populations (Roy et al. 2007; Coetzee et al. 2008; Crawford et al. 2008b). Adults may show some behavioural inertia to dispersal when local environmental conditions become less favourable (Pichegru et al. 2010b), however, prospecting juveniles may respond more rapidly and disperse to colonies or regions where foraging conditions are better (Reed et al. 1999). Changes in the availability of their preferred prey appear to have initiated such a response in African Penguins (Crawford & Dyer 1995; Crawford et al. 2008b), together with other top-predators in the ABE (Crawford et al. 2008d; Okes et al. 2009). Historically, the largest proportion of breeding penguins in the ABE inhabited coastal islands off Namibia, however, following the collapse of pelagic fish stocks there, their core breeding distribution shifted to colonies off South Africa, with the largest colony currently in the Eastern Cape Province (St Croix Island). High levels of connectivity among populations across the range of this species would act to buffer colonies against the potentially deleterious impacts of localised environmental change (Bicknell et al. 2012). Data from mitochondrial and nuclear sequence data (reported in Chapter 3) suggest that historic regional genetic connectivity has been high, but note the resolution of the markers employed is relatively low. Also, mitochondrial sequence data have been shown to be less informative regarding vertebrate population size, history, ecology, and adaptive potential, whereas rapidly evolving nuclear loci are better suited to such investigations (Bazin et al. 2006). Nuclear microsatellite markers provide fine-scale resolution, and reflect more recent, as well as contemporary, levels of

gene-flow (Avise 2004). It is crucial to gain a better understanding of gene-flow and levels of genetic variation between breeding areas to protect this species effectively. Flipper banding data and capture-mark-recapture models have contributed substantially to our understanding of connectivity between breeding regions, and between breeding colonies within regions (Whittington et al. 2005b; Sabarros 2010), but genetic connectivity has yet to be investigated. The consequences of the massive population decline in terms of reduced genetic variability have also not been assessed, and may have an impact on the ability of this species to adapt to ongoing and future changes in its environment.

Endangered species tend to have reduced genetic variation, when compared to abundant, non-threatened relatives (Frankham 2003). For example, the sister-species of the African Penguin, the Magellanic Penguin *Spheniscus magellanicus* although still considered threatened, numbers in the millions, compared to the estimated 21 000 breeding pairs of African Penguins, and is therefore likely to exhibit higher levels of genetic diversity (Baker et al. 2006). The length of time that a population has been large is also important; e.g. the northern elephant seal *Mirounga angustirostrus,* which was heavily exploited during the 19th century - to the extent that it was thought extinct - however some individuals survived, and numbers are now ~250 000 individuals. Despite this large population size, genetic diversity remains extremely low (Hoelzel et al. 1993). Also, species with small effective population sizes, such as the Galápagos Penguin (*Spheniscus mendiculus*), or populations that have exhibited drastic declines in numbers, are expected to possess less genetic variation e.g. the African Penguin colony on Dassen Island off the Western Cape coast of South Africa once supported a population in the order of hundreds of thousands of birds, but now comprises fewer than 5 000 breeding pairs (Crawford et al. 2011). This kind of population bottleneck is likely to have resulted in a genetic bottleneck i.e. a loss of genetic variation (especially rare alleles), although without historical African Penguin samples it is difficult to assess the severity of these events (Luikart et al. 1998; Allendorf & Luikart 2007; Welch et al. 2012b).

Dispersal (effective gene-flow) and genetic drift in seabirds

The demographic and evolutionary trajectories of populations are strongly influenced by the degree of connectivity among them (Bohonak 1999; Dearborn et al. 2003). Frequency-based markers (e.g. allozymes and microsatellites, as opposed to DNA sequence-based markers) are appropriate for inferring population connectivity over ecological timescales (Hellberg 2009; Lowe & Allendorf 2010). Limited effective dispersal among populations will cause allele frequencies in each population to diverge as a result of genetic drift (Wright 1943; Hellberg

2009) and the divergence at neutral, unlinked loci will occur more rapidly in small populations (Broquet et al. 2010). Effective conservation management may depend critically on understanding the metapopulation dynamics of a species e.g. identifying source and sink populations, or establishing the degree of genetic connectivity between populations, which may promote or reduce local adaptation or population differentiation (Avise 2004; Crooks & Sanjayan 2006; Lowe & Allendorf 2010). Nonetheless, dispersal ability has proven to be a poor predictor of the number of effective dispersal events and, consequently, population genetic divergence in vertebrates (Bohonak 1999; Milot et al. 2008; Wiley et al. 2012; Welch et al. 2012a).

That seabirds are often reluctant to disperse despite high levels of vagility, has lead to the term "seabird paradox" (Milot et al. 2008). This phenomenon is partly explained by the benefits of philopatry, non-physical and physical barriers to effective dispersal (Friesen et al. 2007), and the fact that ecological studies of seabird movement may not detect rare dispersal events, or those of juveniles (Milot et al. 2008). Seabirds are generally considered to be highly mobile and many are able to fly great distances without encountering significant barriers to dispersal (Dearborn et al. 2003; Levin & Parker 2012). Penguins are an exception to this general pattern, as they have evolved flightlessness (Elliott et al. 2013), but a number of studies have found that despite their apparently reduced mobility and high levels of observed natal- and breeding-site fidelity, minimal population genetic and phylogeographic structure characterises contemporary populations of most species (Roeder et al. 2001; Akst et al. 2002; Schlosser et al. 2008; Nims et al. 2008; Bouzat et al. 2009; Boessenkool et al. 2009b; Korczak-Abshire et al. 2012). These studies include the three South American *Spheniscus* penguins, which represent all congeners of the African Penguin: The Galápagos Penguin *Spheniscus mendiculus* (Akst et al. 2002; Nims et al. 2008), the Magellanic Penguin *S. magellanicus* (Akst et al. 2002; Bouzat et al. 2009) and the Humboldt Penguin *S. humboldti* (Schlosser et al. 2008).

Microsatellites

The majority of recent population genetic studies of seabirds, and penguins in particular, have employed microsatellite markers. Microsatellite markers evolve rapidly and can provide better resolution than sequence data when studying patterns of gene-flow among populations (Slatkin 1995; Goldstein et al. 1999; Balloux & Lugon-Moulin 2002; Lukoschek et al. 2008). Another advantage to employing microsatellite markers (as opposed to sequence data) is the opportunity to survey multiple independent (unlinked) loci among individuals and combine

these data into multi-locus genotype datasets that represent changes across the nuclear genome (Hellberg 2009). Microsatellite markers do present some technical and analytical challenges (Zink & Barrowclough 2008; Brito & Edwards 2009) relating to their mutation rates, which may vary across loci and even among alleles at the same locus e.g. alleles that contain large numbers of repeats are thought to mutate faster. Similarly, the interpretation of comparative studies across taxa may be influenced by mutation process along different evolutionary lineages (Ellegren 2004). In addition, due to the rapid mutation process, some degree of allele size homoplasy (non-homologous alleles of the same length) is likely to be present at some loci (Shepherd & Lambert 2005; Anmarkrud et al. 2008; Lukoschek et al. 2008). The accuracy of PCR amplification and converting raw data into genotypes can also be influenced by genotyping artefacts, such as null alleles, which should be taken into account when analysing microsatellite data (Hedrick 1999; Brito & Edwards 2009). Although general patterns that emerge from the analysis of sequence data and microsatellite data can be compared, the statistics used in each case are not directly comparable (Brito & Edwards 2009). Comparisons between closely related taxa, using statistics generated from microsatellite markers that have been developed for those species and applied to large samples of individuals are, however, more valid. Standardised indices, which take into account many of the problems encountered in cross-species comparisons have been developed recently (Jost 2008; Ryman & Leimar 2009; Meirmans & Hedrick 2011), and are applied here.

Study goals

The aims of this chapter are to (i) quantify genetic diversity based on microsatellite markers in each of the three breeding regions of African Penguins, and in each of the sampled breeding colonies; and (ii) to investigate whether physical (e.g. geographic isolation) or non-physical (e.g. adult philopatry) barriers act to restrict regional gene-flow, or gene-flow between colonies within or among regions. Regional and colony-level breeding populations of African Penguins have contrasting demographic histories, including the extent of population declines (e.g. Ichaboe Island and Dyer Island), long-term declines followed by a recent (early 2000s) peak in breeding pairs (e.g. Dassen Island), and the recent founding of new populations (e.g. Stony Point and Robben Island). Additionally, colonies differ in the availability of suitable breeding habitat. Based on this, it is expected that colonies that have (a) always been small and (b) have experienced severe population decline will exhibit lower levels of genetic diversity than historically large populations due to genetic drift. Recently

founded populations are expected to have lower genetic diversity (heterozygosity and allelic diversity) than older populations, assuming that the number of founders is small and from a single source; i.e. if founders are from multiple genetically dissimilar sources, it is possible for recently founded populations to have high diversity; e.g. Campbell Island Albatrosses *Thalassarche melanophris* (Burg & Croxall 2001). The complex interactions between breeding dispersal (genetic connectivity through gene-flow), demographic history and genetic drift will affect the degree to which African Penguin populations exhibit these predicted (a and b) patterns of genetic diversity.

Based on banding data, demographic connectivity between breeding colonies, and breeding regions, is likely to be uneven across the range of this species (Whittington et al. 2005b), but levels of effective dispersal between breeding colonies are not accurately known. Based on available evidence – from mitochondrial markers, ringing data and the emerging trend in the literature showing limited population structure in seabirds despite strong philopatry and great distances between colonies - it is expected that genetic connectivity among breeding regions and breeding colonies of African Penguins will be high. A third prediction (c) is that if physical or non-physical barriers to dispersal restrict connectivity among populations, then populations will show a phylogeographic pattern - likely one that is consistent with isolation-by-distance; i.e. the degree of population differentiation will be correlated with geographic distance. It should be noted here that strong gene-flow among populations can mask the effects of genetic drift, and therefore, the genetic signal of bottleneck events.

MATERIALS AND METHODS

African Penguin sample collection

Blood or feathers were sampled from breeding adult African Penguins or young chicks at 12 colonies located throughout their range in the ABE (Chapter 2, Figure 2.2). Blood samples were stored in lysis buffer (100mM Tris, 10mM NaCl, 100 mM EDTA and 0.5% SDS), and feathers were stored in 99% ethanol. A total of 220 samples (189 of which are analysed in this study) were collected between January 2009 and March 2011. The colonies sampled are grouped geographically into three broad breeding regions: Namibia (Ichaboe Island, Mercury Island, Halifax Island and Possession Island), the Western Cape, South Africa (Stony Point, Jutten Island, Boulders Beach, Robben Island, Dyer Island and Dassen Island) and the Eastern Cape, South Africa (Bird Island and St. Croix Island). Details of individuals sampled at each colony are given in the Appendix 3.1 (b). The colonies sampled support the vast

majority of the African Penguin population (>90%) and were selected because they are the biggest colonies i.e. they are all the African Penguin colonies that support >100 breeding pairs, with the exception of Vondeling Island in the Western Cape and Seal Island in the Eastern Cape, which were not sampled. All other African Penguin breeding colonies consisted of fewer than 100 breeding pairs at the time of sampling. Interestingly, the mainland colony at Stony Point grew from ~450 pairs in 2011 to >2000 pairs in 2014; i.e. since sampling took place.

Selection of published microsatellite loci and genotyping

Published microsatellite primers from studies of other *Spheniscus* species were tested for polymorphism among a subset of wild African Penguin samples. Appendices 4.1 and 4.2 list the loci tested during this study, along with associated details. Fluorescent genotyping was used for five published loci and an additional seven loci developed specifically for African Penguins (Labuschagne et al. 2013). Loci PNN01, PNN03, PNN06, PNN08, PNN09, PNN12 and PNN05 were developed via pyrosequencing of a microsatellite-enriched library for African Penguins and the multiplexed PCR amplification conditions for these loci are described in Labuschagne et al. (2012). DNA extraction was conducted using the Qiagen DNeasy© Blood and Tissue Kit as per the manufacturer's instructions (see previous chapter).

PCR amplification and genotyping procedures were performed using fluorescently labelled forward primers. Primer sequences and PCR conditions are given in Appendix 4.2. The fluorescent labels used for each forward primer, the GenBank accession numbers for cloned samples, repeat motifs for each locus and other details are given in Appendix 4.1. Pairs of fluorescently labelled PCR products were pooled for each sample and run against GenescanTM 500 LIZTM internal size standard on an ABI 3130 Genetic Analyser (Applied Bioystems, Inc.). Samples were genotyped using GeneMapper v. 4.0 and Peak Scanner™ Software Version 1.0 (Applied Biosystems).

Microsatellite data analyses

The genotype data for 12 loci from all 189 wild African Penguin samples were analysed in MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004) to check for mistyped allele sizes and deviations from the specified repeat motif for each locus, and to check for null alleles and heterozygote deficiency among loci in each population. GENEPOP v4.1.4 (Rousset 2008) was used to conduct Hardy-Weinberg exact tests for heterozygote deficiency and excess across all loci (global), and within each region and each colony. Some loci did not amplify in

all 189 samples and, therefore, 3.5% of the genotype data was coded as "missing data". This corresponded to 80 "missing" genotypes that were coded as zeros and not included in the MICROCHECKER analyses. MICROCHECKER randomises the observed alleles for each locus within each population to generate random genotype data with which the observed data can be compared. The confidence interval was set to 95% and 1000 iterations were performed to produce graphs of the observed and expected frequencies of allele-specific homozygotes in each population and the frequencies of size differences in alleles i.e. the frequency of genotypes categorised by the size differences (in base pairs) between two alleles of the homozygotes.

Genetic diversity within breeding regions and within breeding colonies

The data analysed included 52 samples from four Namibian colonies, 58 samples from two Eastern Cape colonies and 79 samples from six colonies in the Western Cape (Table 4.3). To investigate genetic diversity within populations, standard estimates including observed and expected heterozygosities (H_O and H_E), Nei's unbiased gene diversity (h), the presence of private alleles and numbers of alleles (Na), were calculated for all three breeding regions and all colonies for each of the 12 loci using GENALEX v6.5 (Peakall & Smouse 2006, 2012) and GENEPOP v4.1.4 (Rousset 2008). The number of effective alleles (Ne), and corrected, nearly unbiased estimators of the number of effective alleles (cNe), expected heterozygosity within populations (defined as breeding regions or breeding colonies, cH_S) and for the total population (cH_T), were also calculated in GENALEX, giving equal weights to all populations independent of real population sizes or sample sizes (Nei & Chesser 1983). Allele frequency histograms for each locus in each breeding region and each breeding colony are given in Appendices 4.14 and 4.15 respectively. GENALEX v6.5 (Peakall & Smouse 2006, 2012) was used to test if loci were in Hardy-Weinberg Equilibrium (HWE) in each breeding region and colony. Similar analyses were conducted in GENEPOP v4.1.4 (Rousset 2008) and FSTAT v.2.9.3.2 (Goudet 1995), which were additionally used to test for linkage disequilibrium (LD), and for evidence of significant heterozygote deficiency and heterozygote excess across all population-locus pairs. LD tests in GENEPOP Version 4.1.4 (option 2, sub-option 1) and FSTAT v.2.9.3.2 (Goudet 1995) were carried out between each pair of loci in each population, with populations defined as breeding regions (a total of 198 comparisons) and breeding colonies (a total of 792 comparisons). For all HW analyses in GENEPOP, the exact P-values were obtained using Markov Chain Monte Carlo (MCMC) simulation of 10 000 dememorization steps, 100 batches, and 10 000 iterations per batch.

FSTAT v2.9.3.2 (Goudet 1995) was also used to calculate rarefied allelic richness (standardized for variation in sample size), another measure of genetic diversity (Petit et al. 1998; Leberg 2002). When testing for HWE in GENEPOP (option 1, sub-option 3), probabilities are based on the exact test with the complete enumeration test conducted for all possible loci.

Fixation indices represent estimates of allele fixation i.e. increased homozygosity relative to HW expectations resulting from inbreeding and genetic drift, and are used to quantify population genetic structure (Nei & Chesser 1983; Meirmans & Hedrick 2011; Whitlock 2011). The underlying assumption is that if fewer migrants are shared between populations, this will be reflected in a higher fixation index, which, therefore, represents an indirect measure of gene-flow. Theoretically, population subdivision into smaller sub-populations results in a decrease in genetic diversity (measured as heterozygosity) in the daughter populations due to their lower effective population size (relative to the parent or total population) and the increased effect of genetic drift (Nei 1987). F-statistics quantify this decrease in heterozygosity relative to what would be expected if no population subdivision had occurred i.e. the expected heterozygosity under panmixia. The three fixation indices developed by Wright (1951) are F_{IS} (inter-individual), F_{ST} (between sub-populations) and F_{IT} (total population). F_{IS} is an average across all sub-populations, and indicates the degree to which heterozygosity is reduced among individuals in a sub-population relative to what would be expected if mating was panmictic in their sub-population. F_{ST} estimates levels of population subdivision based on the reduction in observed heterozygosity (averaged across individuals) in pre-defined sub-populations relative to the expected heterozygosity total population ($F_{SUB\text{-}POPULATION\text{-}TOTAL}$). This allows us to infer what micro-evolutionary forces might play a role in producing the observed pattern e.g. founder effects are likely to result in subpopulations that exhibit different allele frequencies to the source population (Avise 2004). Values of these F-statistics will change depending on how the pre-defined sub-populations are defined in analyses. The statistical significance of estimates of population differentiation are tested by permutation. AMOVAs with 10 000 replicates and 10 000 pairwise population permutations were executed for the overall estimates of fixation indices and pairwise colony estimates.

Population structure: Connectivity among breeding regions and breeding colonies

Spatially explicit analyses

The term 'spatially explicit' here refers to analyses in which populations are pre-defined according to their collection localities. F-statistics, and related measures, are used to study non-random patterns of genotype frequencies among predefined populations that result from factors such as non-random mating, multi-level population subdivision, drift, migration and natural selection (Holsinger & Weir 2009). Regional-level genetic differentiation i.e. among the three broad breeding regions (Western Cape, South Africa; Eastern Cape, South Africa; Namibia), and colony-level genetic differentiation i.e. among all pairs of sampled colonies located throughout the range of African Penguins were investigated separately using a variety of population differentiation estimates. Because multiple comparisons were involved, correction against type I error was made with the Benjamini-Yekutieli (B-Y) method (Narum 2006). Multiple measures of population differentiation based on microsatellite markers exist in the population genetic literature (Nei 1973; Slatkin 1995; Rousset 1997; Neigel 2002; Balloux & Lugon-Moulin 2002; Jost 2008; Meirmans & Hedrick 2011), each with some advantages and disadvantages (Balloux & Lugon-Moulin 2002; Jost 2009; Whitlock 2011). There is on-going debate about which of these are most appropriate for empirical surveys in natural populations, and studies advocate reporting one, some, or all of the available indices (Jost 2008, 2009; Ryman & Leimar 2009; Gerlach et al. 2010; Bird et al. 2011; Meirmans & Hedrick 2011; Whitlock 2011). A subset of indices representing fixation indices, standardised fixation indices and pure estimates of differentiation were calculated at regional- and colony-levels for African Penguins (Bird et al. 2011; Meirmans & Hedrick 2011). Wright's F_{ST} (Cockerham 1973; Weir & Cockerham 1984), and the related measure, R_{ST} (Slatkin 1995), were calculated for the dataset overall, between breeding regions and between pairs of colonies. F_{ST} measures changes in levels of heterozygosity relative to a single panmictic population, whereas R_{ST} measures changes in the variance of allele size relative to what would be expected in a single panmictic population (Figure 4.1). These metrics can be calculated directly from microsatellite data or via AMOVA (and nested AMOVA), which allows for statistical testing i.e. probabilities associated with them can be estimated through permutation methods during AMOVA (Nei 1987; Slatkin 1995; Meirmans 2006). Microsatellite loci contain information about the relative frequencies of particular alleles, and

also about the evolutionary distance among those alleles i.e. allele length differences due to mutation.

Beyond F_{ST}

The R_{ST} statistic takes advantage of differences in allele length to provide additional insight into relationships among populations (Slatkin 1995) i.e. R_{ST} calculations incorporate information about the number of repeat differences between alleles at each microsatellite locus (Holsinger & Weir 2009) and is based on the Stepwise Mutation Model (SMM), whereas F_{ST} is based on the Infinite Allele Model (IAM). The estimation of R_{ST} assumes the SMM when characterizing microsatellite loci, but in practice the variation at microsatellites rarely fulfils the assumptions of the model in natural populations, and R_{ST} can, therefore, be less informative than F_{ST}. GENALEX v6.5 (Peakall & Smouse 2012) calculates F_{ST} in three ways: (1) Wright's F_{ST} for multi-allelic data (Cockerham 1973; Nei 1977), without statistical testing (denoted F_{ST}), (2) corrected F_{ST} (Nei & Chesser 1983; Nei 1987) using cH_S and cH_T (unbiased estimators of H_S and H_T) following Nei and Chesser (1983) and (3) via AMOVA (Excoffier et al. 1992). Genetic differentiation between sub-populations (defined as breeding regions and breeding colonies) was also estimated using Weir and Cockerham's (1984) variant of F_{ST} (denoted h or θ, analogous to (2) above) in GENETIX, FSTAT and GENEPOP, as it accounts for variable sample and population sizes (Weir & Cockerham 1984). R_{ST} is analogous to F_{ST}, and can be calculated via AMOVA in GENALEX (Slatkin 1995; Peakall & Smouse 2012), with statistical significance estimated by random permutation i.e. samples are repeatedly "shuffled" throughout the dataset to simulate panmixia. AMOVA is calculated for each shuffle and the results are compared to the observed value. The null hypothesis for AMOVA is that there is no genetic difference among the pre-defined populations, and can be reasonably rejected if the observed value (e.g. F_{ST} or R_{ST}) differs significantly from the range of values estimated by simulating panmixia by repeatedly randomising samples across the dataset.

One of the principal criticisms of F_{ST} is that the maximum possible value of the statistic (F_{STmax}) decreases with increasing within-population diversity (Meirmans & Hedrick 2011). This problem extends to some related measures, including θ, φ_{ST} (Weir & Cockerham 1984; Excoffier et al. 1992) and G_{ST} (Nei 1987), and has an enormous impact on how results should be interpreted for multi-allelic markers (such as most microsatellites) e.g. if the samples collected from wild populations exhibit more than one allele, even when samples are fixed for different alleles, a maximum of 1.0 is never observed for F_{ST} or G_{ST} (Hedrick 1999; Jost

2008; Bird et al. 2011; Meirmans & Hedrick 2011). In fact, these differentiation measures "cannot exceed the level of within-subpopulation homozygosity, no matter what evolutionary factor is influencing the amount and pattern of variation" (Hedrick 2005). The realization that fixation indices with these mathematical properties systematically underestimate genetic differentiation, especially for highly polymorphic markers such as microsatellites (Hedrick 1999), led to the development of a number of standardization procedures (Bird et al. 2011) for F-statistics (Meirmans 2006) and G-statistics (Nei & Chesser 1983; Hedrick 2005; Meirmans & Hedrick 2011), and a new method (D_{EST}) of estimating population differentiation (Jost 2008). Jost's D is sometimes called a differentiation index, because it measures the degree of deviation from complete divergence, where fixation indices (F_{ST} and its analogues) measure deviations from panmixia (Figure 4.1). The two classes of indices can behave in different ways because they reflect different aspects of genetic diversity: D reflects the proportion of allelic diversity that lies among populations, while F_{ST} is an indicator of the variance of allele frequency among populations. Also, D might be referred to as a distance measure because it is more related to the genetic distance between populations than to the variance in allele frequencies (Whitlock 2011). Standardised measures are more useful for comparisons of genetic differentiation between studies of organisms with different effective population sizes, or between analyses of markers with different mutation rates, because their magnitudes represent the proportion of the maximum possible differentiation based on the level of subpopulation homozygosity observed (Hedrick 2005; Meirmans 2006; Heller & Siegismund 2009).

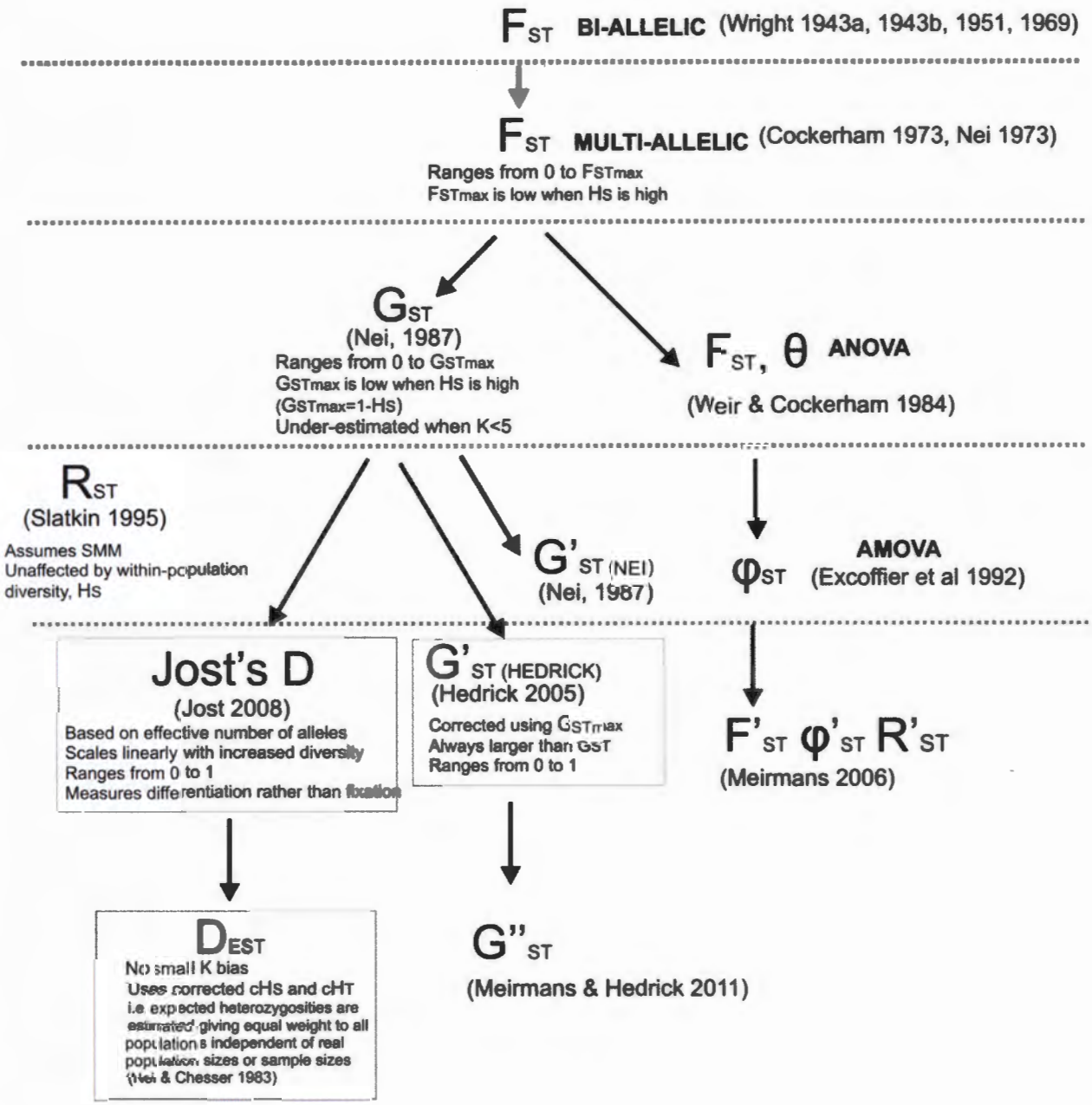
Another problem that arises when estimating population differentiation based on allele frequencies is that genetic estimates are usually based on a relatively small number of samples (compared to the total population size) taken from only a few populations, which represent a much larger metapopulation. For example, in the present study of African Penguins, samples from breeding colonies constitute 0.2 to 1.4% of the total estimated colony size. Extrapolating the allele frequencies observed in the samples collected to estimate expected heterozygosity across the total population (H_T), and within sub-populations (H_S), will indisputably lead to bias. The recognition of this problem led to the development of corrected, nearly unbiased estimators (cH_S and cH_T) of these values (Nei & Chesser 1983; Nei 1987), which should be used in all calculations of standardised fixation indices and pure indices of genetic differentiation. GENALEX was used to calculate F_{STmax}, G_{STmax}, cHS and

cHT, which were in turn used to estimate the standardised and pure measures of population differentiation (F'_{ST}, G'_{ST}, G''_{ST}, D_{EST}).

There is no currently accepted single measure based on multi-allelic genetic markers that best describes population differentiation. Various authors recommend that empirical studies should report both a fixation index (e.g. F_{ST}, R_{ST}, G_{ST}, G'_{ST}, G''_{ST}) and an index of genetic differentiation (e.g. D_{EST}), because they measure subtly different properties of population partitioning (Bird et al. 2011). A hierarchical analysis of molecular variance (AMOVA), and a nested AMOVA (with colonies assigned to breeding regions) based on the African Penguin dataset, were executed in GENALEX v6.5 (Excoffier et al. 1992; Peakall & Smouse 2012) to estimate R_{ST}, F_{ST} and F'_{ST} at various hierarchical levels. When all indices of fixation and differentiation are concordant, one can be confident in the observed pattern and draw robust conclusions, however, when methods yield contrasting results, the pattern and direction of their disagreement can be interpreted as diagnostic of particular phenomena (Bird et al. 2011). A truly informative index would provide similar, or at least congruent, results for all neutral loci i.e. reflecting biologically real differences among populations, rather than differences in the properties of the loci surveyed. Unfortunately, simulation studies have shown that some indices (e.g. G_{ST}), are influenced by mutation rate variation among loci, especially when the mutation rate approaches or exceeds the migration rate (Whitlock 2011) e.g. results can be artificially depressed when G_{ST} is used to compare populations with low migration rates using microsatellite markers with high mutation rates. These low G_{ST} (and F_{ST}) values are often misinterpreted, resulting in the false assumption of high genetic connectivity among populations (Gerlach et al. 2010). G'_{ST} and D are also sensitive to high mutation rates (Ryman & Leimar 2009) and will tend to unity for any locus with a high mutation rate, unlike R_{ST} which explicitly accounts for the mutation process and is thought to be a preferential index for loci with high, or highly variable, mutation rates (Whitlock 2011).

Jost's bias-corrected estimator, D_{EST}, measures 'real' genetic differences between populations and is based on the effective number of alleles, thereby providing more meaningful insight into differentiation (Heller & Siegismund 2009; Jost 2009; Ryman & Leimar 2009; Gerlach et al. 2010). D is intended to measure differentiation in a way that increases as the number of alleles unique to local populations increases (Whitlock 2011). Genetic fixation and genetic differentiation reflect different aspects of population structure i.e. fixation indices provide information about the probability of fixation at each locus in the populations and differentiation gives information on the allelic differentiation between the populations at each

Figure 4.1 Diagrammatic representation of the development of fixation indices, standardised fixation indices and pure differentiation indices in Population Genetic literature.



Assignment tests

Population structure was also investigated using assignment tests, which are a class of clustering methods that explicitly test the likelihood that an individual is a migrant from another population by calculating the probability that its multi-locus genotype originated from a pre-defined locality with a different genetic composition to the known sampling location (Wilson & Rannala 2003; Piry et al. 2004; Morris-Pocock 2012). Assignment tests

locus. Jost (2009) asserts that "D measures the actual relative degree of differentiation of allele frequencies among the demes of a population, while G_{ST} is a useful tool for estimating one of the causes of that structure, the amount of migration between demes". It has been suggested that empirical studies of natural populations should report F_{ST} and G_{ST}, and additionally R_{ST} if microsatellites are employed and the migration rate is low (Whitlock 2011). The development of fixation indices, corrected fixation indices and pure estimates of differentiation by various authors is summarised in Table 4.1.

Interpreting spatially explicit indices of population structure

Jost's D_{EST} is difficult to interpret in terms of basic population genetic parameters, such as population size and gene-flow, because of the way that it is calculated i.e. it does not provide accurate insights into demographic processes (e.g. genetic drift or migration), but rather into the particular mutational characteristics of the loci employed (Ryman & Leimar 2009).

The investigation of demographic processes should ideally be carried out using a measure that is not obscured by genetic processes e.g. R_{ST} (which explicitly takes mutation into account) and G_{ST} – although the practical suitability of these indices may be limited because no group of loci mutates entirely according to the SMM (for R_{ST}; Balloux & Lugon-Moulin 2002) and G_{ST} is dependent on the mutation rate and heterozygosity at particular loci (Balloux & Lugon-Moulin 2002; Ryman & Leimar 2009). It has been suggested that F_{ST} is a better index than G_{ST} when fewer than 20 loci are employed (Gaggiotti et al. 1999). Hedrick's (2005) standardised G'_{ST} is sensitive to similar factors to G_{ST} and, as Ryman & Leimar (2009) state: "there is currently no correction available that accounts for all effects of mutation on G_{ST}". The initial heterozygosities of populations influence the behaviour of both G_{ST} and D, and this effect is much greater for D, especially in the early stages of differentiation (Leng & Zhang 2011, 2013). When initial heterozygosity is low (<0.5), G_{ST} increases faster than D, and the opposite is true when initial heterozygosity is high (Leng & Zhang 2011, 2013).

require the pre-specification of populations. Individual genotype assignment tests were carried out in GENALEX v6.5 (Peakall & Smouse 2012), where the log-likelihood of each individual being assigned to its collection locality (either breeding region or breeding colony) is calculated based on that individual's multi-locus genotype (Moritz et al. 2000; Bouzat et al. 2009; Peakall & Smouse 2012). The assignment test in GENALEX is frequency-based (Paetkau et al. 2004) and assumes random mating within populations. The default "Leave one out" option was employed, which uses population allele frequency estimates based on all samples in a population except the one to be assigned to calculate the log likelihood of that sample being assigned to each population (Peakall & Smouse 2012).

Spatially Independent Analyses

Clustering methods

The term 'spatially independent' here refers to analyses that do not take sampling locality into account i.e. no populations are predefined in the analyses, and are often used to explore population structure where there aren't any clear barriers to gene-flow. Clustering algorithms generally attempt to group individuals into clusters in a way that minimises deviations from HWE and gametic equilibrium within groups (Guillot et al. 2005). The first, and most widely employed, clustering method was implemented in STRUCTURE (Pritchard et al. 2000; Jakobsson & Rosenberg 2007) and has been extended and modified in other software to broaden its utility for example, to allow the user to incorporate geographical sampling information in the priors (e.g., TESS (Chen et al. 2007); GENELAND (Guillot et al. 2005); BAPS (Corander et al. 2008)). The many advantages of clustering methods has led to their broad application in molecular ecology, however, a number of distinct disadvantages have been identified along the way. Initially, the computational requirements for the estimation of K (the number of genetic clusters) represented a significant barrier to the widespread implementation of clustering methods; however, advances in computing technology have largely overcome this problem. The interpretation of the biological significance of K is sometimes controversial (Evanno et al. 2005). One of the most notable drawbacks of clustering methods, especially in the context of this study, is that if population genetic structure is weak, or if genetic differentiation between populations is clinal (e.g. follows a pattern of isolation by distance (IBD)), clustering methods can produce misleading results (Rosenberg 2003; Morris-Pocock et al. 2012).

In studies that investigate population genetic structure, multi-locus genotypes are often employed to estimate membership coefficients (termed *Q*) of individuals to either specified or unspecified sub-populations (clusters). If sub-populations are not specified in advance, the estimation of membership coefficients is carried out simultaneously with that of allele frequencies (and other properties) of a number of abstract clusters (Rosenberg 2003). These clusters are constructed during the estimation procedure, and membership coefficients are assigned to individuals (Rosenberg 2003). Wild African Penguin genotype data were analysed in this way in STRUCTURE Version 2.3.1 (Pritchard et al. 2000) to investigate whether breeding colonies or regions represented spatially independent populations i.e. genetically cohesive populations. STRUCTURE implements a Bayesian algorithm using a model-based Markov chain Monte Carlo (MCMC) simulation with the goal of detecting the true number of genetically homogenous clusters (*K*) i.e. to delineate groups of genetically similar individuals based on their multi-locus genotype, without incorporating any prior information about their population identity (Evanno et al. 2005; Boessenkool et al. 2009b). The analysis detects clusters under the assumptions of HWE and linkage equilibrium within each cluster (Pritchard et al. 2000) and membership coefficients per individual per cluster (*Q*) are estimated, allowing probabilistic assignment of individuals to clusters. In this study, the STRUCTURE analysis was run 20 times for every value of *K* between one and 14 (two more than the total number of colonies sampled). The parameter settings specified the admixture model, with allele frequencies correlated among populations and location information was not given a priori (Pritchard et al. 2000). Also, each run consisted of 100 000 generations, with an additional 10 000 generations discarded as burnin. All other settings were left as default (alpha, lambda and F_{ST} priors). An otherwise identical analysis was run according to a model of no admixture. A final STRUCTURE analysis was run with 100 000 generations as burnin and 1 million generations of MCMC 20 times each for K=1 to K=4 with no admixture and locality data included as prior information (specified sub-populations, LOC PRIOR). The optimal number of clusters (*K*) for each analysis was selected using STRUCTUREHARVESTER (Earl & VonHoldt 2011) to compare the log-likelihood of the data given the number of clusters [ln P(X|K)] (Pritchard et al. 2000; Boessenkool et al. 2009b; Earl & VonHoldt 2011) and the standardised second order rate of change of ln P(X|K), or delta K (ΔK) (Evanno et al. 2005). Because correlated allele frequencies may lead to an overestimation of the number of clusters (Falush et al. 2007), these analyses were repeated using the independent allele frequency model with *lambda* set to 1.0. After the best value of K was determined, CLUMPP version 1.1.2 (Jakobsson & Rosenberg 2007) was used

to combine the results of each of the 20 replicates generated during each of the three analyses, into a final result for each. The "Full Search" option in CLUMPP was employed, with all other settings left as the default. The program DISTRUCT Version 1.1 (Rosenberg 2003) was used to visualise results from the CLUMPP analysis.

GENELAND v3.3.0 (Guillot et al. 2005, 2011) was used to analyse data under both correlated and uncorrelated allele frequency models using spatial parameters for K=1- 12. For each simulation, parameters were set to 10 independent runs with 500 000 MCMC iterations, thinning of 50, no filtering of null alleles, and the delta coordinate (representing the potential error for spatial coordinates) set at 0. All other parameters were set to default values. The 10 runs were post-processed with a burn-in of 100 iterations in order to obtain posterior probabilities of population membership for each individual and each pixel of the spatial domain (200 pixels along the X and Y axes). The consistency of the results across the 10 runs was checked visually.

Ordinations in Reduced Space

The relationships and genetic differentiation among populations were also investigated using Principal Coordinates Analyses (PCoA) and Factorial Correspondence Analyses (FCA) to the colony- and regional-level dataset (based on multi-locus genetic distances between individuals and relatedness estimates among individuals). Multivariate analyses (ordinations in reduced space) are useful for extracting information from genetic markers (Jombart et al. 2009), and do not require rigid assumptions about an underlying genetic model (e.g. the HWE or the absence of linkage disequilibrium). The main application of these methods is to summarise a strongly multivariate dataset into a small set of uncorrelated synthetic variables (Jombart et al. 2009) i.e. to provide a simplified, but meaningful, view of the genetic variability that exists in multi-dimensional space and is impossible to perceive without simplification. PCoA is often used to investigate population genetic structuring among genotypes or populations (Jombart et al. 2009) and can be employed to summarise the Euclidean genetic distance between genotypes or populations, but does not provide a representation of the alleles (Jombart et al. 2009). PCoA is implemented in GENALEX v6.5 (Peakall & Smouse 2012), and can be carried out on any Euclidean distance. In GENALEX, it allows for the investigation of the major patterns present in a multivariate molecular data set i.e. one comprised of many samples and multiple loci, by simplifying numerous multi-dimensional axes of variation into a few synthetic axes that reveal the majority of the separation among distinct groups. In GENALEX it is possible to choose between (a)

converting the genetic distance matrix to a covariance matrix prior to PCoA, and (b) working directly from the genetic distance matrix (Peakall & Smouse 2012). The option to standardise either of these two methods by dividing the respective distance or covariance matrices by the square root of n-1 was implemented for all analyses. The collection localities of samples are not taken into account during PCoA.

An alternative to PCoA of genetic distances is Correspondence Analysis (CA, Greenacre 1966), which can be used to analyse data based on allele counts in each population i.e. alleles are represented (Jombart et al. 2009). Factorial Correspondence Analysis (FCA) was carried out in GENETIX v4.05 (Belkhir et al. 2004). During FCA genetic information is transformed into a graphical display, where each row and column is depicted as a point i.e. individuals are visualised as points in a hyperspace that has as many dimensions as there are alleles for all loci. The GENETIX algorithm seeks the independent (orthogonal) directions in this hyperspace where the "inertia" of the cloud of points is maximised. These points have a centroid that represents the average genotype 'profile', and each profile point contributes to the inertia of the whole cloud. For genotype data, each individual is represented in the hyperspace by its score for each allele at each locus: 0 for missing data (absence), 1 for the presence of an allele in the heterozygous state and 2 for the allele in the homozygous state. The output of FCA in GENETIX represents correspondence between diploid genotypes, and is depicted graphically in 3D, where every point is an individual. The algorithm detects independent directions for each point's inertia multiplied by the square of the distance to the centre of all the co-ordinates. The distance and direction of an individual from the "centre of gravity" (or centroid, also 'origin') is determined by its multi-locus genotype 'profile', and as a result, individuals that are close together, will have similar genotype 'profiles'. When the "with populations" option is activated in GENETIX (Belkhir et al. 2004), the FCA algorithm calculates the 'centre of gravity' of individuals in each population, and tends to exaggerate differences between populations.

Isolation by Distance

The relationship between geographic and genetic distances were tested using Mantel matrix correlations of individuals in Alleles in Space v1.0 (Miller 2005) and GENALEX (Peakall & Smouse 2012). Over land, the straight-line geographic distance between Mercury Island, Namibia (the most north-western colony sampled) and Bird Island, Eastern Cape (the most south-western colony sampled) is 1400km, but it is about 1800km by sea (around Cape Point and Cape Agulhas, the southern tip of Africa). Since African Penguins must disperse in the

marine environment, the extra distance they must travel was taken into account. The data was also tested for a fit to Wright's Isolation by Distance (IBD) model (Wright 1943) using IBD web service Version 3.23 (Jensen et al. 2005) with 30 000 bootstrap replicates, using Slatkin's (Slatkin 1993) similarity index ($M = \frac{\frac{1}{F_{ST}} - 1}{4}$) and M_{max}=1000 (analysis repeated for M_{max}=400).

Phylogenetic relationships between populations and individuals

A phylogenetic tree of individuals was produced in POPULATIONS v1.2.31 (Langella 2001) for comparison with previous work based on mitochondrial sequence data (see previous chapter). Phylogenetic trees based on the allele frequencies present in all colonies were also generated in POPTREE2 (Takezaki et al. 2010) and POPULATIONS. Different models for genetic distances were used to produce phylogenetic trees, which were visualised in TREEVIEW v1.6.6 (Page 2001). F_{ST} values and Nei et al.'s (1983) genetic distance (DA) values were calculated across all 12 loci and used to produce unrooted phylogenies, as suggested by (Takezaki & Nei 1996, 2008). Delta μ^2 (Goldstein et al. 1995b) and Cavalli-Sforza and Edward's chord distance (D_{CE}, Cavalli-Sforza & Edwards 1967) were also used to investigate the evolutionary relationships among colonies. Delta μ^2 is appropriate for use with microsatellites, in that it assumes the SMM and takes into account the size differences among alleles (allele lengths). In contrast, D_{CE} and D_A do not assume any mutation model, but are rather based on the sum of the products of allele frequencies shared between samples (Goldstein & Pollock 1997; Takezaki & Nei 2008). POPTREE2 analyses were based on 10 000 bootstrap replicates, and the distance options "Da," "Dst," and "Dmyu," correspond to DA, DST (sample size bias corrected, Nei 1978) and Delta μ^2 (Goldstein et al. 1995b). A visual representation of the various microsatellite analyses employed is shown in Figure 4.2.

Detecting Genetic Bottlenecks

Two statistical approaches were employed to test for the expected signatures of genetic bottlenecks in African Penguin populations: the first is implemented in the program BOTTLENECK v1.2.02 (Cornuet & Luikart 1996) and the second is the M-RATIO test of Garza and Williamson (2001). The former analysis is based on the theoretical prediction that a population bottleneck results in a faster reduction in allelic diversity than heterozygosity, and this in turn generates an excess of heterozygotes in the post-bottleneck population (Luikart et al. 1998; Williamson-Natesan 2005), compared to what would be expected in a

population at mutation-drift equilibrium. BOTTLENECK tests the significance (based on 1000 permutations) of results under three mutation models (the Two-phase Mutation Model (TPM), the Infinite Alleles Model (IAM), and Stepwise Mutation Model (SMM)), and using four statistical tests (the Sign Test, Standard Differences Test, Wilcoxon Test and Mode-Shift Test). For each population and for each locus BOTTLENECK calculates the expected heterozygosity (H_E) based on the observed number of alleles (k), and the sample size (n), assuming mutation-drift equilibrium (Luikart et al. 1998). The average H_E is compared to the observed heterozygosity (H_O) to establish if there is heterozygosity excess or deficit at each locus (Cornuet & Luikart 1996). For the Mode-Shift or L-shaped test, all the loci are pooled for each population, and alleles are binned by frequency into 10 allele frequency classes to produce an allele frequency distribution. If fewer alleles are found in the rare frequency category than any other category (i.e. the distribution is not approximately L-shaped), then this test ''detects'' a bottleneck (Luikart et al. 1998; Williamson-Natesan 2005). An L-shaped allele frequency distribution is expected under mutation-drift equilibrium and recent bottlenecks will provoke a mode shift.

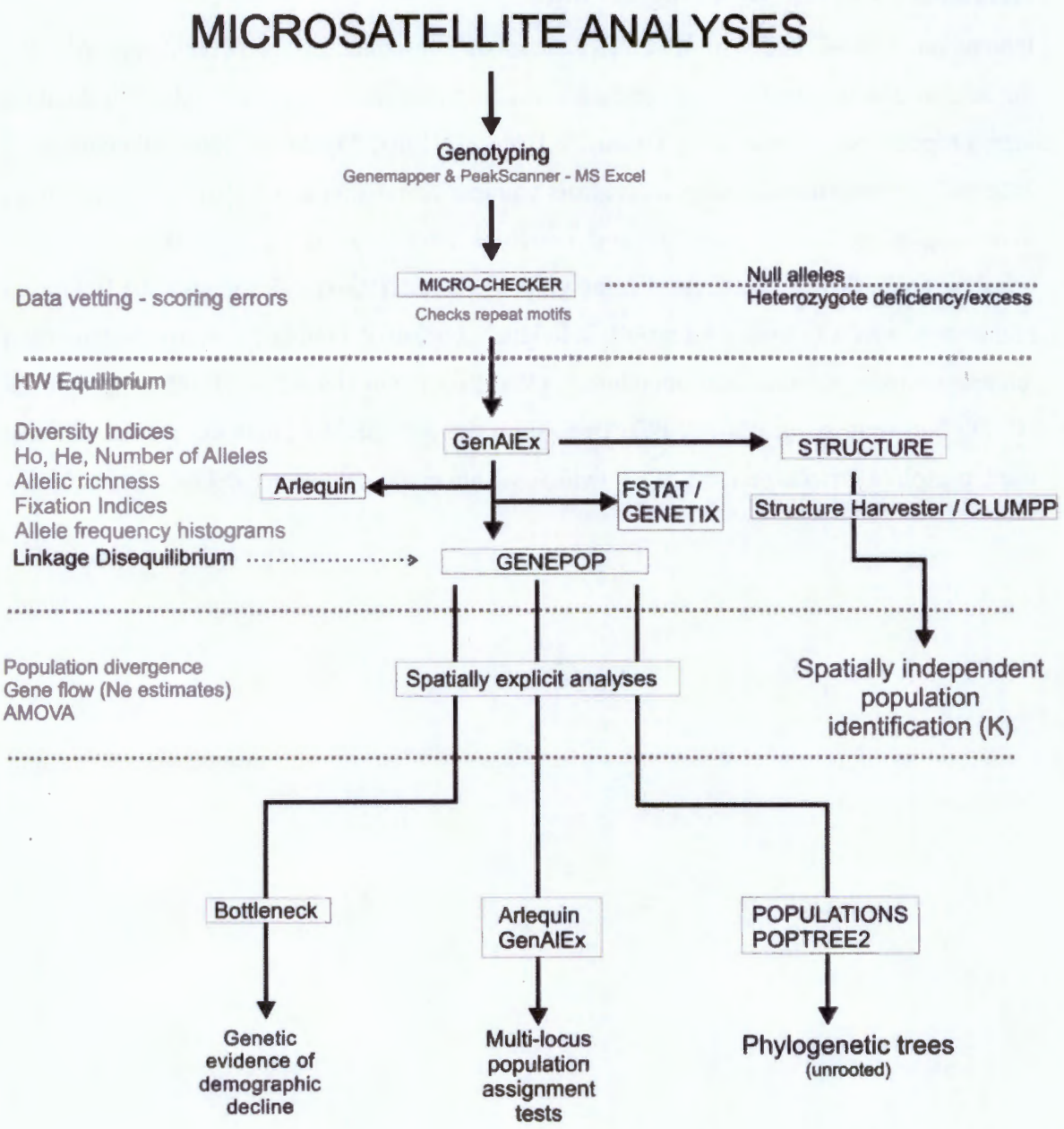
During a genetic bottleneck, the number of alleles is expected to decrease more rapidly than the range in allele sizes, because most of the alleles that are lost by chance will be of intermediate length (Garza & Williamson 2001; Peery et al. 2012). The M-Ratio test was performed to determine whether the African Penguin population declines have resulted in this type of genetic signature (Garza & Williamson 2001). The method calculates the ratio of the total number of alleles to the range in allele sizes (M, using the program M_P_VAL), and the critical value of M (M_C, calculated using CRITICAL_M, Garza & Williamson 2001). M is calculated as $M = \frac{k}{r}$ where k is the number of alleles, and r is the overall range in allele sizes (largest allele minus smallest allele+1). In theory, a declining population will have a smaller M-ratio than a stable population, because k will decrease faster than r in small populations due to genetic drift causing loss of rare alleles (Spear et al. 2006), and only the loss of the smallest or largest allele will lead to a reduction in r. To test for a bottleneck, an expected distribution for M under equilibrium conditions is generated using simulations, and critical value (M_C) is at the lower 95th percentile of that distribution. A bottleneck is ''detected'' whenever a sample or test value of M is lower than this critical value i.e. when the observed average M-ratio is lower than M_C (Garza & Williamson 2001; Busch et al. 2007; Brooke et al. 2011). For the analysis of 189 African Penguins, Θ was set to 10, the average size of non-

1-step mutations is 3.5bp and 90 % of mutations are single step, as suggested by Garza & Williamson (2001).

Relatedness within breeding colonies

Inbreeding is associated with multiple deleterious consequences for populations, including the loss of genetic diversity, and populations comprised of more closely related individuals face a bigger risk of inbreeding (Amos & Harwood 1998; Frankham 2003; Oliehoek et al. 2006). Close relatedness among individuals within a population could also be a consequence of demographic history, isolation and natal-site fidelity in seabirds. Relatedness within breeding colonies was investigated using GENALEX 6.5 (Peakall & Smouse 2012). Genetic relatedness was estimated among all individual African Penguins i.e. individual pairwise relatedness using Queller and Goodnight's (1989) estimator (based on 10 000 permutations, 10 000 bootstrap replicates). A PCoA based on the individual relatedness matrix was then used to explore general patterns of relatedness within breeding regions and breeding colonies.

Figure 4.2 Software programs employed for various microsatellite analyses.



RESULTS

All 12 loci were polymorphic in the African Penguins sampled in this study, except locus PNN05, which was monomorphic at five colonies and locus B3-2, which was monomorphic among Possession Island individuals (Appendix 4.4).

Null alleles and HWE exact tests: Heterozygote deficit and excess per locus

No evidence of scoring error due to stuttering, large allele dropout or of null alleles were detected by MICROCHECKER in any loci among individuals from the Eastern Cape, however, locus B3-2 showed evidence of heterozygote deficit ($P<0.01$, only among samples from the Western Cape, where three private alleles are found for this locus) and three loci in Namibia (PNN09, G2-2 and Sh1Ca9, all $P<0.05$) showed evidence of null alleles (estimation of exact P-values by the Markov chain method). Locus G2-2 in the Western Cape was the only locus to show significant ($P=0.04$) evidence of heterozygote excess. These results were reflected in the global HW exact test for heterozygote deficiency, which was significant overall (all loci in all breeding regions, $P=0.009$). At a regional-level, heterozygote deficiency was significant only in Namibia ($P=0.009$). When populations were defined as breeding colonies, the HW exact test for heterozygote deficiency was again significant overall (all loci in all colonies, $P=0.024$). This pattern was driven primarily by significant heterozygote deficiency in three colonies: Halifax Island (Namibia, $P=0.03$), Mercury Island (Namibia, $P=0.005$) and Dassen Island (Western Cape, $P=0.002$).

Linkage disequilibrium

When testing for linkage disequilibrium (LD) among all 12 loci in all three regions, significant correlations ($P<0.05$) were found between 14 pairs of loci (Appendix 4.5; of a total of 198 possible combinations: 66 per region). At the colony-level, 21 out of 792 locus pairs (66 per colony, 12 colonies) were significantly correlated ($P<0.05$). There was no locus pair that showed significant linkage in more than one region. At the colony-level, two pairs of loci showed significant linkage in two (out of 12) colonies (SH1CA9 and SH2CA21 in Bird and St Croix Islands in the Eastern Cape; PNN01 and PNN12 at Dassen Island in the Western Cape and Bird Island in the Eastern Cape). If these loci were linked, the pattern would be consistent across populations, which it is not, and all loci are assumed therefore in LE (Appendix 4.5).

Genetic diversity within breeding regions and breeding colonies

Private Alleles

Globally, the number of alleles detected at each locus ranged from two to 17 (Appendix 4.6). When analysed at the regional-level (populations defined as breeding regions), all three breeding regions exhibited some private alleles i.e alleles only found in that region (Table 4.1). Fifteen African Penguins exhibited one or more of these private alleles (alleles found only in their breeding region, Table 4.1). Three birds from the Eastern Cape (all from St Croix Island) exhibited alleles that were not found in other breeding region, as did two birds from Namibia and 10 birds from various colonies in the Western Cape. All regional-level private alleles were at a low frequency (≤ 0.025, Table 4.1).

At the colony-level, eight samples exhibited private alleles at various loci i.e. exhibited unique alleles that were not present in any other colony. All private alleles were at a colony frequency of ≤ 0.02 (Table 4.2). The colony-level analyses revealed that all private alleles among the Eastern Cape samples were from St Croix Island (Table 4.2) and, similarly, all those from the Western Cape were from Dassen Island. Halifax and Ichaboe Islands in Namibia also exhibited private alleles (Table 4.2). The mean sample size per colony was 15.2 individuals and the mean frequency of private alleles per colony was 0.02. The mean number of migrants (after correction for sample sizes) based on private alleles was 17.9 (GENEPOP, see Barton & Slatkin, 1986).

Table 4.1 Private alleles exhibited by African Penguins from the three breeding regions (populations defined as breeding regions). The length of the private allele, the locus at which it is exhibited and its frequency in the source region, are all indicated.

Breeding Region	Locus	Allele length	Frequency in region
Eastern Cape	PNN03	380	0.009
	PNN06	310	0.009
	PNN06	316	0.009
Namibia	G2-2	386	0.010
	G3-6	279	0.010
Western Cape	SH1CA9	141	0.013
	SH1CA9	142	0.013
	B3-2	297	0.006
	B3-2	307	0.025
	B3-2	309	0.006
	PNN09	400	0.006

Table 4.2 Private alleles exhibited by African Penguins when populations are defined as breeding colonies. The region in which each colony is found is given in brackets (Namibia – NAM, Western Cape – WC, Eastern Cape – EC). The length of each private allele, the locus at which it is found, and its colony-level frequency are also shown.

Colony (Region)	Locus	Allele length	Frequency in colony
St Croix Island (EC)	PNN03	380	0.016
St Croix Island (EC)	PNN06	310	0.016
St Croix Island (EC)	PNN06	316	0.016
Dassen Island (WC)	B3-2	297	0.020
Dassen Island (WC)	B3-2	309	0.020
Dassen Island (WC)	PNN09	400	0.020
Halifax Island (NAM)	G2-2	386	0.038
Ichaboe Island (NAM)	G3-6	279	0.031
Halifax Island (NAM)	G2-2	386	0.038
Ichaboe Island (NAM)	G3-6	279	0.031

Deviations from HWE

Tests for deviations from HWE were performed for each individual locus in each population (first defined as breeding region, then as breeding colony), and across loci for each population. Regional tests for deviations from HWE revealed that all loci conformed to HW expectations in the Eastern Cape, except locus G3-6. Two loci (PNN03 and PNN09) in Namibia were significantly ($P<0.05$) different from HWE expected values (GENEPOP analyses detected that an additional two loci also deviated from HWE: Sh1Ca9 and PNN12). Three loci deviated significantly ($p<0.05$) from HWE among the Western Cape samples (PNN03, B3-2 and G3-6). Overall, at the regional scale five out of 36 tests (12 loci in three populations) showed significant deviations from Hardy-Weinberg expectations ($P<0.05$), with the Western Cape showing the strongest deviations. The Western Cape and Namibia do not conform to HWE expectations ($P=0.02$ and $P=0.02$ respectively), which causes the overall dataset (all samples from all three regions) to deviate significantly from expectation ($P=0.01$). In Namibia, and the Western Cape to a lesser extent, this seems to be due to heterozygote deficit ($P=0.001$ and $P=0.06$ respectively). Three loci (PNN09, Sh1Ca9 in Namibia and B3-2 in the Western Cape) show evidence of heterozygote deficit.

Colony-level tests for deviations from HWE were carried out and revealed that eight out of 144 tests (12 loci in 12 colonies) showed significant deviations from HW expectations (additionally, loci were monomorphic in a colony in six of 144 tests). No loci deviated from

HWE among samples from Bird Island and St Croix Islands in the Eastern Cape. Similarly, no loci deviated from HWE expectation at Boulders Beach, Dyer Island and Robben Island in the Western Cape. PNN05 was monomorphic at Jutten Island and Stony Point, but all other loci in these Western Cape colonies conform to HWE expectation. At Dassen Island in the Western Cape, three loci significantly deviate from HWE expectations (Sh2Ca21, B3-2 and PNN05). Among samples from Namibian colonies, three loci at Mercury Island deviated significantly from HWE (PNN03 and PNN06, $P<0.01$; PNN08, $P<0.001$). B3-2 and PNN05 were monomorphic at Possession Island, but all other loci do not deviate from HWE. Similarly, at Halifax and Ichaboe Islands in Namibia, PNN05 was monomorphic. PNN09 differed significantly from HWE at Halifax Island ($P<0.01$), as did Sh2Ca21 at Ichaboe Island ($P<0.001$). Deviations from HWE could be driven by heterozygote deficiency, which was investigated for each colony-locus pair (GENEPOP option 1, sub-option 1). Significant heterozygote deficiency was detected at three loci among Dassen Island samples (SH1CA9, $P=0.03$; B3-2, $P=0.0003$; PNN05, $P=0.02$), at two loci among Halifax Island (PNN09, $P=0.002$; G2-2, $P=0.045$) and Mercury Island (PNN09, $P=0.04$; PNN06, $P=0.03$) samples and at one locus each among Stony Point (PNN06, $P=0.049$), St Croix Island (PNN01, $P=0.04$) and Ichaboe Island (SH1CA9, $P=0.0253$) samples. Only three colonies showed evidence of heterozygote excess, and only at one locus each: Stony Point (G2-2, $P=0.025$), St Croix Island (SH1CA9, $P=0.02$) and Dyer Island (G3-6, $P=0.04$). No colonies in Namibia showed evidence of heterozygote excess. There was, however, some evidence indicating a role for null alleles, especially among Namibian colonies (Appendix 4.7).

Heterozygosity, allelic richness and F_{IS}

Mean observed heterozygosities for each locus across all samples (at the regional and colony-level) were generally high ($H_O>0.5$), although two loci exhibited much lower heterozygosity than the other ten loci (PNN05 and B3-2; Appendix 4.8). Interestingly, the suite of published primers employed in this study exhibited higher overall levels of observed heterozygosity (average $H_O=0.632$) than the markers developed specifically for African Penguins (average $H_O=0.57$). At the regional scale, the mean number of alleles per locus ranged from two to 14.3, and some loci showed much higher total heterozygosity (H_T) than others (Table 4.3). These patterns are reflected in the F-statistics and in the colony-level analyses (Appendix 4.9).

Table 4.3 Regional-level sample sizes (N), number of alleles (N_a), number of effective alleles (N_{Ae}), corrected number of effective alleles (cN_{Ae}), observed heterozygosity (H_O), expected heterozygosity in sub-populations (regions, H_S), expected heterozygosity in the total population (H_T), unbiased estimators of H_S and H_T (corrected; cH_S and cH_T; calculated giving equal weights to all populations, independent of real population sizes or sample sizes) and the maximum possible value of G_{ST} (G_{STmax}), given these corrected values of diversity, for all 12 loci and for the total dataset. Note that an overall value of ~0.3 would indicate complete regional population divergence. Standard errors (SE) are estimated by jack-knifing over loci.

Locus	N	N_a	N_{Ae}	cN_{Ae}	H_O	H_S	H_T	G_{STmax}
G2-2	189	6	3.35	3.34	0.73	0.70	0.71	0.22
SH1CA9	184	14.3	6.82	6.59	0.86	0.85	0.86	0.10
SH2CA21	188	10	3.38	3.37	0.70	0.70	0.71	0.21
B3-2	189	4	1.27	1.27	0.19	0.21	0.21	0.71
G3-6	184	7.3	3.33	3.32	0.68	0.70	0.70	0.22
PNN01	186	4	3.12	3.12	0.67	0.68	0.69	0.24
PNN03	186	8.3	2.49	2.49	0.58	0.60	0.60	0.31
PNN06	184	4.7	3.32	3.31	0.69	0.70	0.71	0.22
PNN08	186	5.7	3.42	3.42	0.71	0.71	0.71	0.21
PNN09	186	6.3	3.65	3.65	0.68	0.73	0.73	0.20
PNN12	137	5	2.73	2.73	0.60	0.63	0.64	0.27
PNN05	189	2	1.08	1.08	0.07	0.07	0.07	0.90
Mean	182.3	6.47	3.16	3.14	0.60	0.61	0.61	0.30
Standard Error		0.94	0.41	0.39	0.07	0.07	0.07	0.06

Across all loci, the Western Cape exhibited the highest number of alleles (Na=83, n=79), followed by the Eastern Cape (Na=77, n=58), and then Namibia (Na=73, n=52). Allelic richness for each locus in each breeding region was lowest for locus PNN05 (~1.8 in Namibia, Appendix 4.10 and Figure 4.3) and highest for locus Sh1Ca9 in the Western Cape (~13.9). Allelic richness is notably higher in the Western Cape at three loci (Ah1Ca9, Sh2Ca21 and B3-2). The Eastern Cape exhibits elevated allelic richness at locus PNN06 (Figure 4.4).

At the regional scale, the Western Cape exhibited the highest mean number of alleles per locus (Table 4.4, Na=6.9), although this pattern did not hold for allelic richness, which is standardised for variation in sample size. Mean allelic richness was ~2.4 for all three breeding regions. A similar pattern was present for the colony-level results (Table 4.4, Figure 4.4), for which Dassen Island, St Croix Island and Mercury Island showed the highest mean number of alleles per locus, but allelic richness showed little variation among colonies (although Dassen Island still exhibits the highest allelic richness).

Figure 4.3 Allelic richness calculated per locus in each breeding region of African Penguins.

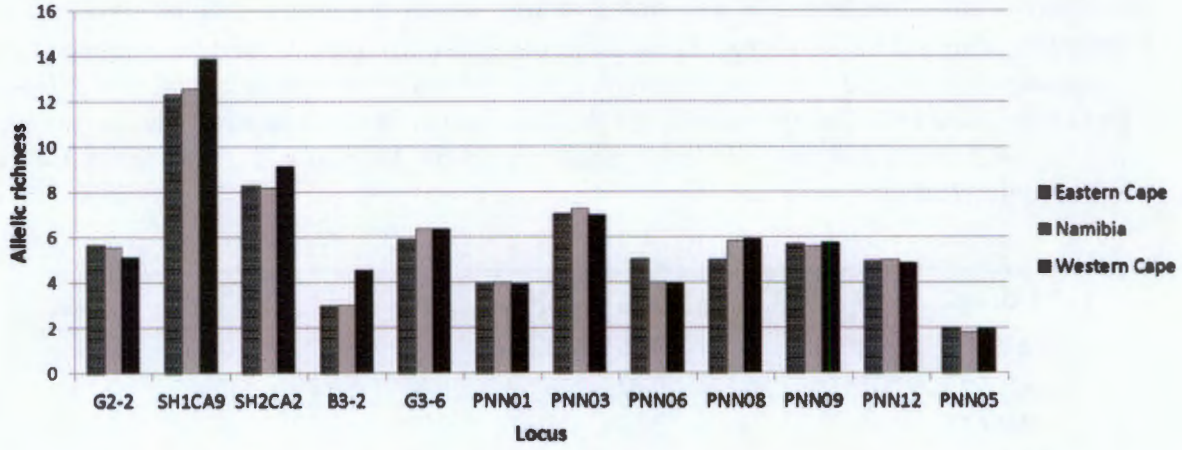
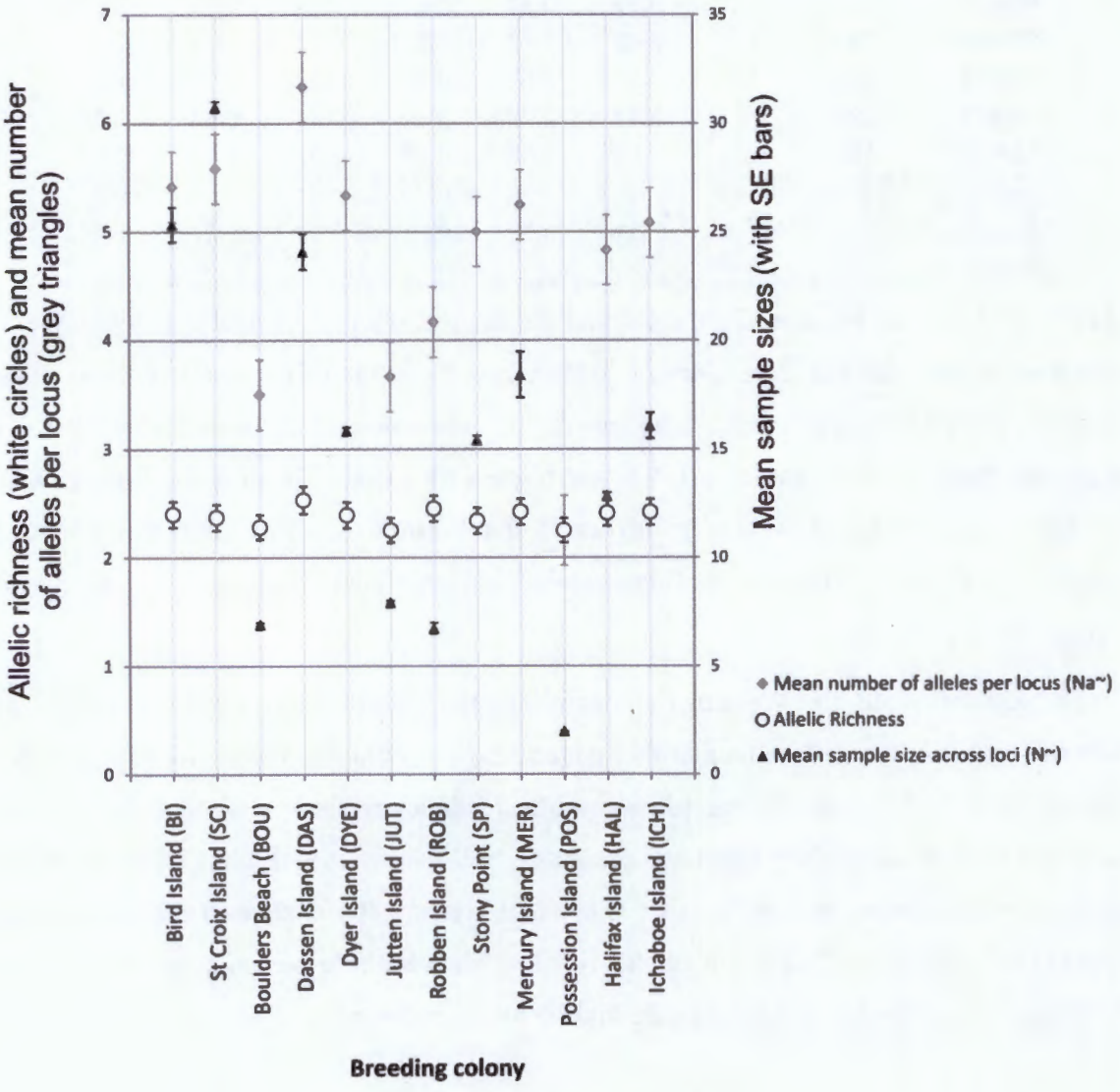


Figure 4.4 Colony-level allelic richness and mean number of alleles per locus (Na) on the left-hand side axis, showing the co-variance of the latter and sample size (right-hand side axis; bars are SE).



Mean observed heterozygosity across all loci was lowest in Namibia (H_O=0.56) and almost identical in the two breeding regions in South Africa (H_O=0.614 and H_O=0.613 in the Eastern Cape and Western Cape respectively). The colony-level results show that most colonies in the Western Cape and all colonies in the Eastern Cape exhibited higher than expected heterozygosities, but only two of the four Namibian colonies showed this pattern. H_O ranged between ~0.5 at Mercury Island (Namibia) and ~0.65 at Robben Island (Western Cape), whereas H_E ranged between 0.43 at Possession Island (Namibia) and 0.64 at Dassen Island in the Western Cape (Figure 4.4, Table 4.4).

Fixation indices, or F-statistics (Wright 1943; Meirmans & Hedrick 2011) are used to investigate genetic structure by considering how genetic variation (expected and observed heterozygosity) is partitioned across the hierarchical components that make up populations. F_{IS} describes the observed proportion of variation (heterozygosity) within individuals relative to what is expected in the sub-population under HWE. F_{IS} (the inbreeding coefficient) measures the deviation of genotype frequencies from what would be expected under panmixia i.e. the divergence between observed heterozygosity to expected heterozygosity (Lowe & Allendorf 2010; Meirmans & Hedrick 2011). F_{IS} values range from –1 to +1, where a negative value indicates heterozygote excess relative to HW expectation (panmixia) and a positive value describes heterozygote deficiency (Durrant et al. 2009). F_{IS} values were calculated for each breeding colony and region to investigate deviations from HWE that might be a result of inbreeding.

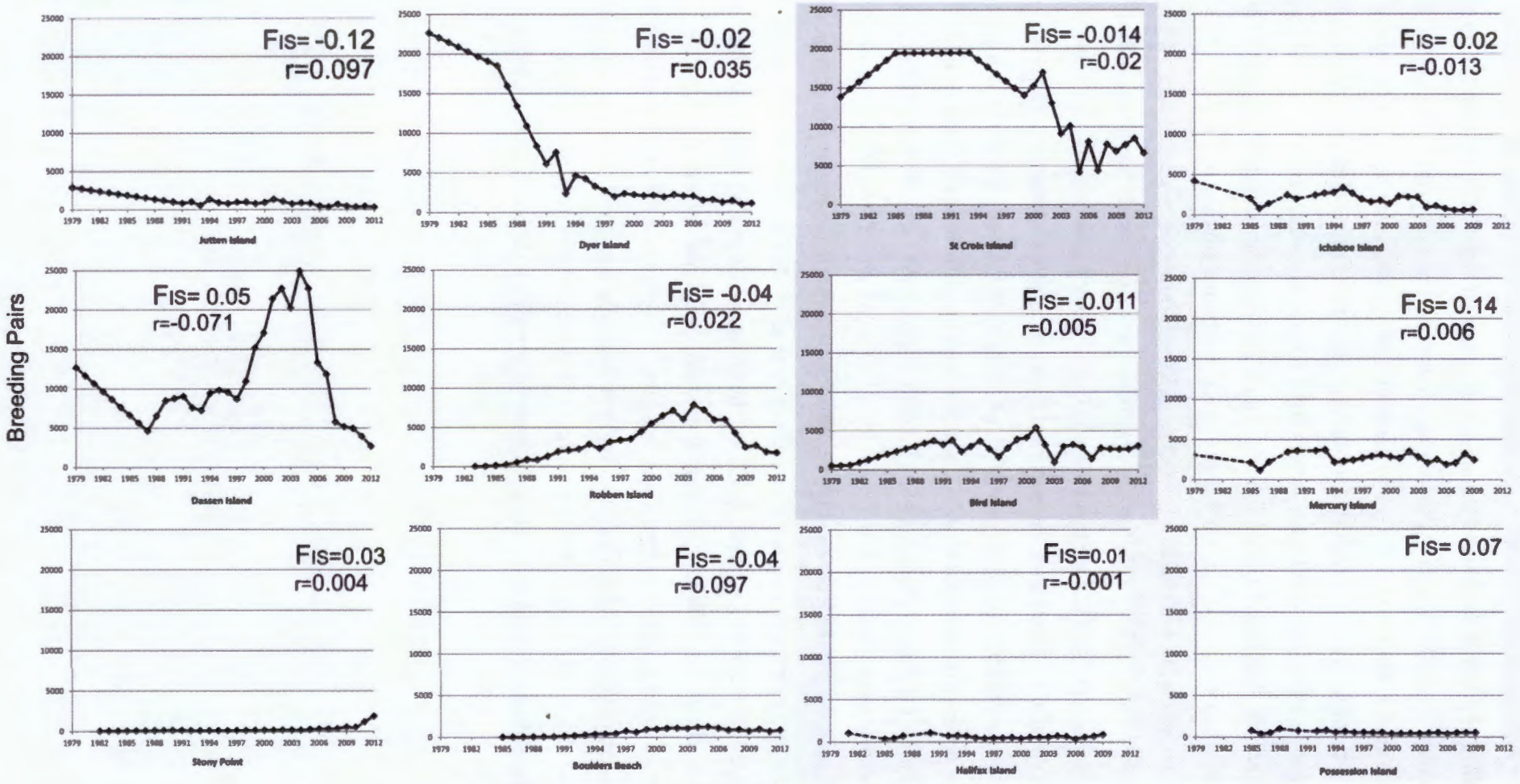
Some clear trends were evident, but overall, F_{IS} values were low i.e expected and observed heterozygosities were similar overall. At the colony-level, all Namibian colonies exhibited positive F_{IS} values, and both colonies in the Eastern Cape exhibited negative values (Table 4.4). Among colonies from the Western Cape, F_{IS} values were mixed, with Dassen Island and Stony Point exhibiting positive values, and all other colonies exhibiting negative values. Mercury and Halifax Islands in Namibia showed the strongest signal of inbreeding. A similar metric, R_{IS}, based on allele size rather than allele identity, showed a different pattern to F_{IS} (Appendix 4.11).

Table 4.4 Genetic diversity estimates for the three geographically disjunct breeding regions of African Penguins, and for the colonies within those regions. F_{IS}-values marked with an asterisk are significant ($P<0.05$; 10 000 bootstrap replicates).

Region	Colony	Sample Size (N)	Mean sample size across loci (N~)	Mean number of alleles per locus (Na~)	Allelic Richness	Number of Private Alleles	Mean H_O across all loci	Mean H_E across all loci	F_{IS}
Eastern Cape		**58**	**56.1**	**6.417**	**2.38**	**3**	**0.61**	**0.60**	**-0.02***
	Bird Island (BI)	27	25.3	5.417	2.4	0	0.62	0.60	-0.01*
	St Croix Island (SC)	31	30.8	5.583	2.37	3	0.61	0.59	-0.01*
Western Cape		**79**	**76.9**	**6.917**	**2.37**	**6**	**0.61**	**0.61**	**0.03***
	Boulders Beach (BOU)	7	6.9	3.5	2.28	0	0.60	0.53	-0.04*
	Dassen Island (DAS)	25	24.1	6.333	2.53	3	0.62	0.64	0.05*
	Dyer Island (DYE)	16	15.8	5.333	2.39	0	0.62	0.59	-0.02*
	Jutten Island (JUT)	8	7.9	3.667	2.25	0	0.63	0.53	-0.12*
	Robben Island (ROB)	7	6.8	4.167	2.45	0	0.65	0.58	-0.04
	Stony Point (SP)	16	15.4	5	2.34	0	0.58	0.58	0.03*
Namibia		**52**	**49.3**	**6.083**	**2.375**	**2**	**0.56**	**0.61**	**0.06***
	Mercury Island (MER)	20	18.4	5.250	2.42	0	0.53	0.60	0.14*
	Possession Island (POS)	2	2.0	2.250	2.25	0	0.54	0.43	0.07
	Halifax Island (HAL)	13	12.8	4.833	2.41	2	0.55	0.58	0.1*
	Ichaboe Island (ICH)	17	16.1	5.083	2.42	2	0.60	0.59	0.02*

African Penguin population trends at each colony (Figure 4.5) based on census data collected annually by the South African Department of Environmental Affairs show that penguin colonies exhibit very different patterns in their recent (since the 1970s) demographic history. Three of the colonies included in the present study were founded (or re-colonised after >100 years) in the 1980s, whereas others have been consistently decreasing for decades (c.f. e.g. Boulders Beach and Dyer Island in Figure 4.5). The demographic history of populations can have a profound effect on heterozygosity because inbreeding is likely to occur in small populations, and the effects of genetic drift will be stronger when effective population size is small. Those colonies that exhibit positive F_{IS} values i.e. all Namibian colonies, Stony Point and Dassen Island, appear to have different demographic histories (Table 4.4, Figure 4.5, Appendix 4.12). Neither the historical population size (Appendix 4.13), nor the proportion of each population (colony) sampled (Appendix 4.14) are correlated with colony-level F_{IS} estimates (data not shown). F_{IS} and F_{IT} were significant and positive overall (Figure 4.6). Across the whole dataset (n=189), with populations defined as breeding regions, fixation indices were generally low (F_{IS}=0.026, F_{IT}=0.033 and F_{ST}=0.007). The positive, significant F_{IS} and F_{IT} values indicate a small, possibly negligible degree of inbreeding, and F_{ST} is close to zero, indicating panmixia at the regional-level. Mean F_{IS} values for each region were -0.024 in the Eastern Cape, 0.032 in the Western Cape and 0.056 in Namibia (Table 4.4), indicating that Namibia shows the strongest signal of local inbreeding (heterozygote deficiency) and the Eastern Cape exhibits the weakest. Analogous estimates based on allele lengths in the overall dataset at a regional scale showed similar results (R_{IS}=0.029, R_{IT}=0.028 and R_{ST} =-0.0004). The raw allele frequency data are presented as histograms with populations defined as breeding regions (Appendix 4.14) and colonies (Appendix 4.15).

Figure 4.5 Population trends (estimated number of breeding pairs) at the 12 African Penguin colonies considered in this study. Namibian colonies have a hashed background, Eastern Cape colonies, grey, and Western Cape, white. The maximum value on all Y-axes is 25 000 pairs. Data is from the South African Department of Environmental Affairs, Oceans and Coasts Division. F_{IS} and Relatedness (Queller and Goodnight (1989) estimator of relatedness, r) is shown for each colony.



Population Structure: Genetic connectivity among breeding regions and colonies

F-statistics, including F_{ST}, are based on allele frequency data, which is most strongly influenced by genetic drift and dispersal, whereas R_{ST} is based on differences in allele length, which come about due to mutation, and subsequent genetic drift. F_{ST} is considered more sensitive to very recent, or contemporary, demographic changes because allele frequencies can vary rapidly in response to such factors. The micro-evolutionary forces that influence allele length (mutation, which generates changes, and drift eliminates novel alleles) should represent slightly older patterns, as these processes take longer to affect allele length in populations. Conventionally, for bi-allelic markers, F_{ST} values <0.05 are interpreted as indicating negligible population divergence, and F_{ST} values >0.25 represent strong differentiation among populations (Nei & Chesser 1983; Meirmans & Hedrick 2011; Whitlock 2011). F_{ST} can be calculated for different loci or averaged over many loci and can be reported for pre-defined populations, or averaged across all populations in a given dataset.

When populations were defined as breeding colonies as opposed to regions, the overall fixation indices changed (F_{IS} =-0.03, F_{IT}=0.02 and F_{ST}=0.05), with a notable increase in the F_{ST} value. This could indicate the Wahlund effect. Deviations from HWE due to an excess of homozygotes (heterozygote deficit), may be the result of a number of factors, including non-random mating, selection, null alleles, inbreeding and population structure (Avise 2004; Allendorf & Luikart 2007). Overlooked population structure in a dataset can lead to reduced heterozygosity relative to HW expectation; i.e. the Wahlund effect, which may result from the pooling of discrete subpopulations, with different allele frequencies, that do not in actuality interbreed as a single panmictic population. Finer scale colony substructure was impossible to investigate, as nest localities were not accurately recorded at breeding colonies.

Regional population connectivity

Spatially explicit analyses

In weakly structured populations, a number of studies have found F_{ST} to be a more accurate estimate of population differentiation than R_{ST}, especially when the number of loci is limited and sample sizes are small (Barlow et al. 2011). In some cases, however, R_{ST} is superior to F_{ST} for example, when the loci analysed predominantly follow the SMM (Balloux & Lugon-Moulin 2002). Based on pairwise population F_{ST} values and Nei's unbiased genetic distance (Table 4.5), there is very little evidence for population genetic structure at the regional scale

in African Penguins across their range. When populations were defined as breeding regions overall F_{ST} was 0.007±0.001, and overall G_{ST} was 0.007 (calculated as G_{ST}=1- H_S/H_T, with multi-locus H_S=0.606 and H_T=0.611) before applying Nei and Chesser's (1983) correction. After correction (multi-locus H_{SNC}=0.612 and H_{TNC}=0.613) G_{STNC} was 0.001. Overall pairwise regional-scale F-statistics were also low, with Weir and Cockerham's estimate F_{STwc}=0.002 (Weir & Cockerham 1984) and Robertson and Hill's estimate RH=0.0017 (both P<0.05, estimated from 1000 bootstrap replicates). Weir and Cockerham's (1984) pairwise F_{ST} was lowest between the Eastern Cape and Namibia (F_{STwc}=0.0006), and was similar between Namibia and the Western Cape, and the Eastern Cape and Western Cape (F_{STwc}=0.00319 and 0.0028 respectively, based on 10 000 bootstrap replicates). Overall F_{ST} and F'_{ST} calculated using AMOVA were low, but significant (Figure 4.6). Interestingly, analogous R_{ST} estimates indicated higher regional population differentiation (3% among regions, Figure 4.6), suggesting slight differences in genetic composition among regions. Regional-scale measures of population differentiation per locus, and overall, are presented in Appendix 4.17 with their associated probabilities. The maximum possible value for G_{ST} (G_{STmax}) was 0.3 overall, because the loci employed exhibited high variation in their number of alleles and in their mean heterozygosities (~0.07 to 0.86, Appendix 4.18).

The overall results were also reflected in the regional pairwise F_{ST}- and R_{ST}-values estimated using AMOVA (Tables 4.6 and 4.7): Low, but significant differentiation was detected between the Western Cape and Namibia based on F_{ST}, and Namibia was significantly differentiated from both South African breeding regions based on R_{ST} (Tables 4.6 and 4.7). Regional pairwise G_{ST} values were low and not significant (zero between Namibia and the Eastern Cape (P=0.4), 0.001 between the Eastern Cape and Western Cape and 0.002 between Namibia and the Western Cape (both P=0.07)). An almost identical pattern was observed for regional pairwise Nei's G'_{ST} (Table 4.8) and Hedrick's G''_{ST} (Appendix 4.19), but not for Hedrick's G'_{ST}, which was low, but significant for all regional pairwise comparisons (G'_{STH}=0.001 between the Eastern Cape and Namibia, 0.006 between the Eastern Cape and Western Cape, and 0.007 between Namibia and the Western Cape; all P=0.002; Table 4.8). D_{EST}, an unbiased estimator of divergence (Jost 2008) was also calculated, as estimates of F_{ST} and its analogues can be misleading when populations with different levels of genetic diversity are compared (Barlow et al. 2011). Regional pairwise differences in Jost's D_{EST} were low and not significant (all $\leq$ 0.005, P>0.072) among African Penguins sampled (Appendix 4.16).

Regional F'_{ST} is smaller than 0.01 in all pairwise comparisons and is significant ($P<0.05$) between Namibia and both the Western and Eastern Cape populations (Table 4.8). AMOVA results further show that 0% of variation is among regions, and 8% among individuals. AMOVA-based R_{ST} estimates revealed a different pattern, with 2% of the variation explained among populations, and 97% among individuals (Figure 4.6).

Table 4.5 Pairwise population F_{ST} values (below the diagonal; p-values in brackets) and Nei's unbiased genetic distance (above the diagonal), with populations defined as breeding regions.

Region	EC	NAM	WC
Eastern Cape (EC)	-	0.001	0.004
Namibia (NAM)	0.005 (0.36)	-	0.006
Western Cape (WC)	0.005 (0.08)	0.006 (0.08)	-

Table 4.6 Pairwise population F_{ST} estimated from an AMOVA (below the diagonal) with populations defined as breeding regions. Probability values (above the diagonal) based on permutations (1000 permutations; 10 000 pairwise population permutations).

	EC	NAM	WC
Eastern Cape (EC)	-	0.069	0.134
Namibia (NAM)	0.004	-	**0.006**
Western Cape (WC)	0.002	**0.007***	-

Table 4.7 Regional pairwise R_{ST} values estimated during AMOVA. R_{ST} values are below the diagonal and probabilities above the diagonal (based on 9 999 permutations and 9 999 pairwise population permutations (three breeding regions, 189 individuals).

(a)	EC	NAM	WC
Eastern Cape (EC)	-	**0.006**	0.377
Namibia (NAM)	0.029*	-	**0.002**
Western Cape (WC)	-0.010	0.044*	-

Figure 4.6 F_{ST}, R_{ST} and related statistics estimated from regional-level (populations defined as breeding regions, n=3), colony-level (populations defined as breeding colonies, n=12), and nested AMOVA (colonies assigned to breeding regions). For each of these categories, the analysis was run with and without the "within individual analysis" suppressed. Probabilities reflect the proportion of 9 999 permutations of the data and 9 999 pairwise population permutations (randomised) produced a statistic greater than that observed.

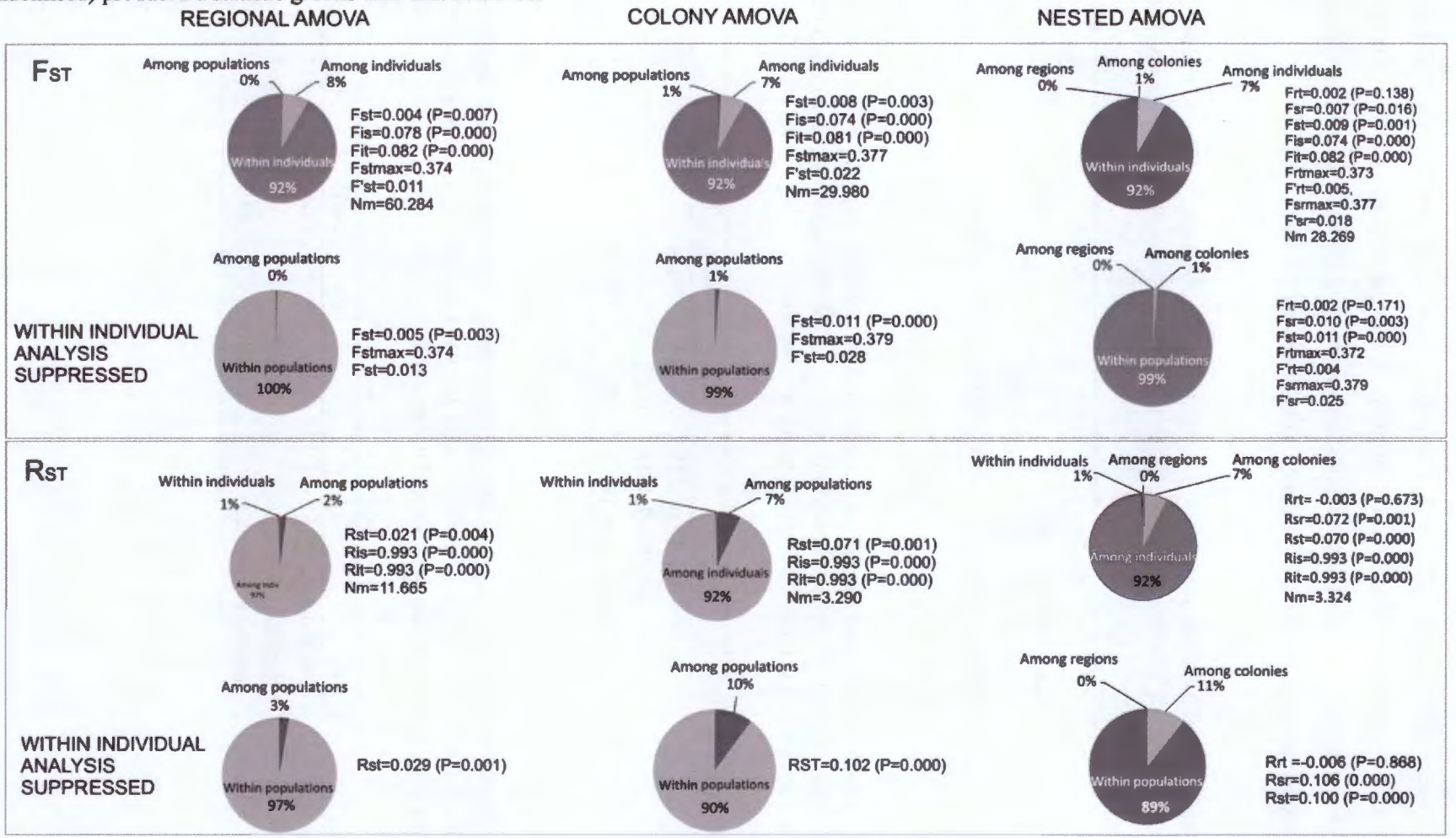


Table 4.8 (a) Regional pairwise F'_{ST} estimated during AMOVA. F'st values are below the diagonal and the associated probabilities above. **(b)** Regional pairwise $G'_{ST(NEI)}$ values (below diagonal) and the associated probabilities (above the diagonal). **(c)** Regional Pairwise Population $G'_{ST(HED)}$ values (below the diagonal) and the associated probabilities (above). All probabilities are based on 9 999 permutations and 9 999 pairwise population permutations. Significant results are marked with an asterisk.

(a) F'_{ST}	EC	NAM	WC
Eastern Cape (EC)	-	**0.038**	0.1
Namibia (NAM)	0.005*	-	**0.003**
Western Cape (WC)	0.002	0.007*	-

(b) $G'_{ST(NEI)}$	EC	NAM	WC
Eastern Cape (EC)	-	0.365	0.072
Namibia (NAM)	0.001	-	0.071
Western Cape (WC)	0.003	0.003	-

(c) $G'_{ST(HED)}$	EC	NAM	WC
Eastern Cape (EC)	-	0.002	0.002
Namibia (NAM)	0.001*	-	0.002
Western Cape (WC)	0.006*	0.007*	-

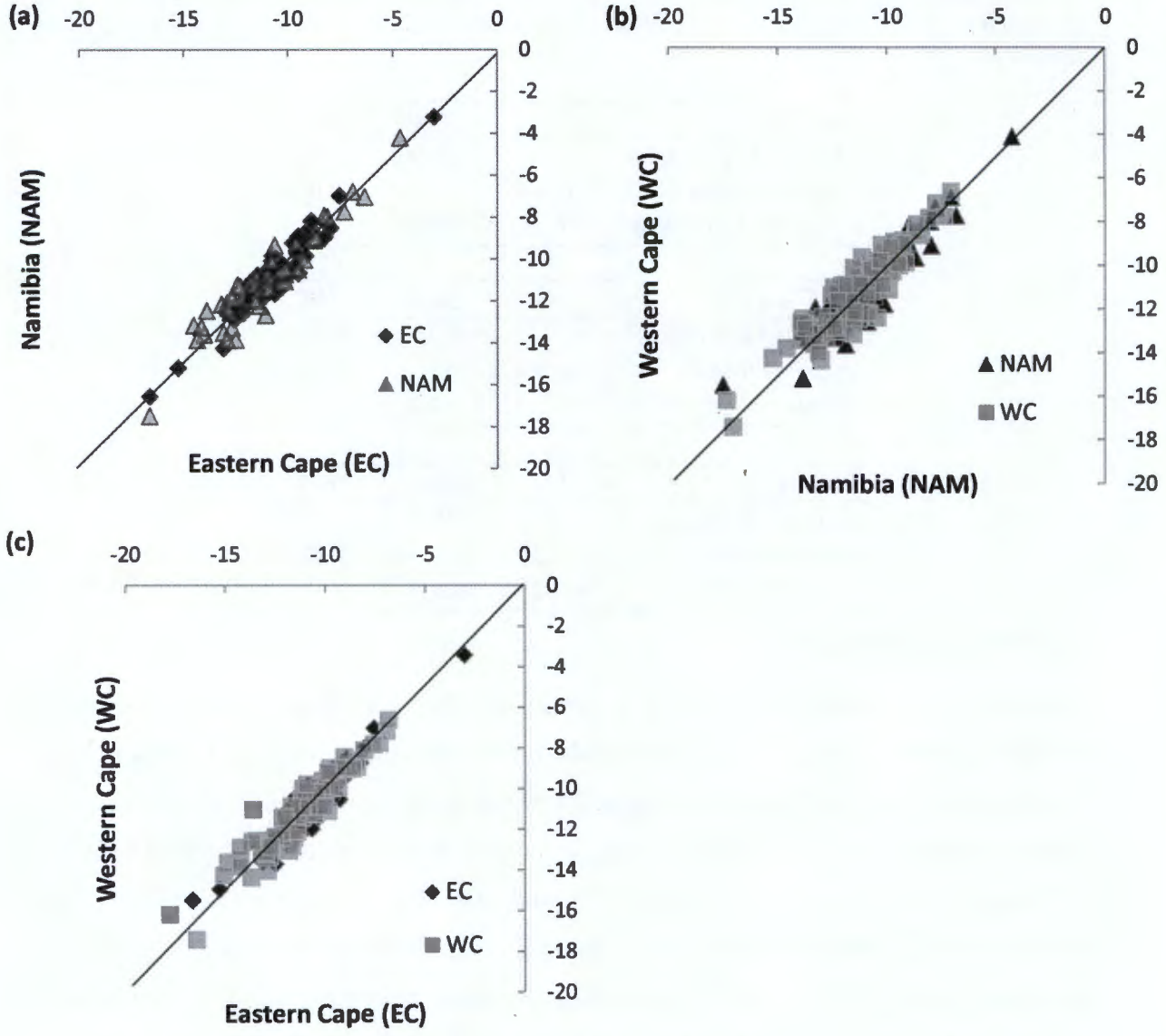
Regional Assignment tests

Assignment tests were carried out to determine whether populations were differentiated enough to allow the assignment of individuals to their breeding region based on their multi-locus genotype. Assignment tests performed very poorly at the regional-level, and correctly assigned only 40% of individuals to the region in which they were collected (Table 4.9). The lowest proportion of correctly assigned individuals was observed for Namibia (36.5%) and the highest in the Western Cape (41%). Figure 4.7 (a) to (c) show the pairwise population assignment between all pairs of breeding regions. These results show that the majority of individuals are almost equally likely to be assigned to either region in the pairwise analysis.

Table 4.9 The numbers of individuals from each breeding region that were correctly assigned to that region based on the multi-locus genotypes of the individuals sampled, and those that were not.

Regional Population	Assigned to 'self' population	Assigned to 'other' population
Eastern Cape	23	35
Namibia	19	33
Western Cape	33	46
Total	75	114
Percentage	40%	60%

Figure 4.7 Regional population assignment tests between (a) Namibia and the Eastern Cape, (b) the Western Cape and Namibia, and (c) the Western Cape and Eastern Cape. Each plot shows the pairwise likelihood of each individual being assigned to one of the two regions on the axes.



Colony-level population differentiation

Spatially explicit colony-level analyses

Overall, the colony-level fixation indices (G_{ST}, G'_{STN}, G'_{STH}, G''_{ST}) and D_{EST} were low and not significant (Appendix 4.20). The overall colony-level G_{ST} was 0.008 using Nei & Chesser's (1983) correction, which is slightly lower than that estimated at the regional-scale. G_{ST} was 0.006 overall (Appendix 4.20), which is low even given that the maximum possible

G_{ST} (based on the dataset) is 0.3 (Appendix 4.21), and also lower than the regional-scale estimate. Hedrick's standardised G-statistics, and D_{EST}, indicated slightly higher levels of overall population genetic structure, but were also not significant (Appendix 4.20). However, the AMOVA-based F_{ST} increased (approximately doubled) when data were analysed at a finer, colony-level scale (F_{ST}=0.01, P<0.001) compared to regional-scale analyses (Figure 4.6). This pattern was more pronounced based on R_{ST} (0.102, P<0.001; Figure 4.6).

Based on hierarchical F-statistics, Dassen Island, Stony Point, Mercury Island and Halifax Island show genetic signal concordant with inbreeding i.e. positive F_{IS}, with Dassen Island showing the strongest signal (Table 4.4, Figure 4.5). Among the pairwise population F_{ST}-values estimated during AMOVA, 14 of the possible 66 (21%) colony pairwise comparisons were significant (P<0.05, Table 4.10). After Benjamini-Yekutieli (B-Y) correction (critical value for 66 comparisons = 0.0105) only six of these remained significant. Of the 15 F_{ST} values that were larger than 0.02, seven involved Mercury Island and colonies from all three breeding regions, and eight involved Robben Island and colonies from all three breeding regions. These results indicate that these two colonies are relatively distinct from the rest. The highest F_{ST} values (~0.05) in descending order were between Dyer Island (WC) and Mercury Island (NAM), between Jutten Island and Robben Island (both WC), and between Jutten Island (WC) and Mercury Island (NAM). This pattern was similar among the pairwise population F_{ST} values calculated by permutation, although only four colony-level pairwise comparisons remained significant (P<0.05, Table 4.11; only one after B-Y correction). For some of the colony-level analyses (G_{ST}, G'_{STN}, G'_{STH}, G''_{ST}, D_{EST}), Possession Island was omitted due to the low sample size there, and two loci were omitted due to missing data (Tables 4.13 and 4.14, Appendix 4.23 and 4.24). Based on this reduced dataset, there was no relationship between locus variability and any of the tested fixation or differentiation indices (Figure 4.8). Also, the highest pairwise F_{ST} values (~0.05) all involved Robben Island (Table 4.11). Colony-level AMOVA-based F_{ST} estimated that 1% of the variation is distributed among colonies, marginally higher than the regional-scale analysis (Figure 4.6).

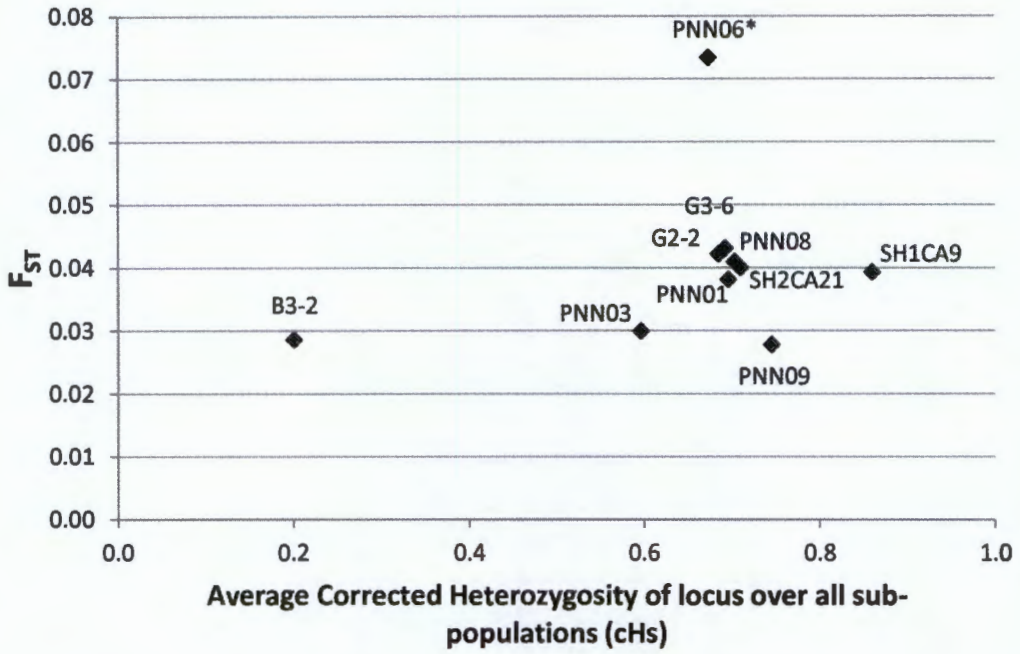
Table 4.10 Pairwise population F_{ST} values (below the diagonal) estimated directly from molecular data from all 12 microsatellite loci during AMOVA (n=189). Populations were defined as collection locality (breeding colony) and significance levels (above the diagonal; $p<0.05$ indicated with an *; B-Y corrected α for 66 comparisons = 0.0105) are based on permutation.

	Breeding colony	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	POS	HAL	ICH
EASTERN CAPE	Bird Island (BI)	-	0.085	0.184	0.281	0.071	0.053	0.128	0.275	0.149	0.463	0.449	0.367
	St. Croix (SC)	0.006	-	0.360	**0.005**	0.149	0.323	**0.022**	0.438	**0.000**	0.452	0.255	**0.014**
WESTERN CAPE	Boulders Beach (BOU)	0.010	0.003	-	0.106	0.281	0.244	0.135	0.446	0.054	0.441	0.217	0.191
	Dassen Island (DAS)	0.002	**0.015***	0.015	-	0.072	0.184	0.346	0.082	**0.018**	0.459	0.070	0.451
	Dyer Island (DYE)	0.010	0.006	0.006	0.010	-	0.385	**0.045**	0.187	**0.000**	0.451	0.271	0.107
	Jutten Island (JUT)	0.019	0.003	0.012	0.009	0.002	-	**0.024**	0.454	**0.002**	0.440	0.150	**0.036**
	Robben Island (ROB)	0.013	**0.028***	0.024	0.003	**0.025***	**0.047***	-	0.059	**0.007**	0.198	0.070	0.444
	Stony Point (SP)	0.003	-0.005	-0.016	0.009	0.007	-0.001	0.024	-	**0.004**	0.439	0.457	**0.037**
NAMIBIA	Mercury Island (MER)	0.006	**0.033***	0.022	**0.013***	**0.05***	**0.046**	**0.036***	**0.026***	-	0.273	**0.004**	0.074
	Possession Island (POS)	-0.022	-0.024	-0.005	-0.028	-0.032	-0.011	0.036	-0.041	0.016	-	0.463	0.454
	Halifax Island (HAL)	-0.003	0.004	0.010	0.011	0.004	0.014	0.023	-0.005	**0.025***	-0.054	-	0.163
	Ichaboe Island (ICH)	0.001	**0.016***	0.010	0	0.010	**0.025***	-0.003	**0.015***	0.011	-0.011	0.008	-

Table 4.11 Colony-level pairwise population matrix of F_{ST} values based on 11 colonies (Possession Island excluded) and 10 loci (PNN05 and PNN12 omitted due to missing data, as were three individuals with missing genotype data, n=184). F_{ST} values are below the diagonal. P-values (significance levels) are above diagonal. Significant values are in bold type (P<0.05); B-Y corrected α for 66 comparisons = 0.0105.

	Breeding colony	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	HAL	ICH
EASTERN CAPE	Bird Island (BI)	-	0.57	0.26	0.35	0.75	0.14	0.11	0.70	0.70	0.81	0.35
	St. Croix (SC)	0.008	-	0.27	**0.04**	0.18	0.26	0.06	0.70	0.23	0.21	0.09
WESTERN CAPE	Boulders Beach (BOU)	0.028	0.026	-	0.078	0.28	0.17	0.14	0.93	0.5	0.19	0.31
	Dassen Island (DAS)	0.011	**0.014***	0.033	-	0.86	0.53	0.33	0.31	**0.04**	0.15	0.49
	Dyer Island (DYE)	0.010	0.015	0.029	0.010	-	0.36	0.23	0.35	0.09	0.41	0.79
	Jutten Island (JUT)	0.027	0.022	0.040	0.021	0.025	-	**0.04**	0.38	0.18	0.18	0.11
	Robben Island (ROB)	0.032	0.033	0.049	0.026	0.031	**0.051***	-	0.12	**0.01**	0.13	0.53
	Stony Point (SP)	0.011	0.011	0.018	0.015	0.017	0.025	0.036	-	0.65	0.71	0.16
NAMIBIA	Mercury Island (MER)	0.010	0.013	0.026	**0.018***	0.021	0.030	**0.045***	0.014	-	0.43	0.31
	Halifax Island (HAL)	0.012	0.017	0.037	0.019	0.019	0.033	0.039	0.016	0.018	-	0.34
	Ichaboe Island (ICH)	0.014	0.016	0.030	0.013	0.012	0.032	0.025	0.021	0.017	0.020	-

Figure 4.8 Plot of F_{ST} versus the average corrected heterozygosity (cH_S) for each locus, based on a reduced dataset of 10 loci, 11 populations (colonies) and 182 African Penguins samples (to eliminate missing genotypes). Values are based on 1 000 permutations of 10 000 pairwise population permutations and 10 000 bootstrap replicates. Total F_{ST} based on this analysis was 0.41±0.004 (standard error, P=0.189). Only locus PNN06 exhibited a significant F_{ST} (P=0.01). A very similar pattern held for G_{ST}, G_{ST}, G'_{STN}, G'_{STH}, G''_{ST}, and D_{EST} (data not shown).



Similarly, 18 of the possible 66 (27%) colony-level pairwise R_{ST} comparisons were significant (P<0.05, Table 4.12). Of the 13 pairwise colony R_{ST} values >0.1, three were between colonies in the same region, five were between colonies in the Western Cape and Namibia, and five involved the Eastern Cape colonies and either Western Cape or Namibian colonies (Table 4.12). The highest AMOVA-based R_{ST} value is between Dyer and Robben Islands in the Western Cape (0.43, Table 4.12 and Appendix 4.22), although this pattern is not reflected among the other fixation indices. The second-highest pairwise R_{ST} was between Dyer Island and Mercury Island (Namibia, R_{ST}=0.34). St Croix Island (Eastern Cape) and Mercury Island in Namibia also showed a high degree of population genetic divergence relative to other colony comparisons (R_{ST}=0.32, F_{ST}=0.03, Appendix 4.22). Based on the R_{ST}-AMOVA, 10% of the molecular variance is distributed among colonies, much higher than the regional-scale estimate (Figure 4.6). Various colony-level fixation indices are not reported in the text, but were calculated and are given in Appendices 4.23 to 4.25.

Table 4.12 Pairwise population R_{ST} values (below the diagonal) estimated directly from molecular data from 12 microsatellite loci during nested AMOVA. Populations were defined as collection locality (breeding colony) and significance levels (above the diagonal; $p<0.05$ indicated with an *; B-Y corrected α for 66 comparisons = 0.0105) are based on permutation.

	Breeding colony	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	POS	HAL	ICH
EASTERN CAPE	Bird Island (BI)	-	**0.000**	0.301	0.402	**0.000**	0.298	0.310	0.257	0.140	0.315	0.194	0.400
	St. Croix (SC)	**0.096***	-	0.337	**0.000**	0.152	0.345	**0.007**	0.180	**0.000**	0.299	0.275	**0.000**
WESTERN CAPE	Boulders Beach (BOU)	-0.024	-0.081	-	0.175	0.096	0.358	0.206	0.274	**0.018**	0.249	0.304	0.243
	Dassen Island (DAS)	-0.013	**0.174***	0.031	-	**0.000**	0.100	0.321	**0.014**	**0.047**	0.377	0.064	0.345
	Dyer Island (DYE)	**0.123***	0.035	0.073	**0.262***	-	0.115	**0.001**	0.276	**0.000**	0.222	**0.037**	**0.000**
	Jutten Island (JUT)	-0.005	-0.076	-0.151	0.054	0.063	-	0.127	0.332	**0.005**	0.372	0.366	0.132
	Robben Island (ROB)	-0.068	**0.255***	0.041	-0.095	**0.432***	0.076	-	0.202	0.279	0.240	0.123	0.380
	Stony Point (SP)	0.013	0.017	-0.078	**0.064***	0.022	-0.062	0.017	-	**0.000**	0.250	0.533	**0.034**
NAMIBIA	Mercury Island (MER)	0.019	**0.319***	**0.132***	**0.039***	**0.336***	**0.155***	-0.035	**0.138***	-	0.211	**0.000**	0.244
	Possession Island (POS)	-0.170	-0.274	-0.321	-0.008	-0.259	-0.309	0.031	-0.318	0.056	-	0.077	0.369
	Halifax Island (HAL)	0.016	-0.039	-0.120	0.060	0.086	-0.107	0.076	-0.029	**0.179***	-0.253	-	0.064
	Ichaboe Island (ICH)	-0.034	**0.175***	0.015	-0.040	**0.232***	0.037	-0.092	**0.048***	0.011	-0.073	0.052	-

Population pairwise G_{ST}, $G'_{ST(Nei)}$ and G''_{ST} values showed very similar patterns (e.g. these indices are significant for identical colony-pairs and similar colony-pairs exhibit the highest values), so the former two are given in the Appendix (Appendices 4.23 and 4.24 respectively) and Hedrick's further standardised G''_{ST} is reported in Table 4.13. Based on these results, the African Penguins sampled at St Croix Island are significantly different from those sampled at two Western Cape colonies: Dassen Island and Robben Island (although not after B-Y correction for multiple comparisons: α=0.0105). Based on these results, Robben Island is also significantly different from Jutten Island in the Western Cape and Mercury Island in Namibia (both G''_{ST}>0.1, P<0.05; after B-Y correction only Robben and Mercury remain significant). No colonies in Namibia were significantly differentiated from any colonies in the Eastern Cape, but some colonies in both of those regions (e.g. Mercury Island and St Croix Island) were significantly different to colonies in the Western Cape (e.g. Robben Island and Dassen Island, Table 4.13; not significant after B-Y correction). Pairwise colony comparisons based on Jost's estimate of differentiation (D_{EST}, Table 4.14) further highlighted the low level of population genetic structure, as all values were low (<0.1) and only five of the 55 possible comparisons were significant at P<0.05 (only one after B-Y correction). Colony-level population pairwise D-statistics (where D = -ln (1-Fst)) and linearised F_{ST} and were also calculated for comparison and are given in Appendix 4.25.

Nested AMOVA

The results of a nested AMOVA, with colonies assigned to breeding regions, and based on F_{ST} (Figure 4.6), showed identical results to regional- and colony-level AMOVAs (0% of variation among regions, and 1% among colonies). The same analysis based on R_{ST} also found 0% of variation partitioned among regions, but a larger 7% among colonies (Figure 4.6). The same analyses for F_{ST} and R_{ST} with the 'within individual' analysis suppressed, showed that, for R_{ST}, 11% of variation is among colonies (Figure 4.6). R_{ST} and F_{ST} show different patterns in terms of population genetic structure across the range of African Penguins at the colony-level. Both statistics are significant, but the overall R_{ST} value is nearly an order of magnitude higher than the overall F_{ST} value (Table 4.15). Neither of the statistics indicates strong population structure at either scale, however, the consistently higher values for colony-level analysis suggest that colony-level processes are more important than those at a regional-scale in driving population differentiation.

Table 4.13 Colony-level pairwise population matrix of G''_{ST} (Hedrick's further standardised G_{ST}) values based on 11 colonies (Possession Island excluded) and 10 loci (PNN05 and PNN12 omitted due to missing data, as were three individuals with missing genotype data, n=184). G''_{ST} values are below the diagonal. Significance levels are shown above diagonal. Significant values are in bold type, B-Y corrected α for 66 comparisons = 0.0105.

	Breeding colony	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	HAL	ICH
EASTERN CAPE	Bird Island (BI)	-	0.575	0.256	0.353	0.744	0.128	0.097	0.698	0.710	0.815	0.354
	St Croix Island (SC)	-0.004	-	0.283	**0.036**	0.181	0.233	**0.045**	0.7	0.232	0.223	0.093
WESTERN CAPE	Boulders Beach (BOU)	0.024	0.018	-	0.091	0.294	0.175	0.141	0.92	0.475	0.182	0.288
	Dassen Island (DAS)	0.005	**0.027**	0.051	-	0.843	0.465	0.303	0.314	**0.045**	0.156	0.502
	Dyer Island (DYE)	-0.013	0.017	0.018	-0.019	-	0.339	0.218	0.36	0.088	0.417	0.789
	Jutten Island (JUT)	0.039	0.019	0.040	0.002	0.012	-	**0.033**	0.356	0.144	0.16	0.09
	Robben Island (ROB)	0.052	**0.064**	0.069	0.016	0.029	**0.108**	-	0.12	**0.009**	0.117	0.493
	Stony Point (SP)	-0.013	-0.011	-0.051	0.009	0.006	0.009	0.052	-	0.639	0.711	0.158
NAMIBIA	Mercury Island (MER)	-0.011	0.011	-0.003	**0.034**	0.032	0.041	**0.112**	-0.011	-	0.424	0.306
	Halifax Island (HAL)	-0.022	0.016	0.041	0.024	0.003	0.045	0.057	-0.018	0.003	-	0.338
	Ichaboe Island (ICH)	0.007	0.025	0.019	-0.002	-0.019	0.052	-0.006	0.028	0.010	0.011	-

Table 4.14 Colony-level pairwise population matrix of Jost's D_{EST} values based on 11 colonies (Possession Island excluded) and 10 loci (PNN05 and PNN12 omitted due to missing data, as were three individuals with missing genotype data, n=184). D_{EST} values are below the diagonal. Significance levels based on 999 permutations in GENALEX 6.5 values are shown above diagonal. Significant values are in bold type B-Y corrected α for 66 comparisons = 0.0105.

	Breeding colony	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	HAL	ICH
EASTERN CAPE	Bird Island (BI)	-	0.575	0.262	0.353	0.743	0.130	0.095	0.698	0.710	0.815	0.354
	St Croix Island (SC)	-0.002	-	0.287	**0.035**	0.180	0.234	**0.042**	0.704	0.233	0.221	0.092
WESTERN CAPE	Boulders Beach (BOU)	0.016	0.011	-	0.094	0.296	0.176	0.141	0.918	0.475	0.186	0.293
	Dassen Island (DAS)	0.003	**0.018**	0.034	-	0.841	0.466	0.305	0.315	**0.046**	0.159	0.501
	Dyer Island (DYE)	-0.008	0.011	0.011	-0.013	-	0.338	0.214	0.357	0.088	0.417	0.789
	Jutten Island (JUT)	0.026	0.012	0.025	0.001	0.008	-	**0.033**	0.357	0.144	0.163	0.090
	Robben Island (ROB)	0.035	**0.043**	0.045	0.011	0.019	**0.073**	-	0.115	**0.009**	0.116	0.493
	Stony Point (SP)	-0.009	-0.007	-0.031	0.006	0.004	0.006	0.035	-	0.639	0.711	0.158
NAMIBIA	Mercury Island (MER)	-0.007	0.007	-0.002	**0.024**	0.021	0.027	**0.078**	-0.007	-	0.424	0.305
	Halifax Island (HAL)	-0.015	0.011	0.026	0.017	0.002	0.030	0.039	-0.012	0.002	-	0.338
	Ichaboe Island (ICH)	0.005	0.017	0.012	-0.001	-0.013	0.034	-0.004	0.019	0.007	0.007	-

Table 4.15 F- and R-statistics generated from a nested AMOVA (individuals assigned to colonies and colonies assigned to regions). Probabilities are based on permutation across the full dataset (number of permutations=999; number of pairwise population permutations=9 999). R=region, T=Total population, S=Sub-population (colony), I=Individuals. Significant values are in bold type.

F-Statistic	Value	P-value
F_{RT}	0.002	0.140
F_{SR}	**0.007**	**0.023**
F_{ST}	**0.009**	**0.002**
F_{IS}	**0.074**	**0.001**
F_{IT}	**0.082**	**0.001**
R_{RT}	-0.003	0.666
R_{SR}	**0.072**	**0.001**
R_{ST}	**0.070**	**0.001**
R_{IS}	**0.993**	**0.001**
R_{IT}	**0.993**	**0.001**

Colony-level assignment tests

The low level of genetic structuring observed among the breeding colonies of African Penguins was also reflected in the colony-level population assignment tests (Table 4.16), where 86% of African Penguins were incorrectly assigned (i.e. were assigned to a colony other than their true collection locality), based on their multi-locus genotypes. St Croix and Jutten Islands had the highest proportion of correctly assigned birds, where Possession and Robben Islands had none.

Spatially independent analyses

Clustering methods

To investigate whether unique genetic clusters could be identified, the program STRUCTURE was used to analyse the full dataset. The results of the multiple STRUCTURE analyses were not affected by model choice or prior settings, and the results used to select the optimal number of clusters (Figure 4.9) are from runs in which admixture and correlated allele frequencies were assumed, and no prior information about sampling locality was provided. Following the ΔK method as described in Evanno et al. (2005), the mean likelihood *L(K)* and standard deviation (*sd[L(K)]*) over 20 runs for each *K* was calculated, followed by the mean difference between successive likelihood values of *K* (the rate of change of the likelihood function with respect to K), *L'(K),* and the second order rate of change (*L''(K)*). *ΔK* was estimated as this value divided by the standard deviation (*sd[L(K)]*) (Evanno et al. 2005). *ΔK* was plotted for each value of K, and the modal value taken to be the real number of populations or the true K (Evanno et al. 2005). The highest value of *ΔK* was for K=2

(Figure 4.9), with a secondary peak at K=6. This analysis was also run using StructureHarvester (Earl & VonHoldt 2011), with identical results. Importantly, though, ΔK cannot find the true number of populations if there is one single panmictic population; i.e. if K equals one. However, none of the multiple STRUCTURE analyses conducted contradict the finding that K=1; i.e. that there are no discrete genetically distinct groups detectable in the dataset, and the mean likelihood was highest for K=1 (Figure 4.9). The smallest value of K that can be statistically analysed using the delta K method is two, and the results show that the majority of African Penguin samples have an equal probability of belonging to either cluster based on their multi-locus genotype (Figure 4.10). This pattern was unchanged when locality information was included as prior information and when a model of no admixture was implemented (Figure 4.10). In GENELAND a single cluster was identified (K=1) using the uncorrelated method (K=1), while the correlated method indicated K=1 and K=2 during the ten independent runs, however the runs with the highest posterior probabilities were those that identified K=1.

Table 4.16 The results of the colony-level population assignment test. The number of individuals from each breeding colony that were correctly assigned to that breeding colony based on their multi-locus genotype are indicated in the "Self Population" column. The number of individuals from each breeding colony that were incorrectly assigned to another colony is given in the "Other Population" column. "% Self" indicates the proportion of all samples from each breeding locality that were correctly assigned.

Population (collection locality)	Number assigned to self-population	Number assigned to other Population	% self
Bird Island (BI)	4	23	15
St Croix Island (SC)	8	23	26
Boulders Beach (BOU)	1	6	14
Dassen Island (DAS)	2	23	8
Dyer Island (DYE)	1	15	6
Jutten Island (JUT)	3	5	38
Robben Island (ROB)	0	7	0
Stony Point (SP)	1	15	6
Mercury Island (MER)	3	17	15
Possession Island (POS)	0	2	0
Halifax Island (HAL)	1	12	8
Ichaboe Island (ICH)	2	15	12
TOTAL	26	163	14
Overall, 86% of African Penguins were incorrectly assigned to 'other' breeding colonies			

Figure 4.9 STRUCTUREHARVESTER (Earl & VonHoldt 2011) results showing (a) likelihood values (L(K), mean ± standard deviation) for all clusters (1 to 4) for all 20 runs of 1 million replicates and (b) the DELTA(K) = |L''(K)| /SD[L(K)] values for all clusters (1 to 14) over all runs.

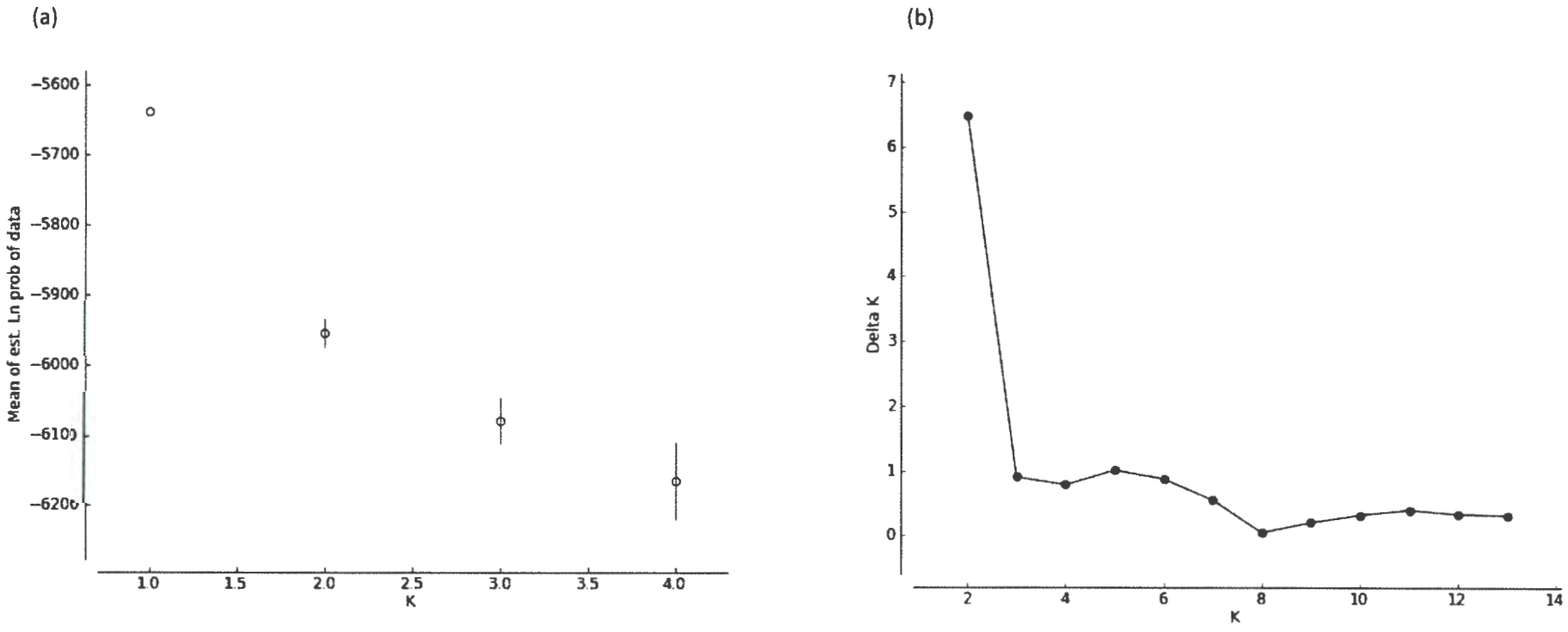


Figure 4.10 Results of STRUCTURE analyses for the best K (K=2), visualised using DISTRUCT. The top panel is the structure analysis with admixture and no prior information provided (20 runs, burnin=10 000, 100 000 replicates, no LOCPRIOR), the middle panel is based on the same analysis, but without assuming admixture. The bottom panel is based on K=2 with no admixture and prior information about sampling locality provided (20 runs, burnin=100 000, 1 million replicates).

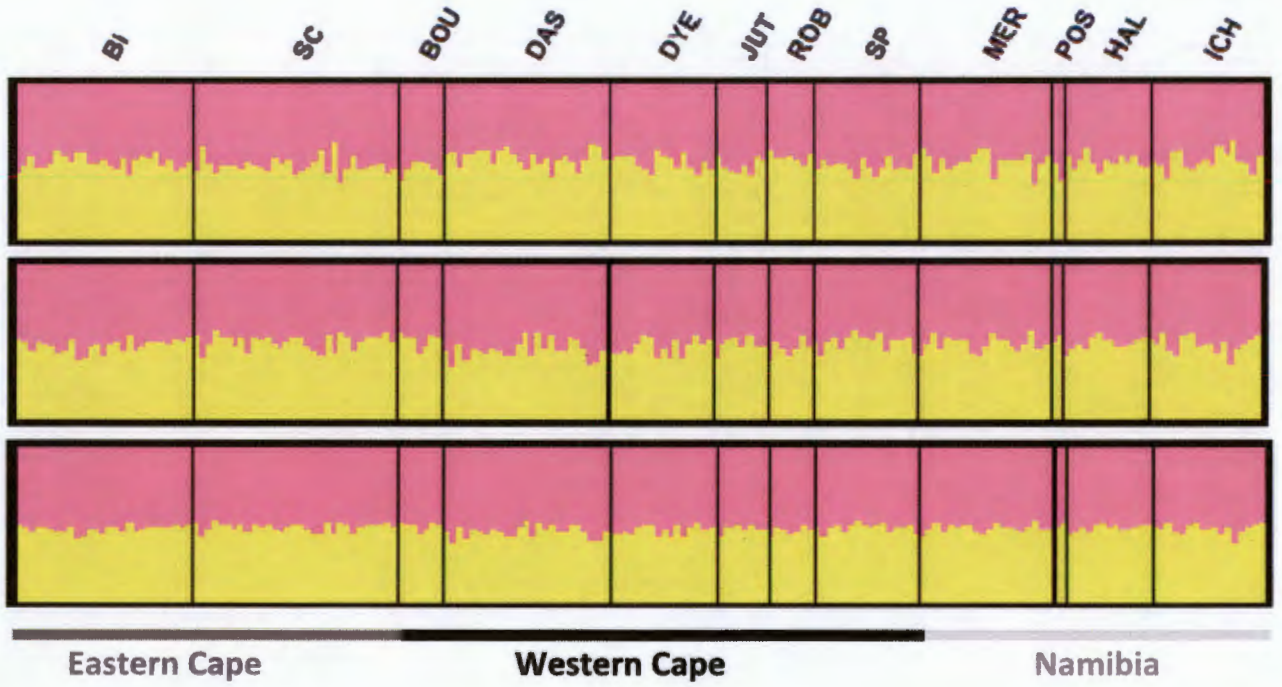
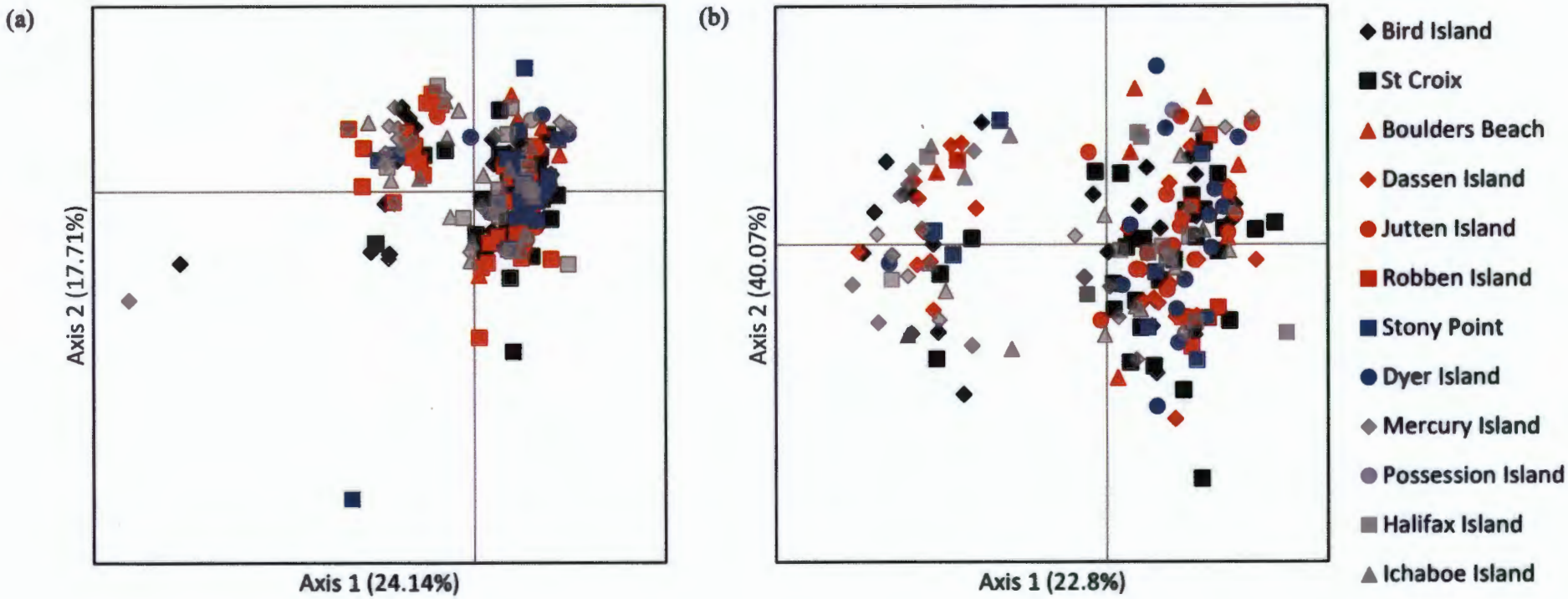


Figure 4.11 The first two axes of the (a) Distance Standardised Principal Coordinates (PCoA) and (b) Covariance Standardised Principal Coordinates (PCoA) based on individual pairwise codominant genotypic distance (genetic distance, 12 loci, 189 individuals, 12 populations). The first three axes of (a) account for 57.12% of the variation in the data, and the first three axes of (b) account for 56.1% of variation.



Ordinations in reduced space

The first two axes of the distance-standardised PCoA (Figure 4.11a) and covariance-standardised PCoA (Figure 4.11b; axes 1 versus 3 showed a similar pattern) based on a genetic distance matrix generated from data for 12 loci and 12 breeding colonies (n=189 individuals) produced two distinct groups and explain 40.07% and 41.85% of the variation respectively. There appears to be only a weak geographic component to the analysis based on genetic distance, however, the pattern does support the results of the STRUCTURE analysis above. A similar analysis based on Queller & Goodnight's (1989) relatedness index (r) calculated from a reduced dataset (n=184, 11 colonies), however, shows a similar division of individuals into two distinct groups, but a much stronger geographic component is evident (Figure 4.19).

Another multivariate analysis that condenses information from a large number of alleles and loci into a few synthetic variables is Factorial Correspondence Analysis (FCA), which can be employed to investigate major patterns of genetic variability based on the multi-locus genotypes of all individuals. Although GENETIX offers the option to incorporate information about sampling locality, all analyses were conducted without this information included. FCA implemented in GENETIX, is modified so that it is appropriate for diploid genetic data. In FCA, all individuals can be thought of as a cloud of points in hyperspace, with as many dimensions as there are alleles for all loci. The GENETIX FCA algorithm finds independent, orthogonal directions in this hyperspace along which the inertia (the number of individuals at a point in hyperspace) multiplied by the square of the distance to the centre of the coordinates (also called centre of gravity) - is maximised. These independent directions are defined by the eigenvectors of the matrix (i.e. linear combinations of original variables vectors) and determine a series of factor axes. No geographical pattern is evident in the FCA plots at either the regional (Figure 4.12) or colony-level (Figure 4.13).

Figure 4.12 Factorial Correspondence Analysis (FCA) reflecting the regional collection locality (Namibia, Eastern Cape and Western Cape) of 189 sampled African Penguins.

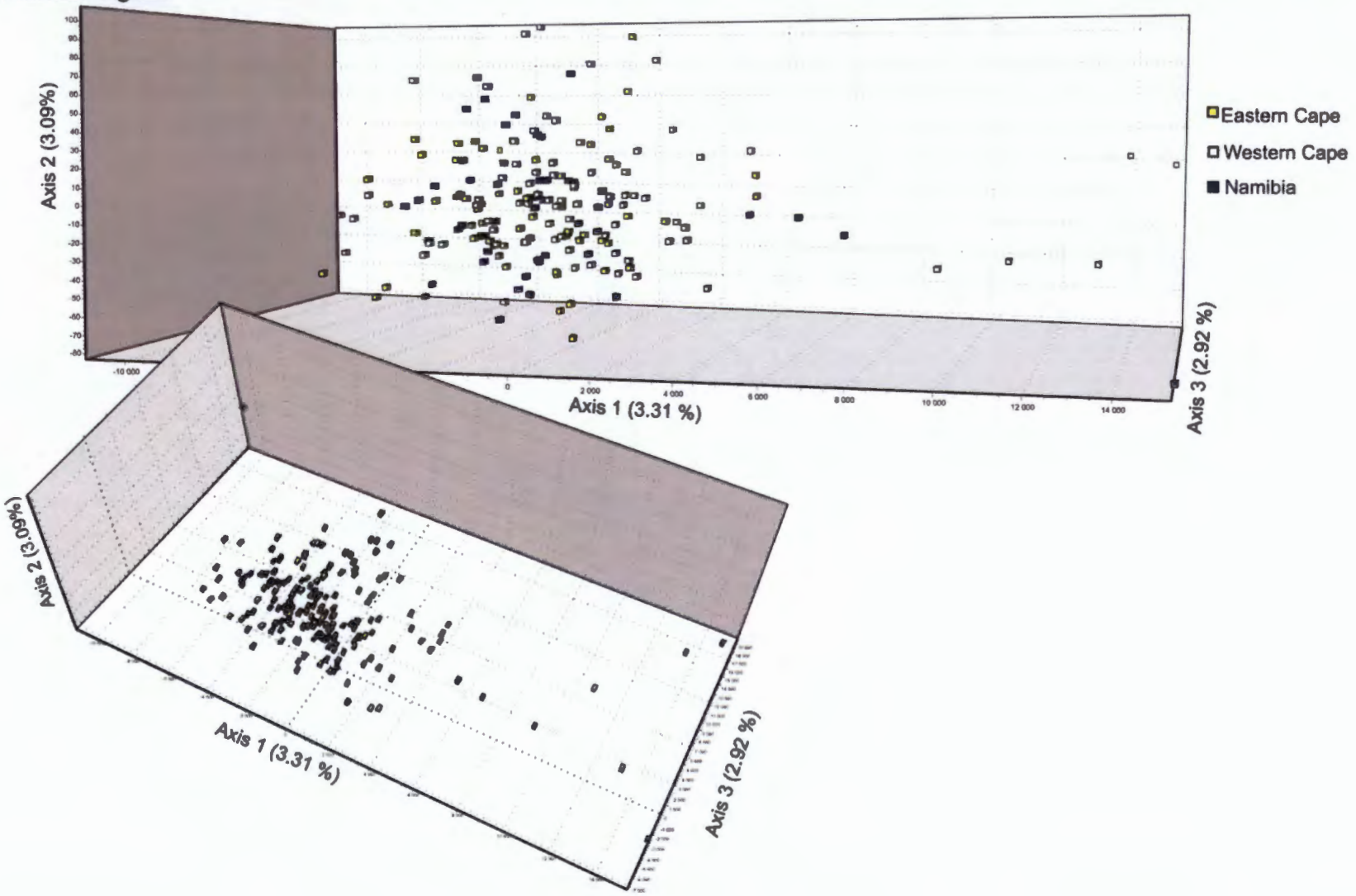
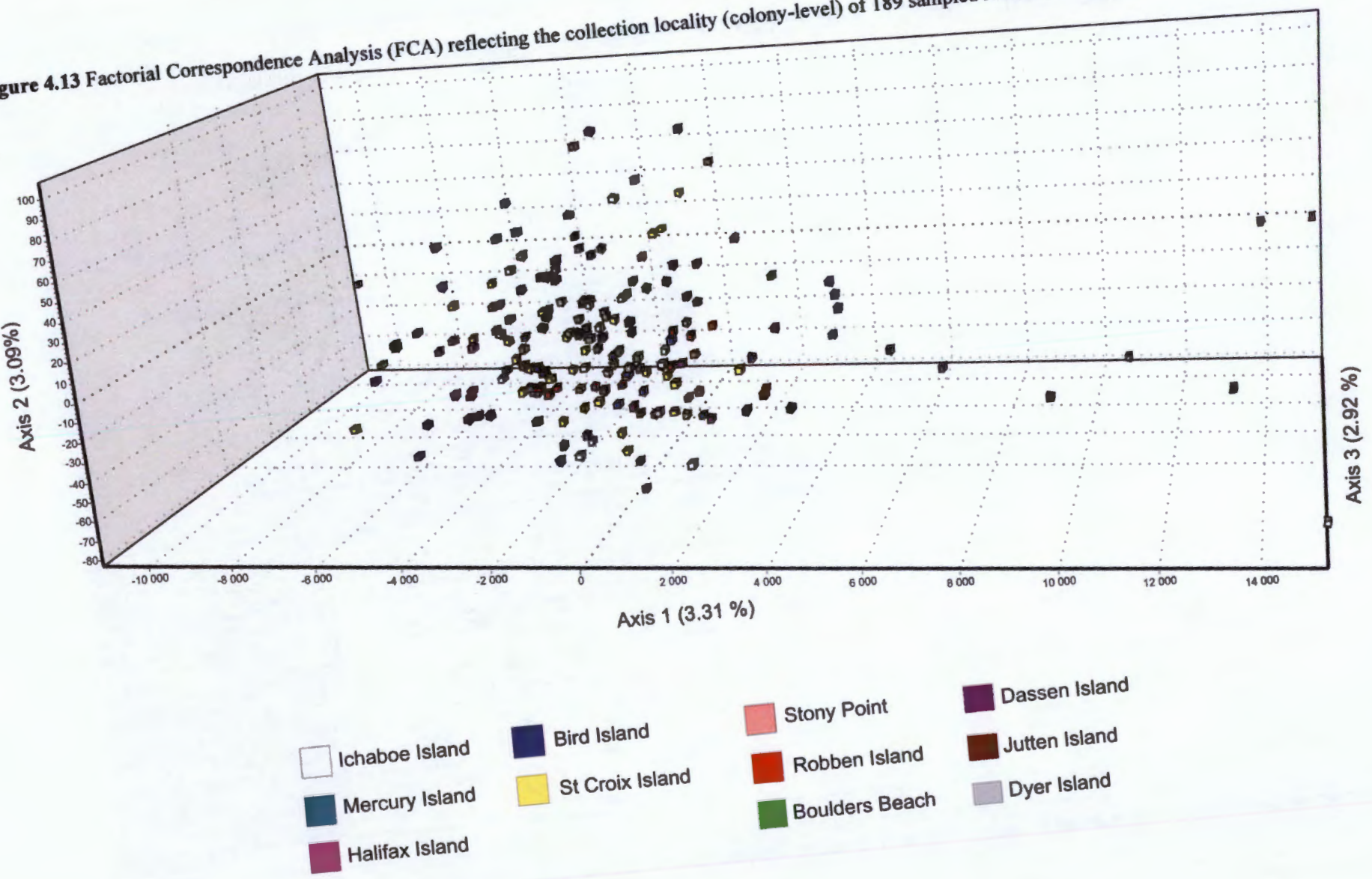


Figure 4.13 Factorial Correspondence Analysis (FCA) reflecting the collection locality (colony-level) of 189 sampled African Penguins.



Mantel tests and isolation by distance

No significant relationship between geographic and genetic distance was detected in any analysis. The Mantel tests conducted in Alleles in Space (AIS) and GENALEX showed very similar results (Figure 4.14), with similar estimates of the correlation of genetic and geographical distances (AIS: r=0.065, P<0.001; GENALEX: R_{xy}=0.054, P=0.012). The three broad groups of individuals in Figure 4.14 reflect the two big geographical gaps between breeding regions i.e. the first group (<250km) is all pairwise comparisons of birds from colonies within the same breeding region; the middle group contains pairwise comparisons of birds from either Namibia and the Western Cape, or the Eastern Cape and Western Cape; the last group (>1500km) contains pairwise comparisons between birds from the Eastern Cape and Namibia. The plot shows that the genetic distance between individuals breeding close together spans the same range as birds breeding nearly 2000km apart i.e. genetic distance and geographic distance are not correlated among samples. This pattern was largely corroborated by isolation by distance (IBD) analyses (e.g. Figure 4.15, Z =-477738.5, r=0.07, P>0.2) based on Slatkin's similarity index (Slatkin 1993). The three broad breeding regions are again discernible in Figure 4.15. Reduced Major Axis (RMA) regression was implemented to investigate the intercept and slope of the relationship between genetic similarity (M) and geographic distance (km). A weak positive trend is present, however this is likely influenced by a small number of outliers, for example, Halifax and Dyer Islands are the least genetically similar breeding colonies (M=-842), but are only a moderate distance apart; Ichaboe Island and Bird Island are the most genetically similar colonies (M=531), but are one of the furthest apart geographically. Among colonies in the same breeding region i.e. colonies that are close together geographically, genetic similarity among colony pairs ranges from M=352 (between Jutten Island and Dassen Island in the Western Cape) to M= -487 (between Bird Island and St Croix Island in the Eastern Cape). Overall, colonies that are geographically proximate are as genetically similar to each other as colonies that are distant. Pairwise colony F_{ST} and Slatkin's similarity index (M) calculated during the isolation by distance analysis (Jensen et al. 2005) are plotted in Appendix 4.26.

Figure 4.14 Mantel test (conducted in GENALEX) for the correlation of linearised genetic and geographical distances between individuals based on all 12 microsatellite loci and 189 African Penguin samples. Low geographic distances represent birds from colonies in the same breeding region and high geographic distances (>1500) represent comparisons between birds from colonies in Namibia and the Eastern Cape.

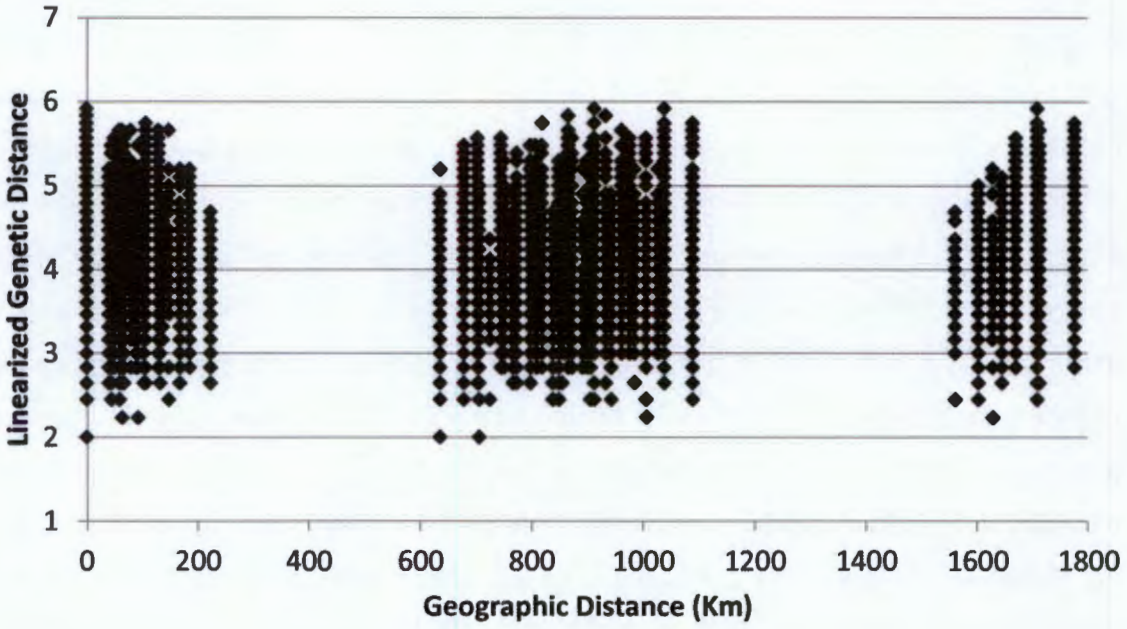
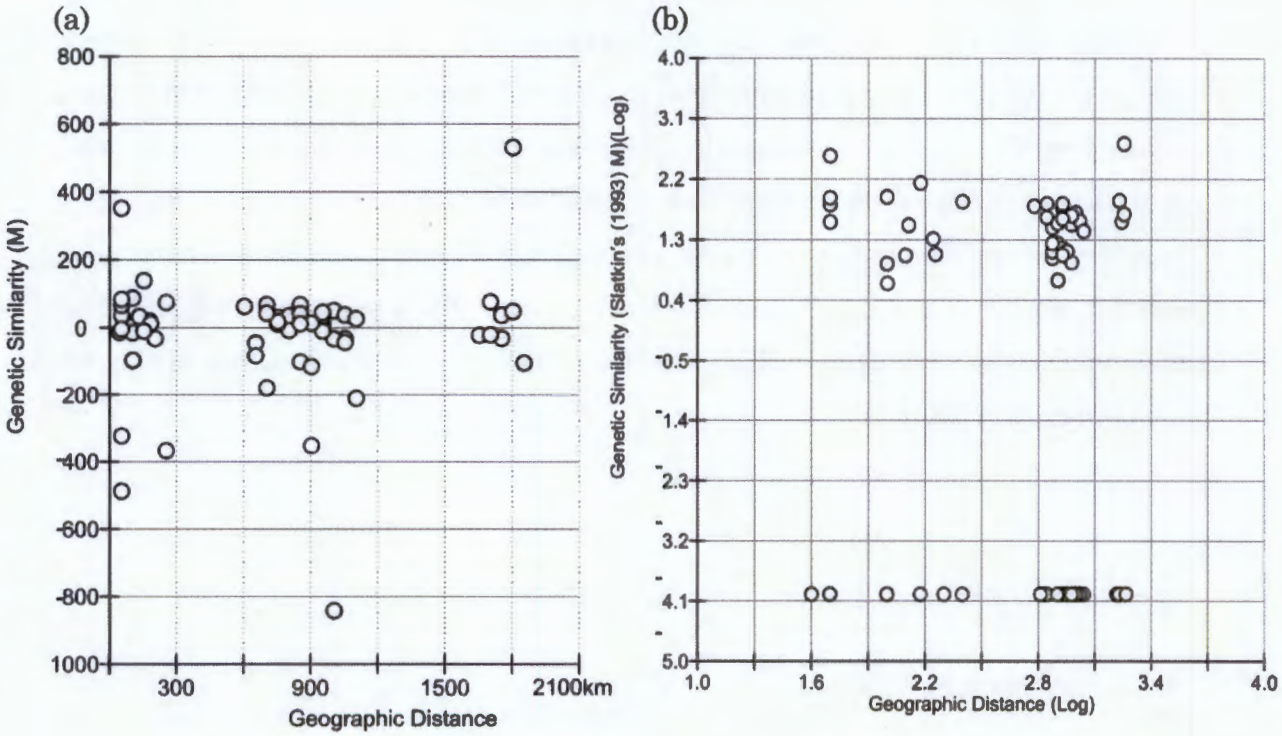


Figure 4.15 (a) IBDWS (Jensen et al. 2005) Mantel Test matrix correlation between Slatkin's M (genetic similarity) and geographic distance based on raw diploid genotypes ("Analysis 1"). (b) The same analysis with both axes Log-transformed ("Analysis 4", as suggested by Slatkin (1993)).



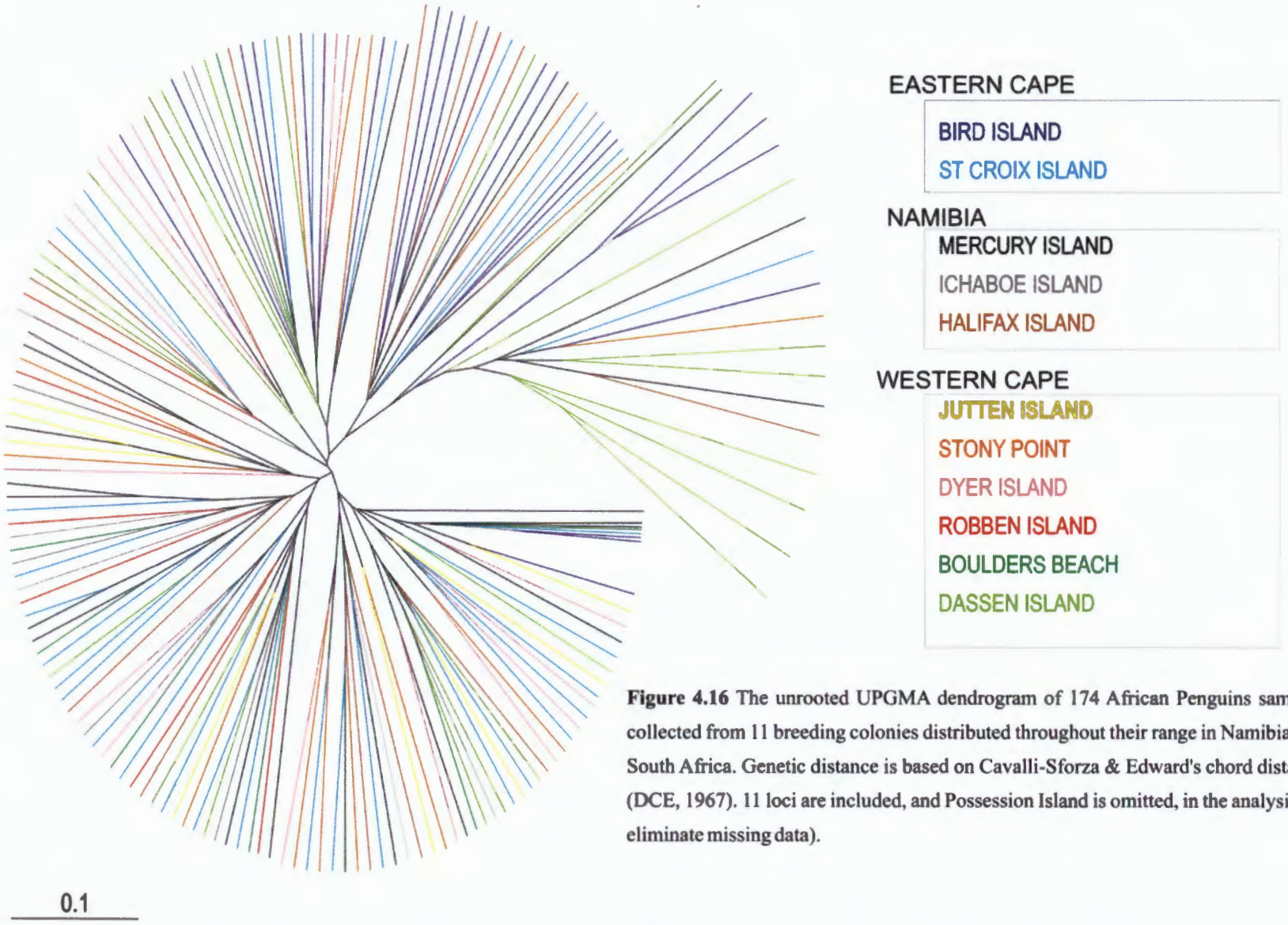


Figure 4.16 The unrooted UPGMA dendrogram of 174 African Penguins samples collected from 11 breeding colonies distributed throughout their range in Namibia and South Africa. Genetic distance is based on Cavalli-Sforza & Edward's chord distance (DCE, 1967). 11 loci are included, and Possession Island is omitted, in the analysis (to eliminate missing data).

Phylogenetic relationships among individuals and populations

The unrooted UPGMA phylogenetic tree (Figure 4.16) strongly reflects the pattern of panmixia, with no spatial pattern evident among the 174 African Penguins included in the analysis.

In the phylogenetic analyses of populations (Figure 4.17 and Appendices 4.27 and 4.28), there appears to be a stronger association among two groups of colonies in the Western Cape, than among any of the other colonies: Stony Point and Dyer Island are closely associated, and Robben Island, Boulders Beach and Jutten Island are also more closely related to each other than they are to any other colonies. The first two genetic distance measures used in the phylogenetic analyses (Figure 4.17 and Appendix 4.27) have been shown to have a high probability of obtaining the correct topology despite bottleneck effects (D_A, D_{CE}; Takezaki & Nei 1996), and the third - Goldstein et al.'s ($\delta\mu^2$, Appendix 4.28) has also proven useful for clarifying evolutionary relationships of closely related populations (Goldstein et al. 1995a, 1995b; Takezaki & Nei 1996, 2008; Goldstein & Pollock 1997).

Detecting Genetic Bottlenecks

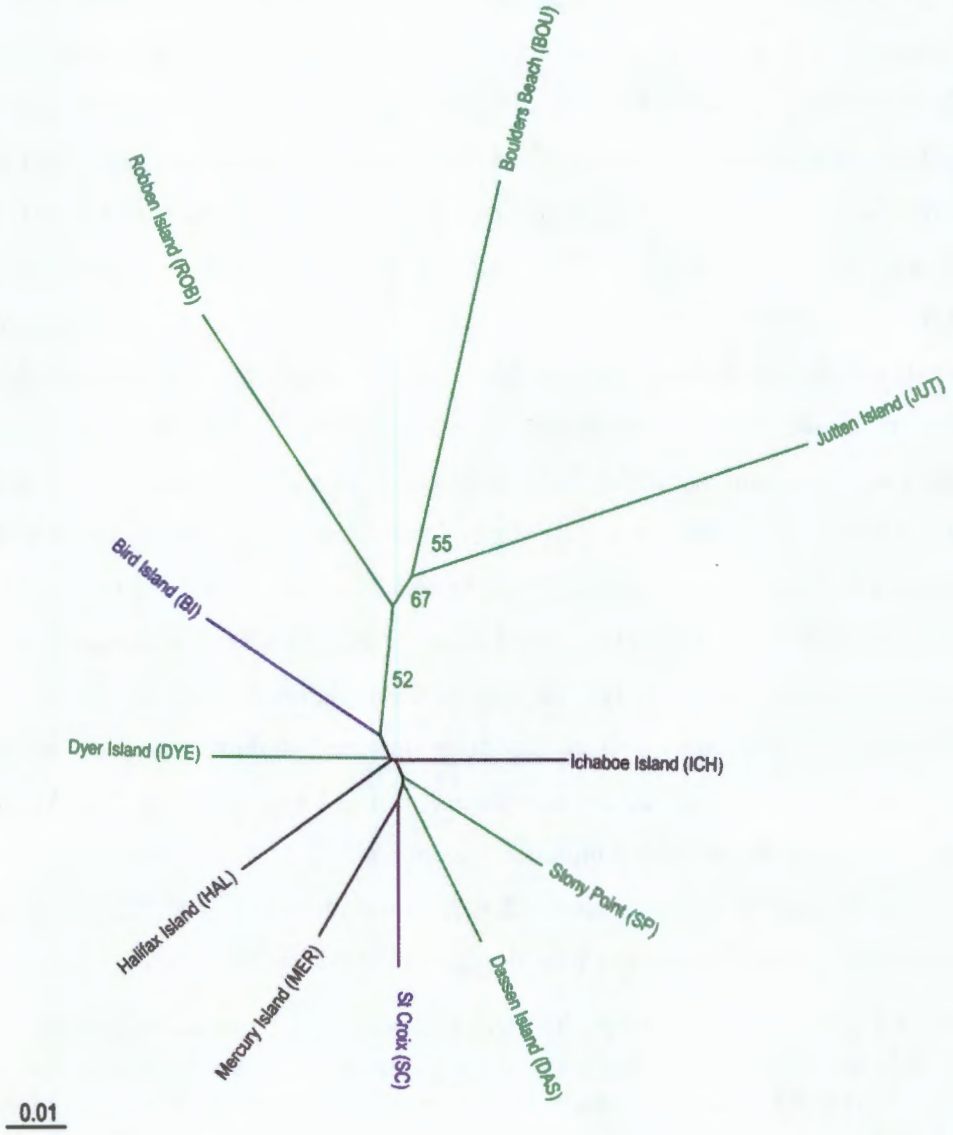
In any population at mutation-drift equilibrium i.e. where the effective size has remained constant in the past, there is approximately an equal chance that a locus shows heterozygosity excess or heterozygosity deficit (Williamson-Natesan 2005). To determine whether a population exhibits a significant number of loci with heterozygosity excess, BOTTLENECK implements a "sign test", a "standardised differences test" (Cornuet & Luikart 1996), and a "Wilcoxon sign-rank test" – considered the most powerful (Luikart et al. 1998; Peery et al. 2012). BOTTLENECK also generates a descriptor of the allele frequency distribution ("mode-shift" test), which deviates from an L-shaped distribution in bottlenecked populations. The four tests implemented by BOTTLENECK were carried out at the regional- and colony-levels, for each of three mutation models. Overall, BOTTLENECK results indicated that a number of colonies and regions were not in mutation-drift equilibrium, and that some exhibited heterozygote excess, which is indicative of a recent bottleneck (Table 4.17). The results, however, were significant for different mutation models in different colonies and regions, indicating weak, sometimes inconsistent signals, and possibly the differential power of genetic bottleneck tests to detect the decline in the population.

heterozygote excess and deficiency significant under IAM (P=0.02 and P=0.04 respectively), but the one-tailed test for heterozygote deficiency significant under the SMM. In the Western Cape, only the one-tailed Wilcoxon test for heterozygote deficiency under the SMM was significant. At the regional-level overall, there is some evidence that African Penguin populations are not in mutation-drift equilibrium, and that Namibia exhibits heterozygote excess, however, all three breeding regions exhibited a normal L-shaped distribution in the Mode-Shift test. The sign test was not significant under any mutation model for any of the 11 breeding colonies analysed. The standardised differences test was significant under the SMM at six of the African Penguin Colonies: St Croix (P<0.0001) and Bird Island (P=0.008) in the Eastern Cape; Mercury Island (P=0.01) in Namibia; and Dyer Island (P=0.0004), Stony Point (P=0.00007) and Dassen Island (P=0.0001) in the Western Cape. The Wilcoxon one-tailed tests provide insight into whether heterozygote excess or deficiency is causing populations to deviate from mutation-drift equilibrium. The Wilcoxon 1-tailed test for heterozygote deficiency was significant under the SMM at St Croix Island (P=0.046) in the Eastern Cape and Dassen Island (P=0.039) in the Western Cape. Significant heterozygote excess was significant under the IAM at Ichaboe (P=0.03) and Mercury (P=0.03) Islands in Namibia. When considering these colony-level results along with the recent demographic histories of individual colonies (Figure 4.5), Dassen and St Croix Islands show the strongest genetic bottleneck signals, which may reflect the steep declines at those colonies. However, all colonies exhibited a normal L-shaped distribution in the Mode-shift test. The M-Ratio test, did not detect a recent decrease in population size overall. M_P_VAL produced an average M of 0.828, with 79% of 10 000 simulations showing a smaller ratio. CRITICAL_M generated a mean M of 0.825, which was larger than the critical M (M_C=0.751).

Table 4.17 Results of the regional BOTTLENECK analyses. If a test was significant under any mutation model (IAM, TPM or SMM), the mutation model is given, along with the associated probability. * indicates that the result is not significant for all tests of heterozygosity excess/deficiency.

	Sign test	Std. differences test	Wilcoxon test	Mode-shift
Eastern Cape	ns	SMM, P=0.0001	*SMM, P=0.03	normal
Western Cape	SMM, P=0.02	SMM, P=0.0001	*SMM, P=0.03	normal
Namibia	SMM, P=0.02	IAM, P=0.02 SMM, 0.003	*IAM, P<0.05 *SMM, P=0.046	normal

Figure 4.17 Unrooted UPGMA dendrogram of African Penguin colonies from all three breeding regions (Namibia in black, Western Cape in green and Eastern Cape in blue), based on Nei's (1983) D_A, calculated from a reduced dataset of 174 individuals and 11 loci - and excluding Possession Island - to eliminate missing data. Numbers at nodes are bootstrap values based on 1000 bootstrap replicates.

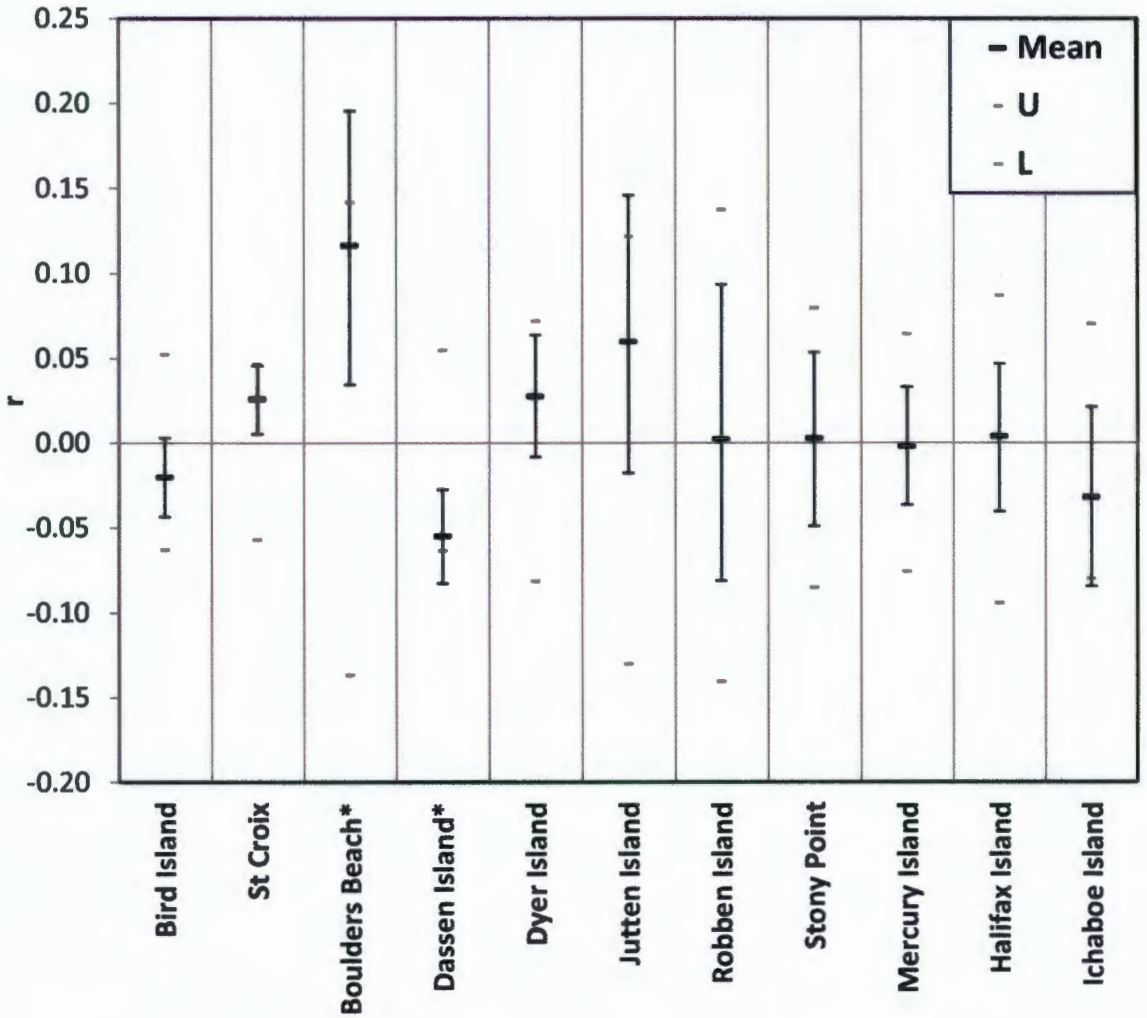


The sign test was not significant for any mutation model in the Eastern Cape, and it was only significant under the SMM in Namibia and the Western Cape (Table 4.17).The standardised differences test was significant under the SMM in all regions, and additionally under the IAM Namibia. The Wilcoxon test was not significant in the Eastern Cape, except for the one-tailed test for heterozygote deficiency under the SMM. In Namibia the Wilcoxon test showed mixed results, with the one-tailed test for heterozygote excess and the two-tailed test for

Relatedness within breeding colonies

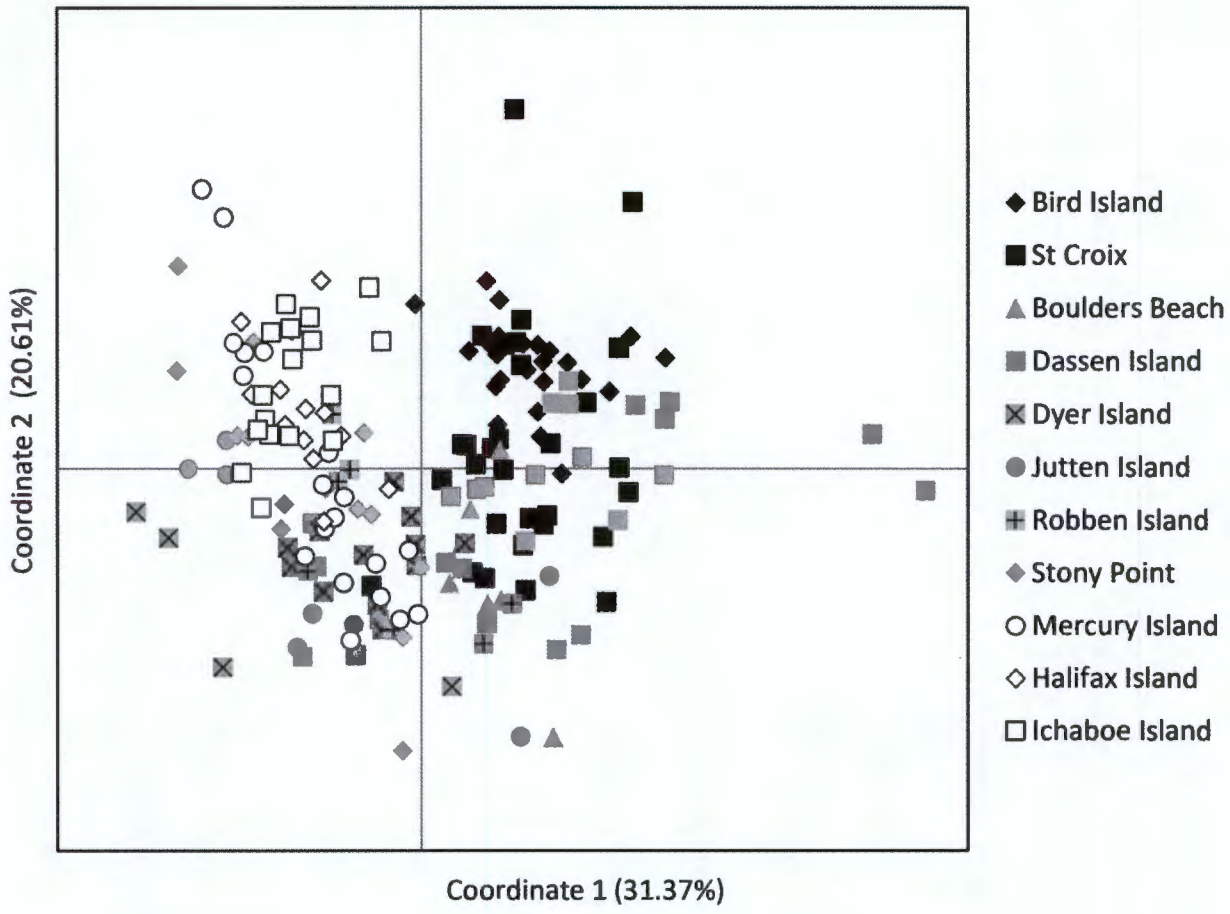
To assess the degree of relatedness within breeding colonies, mean within-population pairwise relatedness were calculated for each colony, and values are highest at Boulders Beach and lowest at Dassen Island (both in the Western Cape), and relatedness values at these colonies are significantly different from what would be expected if there was no difference across colonies (Figure 4.18). The high mean relatedness at Boulders Beach may be due to a founder event there (Figure 4.5), although that pattern is not evident in the other two colonies established in the 1980s (Stony Point and Robben Island). Ichaboe Island in Namibia, Dassen Island in the Western Cape, and Bird Island in the Eastern Cape have lower than expected mean pairwise relatedness values.

Figure 4.18 Mean within-population pairwise relatedness (r) values based on 10 000 permutations and 10 000 bootstrap replicates. Upper (U) and lower (L) confidence limits bound the 95% confidence interval for the null hypothesis of 'No Difference' across the populations as determined by permutation. Asterisks denote colonies with significant differences.



Interestingly, in the covariance-standardised Principal Coordinates Analysis (PCoA) based on the individual pairwise matrix of Queller and Goodnight's (1989) relatedness index (R), there is a spatial pattern that reflects the mean relatedness and demographic history of colonies to some degree (Figure 4.19). Historically large and recently expanding populations (e.g. Bird, St Croix and Dassen Islands) are separated from all colonies in Namibia along coordinate 1. Pairwise comparisons of individuals from Mercury Island are dispersed along coordinate 2, whereas all individuals from Dyer Island fall below the origin of that axis (Figure 4.19). Relatedness within all colonies shows a normal distribution (Appendix 4.29), with the majority of individuals exhibiting relatedness (R) values of 0 or 0.1. Only a few individuals from Mercury, Ichaboe, Boulders Beach and Jutten Island showed R-values >0.7 (Appendix 4.30).

Figure 4.19 Covariance-standardised Principal Coordinates Analysis (PCoA) based on the Queller and Goodnight's (1989) relatedness index (R) for individuals. Colonies located in the Eastern Cape are represented by black markers, those in the Western Cape are grey, and white markers represent Namibian colonies (Possession Island is omitted, n=184)



DISCUSSION

Even before reclassification as Endangered, the African Penguin has been the focus of numerous conservation research programmes. These studies have employed diverse methods in an attempt to understand the drivers and consequences of the long-term and recently steep population decline observed in this species (Crawford et al. 1995; Crawford 1998, 2004; Petersen et al. 2006; Pichegru et al. 2010a; Pichegru 2012; Sabarros et al. 2013). The conservation genetic approach used here is a powerful complementary method to contribute to a better understanding of patterns of connectivity, and the genetic consequences of population decline. The African Penguin population continues to decline overall, and this has ramifications for their prospects of survival into a future with uncertain climatic changes (Crawford et al. 2001; Merino et al. 2010; Rouault et al. 2010), where they will have to be flexible and adaptable in order to persist. Population genetic techniques have been employed in studies of numerous seabirds globally, including other penguin species, and provide a useful comparative framework in which to contextualise the plight of African Penguins among other species struggling to survive.

One of the primary goals of conservation genetics is to identify populations at risk based on the genetic variability or uniqueness of genetic variants among individuals that constitute those populations. Information from these studies can also provide insight into the connectivity between populations and inform decisions about management units and how to prioritise them.

Genetic diversity and genetic drift in declining populations

Genetic drift is the primary determinant of levels of genetic diversity in small populations, especially if those that are isolated i.e. small populations that are not genetically connected to other populations and gene-flow does not act to counter the effects of genetic drift (Wright 1943; Avise 2004; Spielman et al. 2004; Allendorf & Luikart 2007). Strong genetic drift acting on a population affect its evolutionary potential, through the fixation alleles and deleterious mutations (Wright 1943; Kimura 1968; Frankham et al. 2011). Population bottlenecks can also reduce genetic diversity if they result in a genetic bottleneck i.e. some gene variants are completely lost during a population decline (Kimura 1968), which may lead to inbreeding (Spielman et al. 2004; Frankham et al. 2011). It is therefore important to understand how these micro-evolutionary forces are affecting the declining populations of the

threatened African Penguin. It is in this context that the first hypothesis regarding genetic diversity and population size was posed - that African Penguin breeding colonies or breeding regions that support small, and/or reduced, populations will exhibit lower genetic diversity than larger, more stable populations as a result of strong genetic drift and genetic bottleneck effects. This prediction assumes that populations are isolated and, therefore, that genetic drift has played the dominant role in determining genetic diversity.

Deviations from HWE were observed in the overall population of African Penguins. This pattern was driven largely by Namibian colonies, together with Dassen Island in the Western Cape. An explanation may lie in the recent population declines (global and local), which would have allowed for strong genetic drift, especially at Namibian colonies. This interpretation is supported by genetic bottleneck analyses to some degree (discussed later), but there are also notable inconsistencies. Further evidence of the genetic effects of the Namibian population crash (see Chapter 3, Figure 3.1) is that that region exhibits the fewest alleles overall, the fewest rare alleles and the lowest heterozygosity levels. All colonies in that region also display positive F_{IS} values, indicating an excess of homozygotes, likely due to inbreeding effects. It is important to note here that the longevity of African Penguins, and the historically large effective population size, is highly likely to play a role in buffering populations against the loss of diversity. The two colonies with the lowest H_O in the Western Cape were established in the 1980s (Boulders Beach and Stony Point), but the third colony established at the same time (Robben Island), exhibited the highest H_O of all Western Cape colonies. Interestingly, recently founded populations were not consistently characterised by positive F_{IS} values, or marked differences in allelic richness, when compared to older colonies. Dassen Island, which supported the largest penguin colony in the Western Cape historically, and continues to do so, is the only population that exhibits private alleles in this region. Population estimates at St Croix Island, presently the largest African Penguin colony across their range, has seldom fallen below 5 000 pairs, and birds sampled there also exhibited private alleles. Overall, although there is some evidence of the effects of demographic history on genetic diversity within African Penguin populations, the genetic signal is weak and sometimes inconsistent with classic expectations from population genetic theory. Gene-flow, or genetic connectivity, is highly likely the dominant reason for this incongruence, as it counteracts and diminishes the effect of local genetic drift. The high degree of connectivity among populations has resulted in the homogenization of allele

frequencies, and stemmed the rapid loss of alleles associated with genetic bottlenecks and founder events.

The results reported here are interesting because they highlight a gap in our understanding about the dispersal ecology of African Penguins. This apparent paradox of high population connectivity in a philopatric species is likely explained by juvenile recruitment to non-natal colonies that would result in gene-flow among colonies and regions and buffer this species from the effects of genetic drift and local adaptation; this leads to the observed homogenization of genetic diversity such that any breeding region contains 100%, and any colony >89%, of the contemporary range-wide genetic diversity. This would also buffer populations against the impacts of localised changes in prey availability, and could be a part of their life-history that has evolved in response to the inherent variability in the ABE. Some other life-history traits of African Penguins may also buffer populations against reductions in genetic diversity resulting from depressed breeding success in response to sub-optimal local conditions e.g. longevity (long generation-times) and delayed age at first breeding, but the recent, steep population declines suggest that their flexibility is not sufficient to buffer populations against the rate of change in their environment i.e. the cumulative effects of natural variability, competition with fisheries, anthropogenic disturbance and pollution.

Three other *Spheniscus* species have similar life-history traits to the African Penguin, and are also threatened and declining in their respective ranges (Araya 1983; Luna-jorquera et al. 2000; Vargas et al. 2005; Bouzat et al. 2009; Boersma & Rebstock 2014). A second hypothesis regarding levels of genetic diversity among African Penguins relative to its congeners is also of conservation genetic interest. Based on current and historical population size, life-history traits and environmental influences, African Penguin genetic diversity is predicted to be intermediate between Magellanic and Galápagos Penguins, and similar to Humboldt Penguins. Allelic diversity conformed to this pattern, but interestingly, heterozygosity among African Penguins sampled was almost identical to that found among Magellanic Penguins – although Bouzat et al.'s (2009) study was based on only four microsatellite loci (Table 4.18). When comparing genetic diversity calculated from microsatellites between studies, it is important to consider the ascertainment bias that can result from investigators selecting the most polymorphic loci during primer development (Levin & Parker 2012), although some of the primers used in this study are also included in the population genetic studies of other *Spheniscus* penguins.

Fluctuations in effective population size (N_e) influence the strength of genetic drift and, therefore, the rate of loss of genetic diversity. Bird species located towards the slow end of the 'slow–fast' gradient of life histories, such as penguins, are better buffered against such demographic stochasticity i.e. the amplitude of fluctuations in N_e are minimised by the life-history traits of penguins (Milot et al. 2008). In the ABE, the life history traits of African Penguins appear to buffer the loss of genetic diversity, such that they have retained similar levels of heterozygosity as their more abundant sister-species, the Magellanic Penguin. But the behavioural flexibility and phenotypic plasticity of African Penguins may not be sufficient to prevent the effects of further reductions in N_e. Small populations are more susceptible to the effects of genetic drift, selection and inbreeding (Kimura 1968; Spielman et al. 2004; Allendorf & Luikart 2007). As populations get smaller, and genetic variation is lost, the species could theoretically enter an "extinction vortex" (Spielman et al. 2004; Blomqvist et al. 2010), which describes a potential reduction in fitness and population adaptability that results in increased mortality and decreased reproduction, and even smaller populations (Gilpin & Soule 1986).

Table 4.18 Current estimated population sizes of the all four *Spheniscus* penguins, their threat status (EN = endangered, VU = vulnerable, NT = near threatened), number of individuals and loci sampled, and the observed heterozygosity and allelic diversity among those samples (Data from: Nims et al. 2008; Schlosser et al. 2008; Bouzat et al. 2009).

Species	Population size	Number of loci	Sample size	Allelic diversity	H_O
African Penguin (EN)	21 000	12	189	6.5	0.6
Magellanic Penguin (NT)	1 000 000+	4	231	7.11	0.59
Humboldt Penguin (VU)	24 000	12	336	7.8	0.7
Galápagos Penguin (EN)	1 500	5	116	3	0.45

Population structure and connectivity

Microsatellite data was also used to test a number of hypotheses regarding population structure as a proxy for understanding connectivity among African Penguin populations i.e. if restricted gene-flow between breeding colonies or regions has resulted in population subdivision due to physical and/or non-physical barriers, and if so, whether population divergence shows a phylogeographic pattern. Allele frequency histograms (Appendices 4.14

and 4.15) reveal that common or dominant alleles are present at similar frequencies in most colonies and across regions. This basic finding suggests a general pattern of strong genetic connectivity between breeding regions and breeding colonies. The exchange of alleles between populations homogenises allele frequencies, and generally minimises the relative effects of both selection and genetic drift. Gene-flow also maintains and introduces polymorphism into populations, increasing local effective population size, and reducing the rate of fixation of alleles due to random changes in allele frequencies. This opposes the effects of random genetic drift and generates new allele combinations on which selection can potentially act (Balloux & Lugon-Moulin 2002).

Reliable estimates of population differentiation are crucial to understand the connectivity among populations and represent important tools to inform conservation strategies. Various estimates of population differentiation were employed to investigate patterns of structure among populations. While F_{ST} can provide the basis for a measure of genetic distance when divergence is caused by drift, other genetic distance measures have been developed specifically for microsatellites (Goldstein et al. 1995a; Goldstein & Pollock 1997; Balloux & Lugon-Moulin 2002). Genetic differentiation (as measured by fixation indices, standardised fixation indices and a differentiation index) was weak across the range of African Penguins, with the exception of only a few colonies. Neither spatially explicit, nor spatially independent analysis supported the hypothesis that significant population structure exists in this species i.e. that restricted gene-flow among populations has resulted in population divergence. The degree of gene-flow among populations is therefore assumed to have been sufficient to counteract genetic drift acting within populations. There was also no support that population structure followed a pattern of isolation-by-distance, although some measures of population differentiation were significant for colonies at the edges of the species' breeding range (e.g. Mercury Island and St Croix Island). It is difficult to identify the primary process producing genetically distinctive colonies due to incongruence among the results. It does, however, appear that colony-level processes are producing this pattern, although linking this to particular processes is difficult because it likely represents a complex suite of interactions, including demographic history, genetic drift, and gene-flow at particular colonies. There was also no consistent relationship between locus variability and the magnitude of estimates of population subdivision, which indicates that the mutation rates (reflected as locus polymorphism) do not affect their power to discriminate among samples.

All pairwise regional fixation indices, corrected fixation indices, genetic distance measures and pure indices of population differentiation were smaller than 0.01, and many values were significant ($P<0.05$), indicating an overall pattern of weak regional population structure. This pattern holds despite the corrected maximum possible values for G_{ST} and F_{ST} being <0.5 (~0.3 and ~0.4 respectively). Interestingly, this problem (F_{STmax} and G_{STmax}, and their analogues, $\neq 1$) does not affect R_{ST}, which appears to have better resolution, and detected larger significant levels of pairwise regional differentiation. The strongest divergence appears to be between the Western Cape and Namibia ($R_{ST}=0.04$, $P<0.05$), followed by the divergence between the Eastern Cape and Namibia ($R_{ST}=0.03$, $P<0.05$). Despite the low observed levels of divergence, the Eastern Cape and Namibia exhibit slightly higher connectivity than Namibia and the Western Cape overall, which might be as a result of recruitment from Namibia when pelagic fish stocks collapsed there in the 1960s and 1970s. However, ecological connectivity takes a long time to turn into, or become detectable, as genetic connectivity, and the time-frame may, therefore, be too short given the longevity of African Penguins. Physical barriers, demography and life-histories will all contribute to shaping the genetic structure of populations (Aguinagalde et al. 2005; Friesen 2007; Friesen et al. 2007; Bishop et al. 2009; Nishizawa et al. 2011). Because geographical distributions of many species are typically more extended than an individual's dispersal capacity, populations are often genetically differentiated through subtle IBD (Balloux & Lugon-Moulin 2002). Despite their flightlessness, this does not seem to be the case for African Penguins at the regional-level.

Estimates of population differentiation based on F_{ST} (and its analogues) and R_{ST} at the colony-level were consistently higher than at the regional-level, indicating that colony-level processes play a more important role in determining population structure than regional processes. Despite this pattern, overall estimates of F_{ST}, G_{ST}, $G'_{ST(NEI)}$, $G'_{ST(HED)}$, G''_{ST} and D_{EST} were not significant when populations were defined as breeding colonies. Colony-level F_{ST}-based AMOVA estimated that 1% of variation was found among colonies, but this value increased to 7–10% in an analogous R_{ST}-based AMOVA. Seven of the 11 pairwise colony comparisons of F_{ST} and R_{ST} involving Mercury Island were significant, with the next most distinct colonies being Robben Island, St Croix Island and Dyer Island based on that measure. The distinctiveness of these colonies could be due to genetic drift, but there is a possible role for selection i.e. local adaptation to some physical aspect e.g. the topography of the island, that decreases the fitness of immigrants. Mercury Island is probably distinctive due to

reduced gene-flow resulting from its peripheral location. The spatially explicit analyses of colony-level population genetic structure do not appear to show a strong geographic component, nor does it correlate consistently with particular signatures of demographic history. There is some significant structure present among the African Penguin colonies sampled, but it is likely the result of a combination of micro-evolutionary forces, such that one single dominant force does not shape the genetic diversity across the range of this species.

The results of spatially independent analyses corroborate those discussed above, with no strong spatial pattern emerging at either the regional- or colony-level, and clustering algorithms consistently identifying one population (K=1) as the most likely scenario. Patterns of relatedness within colonies, however, do exhibit spatial patterning, with closely related ("inbred") colonies clustering together; often these are colonies in close geographic proximity (Figure 4.19). Some colonies show lower than expected relatedness, possibly due to large historical population sizes and/or migration among colonies. It is also possible that the observed genetic admixture reflects a recent influx of migrant individuals originating from other colonies that are moving in response to deteriorating local environmental conditions. In this scenario, the distinct groups identified in the principal coordinates analysis based on individual genotypic distance (Figure 4.12) could represent historical structure.

Overall, the population genetic pattern observed among African Penguin colonies suggests that they can be viewed as a single genetic metapopulation i.e. spatially structured, interacting breeding sub-populations inhabiting areas of differential productivity, but that are strongly interconnected through migration over the long-term (Matthiopoulos et al. 2005; Bouzat et al. 2009). The genetic structure observed has been shaped by the complex interactions of gene-flow, colony demographic history, genetic drift and the ecological characteristics of the species. No single factor can be easily identified as the dominant driver or predictor of genetic diversity within populations, or of the genetic distinctiveness of populations.

Population bottlenecks

If a population has experienced a recent reduction in its Ne, it may exhibit a correlative reduction of allele numbers and heterozygosities at polymorphic loci (Luikart et al. 1998). Allelic diversity should be lost faster than heterozygosity, such that observed heterozygosity is greater than what is expected under mutation-drift equilibrium (Wright 1931; Cornuet & Luikart 1996; Peery et al. 2012). This heterozygote excess due to the rapid reduction in allelic

diversity compared to heterozygosity is often used as evidence of a recent reduction in population size. Interestingly, heterozygote excess would be more pronounced if gene-flow from other colonies also occurred and thus, both declining and recently recovered populations could show heterozygote excess (Barlow et al. 2011). Also, depending on the way in which a locus evolves, i.e. which mutation model it conforms to best, there are situations where this heterozygosity excess is not observed, despite recent population bottlenecks (Cornuet & Luikart 1996). Few loci are thought to follow the strict SMM, and when depart slightly from this mutation model and towards the IAM, they show the heterozygosity excess expected after a genetic bottleneck (Luikart et al. 1998; Williamson-Natesan 2005). Some of the loci employed in this study may fit different mutation models, but the strongest significant bottleneck signals were detected under the SMM (Table 4.17). At a regional-level, only Namibia showed significant evidence of heterozygote excess, where the Eastern (EC) and Western Cape (WC) exhibited signs of heterozygote deficiency.

Based on these tests conducted at the colony-level, the pattern of heterozygote excess in Namibia seems to be driven by Ichaboe and Mercury Islands. The Wilcoxon sign-rank test detected significant heterozygote excess at Dassen Island (WC) and St Croix Island (EC), possibly as a result of large historical Ne in the former, and immigration in the latter. However, the bottleneck results are not consistent across tests, so cannot be considered conclusive. These results could represent a type II error i.e. failing to detect a bottleneck when there was one (Williamson-Natesan 2005), or it is possible that the observed demographic bottleneck is too recent, or has not been severe enough to cause diversity to decrease substantially in the African Penguin. A population must usually experience an extreme contraction over a large number of generations for bottleneck effects to influence heterozygosity (Milot et al. 2008), but African Penguins have a long generation time, and population sizes at most of the colonies studied are in excess of 500 individuals. Contemporary Ne at most large colonies is likely high enough to reduce the power of bottleneck tests to detect population bottlenecks to below 0.4 (Cornuet & Luikart 1996; Peery et al. 2012). Ironically, it is likely that the same life-history traits that have buffered African Penguins against the loss of genetic diversity may also slow their recovery from a genetic bottleneck.

CONCLUSIONS

The data presented here provide an example of how genetic connectivity (i.e. gene-flow) via long-distance movement and recruitment by juveniles may minimise the potentially

deleterious effects of localised environmental change, although further indirect and direct measures of dispersal are required to better understand this process. The most urgent threats facing African Penguins at present are food availability and predation, but as colonies decline, it is likely that genetic threats will come into play, and are already detectable at some colonies. From a conservation genetic and conservation management perspective, the most important goal is to maintain as high a N_e as possible to minimise genetic drift and inbreeding, and avoid the 'extinction vortex'. Genetic monitoring is strongly recommended, especially at smaller African Penguin colonies. To this end, it may be better to conserve a few large colonies across the species range, rather than small colonies. Further research into adaptive genetic diversity is also recommended, as there may be important differences between regions and/or colonies at coding loci.

CHAPTER 5: Population genetic considerations on the conservation breeding and captive management of African Penguins *Spheniscus demersus*:

"What's the use of a fine house if you haven't got a tolerable planet to put it on?"

Henry David Thoreau (Familiar letters, p.416)

SUMMARY

The number of species to become extinct in the wild is likely to increase rapidly in the near future, as their natural habitats are degraded, fragmented and destroyed (Frankham 2008). Conservation breeding is increasingly part of management plans for endangered species, and has been suggested as a possible intervention to help slow the population decline of African Penguins. The main factor thought to be causing their rapid decline is a reduction in food availability and conservation action is urgently required to halt further decline. Supportive breeding through the captive rearing of wild-sourced chicks, and hatching of wild-sourced eggs, to be released into the wild has been attempted and shows some signs of success, but the effectiveness of this approach depends on effective monitoring, and the feasibility of raising and releasing sufficient numbers of chicks to buffer wild populations against current threats. Captive breeding in zoos and aquariums could provide the necessary numbers, but the potentially far-reaching genetic implications of such a strategy have not been investigated. This study compares genetic diversity at 12 microsatellite loci among 119 captive African Penguins from four captive **'source-populations'** to that among 189 wild individuals from 12 colonies distributed throughout their natural range.

All captive populations exhibited lower than expected heterozygosity, and each contained private alleles. Mean population relatedness was significantly higher than expected among all captive populations, and relatedness largely reflected what is known from studbook-based pedigree information. Wild African penguins exhibited 17 private alleles (only found in the wild), whereas captive birds only exhibited seven. Namibian and captive populations exhibited positive F_{IS}, whereas these were predominantly negative among Eastern Cape and Western Cape colonies. Spatially explicit and independent analyses confirm that structure among captive populations is stronger than among wild populations, and that there is significant, weak to intermediate population divergence between captive populations and wild populations. This pattern appears to be driven by genetic composition at the level of breeding colonies, rather than at broader regional scales. Although a large proportion of wild genetic diversity is represented among captive birds, there is some evidence that the negative genetic effects associated with captivity are influencing African Penguins held in captive institutions.

INTRODUCTION

Captive breeding has recently been suggested as a possible additional strategy to the multiple conservation interventions carried out to date in an attempt to reverse the population decline of the Endangered African Penguin *Spheniscus demersus* across its range in Namibia and South Africa (DEA 2013). The goal of conservation programmes is to ensure the survival of species or populations by reducing the threats thought to compromise their long-term persistence. In most cases, conservation interventions target threats to wild populations *in situ,* but pristine natural habitat available to many species is becoming increasingly limited due to direct or indirect human impacts; e.g. overexploitation, pollution and introduced predators or disease (Frankham 2008; Marsden et al. 2013). By providing a source of individuals to supplement or re-establish natural populations of many Endangered species, *ex situ* conservation breeding programmes may contribute to ensuring their long-term survival in the wild (Marsden et al. 2013). Over the last few decades, there has been an increasing trend in the number of management plans that employ elements of captive breeding or rearing in attempts to restore or bolster wild populations (Redford et al. 2011). The degree of intervention falls along a continuum from "lightly" to "intensively" managed and the classification of "wild" and "captive" populations is not necessarily binary (Redford et al. 2011). The risk of extinction increases with time for declining species, so implementing conservation actions sooner is better when trying to prevent population declines (D'Elia 2010). As their natural habitats are transformed or destroyed, it is inevitable that an increasing number of species will shift along this continuum and become more reliant on human intervention and require intensive demographic, health, and genetic management (Redford et al. 2011). Preparing healthy captive populations for reintroduction is a precautionary action that may ultimately save many species, but is also fraught with difficulties (Snyder et al. 1996; Fraser 2008). At least 25 animal species have so far been preserved in captive institutions after becoming extinct in the wild (Frankham 2008). In 2013, the IUCN Red List of Threatened Species (v.2013.1) classified 61 species as Extinct in the Wild (EW), and over ten thousand as either Endangered (EN, >6000) or Critically Endangered (>4000).

African Penguin conservation management interventions

The conservation status of the African Penguin has deteriorated in recent years and multiple conservation interventions have been instituted to ameliorate the effects of various threats that might be causal factors in the species decline (Crawford et al. 2011). Breeding colonies

of African Penguins are not all managed by a single conservation authority across their range in Namibia and South Africa, and as a result, most conservation-directed interventions have not been applied to all colonies. Artificial nests and burrows have been installed at some colonies, with varying effectiveness in terms of breeding success and chick survival (Sherley et al. 2012; Pichegru 2012). Several long-term studies investigating the effectiveness of reducing fishing near breeding colonies during the African Penguin breeding season have also been carried out i.e. to investigate the conservation benefits of Marine Protected Areas to seabirds breeding within them (Pichegru et al. 2010a; Crawford et al. 2011). Culling of Cape Fur Seals *Arctocephalus pusillus* and Kelp Gulls *Larus dominicanus* has been carried out at colonies where predation has been shown to impact on African Penguin survival and breeding success (Pichegru 2012). Predator-proof fencing has been erected at some mainland colonies to prevent predation by wild predators e.g. leopards *Panthera pardus*, Black-backed jackals *Canis mesomelas* and mongoose e.g. the Cape Grey mongoose *Galerella pulverulenta*. The effects of extreme weather events e.g. floods, storms and heat-waves are more difficult to manage, but an attempt has been made through the work of the "Chick Bolstering Project", a cooperative initiative that sees abandoned African Penguin chicks collected after extreme weather events, and taken to rehabilitation centres in South Africa. These chicks are hand-raised to a healthy weight and body condition before being released back into the wild. Late-season chicks are also collected by the relevant management authorities towards the end of the breeding season, when their prospects for survival are poor. An extension of this program is to hatch abandoned eggs collected at wild African Penguin colonies. The recently established "Chick-rearing Unit" based at the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB, Cape Town, South Africa) has begun incubating eggs abandoned in the wild in 2012 and hatching, then raising the chicks to be released. All captive-reared African Penguin chicks are fitted with a flipper-band, and more recently with GPS transponders in an attempt to track their dispersal and estimate survival and subsequent reproductive contribution to wild populations (Sherley et al. 2013). Preliminary Population Viability Analysis (Lacy 1993, 2000) models suggest that reintroduction at appropriate levels should improve the medium-term persistence of the Western Cape African Penguin colonies (analysed in isolation i.e. no dispersal from other regions), assuming relatively low levels of mortality among the released birds and a large captive population (approximately 15x larger than presently registered in the African regional studbook) (Harriet Davies-Mostert, October 2012, pers. Comm). Another management intervention that has been proposed is to attempt to establish a new breeding colony of

African Penguins on the south-eastern coast of South Africa, where food availability is thought to be higher than at most of their current breeding localities.

Despite all of these management strategies, numbers of African Penguins continue to fall at the majority of their breeding colonies. The conservation community has, therefore, begun to prepare formal guidelines for the establishment of a conservation breeding programme as part of the South African National African Penguin Biodiversity Management Plan (APBMP), with the aim of further supplementing wild populations. SANCCOB and the Two Oceans Aquarium (Cape Town, South Africa) have been releasing captive-bred African Penguins into the wild for a number of years, but the dispersal and reproductive success of these individuals, together with the genetic consequences of doing so, are unknown. *Spheniscus* penguins generally breed well in captivity and can likely provide a significant source of individuals to "bolster" natural populations, provided that individuals survive and reproduce in the wild. An expansion of current African Penguin conservation breeding activities may have a significant impact on wild populations, but captive populations must be appropriately managed to avoid possible adverse ecological and genetic changes that could jeopardise the ability of released birds to improve the conservation status of wild African Penguins (Gilpin & Soule 1986; Frankham 2008).

The genetic composition of captive African Penguin populations relative to wild populations is currently unknown, but if there are no significant differences between wild and captive birds at neutral markers, it is unlikely that the adaptive potential of captive populations has been affected (Frankham 2008; Williams & Hoffman 2009; Jamieson 2011; Witzenberger & Hochkirch 2011; Marsden et al. 2013). In such a case, and provided captive populations are carefully managed, the captive breeding and release of African Penguins might prove to be a useful component of a multi-faceted conservation strategy to ensure the continued survival of this species in the wild.

Conservation breeding

Captive breeding is being increasingly employed as a conservation management tool for Endangered species (Seddon et al. 2007; Fraser 2008; Witzenberger & Hochkirch 2011), and is more accurately referred to as 'conservation breeding' or 'supportive breeding' when its purpose is reintroduction (Wang & Ryman 2001; Allendorf & Luikart 2007). Unfortunately, bringing rare or endangered wild animals into captivity often has a negative effect on their survival and breeding success (fitness) when they are later reintroduced (Williams & Hoffman 2009). An estimated 11-13% of captive-sourced reintroduced populations

successfully re-established self-sustaining, viable populations in the wild (Beck et al. 1994; Fischer & Lindenmayer 2000) compared to 31% of wild-born translocations. Additionally, captive-born individuals are less likely to survive and reproduce in the wild compared to wild-caught individuals (Williams & Hoffman 2009). The re-introduction of wild-caught, hand-reared African Penguin chicks, those hatched in captivity from eggs collected in the wild, and captive-bred individuals from zoos and aquaria, may therefore have variable influence on fitness and genetic diversity in wild colonies (Wang & Ryman 2001).

Initially, in the 1990s, research into reintroduction biology was fragmented, *ad hoc* and often retrospective (Seddon et al. 2007). As a result of the failure of the majority of wildlife reintroduction attempts, more emphasis has been placed on gaining the knowledge needed to improve the success rate of reintroduction programs. In 1992, the IUCN's Reintroduction Specialist Group (RSG) began to formulate guidelines for wildlife reintroductions (IUCN Reintroduction Specialist Group 1998) amid growing concerns that many reintroduction attempts were ill-conceived and were unlikely to benefit the target species (Snyder et al. 1996). The RSG has since been encouraging stronger, better-planned research and monitoring components of captive breeding programs, in order to study the variables that are important for a successful outcome. Post-release monitoring is often neglected or poorly planned because, although reintroductions initially generate publicity when animals are being moved and released, the subsequent fates of reintroduced animals often attract less attention (Seddon et al. 2007). The limitations of captive breeding for reintroduction are dealt with in detail by Snyder et al (1996), and, more recently, some authors have raised specific concerns regarding genetic diversity in captive populations and adaptation to captivity (Araki et al. 2007; Frankham 2008; Fraser 2008; Williams & Hoffman 2009; Witzenberger & Hochkirch 2011; Christie et al. 2012).

Population genetics and conservation breeding

Conserving genetic diversity within species is a prominent goal of conservation biology (Moritz 2002; Frankham 2005), and the tools of population genetics have great potential to inform conservation planning in South Africa (e.g. see discussion of its role in marine protected area planning in South Africa in: von der Heyden 2009). Conserving evolutionary patterns of genetic diversity, and the processes that have generated them, should be prioritised because maintaining genetic diversity will maximise the potential of populations to evolve to cope with environmental changes by providing the raw material for natural selection to act on (Lande 1988; Lande & Shannon 1996; Fraser 2008; Chevin et al. 2010).

As discussed in Chapter 4, genetic variation enhances the probability of population persistence, and its adaptability, in a changing environment because the mean phenotype tracks the moving optimum increasingly closely (Lande & Shannon 1996). For species that cannot shift their geographic distribution to where conditions are optimal (such as African Penguins), the maintenance of additive genetic variation and the associated adaptive potential may well be critical to their survival (Lande & Shannon 1996).

Captive populations of Endangered species are often necessarily small and are often established by only a few founders, which can result in the loss of genetic diversity, and also inbreeding depression and the accumulation of deleterious mutations (Woodworth et al. 2002; Frankham 2008). Genetic diversity is lost rapidly in small, isolated populations, either passively through genetic drift or actively as a result of selection (Amos & Harwood 1998; Amos & Balmford 2001; Blomqvist et al. 2010; Jamieson 2011). In addition to these changes, adaptation to captivity can also occur, which may reduce an animal's ability to persist once released into the wild (Swinnerton et al. 2004; Araki et al. 2007; Frankham 2008; Hedrick & Fredrickson 2008). Genetic adaptation to captivity is caused by selection in the captive environment that favours genetic variants that are different to those favoured under natural conditions (Frankham 2008; Williams & Hoffman 2009). This raises an issue which has been shown for multiple species: characteristics selected for under captive conditions are often disadvantageous in the natural environment, and adaptation to captivity reduces reproductive fitness in the wild, thereby contributing (among other factors) to the low success rate of reintroduced animals (Frankham 2008). The exact mechanisms involved in lowering the fitness of re-introduced individuals are not well understood, but hypotheses include the environmental effects of captive rearing, inbreeding among close relatives, relaxed natural selection, and unintentional domestication selection i.e. genetic adaptation to captivity (Frankham 2008; Williams & Hoffman 2009; Witzenberger & Hochkirch 2011; Christie et al. 2012). These negative genetic factors may amplify the effects of demographic declines in the wild and increase the extinction risk of populations (Gilpin & Soule 1986; Brook et al. 2002; Ballou et al. 2010; Blomqvist et al. 2010). A number of studies have investigated ways to reduce the effects of negative genetic factors through careful captive management (Williams & Hoffman 2009; Jamieson 2011). Current global awareness of the importance of conserving biological diversity has meant that genetic monitoring is becoming more common for many species, and especially threatened species in zoos and aquariums (Witzenberger & Hochkirch 2011; Jansson et al. 2013). Without financial impediments, molecular methods could be used to accurately quantify genetic diversity among captive populations (including the founders in

an ideal situation), and continually monitor individuals over multiple generations. This information could feed into management plans so that the retention of all the founder genetic diversity could be maximised (Ballou et al. 2010).

Globally, the focus of zoos and aquariums has shifted from collecting and exhibiting exotic animals, to actively contributing to conservation, research and public education (Diebold et al. 1999; Witzenberger & Hochkirch 2011). Zoos and aquariums are becoming centres for *ex situ* conservation by breeding species outside their natural habitat, and without their input, a number of mammalian and avian species would be extinct in the wild (D'Elia 2010). On an increasingly transformed planet, the harsh reality is that captivity may be the only ''habitat'' left for some species in the future (Gibbs et al. 2008). In South Africa the majority of zoos, aquariums and rehabilitation centres are members of the World Association of Zoos and Aquariums (WAZA) or the African Association of Zoos and Aquaria (PAZAAB) and are increasingly contributing to conservation efforts and conservation research. In managing their captive populations, especially if the intention is future reintroductions, these institutions should aim to preserve the maximum genetic variability for a species, and minimise that species' adaptation to the captive environment.

Captive populations should ideally preserve as much 'wild' genetic diversity as possible, and assessing current levels of 'wild' genetic diversity provides a baseline against which the success of captive management at slowing unwanted evolutionary change can be measured (Lacy 1987, 2009). Unfortunately, very few published studies compare levels of genetic diversity in captive and wild populations, and most are based on neutral rather than adaptive variation (Marsden et al. 2013). The genetic composition of wild and captive populations can be quantified based on the alleles present at different loci for a representative sample of individuals, and the distribution of those alleles among individuals. Common metrics are 'allelic diversity,' which has been shown to be linked to a population's long-term adaptability, and heterozygosity, which is important for more immediate individual health (Allendorf & Phelps 1981; Ferson & Burgman 2002; Ballou et al. 2010). Allelic diversity is lost faster than heterozygosity in small, declining, isolated populations, but heterozygosity is lost at a similar rate to additive quantitative variation (those traits that are related to the overall fitness of individuals) and is therefore a reflection of population adaptability (Lande & Shannon 1996). As a result, management plans that aim to maintain heterozygosity will concurrently maintain additive genetic diversity (Ballou et al. 2010), although the value of unique alleles should not be disregarded (Vrijenhoek 1991; Allendorf & Luikart 2007).

Considerations pertaining to captive population size and diversity by and large do not impact the 'bolstering' work (i.e. the collection, rehabilitation, and release of eggs and chicks from wild colonies), but do need to be taken into account if birds born and raised in captivity over multiple generations are to be released into the wild. The purpose of supplementing wild African Penguin populations is not primarily to prevent the imminent extinction of declining colonies, but rather to maintain the genetic diversity and fitness at breeding colonies until the threats to them are better understood, and action has been taken to ameliorate them such that colonies can again be stable and self-sustaining (Fraser 2008). It follows that there must be a reasonable amount of certainty that released individuals will establish, survive and reproduce in the wild, and not have a negative impact on the wild population in any way. Chapter 4 of this thesis quantified genetic diversity and population structure among wild African Penguins, and found that their life-history traits seem to have buffered the species against the loss of genetic diversity to a large extent, despite recent population declines. Although there is variation across their range, the overall population sizes at colonies, and the strong genetic connectivity among most of them, appear to have also ameliorated the negative effects of inbreeding (Brook et al. 2002). Unfortunately, numbers in the wild are still declining, and the genetic risks associated with small population sizes are likely to impact African Penguins in the future (Spielman et al. 2004).

Pedigree- versus molecular genetic analyses

In this chapter, neutral genetic markers are used to investigate the degree of similarity between captive and wild populations, so as to minimise the chances that captive-bred birds are genetically affecting fitness in wild populations upon reintroduction. Ideally, such an analysis would be based on a large suite of neutral and adaptive genes (those directly involved in survival and reproduction), but in many cases the genes involved are unknown and molecular analyses that measure diversity at the individual level across the entire genome are not feasible. Managing populations based on the diversity of a few neutral markers will not preserve diversity across the entire genome; this is because high diversity will only be maintained at the monitored loci and may be lost at others (Ballou et al. 2010). However, managing genome-wide genetic diversity has been attempted using pedigree data i.e. when pedigrees are known, kinships can be calculated for all individuals, as can inbreeding coefficients, which provide genome-wide estimated or average levels of diversity in individuals relative to the source population. Genetic management using these methods has been shown to be effective at maintaining genetic diversity (Fernández et al. 2005; Oliehoek

et al. 2006; Pemberton 2008; Ivy & Lacy 2012). The main difference between molecular- and pedigree-based methods for conserving genetic diversity is that the former provides empirical estimates of real levels of diversity at relatively few loci, where the latter provides a statistical measure of average genome-wide diversity relative to the founder population. If the goal is to preserve genetic diversity of the founder population, then pedigree-based methods will be effective (Ballou et al. 2010), but if the goal is reintroduction, a captive population should be as similar to the wild population as possible, and molecular-based methods are required.

Pedigree analyses apply Mendelian principles to generate theoretical genetic estimates from pedigree relationships among individuals, and have been carried out for African Penguins based on the African regional studbook data (Tracy Shaw, National Zoological Gardens, pers. Comm.). Based on the pedigree structure, genome-wide parameters e.g. mean heterozygosity, probabilities of shared alleles between individuals, probabilities of allele loss, and gene diversity or other estimates of diversity can be estimated (Fernández et al. 2005; Ivy et al. 2008; Caballero et al. 2010). Molecular genetic analyses quantify genetic diversity directly, albeit at a limited number of loci. The empirical (molecular genetic) and theoretical (pedigree) approaches differ in their goals and in the way they characterise genetic structure, but they can be complementary. Pedigree analyses assume (in the absence of information about wild populations) that the starting (founder) population is genetically healthy, and estimates how quickly and how much the population has diverged from that starting baseline (Lacy et al. 1995). The recommendations from pedigree analyses aim to minimise further genetic 'decay' by manipulating breeding pairs in an attempt to stop undesired evolution in captive populations. The molecular genetic approach, however, allows for a comparison of genetic diversity in wild and captive populations i.e. the baseline is the wild population, not the founding captive population. Both approaches can contribute to a genetic management plan for captive African Penguins.

Until more is known about the impact of introducing captive-bred birds into the wild, the feasibility of an African Penguin captive breeding program will remain uncertain. To this end, the present study addressed a number of questions regarding captive African Penguin populations: (i) How different are wild and captive populations, and what proportion of 'wild' genetic diversity is represented in captivity? (ii) Are there clear patterns of genetic relatedness in the dataset that can be explained by studbook pedigrees? And (iii) is there any evidence that genetic drift is influencing captive populations to a greater extent than wild populations? To answer these questions, levels of genetic diversity were investigated among

four South African captive populations, and compared to wild colonies and regions. Relatedness and inbreeding coefficients were estimated, and compared to analogous estimates from the South African studbook data. And lastly, population structure was investigated among captive African Penguin populations for comparison with wild populations, and divergence between wild and captive populations was estimated. The relevance of these results to the establishment of a conservation breeding program for African Penguins is discussed.

MATERIALS AND METHODS

Sample collection

A total of 119 African Penguins from four captive institutions were genotyped at 12 microsatellite loci for comparison with the wild birds (n=189) analysed in Chapter 4. All sample collection and genotyping methods are as previously described (Schlosser et al. 2003; Labuschagne et al. 2013). Appendix 5.1 gives the details of the captive individuals included in this study. A dataset comprised of only captive African Penguin samples was used for part of the analyses (n=119), and a second combined dataset consisting of both wild and captive African Penguins (n=308) was used to determine what proportion of 'wild' genetic diversity is currently 'secured' in captivity, and how genetically distinctive captive populations are from wild populations. Pedigree data was extracted from the fourth Edition of the African Penguin Studbook (Tracy Shaw, National Zoological Gardens, Pers. Comm. 2013). The studbook uses the Single Population Analysis and Record Keeping System (SPARKS, v.1.6) and PM2000 (Lacy et al. 2002). The first data entry for the regional African Penguin Studbook is 1980 and the data presented in this chapter is current through to 1 March 2012.

Microsatellite analysis

The genotype data for 12 loci from all 119 wild African Penguin samples were analysed in MICROCHECKER Version 2.2.3 (Van Oosterhout et al. 2004) to check for mistyped allele sizes and deviations from the specified repeat motif for each locus, and to check for null alleles and heterozygote deficiency among loci in each population. The genotype data for captive African Penguins was analysed separately from, and also in combination with, wild African Penguin data (n=308). All microsatellite-based analyses were conducted using the same software and parameter settings as described in Chapter 4. Because multiple comparisons were involved, correction against type I error was made with the Benjamini-Yekutieli (B-Y) method (Narum 2006). The B-Y corrected critical α-value for 120 comparisons was 0.009).

Two STRUCTURE (Pritchard et al. 2000) analyses were run: the first based only on the 119 captive African Penguins from four captive institutions, and the second based on a combined data set comprised of these captive birds and 189 wild-caught African Penguins (n=308, 16 'populations'). For the first analysis, burnin was set to 100 000, with 1 million MCMC iterations repeated 20 times for each value of K (K=1 to K=6, two more than the number of 'populations'). For the second analysis, burnin was also set to 100 000, with 250 000 MCMC iterations repeated 20 times for each value of K (K=1 to K=18). No prior information about collection locality was included and all other settings were left as default. The STRUCTURE results were analysed using STRUCTUREHARVESTER (Earl & VonHoldt 2011), to select the most likely value of K for both analyses (Figures 5.11 and 5.12).

RESULTS

Deviations from Hardy-Weinberg Equilibrium (HWE)

When captive African Penguins were grouped according to their captive institution (four populations, n=119, 12 loci), only one locus deviated from HWE among birds from the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB; B3-2, $P<0.05$), whereas two loci were monomorphic (B3-2 and PNN05) and another deviated from HWE (PNN03, $P<0.001$) among birds from the Two Oceans Aquarium (TOA). In the National Zoological Gardens (NZG) population, two loci deviated from HWE (SH2CA21 and B3-2, $P<0.01$) and one was monomorphic (PNN05). African Penguins from uShaka Marine World showed the strongest deviation from HWE, with eight of the 12 loci deviating significantly (G2-2, SH1CA9, G3-6, PNN12, $P<0.001$; PNN09, $P<0.01$; SH2CA21, B3-2, PNN03, $P<0.05$). Overall, only NZG ($P<0.002$) and uShaka ($P< 0.00001$) deviated significantly from HWE. A global test for heterozygote deficiency based on this dataset returned a highly significant result ($P=0.0002$), though this seems to be driven by the uShaka population, which was the only captive population to exhibit significant heterozygote deficit ($P<0.00001$). Neither global nor population-level tests returned significant results for heterozygote excess. When populations were defined as breeding regions (Eastern Cape, Namibia, Western Cape and 'captive'), there were no significant deviations from HWE in the Eastern Cape, two loci deviated from HWE in Namibia, three in the Western Cape, and five among captive African Penguin samples.

Genetic diversity within captive African Penguin populations

The lowest H_O was found among individuals housed at the NZG (H_O=0.52), despite the mean sample size per locus being one of the highest. TOA and uShaka exhibited intermediate observed heterozygosity (among captive populations), and SANCCOB had the highest. All captive populations exhibited lower than expected heterozygosity. SANCCOB also exhibited the highest mean allelic richness (AR=4.75), followed by TOA and uShaka, with NZG birds exhibiting the lowest (AR=4.33, 4.24 and 3.9 respectively). All captive populations exhibited unique alleles (private alleles, Table 5.1); i.e. not found any other captive institutions. Twenty-three individuals from uShaka exhibited at least one of the five private alleles, and the same was found for six individuals from SANCCOB, two from TOA and three from NZG. Appendix 5.2 lists the individuals from each captive institution that exhibited private alleles (i.e. those alleles not found in the wild population).

Table 5.1 The frequencies of private alleles found in each captive population (SANCCOB = Southern African Foundation for the Conservation of Coastal Birds, TOA = Two Oceans Aquarium, NZG = National Zoological Gardens and uShaka = uShaka Marine World).

Captive institution	Locus	Allele	Frequency in captive population
SANCCOB	SH1CA9	126	0.063
	SH1CA9	140	0.063
	G3-6	263	0.031
	PNN03	386	0.031
TOA	SH1CA9	144	0.083
	PNN08	145	0.083
NZG	G3-6	279	0.041
uShaka	SH1CA9	116	0.019
	B3-2	301	0.123
	G3-6	261	0.048
	PNN09	376	0.047
	PNN12	244	0.019

Comparison of genetic diversity in wild and captive African Penguin populations

H_O and H_E among all wild African Penguins (n=189; 0.60±0.07 and 0.61±0.07 respectively) and all captive African Penguins (n=119; 0.57±0.06 and 0.62±0.06 respectively) were similar, but the fixation index among captive birds (F_{IS}=0.08±0.04) was more than double that of wild birds (F_{IS}=0.03±0.01). Also, the patterns of genetic diversity composition and distribution appear to be different at regional- and colony-scales. Among wild colonies of

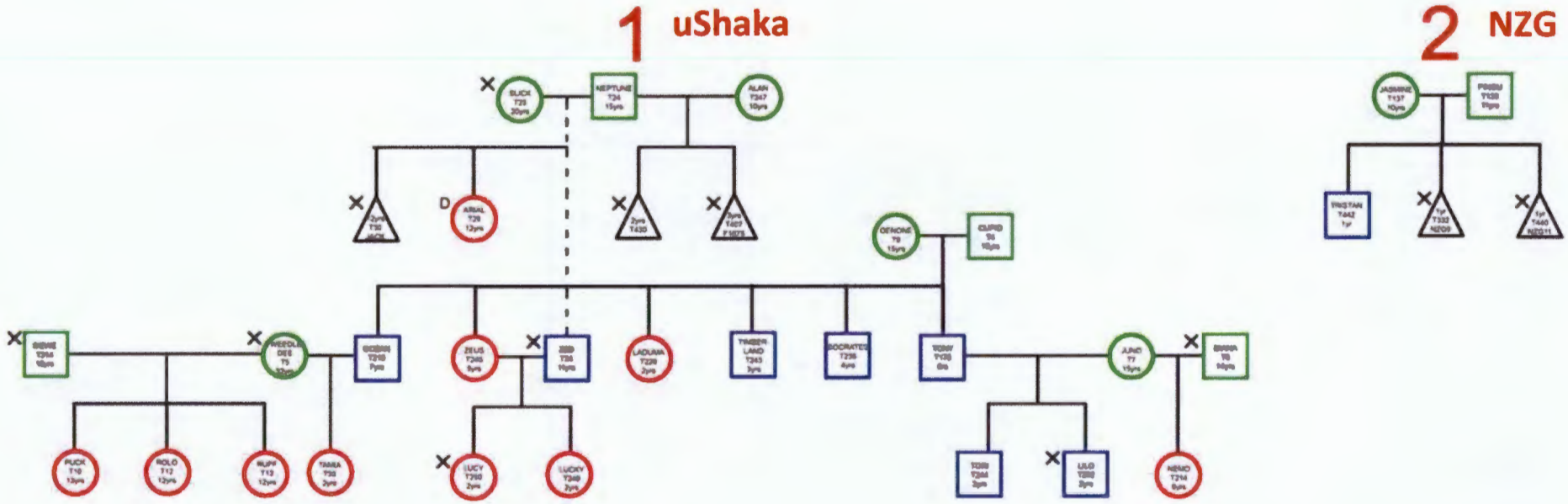
African Penguins, mean colony H_O ranged from 0.56 in Namibia to 0.62 in both the Western Cape (WC) and Eastern Cape (EC). The mean H_O across captive populations was more similar to that found among Namibian colonies (captive H_O=0.58). Both colonies in the EC exhibited higher than expected heterozygosity, as did the majority of WC colonies, which is reflected in their negative F_{IS}-values (Table 5.2). All Namibian and captive populations showed positive F_{IS} values, which were significant at Mercury Island, Halifax Island, and among birds from uShaka. Given the putative relationships among individuals sampled at uShaka (Figure 5.1), this may indicate roles for inbreeding and genetic drift at some Namibian colonies. Elevated numbers of alleles and heterozygosity might be expected from the SANCCOB 'population' because all the birds kept at that rehabilitation facility are wild-caught birds i.e. they are not thought to be closely related to one another. SANCCOB's birds are also predominantly from the Western Cape, because that is where the rehabilitation centre is situated. uShaka had the highest sample size of all populations sampled, but only 17 of these individuals had wild parents, whereas 11 NZG birds included in this study had wild parents.

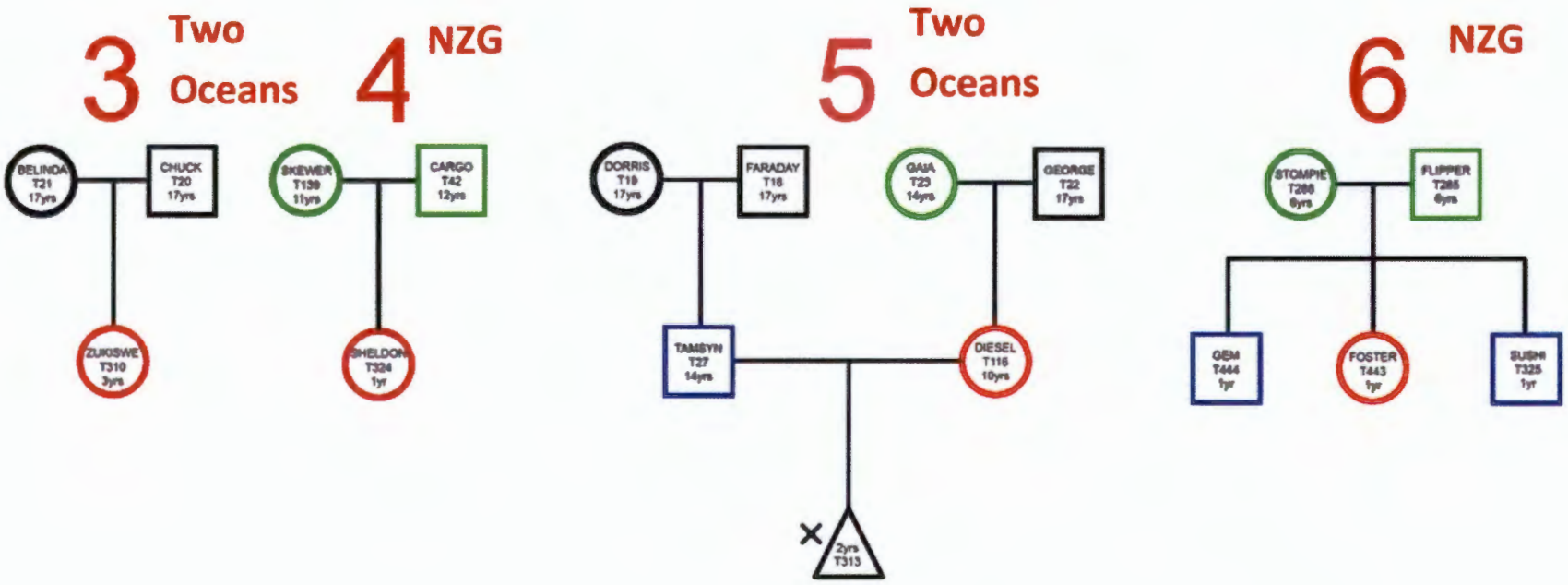
The pedigrees based on studbook data (Figure 5.1) show the putative relationships among samples, and also inform how genetic results are interpreted. 93% of the captive African Penguins sampled from NZG, uShaka and TOA fall into 12 families. The two biggest families are family 1 from uShaka, and family 12 from NZG. A list of the captive African Penguins included in this study, their studbook numbers and the institutions where they were housed at the time of sampling are given in Appendix 5.1. Some birds from NZG have been moved to Bayworld Aquarium (East London, South Africa) since the time of sampling. Regional-scale allelic patterns observed among the four African Penguin breeding regions, i.e. three wild breeding regions, and one 'region' comprising all captive birds, are presented in Appendix 5.3. When captive and wild populations are sorted in ascending order of observed heterozygosity (Figure 5.2), Namibian colonies, captive institutions and recently founded populations clearly represent populations depauperate in genetic diversity. Eastern Cape and Western Cape populations (including SANCCOB) appear generally more diverse.

Table 5.2 Average sample size (N), number of alleles per locus (N_A), number of effective alleles (N_{AE}), observed (H_O), expected (H_E) and unbiased (uH_E) heterozygosities, and the inbreeding coefficient (FIS) based on 12 microsatellite loci. Birds from 12 wild African Penguin colonies and 4 South African captive institutions are included (n=308). Significant values are indicated in bold. SANCCOB = Southern African Foundation for the Conservation of Coastal Birds, TOA = Two Oceans Aquarium, NZG = National Zoological Gardens and uShaka = uShaka Marine World.

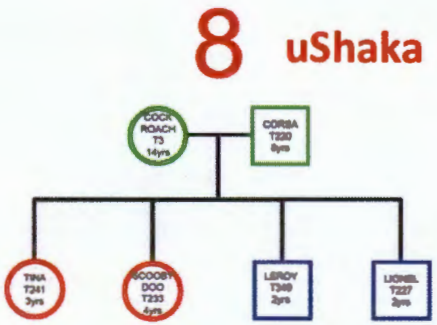
	Colony / institution	Mean N	N_A	N_{AE}	H_O	H_E	uH_E	F_{IS}
CAPTIVE	SANCCOB	15.9	5.2	3.1	0.63	0.62	0.64	0.010
	TOA	11.9	4.4	3.0	0.57	0.57	0.59	0.038
	NZG	36.8	5.0	2.6	0.52	0.54	0.55	0.053
	uShaka	53.3	5.4	3.0	0.59	0.62	0.63	**0.054**
	Average	29.5	5.0	2.9	0.58	0.59	0.60	0.039
EASTERN CAPE	Bird Island (BI)	25.3	5.4	3.1	0.62	0.6	0.61	-0.011
	St Croix Island (SC)	30.8	5.6	2.9	0.61	0.59	0.6	-0.014
	Average	28.1	5.5	3.0	0.62	0.60	0.61	-0.013
WESTERN CAPE	Boulders Beach (BOU)	6.9	3.5	2.5	0.60	0.53	0.58	-0.038
	Dassen Island (DAS)	24.1	6.3	3.5	0.62	0.64	0.65	0.047
	Dyer Island (DYE)	15.8	5.3	3.0	0.62	0.59	0.61	-0.019
	Jutten Island (JUT)	7.9	3.7	2.6	0.63	0.53	0.57	-0.119
	Robben Island (ROB)	6.8	4.2	3.2	0.65	0.58	0.62	-0.043
	Stony Point (SP)	15.4	5	2.8	0.58	0.58	0.6	0.032
	Average	12.8	4.7	2.9	0.62	0.58	0.61	-0.023
NAMIBIA	Mercury Island (MER)	18.4	5.3	3.1	0.53	0.6	0.62	**0.141**
	Possession Island (POS)	2	2.3	2.0	0.54	0.43	0.57	0.071
	Halifax Island (HAL)	12.8	4.8	3.1	0.55	0.58	0.61	**0.097**
	Ichaboe Island (ICH)	16.1	5.1	3.2	0.6	0.59	0.61	0.019
	Average	12.3	4.4	2.8	0.56	0.55	0.60	0.082

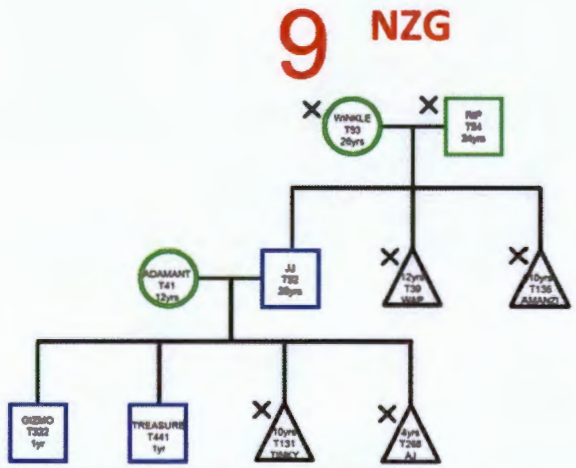
Figure 5.1 Captive African Penguins samples that fall into one of 12 families based on studbook data. Some of the birds included in this study had wild parents and have not bred successfully and will, therefore, not be represented in the pedigrees below (e.g. T217-T219, 'Basil' and 'Bently', all from uShaka). Also the offspring of some birds have all been released into the wild, so have not contributed to the pedigree. All birds from SANCCOB have wild parents, and are therefore not included. 95 of the remaining 103 samples fall into 12 families. Families 1, 7, 8, 10 and 11 are comprised of uShaka birds; families 2, 4, 6, 9 and 12 contain all samples from the NZG; families 3 and 5 represent all Two Oceans birds except for two ('Neptune' – who was transferred from uShaka and had contributed to family 1, and his partner 'Alan' who was transferred from SANCCOB – whose offspring have all died or been released). Black crosses adjacent to symbols mean that samples were unavailable, so those individuals are not included in this study. Red circles represent known females, blue squares are males and triangle unknown sex. Individuals with wild parents are in green. The letter D signifies 'deceased'.

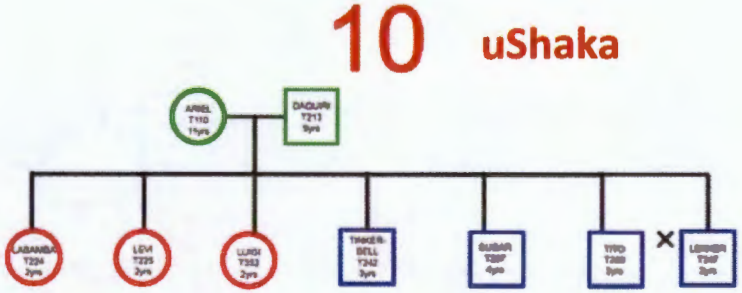


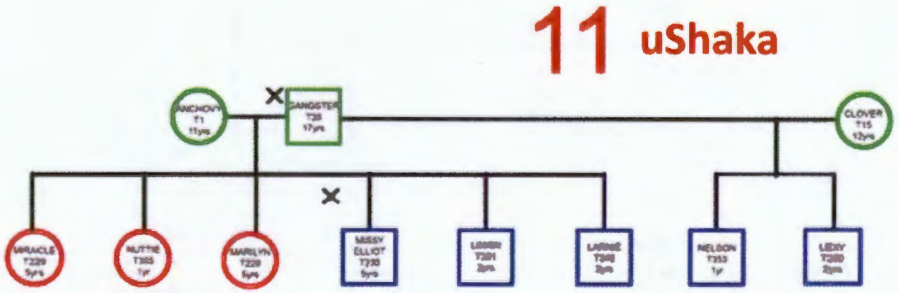

3
Two Oceans
4
NZG
BELINDA T21 17yrs
CHUCK T20 17yrs
ZUKISWE T310 3yrs
SKEWER T139 11yrs
CARGO T42 12yrs
SHELDON T324 1yr
5
Two Oceans
DORRIS T19 17yrs
FARADAY T16 17yrs
GAIA T23 14yrs
GEORGE T22 17yrs
TAMSYN T27 14yrs
DIESEL T116 10yrs
2yrs T313
6
NZG
STOMPIE T288 6yrs
FLIPPER T285 6yrs
GEM T444 1yr
FOSTER T443 1yr
SUSHI T325 1yr


7 uShaka
PEPPER T316 27yrs
TEABAG T315 26yrs
PEANUT T9 13yrs
RASCAL T11 13yrs
SLIMER T17 12yrs
TEDDY T239 3yrs
MALAIKA T231 4yrs
SNOOP DOG T235 4yrs
NONU T364 1yr


8 uShaka
COCK ROACH T3 14yrs
CORSA T230 6yrs
TINA T241 3yrs
SCOOBY DOO T233 4yrs
LEROY T349 2yrs
LIONEL T227 2yrs


9 NZG
WINKLE T93 26yrs
RIP T84 26yrs
ADAMANT T41 12yrs
JJ T82 26yrs
12yrs T39 WAR
10yrs T136 AMANDI
GIZMO T322 1yr
TREASURE T441 1yr
10yrs T131 TRACY
4yrs T268 JJ


10 uShaka
ARIEL T110 11yrs
DAQUIN T213 9yrs
LABAMBA T234 2yrs
LEVI T235 2yrs
LUIGI T383 2yrs
TINKER BELL T242 3yrs
SUGAR T237 4yrs
TYPO T369 5yrs
LERNER T367 2yrs


11 uShaka
ANCHOVY f1 11yrs
GANGSTER T39 17yrs
CLOVER T15 12yrs
MIRACLE T229 5yrs
NUTTIE T365 1yr
MARILYN T229 5yrs
MISSY ELLIOT T233 5yrs
LERNER T391 2yrs
LARNE T249 3yrs
NELSON T353 1yr
LENNY T260 2yrs

12 NZG

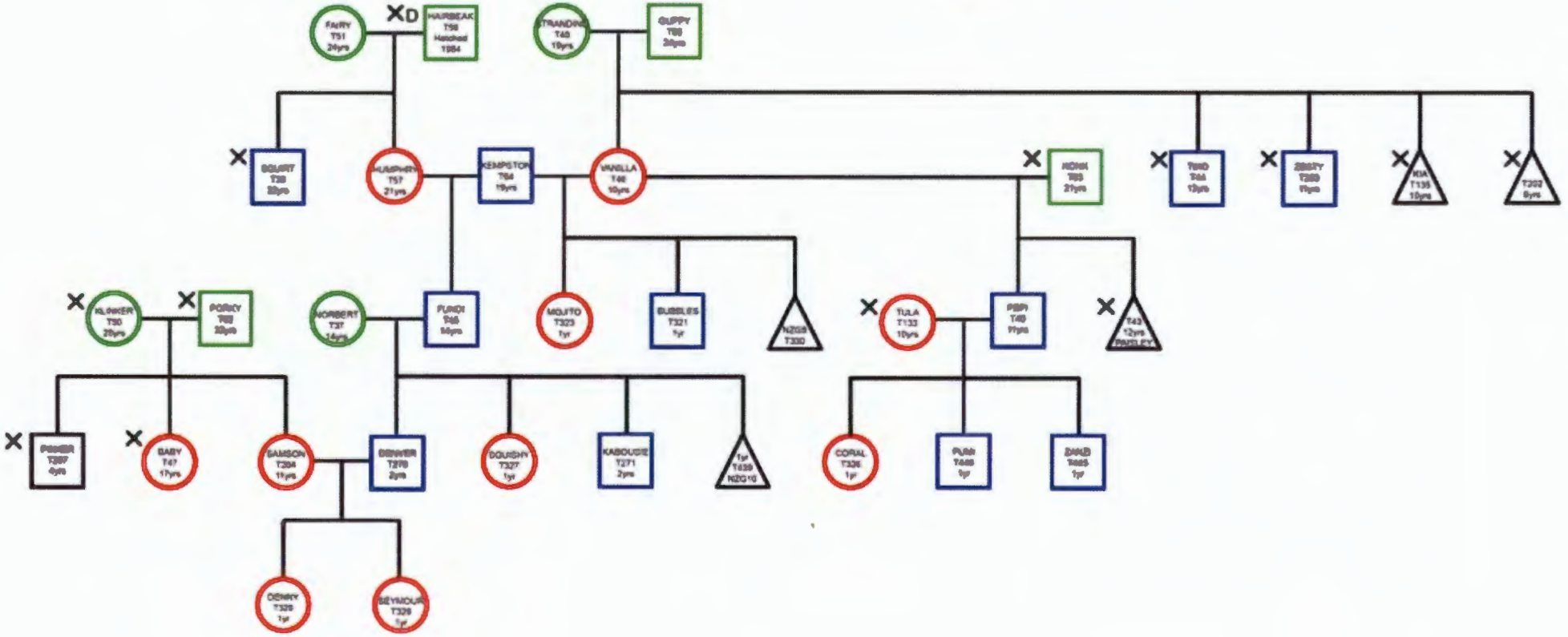
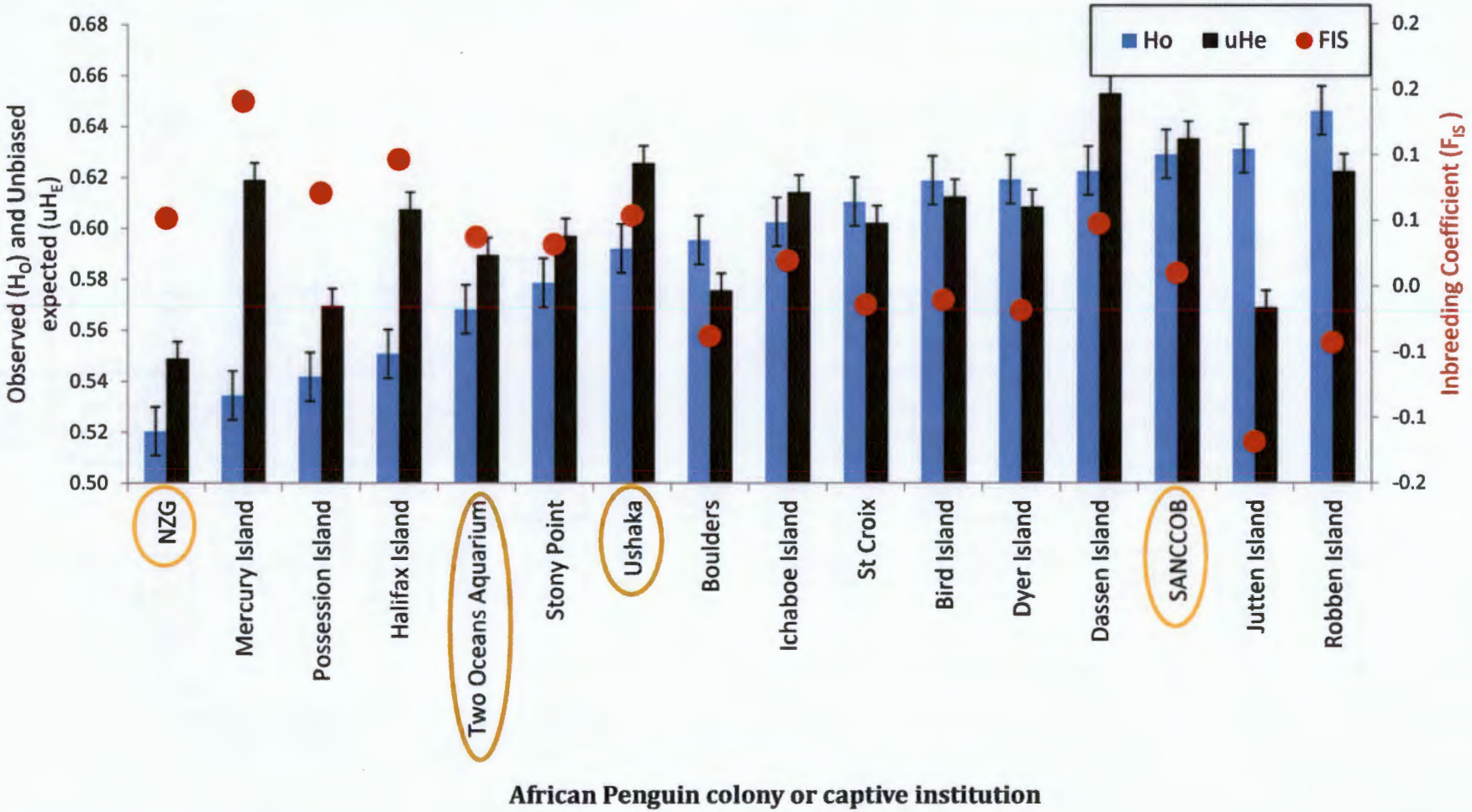


Figure 5.2 Observed and expected heterozygosities, and the associated F_{IS} values for each of the wild colonies sampled, and for four captive institutions (Circled: SANCCOB, National Zoological Gardens (NZG), uShaka Marine World and the Two Oceans Aquarium; error bars are standard error).



Private alleles

When populations were simply defined as 'wild' and 'captive', the wild population exhibited 17 private alleles, whereas the captive population only exhibited seven (Table 5.3). A total of 67 wild birds exhibited at least one allele that was not detected in captivity (EC n=22, WC n= 28 and Namibia n=17). Only 22 of the captive individuals possessed one or more alleles that were not found in the wild. The allele frequency histograms for 'wild' versus 'captive' populations are presented for each locus in Appendix 5.4.

Table 5.3 Private alleles in all wild samples versus all captive samples (n=308, 2 populations)

	Locus	Allele	Frequency
WILD	G2-2	370	0.013
	G2-2	386	0.003
	SH1CA9	135	0.038
	SH1CA9	137	0.030
	SH1CA9	139	0.005
	SH1CA9	141	0.005
	SH2CA21	112	0.013
	SH2CA21	124	0.008
	B3-2	297	0.003
	B3-2	299	0.053
	B3-2	309	0.003
	G3-6	275	0.014
	PNN03	380	0.003
	PNN06	310	0.003
	PNN06	316	0.003
	PNN08	143	0.022
	PNN09	400	0.003
CAPTIVE	SH1CA9	116	0.008
	SH1CA9	144	0.008
	B3-2	305	0.038
	PNN08	145	0.009
	PNN12	244	0.009
	PNN12	248	0.070
	PNN12	258	0.022

When populations were defined as breeding colonies and captive institutions, only St. Croix Island (EC), Dassen Island (WC), and Halifax Island (Namibia), exhibited alleles that are not found in any other wild or captive population. The Two Oceans Aquarium (TOA) and uShaka exhibit four alleles that are not found in any other wild or captive population (Table 5.4). Each of these private alleles is found in only one individual, except for allele 116 at locus SH1CA9 in uShaka, which was found in two individuals (studbook numbers: T241 and

T351). The allele frequency histograms for captive African Penguins are presented in Appendix 5.5.

Allelic richness was calculated at the 'regional-level' (with populations defined as breeding regions: Namibia, Western Cape, Eastern Cape and 'captive'). The Eastern Cape had the highest allelic richness (A_R=2.5) followed by the Western Cape and Namibia (both 2.4) and the captive birds showed the lowest (A_R=2.3).

Table 5.4 Private alleles identified in wild and captive populations. Analysis is based on 308 individuals genotyped at 12 loci from 12 wild penguin populations and 4 captive institutions.

Population	Locus	Allele	Frequency
St Croix (Eastern Cape)	PNN03	380	0.016
St Croix (Eastern Cape)	PNN06	310	0.016
St Croix (Eastern Cape)	PNN06	316	0.016
Dassen Island (Western Cape)	B3-2	297	0.020
Dassen Island (Western Cape)	B3-2	309	0.020
Dassen Island (Western Cape)	PNN09	400	0.020
Halifax Island (Namibia)	G2-2	386	0.038
Two Oceans Aquarium (TOA)	SH1CA9	144	0.083
Two Oceans Aquarium (TOA)	PNN08	145	0.083
uShaka Marine World	SH1CA9	116	0.019
uShaka Marine World	PNN12	244	0.019

When all wild samples were compared to all captive samples the percentage of 'wild' alleles per locus found among captive samples ranged from 57% to 100% (mean across 12 loci 75.43%) based on presence/absence. The proportion of 'wild' alleles 'secured' in each captive population (Table 5.5) ranged from 57% at TOA, to 70% at SANCCOB and uShaka Marine World. An average of 66% of alleles found in the wild are represented in captive populations.

Estimates of relatedness among captive individuals

As in the genetic diversity analyses, relatedness was first calculated for the captive population(s) alone, and then for the full dataset. Individual pairwise relatedness estimates among all captive African Penguins (n=119, 7021 pairwise comparisons) were calculated using three methods (Table 5.6): Ritland's estimator (RI, 1996), Lynch & Ritland's mean estimator (LRM, 1999) and Queller and Goodnight's mean estimate (QGM, 1989). For all three analyses, some individual pairwise estimates exceeded 0.8 (Table 5.6). Mean population relatedness estimates were significantly higher than expected for all captive

populations (P<0.003), with TOA exhibiting the highest value, and NZG and uShaka the most significant (Figure 5.3). Interestingly, birds from uShaka comprise more than half of the ten most closely related pairs of individuals (based on the three relatedness estimates, Appendix 5.6).

Table 5.5 Proportion of 'wild' alleles found among captive populations (presence/absence) for each locus. SANCCOB = Southern African Foundation for the Conservation of Coastal Birds, TOA = Two Oceans Aquarium, NZG = National Zoological Gardens and uShaka = uShaka Marine World.

Captive institution	LOCUS												
	G22	SH1 CA9	Sh2Ca 21	B32	G36	PNN 01	PNN 03	PNN 06	PNN 08	PNN 09	PNN 12	PNN 05	mean
SANCCOB	0.57	0.53	0.64	0.43	0.67	1	0.67	0.67	0.71	0.71	0.8	1	0.70
TOA	0.57	0.47	0.36	0.14	0.44	1	0.56	0.50	0.57	0.71	1	0.5	0.57
NZG	0.71	0.58	0.55	0.14	0.56	1	0.78	0.67	0.71	0.71	1	0.5	0.66
uShaka	0.71	0.53	0.73	0.29	0.56	1	0.56	0.67	0.57	0.86	1	1	0.70
mean	0.64	0.53	0.57	0.25	0.56	1	0.64	0.63	0.64	0.75	0.95	0.75	0.66

Table 5.6 Mean pairwise relatedness estimates of captive African Penguins calculated using three methods: Ritland's estimator (RI, 1996), Lynch & Ritland's mean estimator (LRM, 1999) and Queller and Goodnight's mean estimate (QGM, 1989). The minimum and maximum pairwise values for each of these estimates are also presented.

	Ritland (RI)	Lynch & Ritland (LRM)	Queller & Goodnight (QGM)
Mean ± SE	-0.005±0.067	-0.008±0.146	-0.008±0.213
Range	-0.143 to 0.945	-0.426 to 0.842	-0.594 to 0.833

Mean population relatedness among captive and wild African Penguins

Mean population relatedness was estimated from individual pairwise relatedness matrices using the methods of Ritland, (1996), Lynch & Ritland (1999), and Queller & Goodnight (1989). Mean within-population pairwise relatedness values, and the associated probabilities, were estimated based on 10 000 permutations and 10 000 bootstrap replicates. Mean relatedness within both wild and captive populations was significantly higher than expected (P<0.001) based on Lynch & Ritland's (1999) estimate, and the captive individuals were on average slightly more closely related than wild birds (Figure 5.4).

Figure 5.3 Mean population relatedness estimates (Lynch & Ritland, 1999) for each of the captive populations. Blue bars are mean relatedness; error bars represent the 95% confidence interval about the mean values as determined by bootstrap resampling. The upper (U) and lower (L) confidence limits bound the 95% confidence interval about the null hypothesis of 'no difference' across the captive populations as determined by permutation.

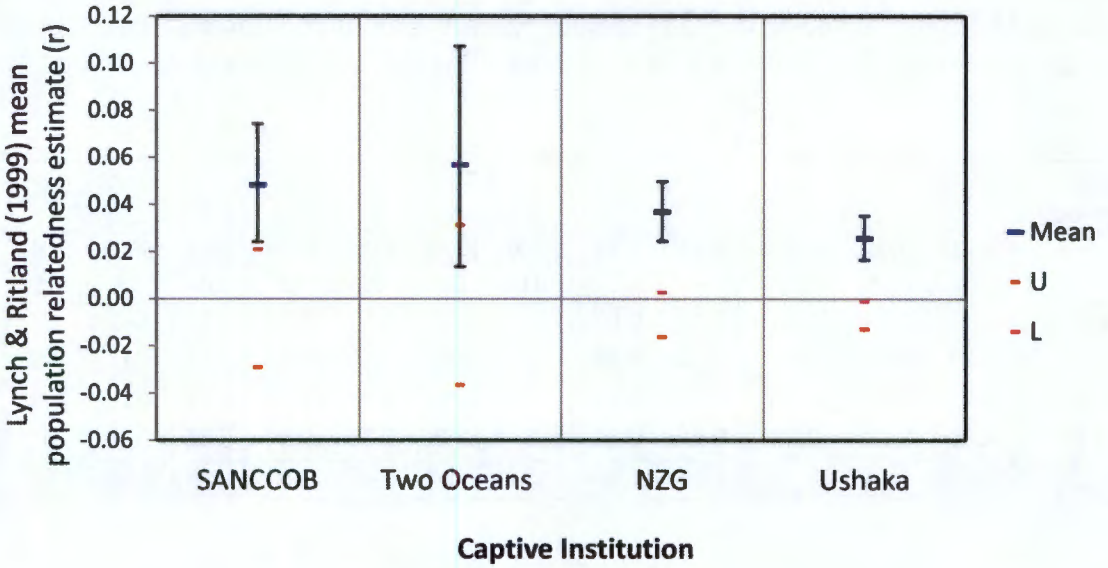
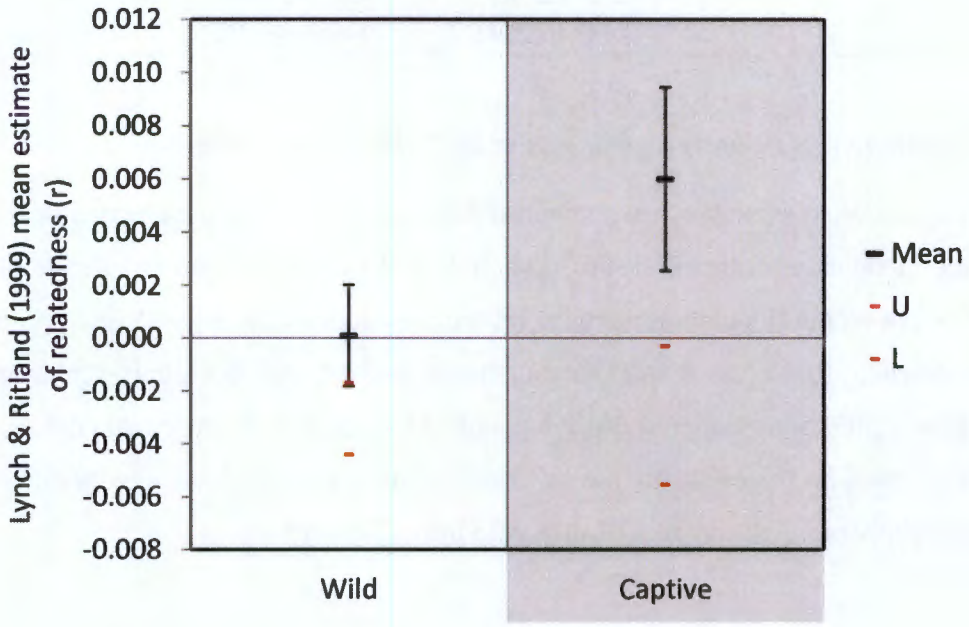


Figure 5.4 Mean population relatedness among wild (n=189) and captive (n=119) African Penguin populations based on **Lynch & Ritland's (1999) relatedness (r)**. Upper (U) and lower (L) confidence limits bound the 95% confidence interval of the null hypothesis of 'no difference' across the populations (determined by permutation), and the upper and lower error bars bound the 95% confidence interval about the mean values (determined by bootstrap resampling).



The differences between mean regional relatedness values were small, but significant. Based on Lynch & Ritland's (1999) mean relatedness (Figure 5.5), all breeding 'regions' (including captive birds) were significantly more highly related than expected ($P<0.02$), with the exception of the Western Cape population (ns). Analogous analyses with populations defined as breeding colonies and captive institutions, showed divergent patterns of relatedness among colonies in different regions, and among colonies within regions (Figure 5.6a and b). Based on Lynch & Ritland's (1999) relatedness (Figure 5.4), all captive populations are significantly more closely related to each other than expected ($P<0.004$), as were Bird Island ($P=0.037$) and Jutten Island ($P=0.009$). According to Queller and Goodnight's (1989) estimator, Dassen Island exhibits significantly lower than expected relatedness ($P=0.001$, Figure 5.6b), but Jutten, NZG and uShaka remain significantly highly related ($P=0.049$, $P=0.0001$ and $P=0.007$ respectively).

Figure 5.5 Mean population relatedness among wild African Penguin breeding regions (n=189) and all captive individuals (n=119) based on Lynch & Ritland's (1999) relatedness (r). Upper (U) and lower (L) confidence limits bound the 95% confidence interval of the null hypothesis of 'no difference' across the populations (determined by permutation), and the upper and lower error bars bound the 95% confidence interval about the mean values (determined by bootstrap resampling).

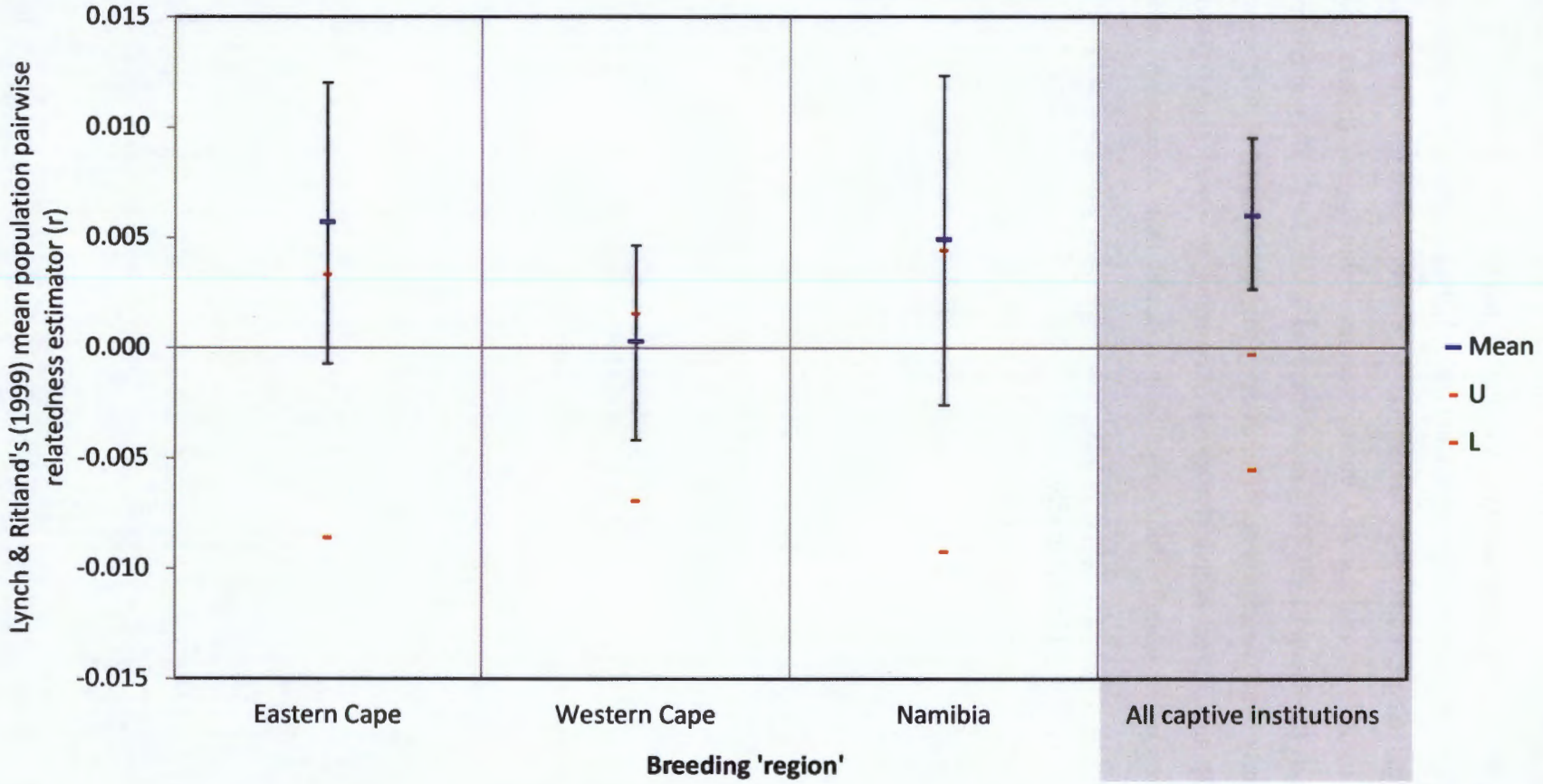
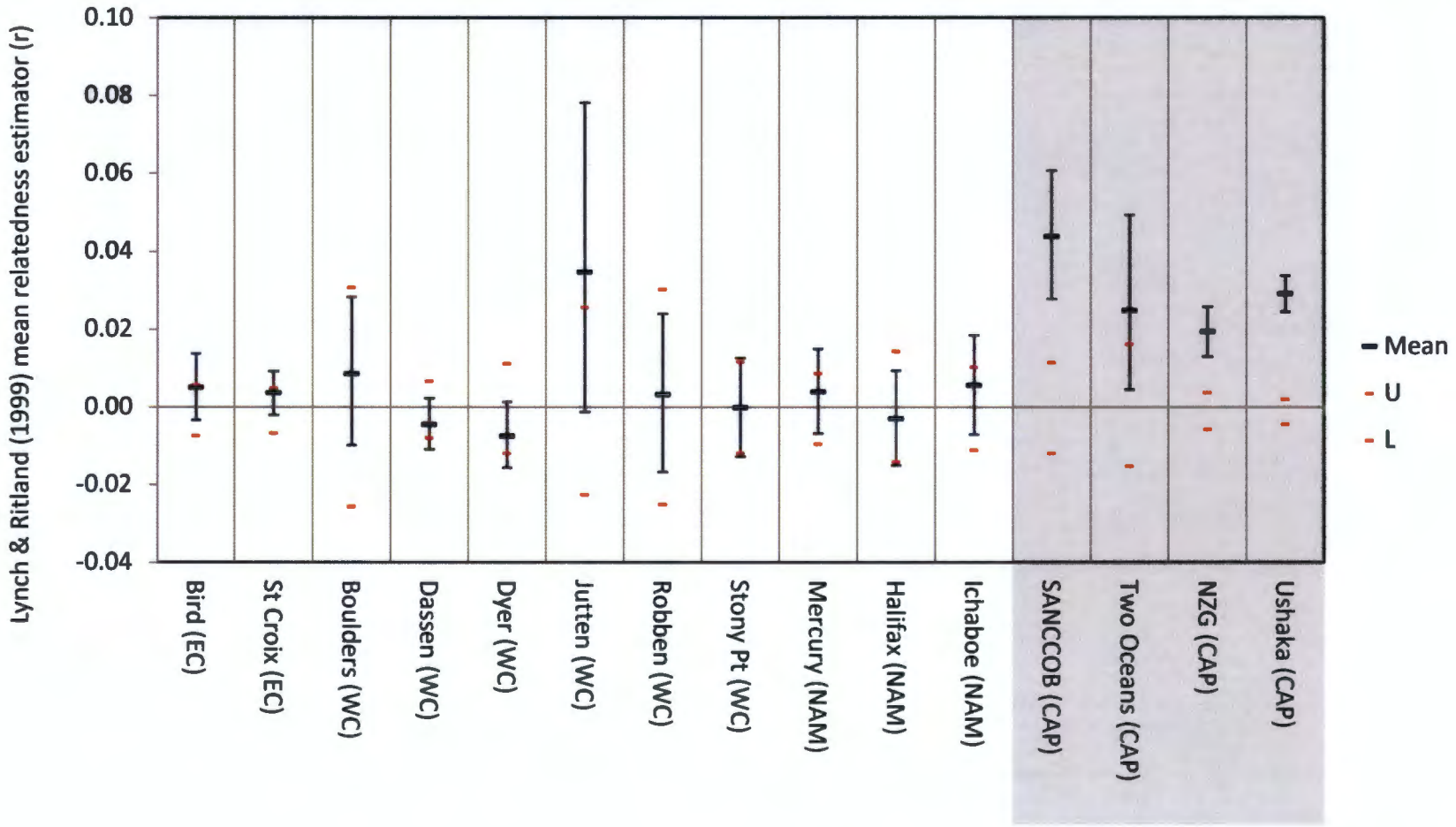
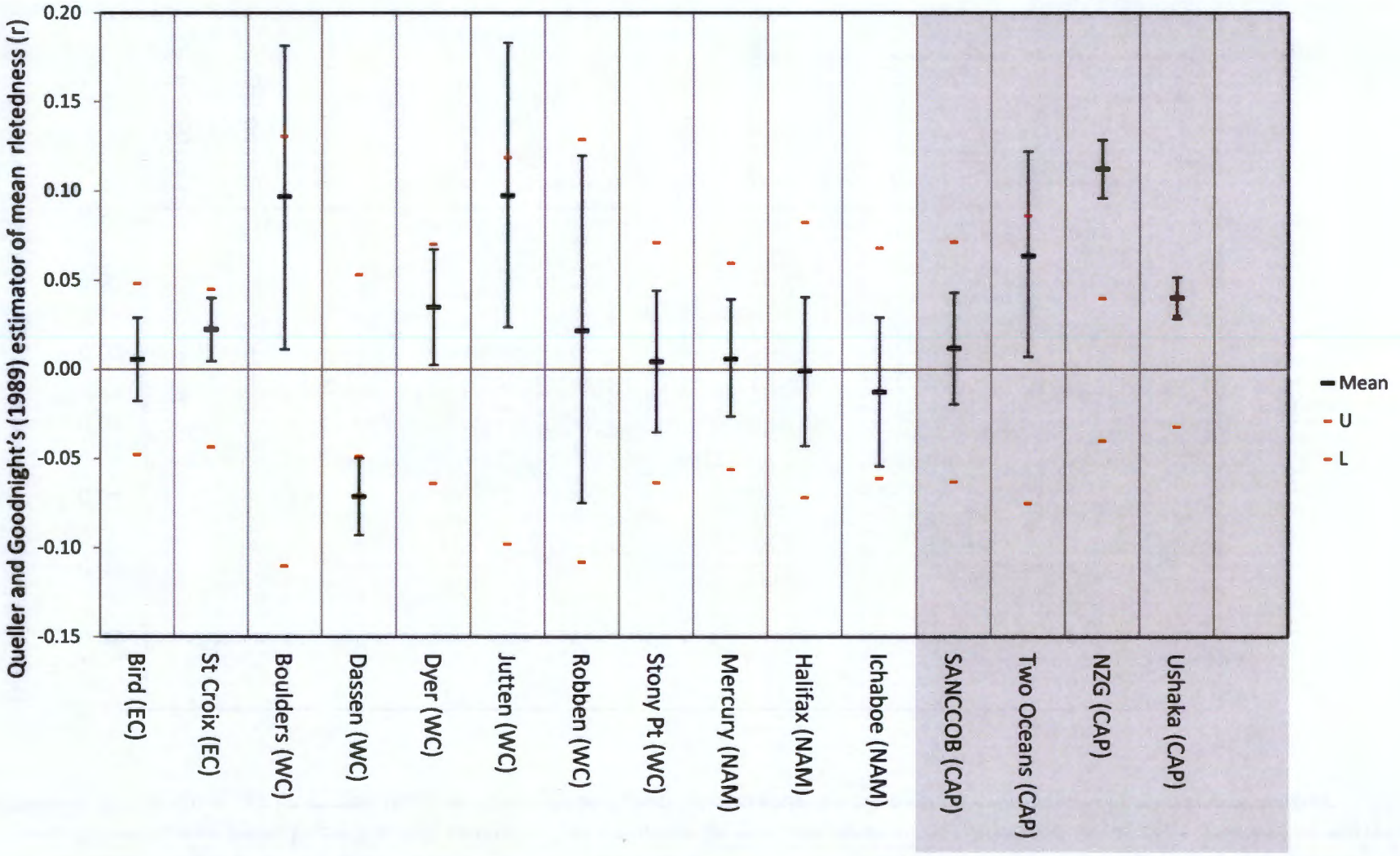


Figure 5.6 Mean within-population relatedness values based on (a) Lynch & Ritland's (1999) and (b) Queller and Goodnight's (1989) estimators. Upper (U) and lower (L) confidence limits bound the 95% confidence interval about the null hypothesis of 'No Difference' across the populations as determined by permutation, and the upper and lower error bars bound the 95% confidence interval about the mean values as determined by bootstrap resampling.

(a)



(b)


Queller and Goodnight's (1989) estimator of mean rletedness (r)
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
Bird (EC)
St Croix (EC)
Boulders (WC)
Dassen (WC)
Dyer (WC)
Jutten (WC)
Robben (WC)
Stony Pt (WC)
Mercury (NAM)
Halifax (NAM)
Ichaboe (NAM)
SANCCOB (CAP)
Two Oceans (CAP)
NZG (CAP)
Ushaka (CAP)
Mean
U
L

Population structure and connectivity

A number of analyses (spatially explicit and spatially independent, as defined in Chapter 4) were conducted to determine the role of genetic drift in captive populations as compared to their wild counterparts.

Population structure among captive African Penguin populations

Spatially explicit analyses

Fixation indices, and their derivatives, were calculated with populations defined as the four captive institutions. All G_{ST} and corrected G_{ST} estimates, as well as D_{EST}, were highly significant ($P<0.0001$). G_{STmax} was 0.3±0.06 after correction, and overall G_{ST} was 0.03. F_{ST} was estimated as 0.05±0.01 ($P<0.0001$) across all loci and all populations, and G''_{ST} was estimated at 0.105±0.045. Pairwise population differentiation estimates were all highly significant ($P<0.01$), and consistently showed that the TOA and uShaka populations were the most different from each other (Tables 5.7 to 5.9).

Table 5.7 Pairwise population G_{ST} estimates (below the diagonal) and the associated probabilities (above the diagonal) based on 9 999 permutations of 9 999 pairwise population permutation and 10 000 bootstrap replicates.

	SANCCOB	Two Oceans	NZG	uShaka
SANCCOB		0.003	<0.001	<0.001
Two Oceans	0.019		0.009	<0.001
NZG	0.019	0.013		<0.001
uShaka	0.022	0.032	0.022	

Table 5.8 Pairwise population G''_{ST} estimates (below the diagonal) and the associated probabilities (above the diagonal) based on 9 999 permutations of 9 999 pairwise population permutation and 10 000 bootstrap replicates.

	SANCCOB	Two Oceans	NZG	uShaka
SANCCOB		0.004	<0.001	<0.001
Two Oceans	0.096		0.007	<0.001
NZG	0.091	0.062		<0.001
uShaka	0.117	0.156	0.107	

Table 5.9 Pairwise population estimates of Jost's D (below the diagonal) between the four captive populations of African Penguins, and the associated probabilities (above the diagonal).

	SANCCOB	Two Oceans	NZG	uShaka
SANCCOB		0.004	<0.001	<0.001
Two Oceans	0.061		0.007	<0.001
NZG	0.056	0.036		<0.001
uShaka	0.077	0.101	0.065	

AMOVA-based F_{ST} was estimated based on the four captive populations to investigate the hierarchical partitioning of genetic diversity. F_{STmax} was 0.4, and overall F_{ST} was 0.04 (F'_{ST}=0.1) indicating significant moderate population structure (P<0.0001). AMOVA revealed 4% of the variation in allele frequencies is among captive populations (Figure 5.7). Pairwise population F_{ST}-values were >0.1 for all comparisons involving uShaka (Table 5.10), and TOA and NZG are the least differentiated. In contrast, the R_{ST}-based AMOVA for the same dataset (Figure 5.7) estimated overall R_{ST} to be -0.01, with none of the variance in allele sizes explained among populations. None of the pairwise population comparisons of R_{ST} were significant, and all were less than zero. This pattern is strikingly different to what was observed among wild populations, where R_{ST}.values were much higher than F_{ST}-values.

Table 5.10 Pairwise population estimates of corrected F_{ST} (F'_{ST}, from AMOVA) for all captive African Penguin populations

	SANCCOB	Two Oceans	NZG
Two Oceans	0.09		
NZG	0.09	0.06	
uShaka	0.11	0.15	0.10

Spatially independent analyses of population structure among captive populations

An assignment test based on the four captive populations correctly assigned 82% (97 out of 119 individuals) of captive individuals to their institutions based on their multi-locus genotypes, with over 90% of individuals assigned correctly to the uShaka population. These values are much higher than those calculated for wild populations (Table 4.16). The FCA (Figure 5.8) and PCoA (Figure 5.9) show the multivariate distribution of individuals based on the multi-locus genetic distances between them. Both analyses show some overlap between all captive institutions, but TOA and NZG are distinct from uShaka outside areas of overlap. In contrast to similar analyses based on wild populations (Chapter 4), more structure is evident based on these analyses.

Figure 5.7 Summary of AMOVA results from analyses based on different populations, and population subdivisions of the datasets. Within individual analyses were suppressed for all AMOVAs, and significant values are marked with an asterisk (probability values range from P<0.00001 to 0.05).

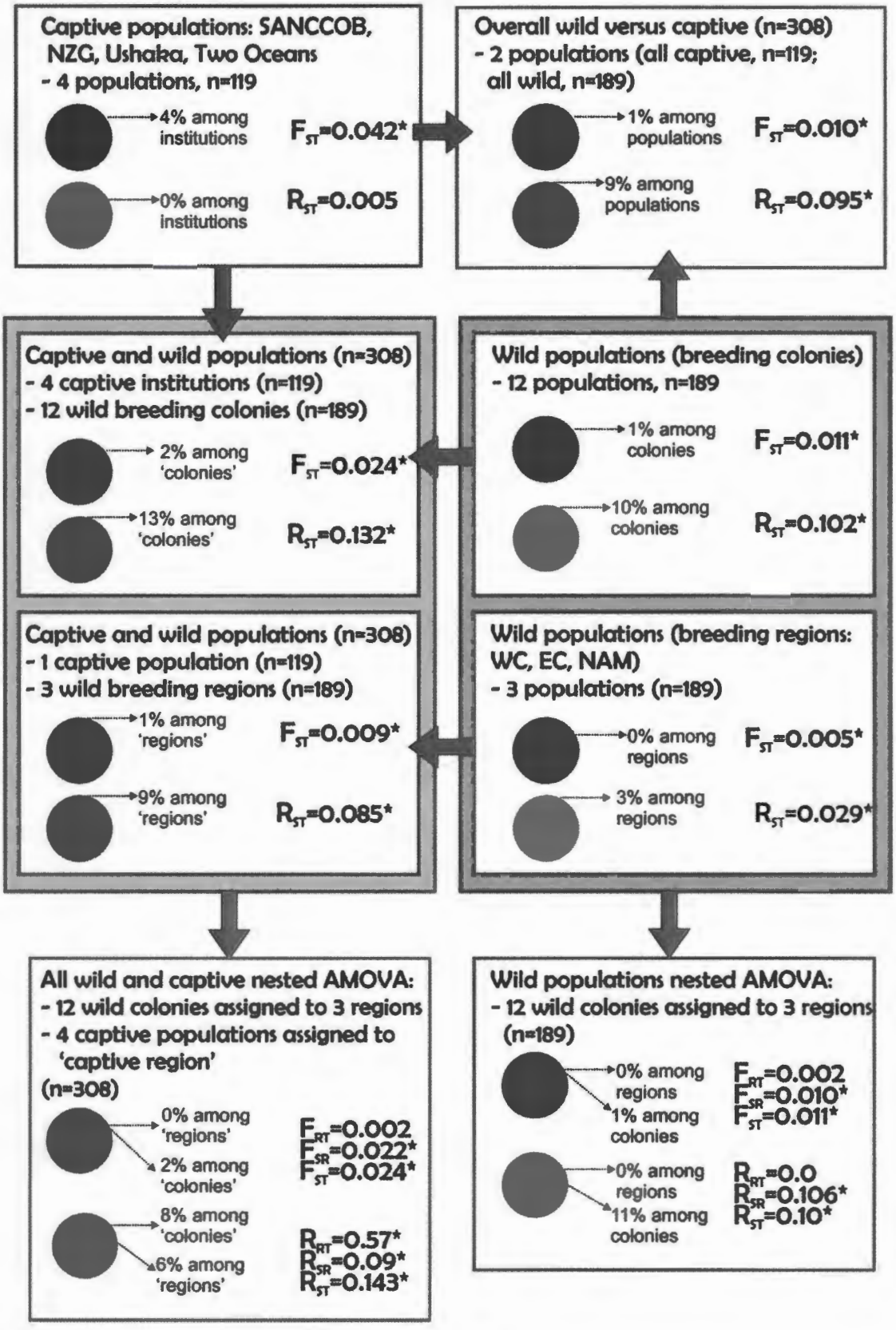


Figure 5.8 Correspondence analysis showing the distribution of captive individuals (n=119) relative to each other in Euclidean space based on the first three axes. The amount of variation in the dataset explained by each axis is shown on the axes. The shaded area indicates strong overlap among captive populations.

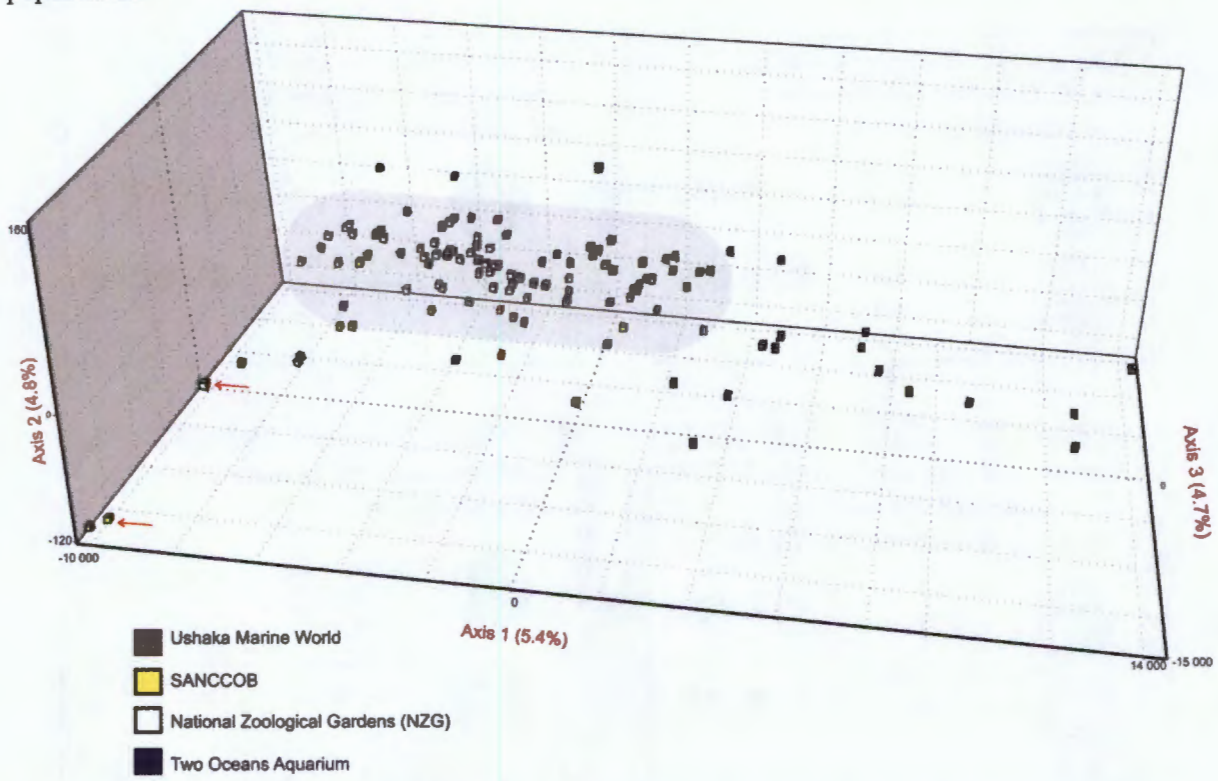
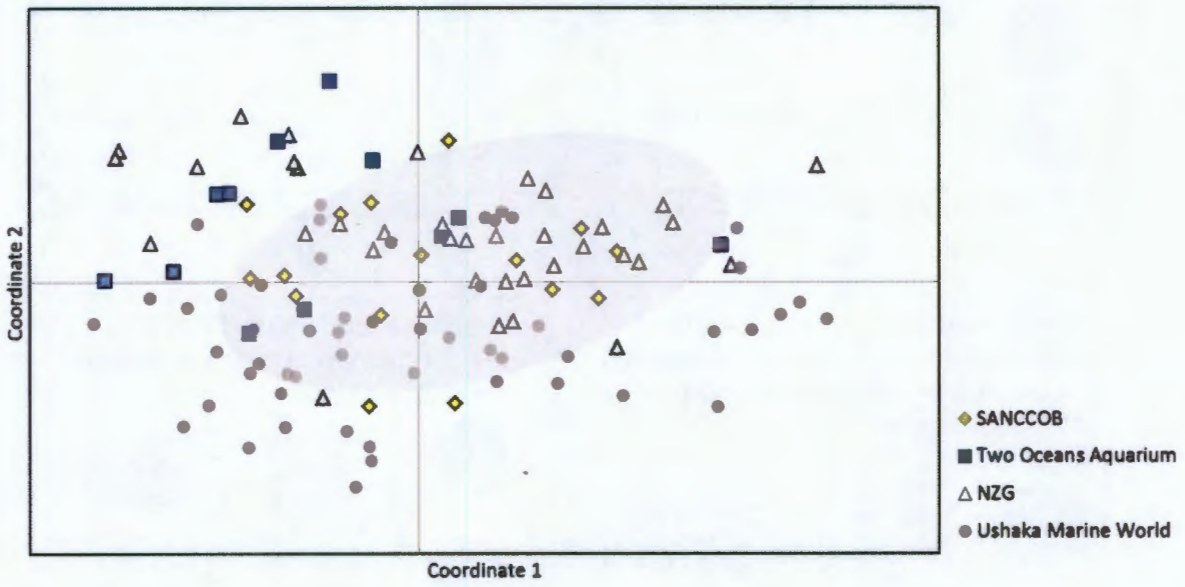


Figure 5.9 Covariance-standardised Principal Coordinates analysis based on pairwise genetic distance matrix of individual African Penguins from four captive institutions in South Africa. The first two axes explain 17.15% and the shaded area indicates strong overlap among captive populations.



Population structure among and within captive and wild populations

Wild versus captive overall

When the dataset was broadly divided into 'wild' and 'captive' populations (n=189 and n=119 respectively), G_{STmax} was 0.24, and the estimated overall G_{ST} was 0.004 (P<0.0001). F_{STmax} was 0.37, and overall F_{ST} was 0.01 (P<0.0001; F'_{ST}=0.03), indicating significant, but low differentiation between the two populations based on differences in allele frequencies. Only 1% of variance in allele frequencies was explained among populations. Other frequency-based estimates of population divergence showed similar patterns (e.g. G''_{ST}=0.02±0.007 and D_{EST}=0.01±0.005, both P<0.0001). AMOVA-based R_{ST}-estimates based on this dataset (n=308, two populations) showed that 9% of the variation in allele sizes was explained among populations (Figure 5.7). Overall R_{ST} was estimated as 0.09 (P=0.0001). An assignment test correctly assigned 70% of individuals to 'wild' and 'captive' populations. The stronger signal of genetic difference between wild and captive penguins at this scale appears to be the composition of alleles rather than their frequencies in each population. This might be expected because alleles are lost faster than heterozygosity during a population bottleneck – represented in this case by bringing a relatively small number of individuals into captivity.

Population structure at level of breeding region and all captive birds

When wild populations were defined as breeding regions and all captive populations grouped as one population, an among group comparison of H_O (EC=0.63, WC=0.59, Namibia=0.61, Captive=0.54) and allelic richness found overall significant differences among regions in A_R, but not H_O (A_R P=0.012; H_O P=0.17). The F_{ST}-based AMOVA of these four populations generated an F_{STmax} of 0.38 and an overall F_{ST} of 0.009 (P=0.0001, F'_{ST}=0.02). This low, but significant population structure was also reflected among other estimates of population subdivision: G''_{ST}=0.014 and D_{EST}=0.008 (both P=0.0001). The F_{ST}- and R_{ST}-based AMOVA results showed different patterns with regard to the partitioning of variance (Figure 5.7). F_{ST} showed only 1% difference among populations, where R_{ST} showed 9% (Figure 5.7). All pairwise F_{ST} (Table 5.11) and corrected pairwise F_{ST} (Table 5.12) comparisons involving the captive population were highly significant, and the highest differentiation was observed between the Namibian population and the captive population. The R_{ST}-based AMOVA generated an overall R_{ST} of 0.08 (P=0.0001). Pairwise R_{ST} values (Table 5.13) showed a very similar pattern to F_{ST}-based estimates, with highly significant differences found between the captive population and each of the three wild breeding populations. Namibia was also

significantly different from the WC and EC populations, but the differences were approximately 10-fold smaller than those between Namibia and the captive population.

Table 5.11 Pairwise population estimates of F_{ST} (above diagonal) generated during AMOVA (9 999 permutations of 9 999 pairwise permutations and 10 000 bootstrap replicates) conducted on a dataset partitioned into regional breeding populations (Namibia, Western Cape and Eastern Cape; n=189) and the captive population (n=119). Probabilities are above the diagonal (bold $P < 0.05$).

	EC	WC	NAM	Captive
Eastern Cape (EC)	-	0.128	0.066	**<0.001**
Western Cape (WC)	0.002	-	**0.006**	**<0.001**
Namibia (NAM)	0.004	0.007	-	**<0.001**
Captive (all institutions)	0.009	0.007	0.018	-

Table 5.12 Pairwise population estimates of F'_{ST} generated during AMOVA (9 999 permutations of 9 999 pairwise permutations and 10 000 bootstrap replicates) conducted on a dataset partitioned into regional breeding populations (Namibia, Western Cape and Eastern Cape; n=189) and the captive population (n=119).

	EC	WC	NAM	Captive
Western Cape (WC)	0.006	-		
Namibia (NAM)	0.010	0.018	-	
Captive (all institutions)	0.025	0.020	0.048	-

Table 5.13 Pairwise R_{ST} values (below diagonal) estimated during AMOVA (9 999 permutations of 9 999 pairwise permutations and 10 000 bootstrap replicates) and based on a dataset partitioned into regional breeding populations (Namibia, Western Cape and Eastern Cape; n=189) and the captive population (n=119). Probabilities associated with R_{ST} estimates are above the diagonal (bold $P < 0.05$).

	EC	WC	NAM	Captive
Eastern Cape (EC)	-	0.382	**0.009**	**<0.001**
Western Cape (WC)	0.000	-	**0.001**	**<0.001**
Namibia (NAM)	0.029	0.044	-	**<0.001**
Captive (all institutions)	0.073	0.073	0.239	-

Population structure at level of colonies and captive-institutions

Fixation indices and their derivatives were estimated based on a reduced dataset of 11 loci and 290 individuals to eliminate all missing data at this finer scale of analysis: 16 populations, defined either as breeding colonies or captive institutions. G_{STmax} was 0.39±0.07, and overall G_{ST} was low and not significant at 0.007. F_{ST} indicated stronger differentiation (F_{ST}=0.05±0.005), but was also not significant. G''_{ST} and Jost's D were also not significant

overall. These values, however, do not reflect the striking patterns observed in the pairwise population values (Table 5.14 and 5.15). Pairwise F_{ST} and D_{EST} values for all captive institutions except SANCCOB showed significant differentiation ($P<0.05$) from five or more wild breeding colonies. The uShaka population is consistently significantly differentiated from all other populations. The NZG population is significantly differentiated from some breeding colonies in all three wild breeding regions (Tables 5.14 and 5.15). The TOA population is significantly differentiated from all three of the other captive institutions, and some colonies in the Eastern Cape and Western Cape of South Africa. After B-Y correction, the number of significant differences among coloniesis reduced, but the general pattern still holds.

The F_{ST}-based AMOVA of the full dataset (16 populations, n=308, 12 loci) generated an F_{STmax} of 0.38 and an overall F_{ST} of 0.02 ($P<0.0001$, $F'_{ST}=0.06$). Overall F_{IS} was 0.07 ($P<0.0001$), and 2% of the variance was explained among populations (Figure 5.7). An analogous R_{ST}-based AMOVA produced a highly significant value of 0.11 ($P<0.0001$) and showed a much higher proportion of the variation in allele size was distributed among populations (13%) compared to the variation in allele frequency (F_{ST}-based AMOVA above, Figure 5.7).

Nested F_{ST} and R_{ST} AMOVAs

The nested F_{ST}-based AMOVA of the full dataset (16 populations, 4 'regions', n=308, 12 loci) generated an overall significant F_{ST}-value of 0.022 ($P<0.0001$, Figure 5.7). Regional population structure was low ($F_{RTmax}=0.37$, $F'_{RT}=0.006$) compared to colony- and institution-level structure within those regions ($F_{SRmax}=0.384$, $F'_{SR}=0.053$). All hierarchical F-statistics were significant ($P<0.05$), although 0% of variation was explained among regions, 2% was explained among colonies (colonies and captive institutions) and 7% among individuals (Figure 5.7). All AMOVAs reported above were also conducted with all 'within-individual' analyses suppressed and Figure 5.7 summarises the results of these AMOVAs for each dataset, and clearly shows the different patterns detected using F_{ST}- and R_{ST}-based analyses, and how the inclusion of captive birds at all scales increases population structure among African Penguins.

All captive populations were highly significantly differentiated from each other based on pairwise values of F_{ST} estimated during the nested AMOVA ($P<0.007$; Table 5.16), even after B-Y correction. Only 11 of the possible 66 pairwise comparisons of wild colonies were significant (16%; seven involving Mercury Island), whereas 32 of the possible 48 pairwise

comparisons of captive versus wild populations were significant (67%; Table 5.16). This pattern was also detected among pairwise R_{ST}-estimates (Table 5.17). Eighteen significant pairwise R_{ST}-values were detected among wild populations ($P<0.05$, breeding colonies, out of a total of 66 comparisons between wild colonies), and seven of these again involved Mercury Island.

None of the captive populations were significantly differentiated from each other based on R_{ST}-values from this nested AMOVA. However, even after B-Y correction, all four captive populations were significantly differentiated from Mercury Island in Namibia (Table 5.17). All except TOA were significantly differentiated from Bird Island in the Eastern Cape, Dassen Island in the Western Cape and Ichaboe Island in Namibia. After B-Y correction, 15 out of 48 comaprisons (~31%) between captive and wild populations were significant, compared to 13 out of 66 (~20%) between wild colonies.

Table 5.14 Population pairwise F_{ST}-values (below diagonal) among wild and captive African Penguin populations (n=308, 16 populations). Probabilities (above diagonal) are based on 10 000 bootstrap replicates and significant values are in bold and red type (P<0.05). The Benjamini-Yekutieli corrected critical α-value for 120 comparisons is 0.009 (red type).

	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	POS	HAL	ICH	SANC COB	Two Oceans	NZG	uShaka
Bird Island (BI)	-	0.287	0.221	0.314	0.684	0.105	0.084	0.607	0.708	0.933	0.830	0.229	0.506	**0.023**	**0.007**	**<0.001**
St Croix (SC)	0.011	-	0.271	0.063	0.130	0.255	0.046	0.678	0.245	0.795	0.196	0.176	0.190	0.397	**0.045**	**<0.001**
Boulders (BOU)	0.028	0.025	-	0.069	0.311	0.173	0.136	0.917	0.533	0.584	0.172	0.386	0.329	0.052	0.457	**0.024**
Dassen (DAS)	0.012	0.013	0.033	-	0.704	0.470	0.269	0.275	0.085	0.957	0.180	0.452	0.606	**0.003**	**0.002**	**0.001**
Dyer (DYE)	0.012	0.017	0.030	0.012	-	0.226	0.235	0.312	0.096	0.863	0.338	0.679	0.351	**0.022**	**0.016**	**<0.001**
Jutten (JUT)	0.028	0.022	0.040	0.021	0.028	-	**0.042**	0.383	0.158	0.511	0.165	0.144	0.516	0.211	0.281	**0.001**
Robben (ROB)	0.033	0.033	0.048	0.027	0.031	**0.051**	-	0.129	**0.020**	0.442	0.136	0.529	0.394	**0.025**	**0.049**	**0.008**
Stony Point (SP)	0.013	0.011	0.018	0.016	0.019	0.025	0.036	-	0.634	0.920	0.698	0.173	0.781	0.114	0.752	**0.002**
Mercury (MER)	0.011	0.013	0.026	0.017	0.022	0.030	**0.044**	0.014	-	0.924	0.410	0.362	0.242	0.062	**0.033**	**<0.001**
Possession (POS)	0.049	0.057	0.081	0.049	0.057	0.080	0.090	0.050	0.056	-	0.981	0.812	0.833	0.566	0.627	0.636
Halifax (HAL)	0.012	0.017	0.037	0.019	0.021	0.033	0.039	0.016	0.019	0.044	-	0.297	0.604	0.076	0.153	**0.002**
Ichaboe (ICH)	0.017	0.016	0.030	0.015	0.016	0.032	0.027	0.023	0.018	0.061	0.022	-	0.300	0.249	**0.003**	**<0.001**
SANCCOB	0.013	0.015	0.029	0.012	0.018	0.022	0.028	0.013	0.018	0.061	0.017	0.019	-	**0.040**	0.125	**0.001**
Two Oceans	**0.027**	0.015	0.049	**0.031**	**0.033**	0.032	**0.052**	0.027	0.028	0.073	0.032	0.024	**0.030**	-	**0.013**	**<0.001**
NZG	**0.018**	**0.012**	0.023	**0.020**	**0.023**	0.023	**0.037**	0.010	**0.018**	0.065	0.019	**0.028**	0.016	**0.028**	-	**<0.001**
uShaka	**0.023**	**0.028**	**0.037**	**0.018**	**0.029**	**0.043**	**0.041**	**0.026**	**0.028**	0.065	**0.028**	**0.031**	**0.027**	**0.050**	**0.032**	-

Table 5.15 Population pairwise D_{EST}-values (below diagonal) among wild and captive African Penguin populations (n=308, 16 populations). Probabilities (above diagonal) are based on 10 000 bootstrap replicates and significant values are in bold type (P<0.05). The Benjamini-Yekutieli corrected critical α-value for 120 comparisons is 0.009 (red type).

	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	POS	HAL	ICH	SANC COB	Two Oceans	NZG	uShaka
Bird Island (BI)	-	0.287	0.233	0.314	0.675	0.097	0.076	0.607	0.714	0.924	0.837	0.233	0.507	**0.026**	**0.006**	**<0.001**
St Croix (SC)	0.004	-	0.283	0.062	0.124	0.233	**0.038**	0.683	0.260	0.816	0.209	0.180	0.189	0.413	**0.043**	**<0.001**
Boulders (BOU)	0.013	0.009	-	0.086	0.330	0.176	0.136	0.904	0.495	0.553	0.169	0.382	0.333	0.050	0.433	**0.030**
Dassen (DAS)	0.004	0.013	0.030	-	0.688	0.420	0.264	0.279	0.089	0.942	0.195	0.456	0.602	**0.004**	**0.001**	**<0.001**
Dyer (DYE)	-0.006	0.012	0.008	-0.006	-	0.219	0.235	0.311	0.090	0.871	0.340	0.679	0.349	**0.024**	**0.011**	**<0.001**
Jutten (JUT)	0.024	0.010	0.020	0.002	0.013	-	**0.042**	0.359	0.135	0.560	0.152	0.134	0.486	0.199	0.225	**0.001**
Robben (ROB)	0.032	**0.034**	0.034	0.011	0.014	**0.058**	-	0.122	**0.015**	0.442	0.125	0.515	0.386	**0.021**	**0.027**	**0.005**
Stony Point (SP)	-0.005	-0.005	-0.025	0.006	0.005	0.005	0.028	-	0.626	0.898	0.703	0.173	0.780	0.116	0.753	**0.002**
Mercury (MER)	-0.006	0.005	-0.003	0.015	0.019	0.021	**0.060**	-0.006	-	0.911	0.406	0.355	0.238	0.063	**0.029**	**<0.001**
Possession (POS)	-0.077	-0.047	-0.024	-0.090	-0.060	-0.007	0.002	-0.083	-0.079	-	0.971	0.770	0.815	0.586	0.625	0.639
Halifax (HAL)	-0.013	0.008	0.021	0.010	0.005	0.023	0.031	-0.009	0.002	-0.114	-	0.298	0.610	0.077	0.143	**0.001**
Ichaboe (ICH)	0.008	0.009	0.005	<0.001	-0.007	0.022	-0.004	0.014	0.004	-0.063	0.007	-	0.301	0.250	**0.002**	**<0.001**
SANCCOB	-0.001	0.008	0.007	-0.004	0.004	-0.001	0.005	-0.011	0.008	-0.054	-0.006	0.006	-	**0.043**	0.106	**<0.001**
Two Oceans	**0.032**	0.001	0.050	**0.048**	**0.040**	0.018	**0.068**	0.021	0.027	-0.028	0.029	0.010	**0.034**	-	**0.010**	**<0.001**
NZG	**0.023**	**0.011**	<0.001	**0.031**	**0.028**	0.010	**0.040**	-0.007	**0.019**	-0.025	0.012	**0.042**	0.012	**0.034**	-	**<0.001**
uShaka	**0.049**	**0.067**	**0.044**	**0.035**	**0.058**	**0.076**	**0.066**	**0.044**	**0.058**	-0.030	**0.048**	**0.061**	**0.053**	**0.110**	**0.072**	-

Table 5.16 Pairwise population F_{ST} values estimated during a nested AMOVA (n=308). Data from 16 populations and 4 breeding regions (including "captive") were included and results are based on 9 999 permutations of 9 999 pairwise population permutations. F_{ST} values are below the diagonal and significance values based on 10 000 bootstrap replicates are above. Negative F_{ST} values have been converted to zeroes and significant values are in bold type ($P<0.05$). The Benjamini-Yekutieli corrected critical α-value for 120 comparisons is 0.009 (red type).

	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	POS	HAL	ICH	SANC COB	Two Oceans	NZG	uShaka
Bird Island (BI)	-	0.114	0.278	0.420	0.102	0.073	0.198	0.404	0.301	0.452	0.451	0.448	0.002	**0.029**	<0.001	<0.001
St Croix (SC)	0.006	-	0.364	0.007	0.155	0.308	0.024	0.441	<0.001	0.450	0.301	**0.019**	0.001	0.441	**0.033**	<0.001
Boulders (BOU)	0.006	0.003	-	0.150	0.288	0.199	0.139	0.443	0.134	0.450	0.311	0.286	0.066	0.080	0.433	0.073
Dassen (DAS)	0	**0.015**	0.011	-	0.097	0.242	0.460	0.136	**0.039**	0.461	0.123	0.451	0.007	0.003	<0.001	<0.001
Dyer (DYE)	0.008	0.006	0.006	0.008	-	0.347	**0.050**	0.224	<0.001	0.455	0.344	0.141	0.003	**0.014**	0.009	<0.001
Jutten (JUT)	0.017	0.004	0.015	0.006	0.003	-	**0.024**	0.442	0.003	0.443	0.203	0.056	**0.021**	0.174	0.196	0.003
Robben (ROB)	0.009	0.028	0.024	0	**0.025**	**0.049**	-	0.084	**0.023**	0.224	0.119	0.458	0.051	0.013	0.004	0.004
Stony Point (SP)	0.001	0	0	0.007	0.005	0	0.020	-	0.008	0.450	0.451	0.073	**0.029**	0.196	0.454	0.001
Mercury (MER)	0.002	**0.032**	0.014	**0.010**	**0.047**	**0.040**	**0.029**	**0.022**	-	0.463	**0.016**	0.170	<0.001	0.002	<0.001	<0.001
Possession (POS)	0	0	0	0	0	0	0.031	0	0	-	0.456	0.460	0.462	0.452	0.446	0.448
Halifax (HAL)	0	0.003	0.006	0.008	0.003	0.011	0.018	0	**0.02**	0	-	0.272	**0.035**	0.106	0.121	0.005
Ichaboe (ICH)	0	**0.015**	0.007	0	0.008	0.023	0	0.012	0.006	0	0.004	-	0.005	**0.041**	<0.001	<0.001
SANCCOB	**0.023**	**0.028**	0.022	**0.02**	**0.029**	**0.031**	0.024	**0.019**	**0.038**	0	**0.018**	**0.025**	-	0.006	<0.001	<0.001
Two Oceans	**0.018**	0	0.027	**0.028**	**0.029**	0.014	**0.045**	0.008	**0.037**	0	0.014	**0.02**	**0.034**	-	0.006	<0.001
NZG	**0.025**	**0.009**	0.001	**0.032**	**0.019**	0.009	**0.043**	0	**0.049**	0	0.009	**0.042**	**0.035**	**0.027**	-	<0.001
uShaka	**0.032**	**0.035**	0.018	**0.026**	**0.027**	**0.042**	**0.041**	**0.024**	**0.055**	0	**0.023**	**0.040**	**0.040**	**0.057**	**0.042**	-

Table 5.17 Pairwise population R_{ST} values estimated during a nested AMOVA (n=308). Data from 16 populations and 4 breeding regions (including "captive") were included and results are based on 9 999 permutations of 9 999 pairwise population permutations. R_{ST} values are below the diagonal and probabilities above. Negative R_{ST} values have been converted to zeroes and significant values are in bold type (P<0.05). The Benjamini-Yekutieli corrected critical α-value for 120 comparisons is 0.009 (red type).

	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	POS	HAL	ICH	SANC COB	Two Oceans	NZG	uShaka
Bird Island (BI)	-	<0.001	0.307	0.410	<0.001	0.298	0.303	0.251	0.141	0.322	0.192	0.394	0.004	**0.044**	<0.001	<0.001
St Croix (SC)	**0.096**	-	0.340	<0.001	0.144	0.344	0.006	0.176	<0.001	0.294	0.268	<0.001	0.354	0.352	0.143	0.142
Boulders (BOU)	0	0	-	0.179	0.096	0.358	0.216	0.274	**0.020**	0.258	0.304	0.243	0.302	0.278	0.122	0.280
Dassen (DAS)	0	**0.174**	0.031	-	<0.001	0.095	0.326	**0.013**	**0.049**	0.379	0.071	0.343	0.002	**0.013**	<0.001	<0.001
Dyer (DYE)	**0.123**	0.035	0.073	0.262	-	0.113	<0.001	0.273	<0.001	0.225	**0.037**	<0.001	0.440	0.293	0.316	0.183
Jutten (JUT)	0	0	0	0.054	0.063	-	0.121	0.342	0.004	0.367	0.363	0.137	0.286	0.282	0.144	0.325
Robben (ROB)	0	**0.255**	0.041	0	0.432	0.076	-	0.206	0.274	0.243	0.125	0.379	0.006	**0.032**	<0.001	**0.011**
Stony Point (SP)	0.013	0.017	0	0.064	0.022	0	0.017	-	<0.001	0.259	0.540	**0.031**	0.492	0.517	0.059	0.157
Mercury (MER)	0.019	**0.319**	**0.132**	**0.039**	**0.336**	**0.155**	0	**0.138**	-	0.202	<0.001	0.241	<0.001	<0.001	<0.001	<0.001
Possession (POS)	0	0	0	0	0	0	0.031	0	0.056	-	0.078	0.375	0.210	0.271	0.187	0.251
Halifax (HAL)	0.016	0	0	0.060	0.086	0	0.076	0	**0.179**	0	-	0.069	0.364	0.299	**0.042**	0.113
Ichaboe (ICH)	0	0.175	0.015	0	0.232	0.037	0	0.048	0.011	0	0.052	-	0.002	**0.014**	<0.001	<0.001
SANCCOB	0.084	0	0	0.184	0.010	0	0.296	0	**0.286**	0	0	0.170	-	0.308	0.359	0.209
Two Oceans	0.051	0	0	0.135	0.016	0	0.209	0	**0.235**	0	0	0.119	0	-	0.519	0.286
NZG	0.169	0.014	0.037	0.306	0	0.031	0.483	0.051	**0.424**	0	0.069	0.304	0	0	-	0.445
uShaka	**0.133**	0.011	0	**0.213**	0	0	0.225	0.009	**0.356**	0	0.012	0.205	0	0	0	-

Spatially Independent analyses

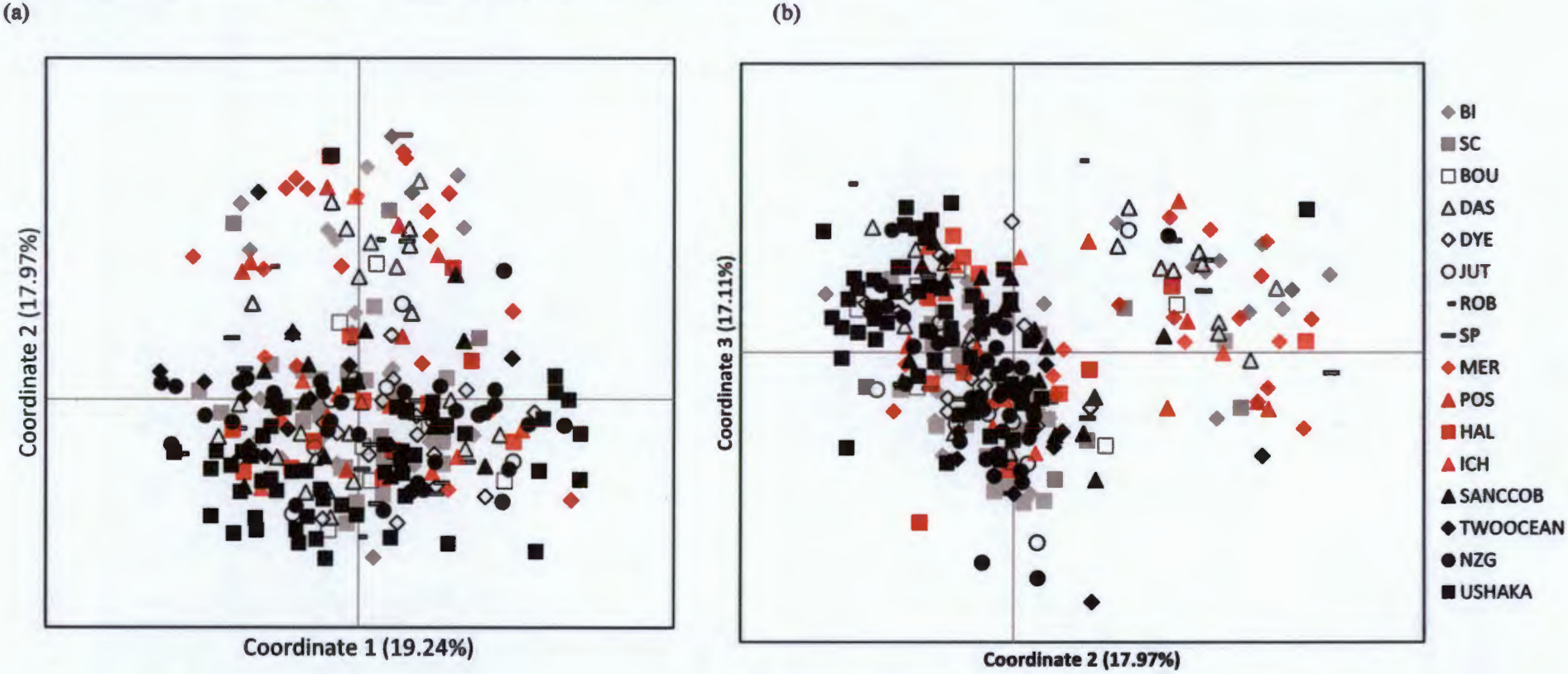
A population assignment test revealed that individuals from three of the four captive facilities (Two Oceans Aquarium, SANCCOB and uShaka Marine World) were assigned correctly to their facility most often based on their multi-locus genotype (Table 5.18). Jutten Island in the Western Cape was the only African Penguin colony to which a higher percentage of individuals were correctly assigned than in a captive institution (38%, Table 5.18). The elevated number of individuals correctly assigned to captive institutions highlights the increased population structure observed among captive populations.

Table 5.18 Summary of population assignment outcomes to 'Self' or 'Other' population (with 'leave one out' option). SANCCOB = Southern African Foundation for the Conservation of Coastal Birds, TOA = Two Oceans Aquarium, NZG = National Zoological Gardens and uShaka = uShaka Marine World.

Population or Institution	'Self'	Other Pop	% correctly assigned to self
Bird Island	4	23	15
St Croix	4	27	13
Boulders Beach	0	7	0
Dassen Island	1	24	4
Dyer Island	1	15	6
Jutten Island	3	5	38
Robben Island	0	7	0
Stony Point	1	15	6
Mercury Island	3	17	15
Possession	0	2	0
Halifax Island	1	12	8
Ichaboe Island	2	15	12
SANCCOB	10	6	**63**
TOA	5	7	**42**
NZG	12	25	**32**
uShaka	33	21	**61**
Total	80	228	
Per cent	26%	74%	

A Principal Coordinates Analysis (PCoA) based on genetic distance between 308 captive and wild African Penguins (Figure 5.10) shows that the captive population is representative of a large proportion of genetic diversity found among wild individuals, but there is a subset of genetic diversity across all three wild breeding regions that is not found among captive individuals.

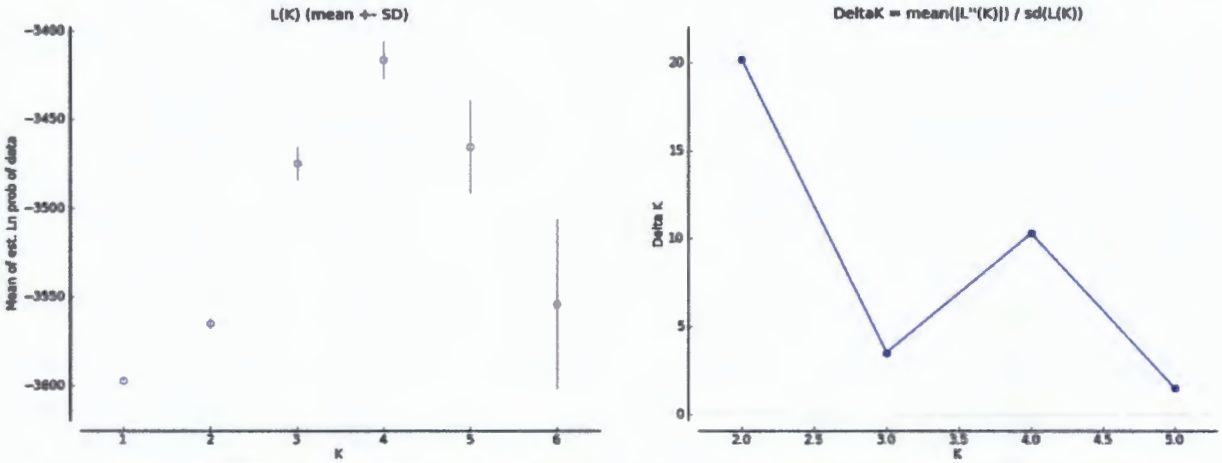
Figure 5.10 Principal Coordinates analysis (PCoA) based on genetic distance between 308 captive and wild African Penguins showing their collection localities. Numbers on the axes represent which coordinates are plotted and the percentage of variation they explain: (a) axis 1 versus 2 and (b) axis 2 versus axis 3. Populations are colour coded by regional collection locality: Eastern Cape colonies (grey symbols), Western Cape colonies (white symbols with black borders), Namibian colonies (red symbols) and captive populations (black symbols).



Abbreviations: BI (Bird Island), SC (St Croix Island), BOU (Boulders Beach), DAS (Dassen Island), DYE (Dyer Island), JUT(Jutten Island), ROB (Robben Island), SP (Stony Point), MER (Mercury Island), POS (Possession Island), HAL (Halifax Island), ICH (Ichaboe Island), SANCCOB (Southern African Foundation for the Conservation of Coastal Birds, rehabilitation centre, Cape Town, Western Cape Province of South Africa), TWOOCEAN (Two Oceans Aquarium, Cape Town), NZG (National Zoological Gardens of South Africa, Pretoria, Gauteng Province of South Africa), USHAKA (uShaka Marine World, Durban, KwaZulu Natal Province of South Africa).

Delta K for both analyses indicated that the most likely number of genetic clusters was two (Figure 5.11 and 5.12), as in Chapter 4. Importantly, ΔK cannot find the true number of populations if there is one single panmictic population; i.e. if K equals one. However, none of the STRUCTURE analyses conducted contradict the finding that K=1; i.e. that there are no discrete genetically distinct groups detectable in the dataset, and the mean likelihood was highest for K=1. CLUMPP (Jakobsson & Rosenberg 2007) was used to summarise all runs for the optimal value of K and DISTRUCT (Rosenberg 2003) to visualise those results (Figure 5.13 and 5.14). Cluster analysis among captive individuals reflected the results of the spatially explicit analyses in that no strong genetic structuring was evident across captive populations. When analysed together with the wild individuals, a similar pattern is evident in that the majority of individuals are equally likely to belong to either of the two clusters. There is no spatial pattern to cluster identity (Figure 5.14).

Figure 5.11 Output from STRUCTUREHARVESTER based on 119 captive African Penguins from 4 institutions. Results are based on 20 runs of 1 million repetitions for K=1 to K=6.**Figure 5.12** Output



from STRUCTUREHARVESTER based on 308 wild and captive African Penguins from 16 **'populations' (wild colonies and captive institutions)**. Results are based on 20 runs of 250 000 repetitions for K=1 to K=18.

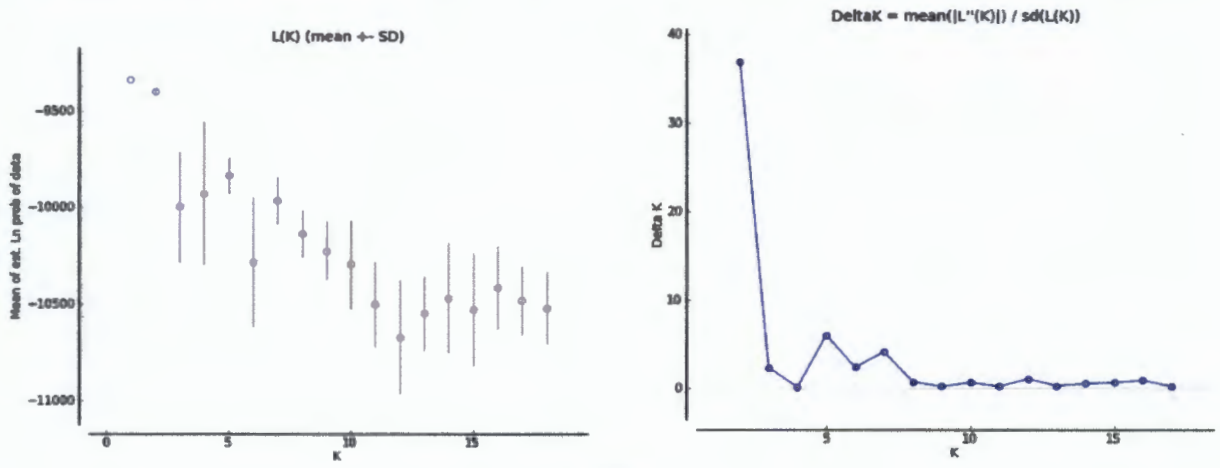


Figure 5.13 Cluster identity plots for the STRUCTURE analysis of captive birds only (n=119). Results are shown for K=2. Results are based on the average of 20 runs of 1 million iterations each, for each value of K. Each vertical bar represents one individual.

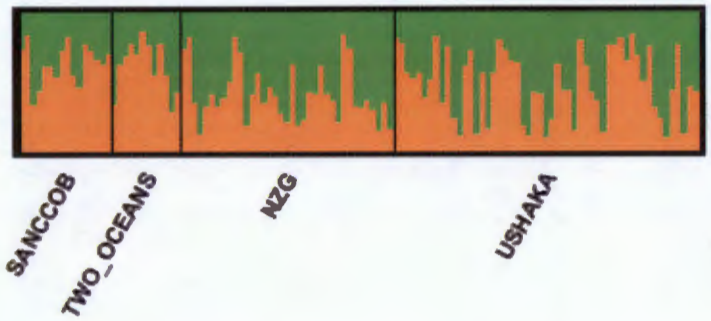
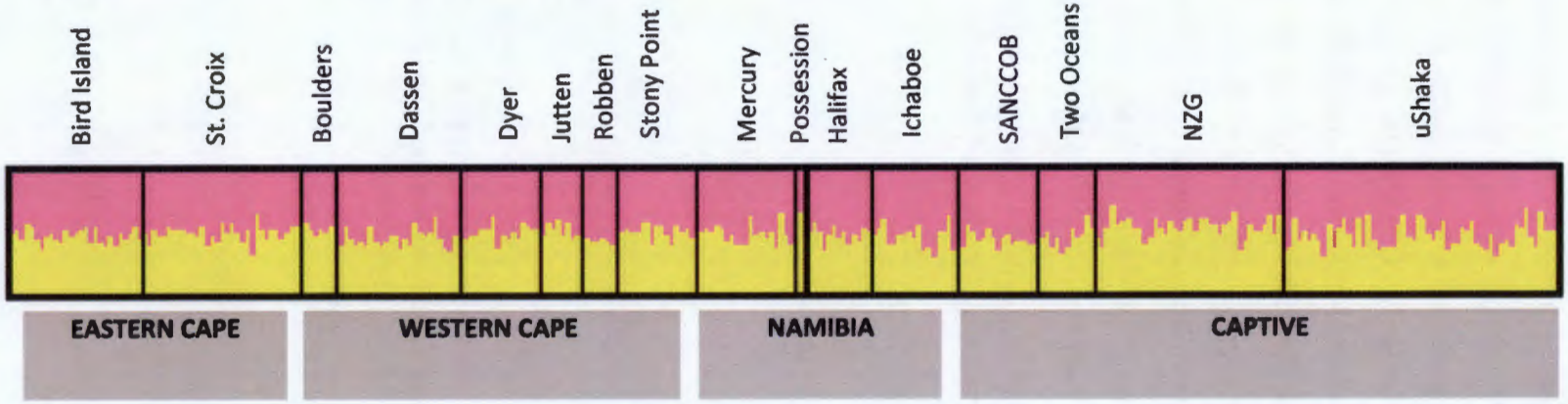


Figure 5.14 Average cluster identity of 308 African Penguins (wild and captive birds) for the best K-value (K=2). Results are based on the average of 20 runs of 1 million iterations each. Each vertical bar represents one individual.



DISCUSSION

Following range-wide declines, less than 5% (~24 thousand pairs) of the pre-1850s African Penguin population size is estimated to remain in the wild (Crawford et al. 2011). The conservation status of the African Penguin is likely to worsen in the future, largely because the drivers of this species' range-wide population decline are not fully understood (Crawford et al. 2008c, 2011). Any conservation intervention with the potential to improve the long-term prospects of survival of African Penguins in the wild deserves consideration. An intervention that has recently been suggested involves expanding the 'chick bolstering' programme (hand-rearing wild-collected chicks and eggs) to include captive breeding of African Penguins in zoos and aquaria followed by their reintroduction into natural habitats. A conservative estimate of global holdings of African Penguins is over 2500 individuals: 95 ISIS (International Species Information System) member institutions across South Africa, Europe, North America and Asia held 2272 individuals in 2012 (www.isis.org). The North American Regional studbook extends as far back as 1913, with over 3000 entries, and the current North American population is estimated at over 800 birds housed across 53 institutions. The global captive population, therefore, has considerable potential to contribute to a re-introduction programme for African Penguins, and may harbour some historical genetic diversity that has been lost in the wild. The genetic diversity represented by the global population, and the genetic health of the animals, has however not been assessed. Using studbook and molecular genetic data, this study assessed the captive population in South African zoos and aquaria. Aside from the logistical and economic constraints associated with launching a nation-wide, or possibly global, conservation breeding program for African Penguins, there are genetic aspects that first require investigation.

One of the most important determinants of the evolutionary consequences of conservation breeding is the number of generations spent in captivity i.e. the number of generations exposed to an altered selective regime (Wang & Ryman 2001; Robert 2009). Even a few generations of domestication appear to have negative fitness effect in the wild and, consequently, the long-term use of captive-bred populations to supplement wild populations should be carried out with caution (Araki et al. 2007). For long-lived species, such as African Penguins, it should be possible to keep the number of generations in captivity to a minimum by carefully managing husbandry, thereby minimizing genetic adaptation to captivity. In the case of supplement breeding for bolstering populations, birds should be kept at rehabilitation centres for as short a time as is possible before being released back into the wild. Supplement

breeding, as is carried out by SANCCOB and the "chick bolstering project" theoretically poses minimal threat to the genetic integrity of African Penguins, however, further research is strongly recommended to quantify successful establishment of captive-reared individuals. The motivation to release captive-bred penguins is to boost their abundance in the wild, which will hopefully stem the loss of wild genetic diversity and reduce the extinction risk of wild colonies. However, research into the dispersal, survival and reproduction of captive-bred birds, and the fitness implications for wild colonies, is lacking. The effectiveness of captive breeding programs at improving the status of wild African Penguin populations critically depends on whether or not released birds survive and reproduce successfully. It is therefore advisable for captive institutions to first investigate - using tagging and tracking studies - if captive-bred individuals survive, where they disperse post-release, and if they reproduce. Also, depending on the genetic composition of captive populations, it may be advisable for multiple institutions to carry out simultaneous captive-breeding programmes to prevent particular genetic lineages from dominating among released birds (Fraser 2008).

The active contribution of South African zoos and aquaria to African Penguin conservation is undoubtedly a positive development in the battle to ensure the survival of this species. However, at least initially, the most valuable contribution by these institutions may be further research into genetic diversity, as well as the dispersal, survival and reproductive success of captive-bred individuals. In addition, experimental and developmental studies aimed at improving our understanding of disease resistance, physiological thresholds, and the effects of environmental change on individual birds will be critical to future conservation efforts.

Two critical factors to consider when attempting to maximise retention of genetic diversity in captive populations are population size (specifically genetically effective population size, N_e) and isolation or a lack of gene-flow (Carroll & Fox 2008). Economic constraints and limitations of available space in captive breeding or supplement breeding institutions restrict the breeding population size, which in turn has implications for how fast genetic diversity is lost due to inbreeding, or how quickly the population exhibits the negative effects of inbreeding (Wright 1921; Brook et al. 2002; Jamieson 2011). If it is possible to occasionally incorporate new wild individuals and/or individuals from other institutions into the breeding program, this would ameliorate the effects of isolation (Williams & Hoffman 2009). Also, the size and genetic diversity of the founder population determines the composition of the captive population, and ideally founders should have included individuals from several populations to avoid a small number of genetic lineages dominating the captive population (Witzenberger &

Hochkirch 2011). Many of the African Penguins currently in captivity in South Africa are wild-caught, and the oldest known captive lineages only extend back five to six generations, which means that the loss of genetic diversity may have been kept to a minimum to date.

With respect to genetic diversity, all captive populations exhibited lower than expected heterozygosity and were more closely related than expected. The captive population also exhibits fewer private alleles overall compared to the wild population, which may indicate possible adaptation to captivity, or, more likely, the loss of rare alleles through genetic drift acting in captive and declining wild populations. Captive birds also showed depressed allelic richness compared to wild populations. 'Wild' alleles are fairly well represented in captivity, although they will only persist if captive populations are carefully managed. Inbreeding will inevitably lead to the fixation of some alleles, and the loss of others, and over even a few generations, the captive population could diverge from the wild population as it loses diversity.

In terms of population structure, allele frequencies among captive populations of African Penguins were moderately (F_{ST}=0.05, G_{ST}=0.032), but highly significantly differentiated. uShaka and Two Oceans Aquarium are most differentiated from each other (F_{ST}=0.15, D_{EST}=0.1), possibly because the uShaka population comprises a few large, closely related families (Figure 5.1). In fact, the three highest pairwise population differentiation estimates are between uShaka and each of the other three captive populations (Tables 5.7 to 5.9). The majority (82%) of captive individuals were correctly assigned to their institution. The use of differences in the variance of the repeat number of the alleles in each population is less sensitive to structure among captive populations than among wild populations. The broad comparison between captive and wild populations showed weak, but significant differentiation of allele frequencies (F_{ST}=0.01), but an almost ten-fold stronger, significant differentiation in terms of genetic composition (R_{ST}=0.092). However, only 70% of individuals were correctly identified as "wild" or "captive".

At a 'regional' scale, the captive population shows elevated levels of population differentiation compared to wild regional populations i.e. it is more different from wild regional populations than they are from each other based on R_{ST} and F_{ST} estimates (Tables 5.11 to 5.13). Overall R_{ST} is again higher than F_{ST} (0.08 compared to 0.009 respectively, both significant). This emphasises that the repeat number of allele statistic (R_{ST}) detects more structure than traditional frequency-based F_{ST} among wild populations, suggesting differences in allelic composition, but this pattern is reversed among captive populations.

Pairwise population comparisons revealed that the captive population is most differentiated from the Namibian population (R_{ST}=0.24). The Namibian population is underrepresented in captivity, which should be taken into account in the genetic management of African Penguins, and is particularly relevant in terms of a reintroduction program.

At a finer scale there is stronger differentiation among captive populations, and between populations in captive institutions and wild breeding colonies. Overall, R_{ST} was again higher than traditional frequency-based estimates (0.143 compared to F_{ST}=0.024, both significant; Figure 5.7), but striking patterns of significant differences were detected in pairwise population comparisons (Tables 5.16 and 5.17). Overall the inclusion of captive populations elevated population structure at coarse and fine scales of analysis (Figure 5.14). This pattern is emphasised by the results of the population assignment tests, where an average of 50% of captive individuals were correctly assigned to their captive institution, where only 10% of wild birds were assigned to their breeding colony based on their multi-locus genotype.

Conservation breeding for reintroduction

Potentially rapid genetic changes can occur in captive populations that may compromise the goals of conservation breeding programs. Careful genetic management is required to avoid the future loss of genetic diversity, maintain individual animal health and ensure population persistence (Ballou et al. 2010). Captive populations that are not intended for reintroduction into the wild are traditionally managed based on studbook data with the goal of minimizing the loss of founder genetic diversity. However, if the goal of captive breeding is reintroduction or supplementation of wild populations, captive populations must retain the wild characteristics required to survive and reproduce in their natural environment, and should also exhibit high levels of genetic variation to allow them to adapt to future environmental changes (Woodworth et al. 2002; Lacy 2009). It is therefore important to understand the genetic status of wild African Penguin populations, and to try and ensure that 'wild genetic diversity' is well represented among captive populations (Marsden et al. 2013). Molecular genetic markers, specifically microsatellite loci, are increasingly being employed to investigate inbreeding and relatedness among captive populations for comparison with wild populations (Marsden et al. 2013). Assaying and comparing genetic diversity within/between wild and captive populations based purely on microsatellite markers, however, is not advisable. As mentioned before, there may be important differences in adaptive genetic diversity that affect the fitness of individuals in different environments.

Range-wide adaptive genetic diversity should, therefore, also be investigated and considered in captive-breeding programmes.

The IUCN Species Survival Commission Guidelines for Reintroduction state that: "The potential negative effects of removing individuals from wild or captive populations should be assessed; where captive or propagated populations are sources, the holding institutions should ensure that their collection plans, institutionally and regionally, are designed to support such removals for conservation translocations" and that "Captive or propagated individuals should be from populations with appropriate demographic, genetic, welfare and health management, and behaviour" (IUCN Species Survival Commission 2012, section 5.1.4). Although it is not accurately known what environmental changes will impact African Penguins in the future, it is certain that environmental change is occurring globally, and that maximizing genetic variation among African Penguins will improve their capacity to adapt to future changes. We also know that high connectivity among wild breeding regions and breeding colonies appears to buffer the loss of genetic diversity among declining seabird populations (Taylor et al. 2011a; Ramírez et al. 2013), including African Penguins (Chapter 4).

To maintain captive populations that are genetically similar to wild populations, two primary drivers of genetic change in captivity must be avoided: genetic drift and artificial, unintended selection for traits that confer high fitness in the captive environment (Lacy 2009); i.e. genetic drift, inbreeding and potential adaptation to captivity. There is some evidence that these factors are already influencing the captive populations investigated in this study e.g. deviations from HWE and elevated inbreeding coefficients, which may be associated with inbreeding depression and negative effects on fitness. Several possible explanations exist for the significant heterozygote deficiency detected among captive birds (especially evident among those from uShaka Marine World): the Wahlund effect (erroneous lumping of subdivided populations), strong genetic drift, inbreeding, hitchhiking or synteny, null alleles and selection for homozygotes (Rooney et al. 1999; Spong et al. 2000). These results could also reflect that the captive samples comprised many more related individuals than the wild samples. If genetic drift were playing a dominant role, we expect to find lower levels of genetic diversity than observed in the wild. We cannot, however discount the possibility that genetic drift is also occurring in wild populations, and depressing genetic diversity there. LD tests would have detected the effects of hitchhiking or synteny, and the possibility of such a high proportion of loci being affected is remote (similarly, null alleles). Based on the studbook data, and also the fact that the data were analysed at multiple 'population'-levels,

the Wahlund effect is also unlikely to be causing the observed pattern. The most plausible explanations in the case of captive African Penguins are genetic drift and inbreeding. Genetic drift can be strong in captive populations, because they are often founded by only a few individuals, and are usually maintained at a small total size (Lacy 2009). The pedigree data (Figure 5.1) indicate elevated consanguious matings among uShaka's penguins, which could be the cause of the significant observed heterozygote deficiency. This is also likely to be causing the NZG population, and 42% of loci among the captive population overall, to significantly deviate from HWE.

Studbook (pedigree) -based versus molecular genetic analyses of African Penguins

Some discrepancies were detected between metrics derived from studbook and genetic marker data. Two important parameters estimated during pedigree-based genetic analyses of captive populations are the mean kinship (MK, Ballou & Lacy 1995) and the kinship coefficient between any two individuals. The latter represents the probability that an allele sampled at random will be identical to one sampled from the same locus in the second individual, and also represents the inbreeding coefficient of any offspring that a pair of individuals produce (Ivy & Lacy 2012). An individual's MK is the mean of its pairwise kinships to all individuals in the population i.e. individuals with few close relatives will exhibit a low MK and should theoretically carry rare alleles (Leus & Lacy 2009; Ivy & Lacy 2010). The overall population MK (the mean of all pairwise kinships) represents the expected average inbreeding of the next generation if mating is random, and if it is minimised, inbreeding will also theoretically be minimised. One minus MK is equal to the mean heterozygosity expected under Hardy-Weinberg equilibrium (HWE), which is expressed as a fraction of the genetic diversity of the population from which the founders were sampled. Thus, minimizing MK among captive African Penguins is equivalent to maximizing gene diversity.

The population mean kinship based on the African Regional African Penguin Studbook (4th Edition, March 2012) is 0.018, and the mean heterozygosity expected under HWE is, therefore, 0.982. Mean relatedness among captive populations based on molecular genetic data ranged from 0.026 (uShaka Marine World) to 0.057 (Two Oceans Aquarium, Figure 5.3). Positive F_{IS} values among captive populations also indicate that pedigree-based analyses are under-estimating the effects of inbreeding on captive populations. Among published studies, the correlation between pedigree inbreeding coefficients and marker-based estimates

of inbreeding is low (Pemberton 2008). Unbiased expected heterozygosity based on molecular genetic data is much lower than 1-MK, at ~0.6. The estimated number of founders based on studbook data (African Regional Studbook, 4th Edition, March 2012) is 56 individuals, with the current population representing 27 'founder genome equivalents' i.e. although the population descends from 56 presumably-wild founders, much of the genetic variability of those wild animals has been lost over time, with the current "gene diversity" estimated as being that which would be represented in 27 wild-caught animals, and the studbook contains >210 individuals (approximately 45 of these are founders) housed in 12 institutions. Based on molecular genetic data, the current captive populations contain on average 66% of the alleles detected among wild birds.

The ratio of effective population size to total population size ($N_e/N = 0.28$) estimated from studbook pedigree analysis (Lacy 2009; Ivy & Lacy 2010) of African Penguins is similar to that of the Arabian Oryx (0.3), which is considered to be an exemplary example of a successful captive breeding programme. Among captive populations of African Penguins included the African Regional Studbook, there is certainly individual variation in breeding attempts and breeding outputs. About 67% of the 210 birds recorded in the studbook (March 2012) hatched in captivity. The oldest male is 34 years old, similar to the oldest female, although birds rarely breed successfully when they are >29 years old. According to the studbook data, much of the breeding has been due to a few prolific animals i.e. the five most reproductively active males have sired 120 chicks, and the five most successful females 109 chicks (maximum of 31 for males and females) since 1980. According to hatch seasonality in the studbook, most captive African Penguins breed at the same time as their wild counterparts, indicating that adaptation to captivity may play only a minimal role in shaping genetic diversity in captive populations.

Overall, the molecular genetic- and pedigree-based assessments of the captive African Penguin population in South Africa are positive, and a large proportion of wild and founder genetic diversity appears to be conserved among captive birds. However, some captive populations are more representative of wild populations than others, and some wild populations are not well represented (e.g. Mercury Island in Namibia). The best captive born candidates for reintroduction are those individuals that will benefit the genetic diversity of the wild population, but are genetically well represented in the captive population (Leus & Lacy 2009). Investigating genetic diversity loss in captive populations is important because it is a

measure of the accumulated inbreeding that can depress fitness of individuals, and a measure of the loss of the population's potential for future adaptive evolution.

Recent molecular advances have the potential to accurately describe relatedness among wild-caught founder individuals. Where ascertaining relationships among wild-caught animals was previously considered almost impossible (leading to captive population founders nearly always being assumed to be unrelated), it is now possible to quantify their relatedness. Founders no longer need to be the baseline population for future kinship calculations – especially if data are available from wild populations. This means that captive breeding programs, instead of merely retaining the genetic variation that was present in the founders, can manage populations so that they are representative of the wild population. This should be the goal of captive management for African Penguins, if captive-bred birds are to be released into the wild as part of a broader conservation strategy. Captive populations should be grown as quickly as possible, because slower growth generally increases the likelihood that founders will die before contributing sufficient offspring to the breeding program. This appears to have been largely accomplished (based on studbook data and molecular estimates of genetic diversity), with the number of captive-born and wild-born African Penguins in captivity having more than doubled since the year 2000, and continue to grow. Importantly, although the probability that a particular founder's alleles will be retained in the population increases as it produces more offspring, genetic diversity will be maximised if all founders produce an equivalent number of offspring.

It is evident from the studbook data that in most cases, although the population has grown quickly, captive breeding pairs have not contributed equally to the population growth. The movement of birds (or eggs) between institutions will be crucial to reducing the population differentiation among captive populations, and will minimise the need to add wild birds to the current captive population. Promoting higher genetic diversity in captive populations will increase the probability that some birds will survive and reproduce in the wild (Kleiman 1989). South African captive populations will have to either (a) continue to grow rapidly to reach the population sizes recommended for maintaining quantitative variation and sustainable genetic health (Kleiman 1989; Ballou et al. 2010; Jamieson & Allendorf 2012, 2013; Frankham et al. 2013), or (b) grow 'artificially' i.e. by improving international institutional cooperation in moving birds between breeding facilities, the genetic diversity of the source population for reintroduction would increase, and the loss of alleles through genetic drift would decrease.

Conservation and welfare organisations, together with aquaria have released captive-bred African Penguins into the wild for a number of years. However, such welfare-motivated reintroductions, while well-intentioned, can potentially cause suffering to individual birds, and result in mortality if they are not based soundly on scientific principles. In the event that such releases are successful in terms of the individual's welfare, they may fail in terms of their conservation value, which may be zero, or even negative. The greatest opportunity for rehabilitation programs to contribute to conservation lies in the potential for research, i.e to develop techniques and refine concepts that will improve the chances of success of future reintroduction programs. Before any conservation breeding program is initiated, careful planning must be undertaken guided by the IUCN technical guidelines for the management of *ex situ* populations for conservation (www.iucn.org), together with the guidelines for the management of captive penguins (Diebold et al., 1999). Interestingly, captive breeding has also been suggested for the other Endangered *Spheniscus* species, the Galápagos Penguin (Vargas et al. 2007). This recommendation was made based on the results of a modelling study, which indicated a 10% probability of extinction within 50 years for that species after population size dropped below 500 individuals. Similar population viability assessments have been carried out for African Penguins (Whittington et al. 2000; Kemper 2006), and are currently in the process of being updated.

CONCLUSIONS

Despite years of protection African Penguin populations have failed to recover. This suggests that the root cause(s) of their decline in the wild is yet to be significantly addressed. The species may take many hundreds of years to naturally return to anything resembling their historical numbers, if ever. Indeed, the carrying capacity of the Agulhas-Benguela Ecosystem for African penguins has declined drastically over the last century (Crawford et al. 2007c). Increased competition and predation, and reduced prey resources, among other threats, are limiting the ability of African Penguins to efficiently track shifts in their prey resources. Actively supplementing populations may help to maintain wild populations at sustainable numbers, and may also contribute to the establishment of new, potentially stable populations (Schultz et al. 2011). Further research into the fate of captive-bred and hand-reared birds in the wild is required, as is a thorough investigation of the consequences of captivity in terms of adaptive genetic diversity.

In conclusion, having assessed the genetic status of the captive African Penguin population relative to wild populations using neutral markers, it is recommended that captive populations

be managed more carefully to improve genetic representation of 'wild' diversity among captive birds. Genetic monitoring should be routine, and the incorporation of individuals from the global captive African Penguin population is strongly advised. Captive breeding is an intensive and expensive approach that should be seen as a short-term solution i.e. to be implemented until the primary causes of African Penguin population declines in the wild are better understood, and effective strategies to reverse them have been identified.

CHAPTER 6: Synthesis of findings and future research directions

"The animals of the world exist for their own reasons. They were not made for humans any more than black people were made for white, or women created for men."

(Alice Walker, in Spiegel 1996, p.14)

Conservation genetics has great potential to contribute to the conservation of threatened, endemic seabirds in the Agulhas-Benguela Ecosystem (ABE) at a number of ecological and evolutionary scales. This thesis demonstrates that molecular techniques can be highly informative (i) for broad taxonomic questions, (ii) when trying to understand genetic connectivity among wild populations and (iii) when planning for the possible future reintroduction of captive-bred or captive–reared individuals.

The DNA sequence-based comparative genetic results all reveal a general pattern of genetic homogeneity, suggesting extensive regional connectivity, among the three study species, which are similar in many aspects of their foraging and breeding biology, but markedly different in, among others, their dispersal ecology and mobility. The lack of population or phylogeographic structure within these species, despite known natal-site and mate-fidelity, and large distances between breeding regions, suggests that these species behave as metapopulations, and that sub-populations of the species are strongly connected through long-term gene-flow. This tight connectivity is most likely mediated via juvenile dispersal, which may have buffered these species against genetic diversity loss and population differentiation, and is potentially a life-history characteristic that has evolved in response to high levels of variability inherent in the ABE. The closest extant relatives of the African Penguin *Spheniscus demersus* have also been extensively studied in a conservation genetic framework, and show similar results, although some evidence exists for a pattern of isolation-by-distance population structure among Magellanic Penguins *S. magellanicus.* Similarly, for Cape Gannets *Morus capensis,* their close relatives and ecological analogues in other ecosystems, the boobies, generally only show population structure where large physical barriers (historical or contemporary) exist; e.g. continents, the Isthmus of Panama, or vast stretches of open ocean between populations. Non-physical-barriers e.g. strong philopatry and restricted dispersal may also play a role in generating spatial patterns of genetic differentiation in boobies and other seabirds (Greenwood & Harvey 1982; Klages 1994; Steeves et al. 2005b; Alcaide et al. 2009; Morris-Pocock et al. 2010a; Hailer et al. 2011; Taylor et al. 2011a). Based on comparative studies of inshore and pelagic booby species, however, foraging mode appears to play a role in shaping the geographic distribution of genetic diversity. The preliminary results for inshore-foraging Bank Cormorants

Phalacrocorax neglectus, may provide further support for this hypothesis, and should be further investigated.

African Penguins and Cape Gannets are likely to represent colonization events from South America and Australasia respectively (Patterson et al. 2011; Ksepka & Thomas 2012), and the minimum ages of these species are known with some certainty (0.5-0.8 Mya and ~1.1 Mya respectively). The age of the Cape Cormorant *Phalacrocorax capensis* lineage is less certain, and the cormorant phylogenies presented in Chapter 2 indicate that the closest relative of Cape Cormorants is either the White-breasted *Phalacrocorax lucidus* or Bank Cormorant, and suggest that the Cape Cormorant lineage has evolved *in situ* in the ABE. This may explain the Cape Cormorant's higher resilience (demographically, when compared to the other two focal species) to changes in food availability. For African Penguins, there appears to have been multiple waves of dispersal during the Cenozoic (Thomas & Ksepka 2013), possibly during times of glaciation when penguins on the South American Atlantic and Pacific Coasts were forced northwards by ice and sea-level changes (possibly combined with the northward migration of the subtropical convergence). Similar drivers may explain the colonization of the ABE by Cape Gannets. All three of the focal species have shifted their core breeding distributions in response to changes in their environment over recent decades, and have likely had to do this over evolutionary time too. Such range shifts have been observed in a number of seabird species worldwide over the past few decades; e.g. a global meta-analyses documented significant range shifts towards the poles (Parmesan & Yohe 2003); seabirds in the California and Humboldt upwelling ecosystems shifted their ranges recently, purportedly due to environmental change (Ainley and Divoky 2001); in Western Australia some tropical seabirds have extended their ranges southwards (Dunlop and Wooler 1986), likely as a result of changes in ocean temperatures and climatic conditions (Chambers et al. 2005). Ongoing change in the ABE represents an ecological trap for seabirds because there is no suitable breeding habitat farther south than Dyer Island in the Western Cape.

A large number of population genetic and phylogeographic studies have been carried out on seabird populations around the world. These studies add to a growing body of knowledge regarding seabird evolution and ecology, and provide a perspective that greatly complements traditional ecological research. The motivation to better understand seabird species and communities is driven by two main factors: (i) they are convenient to study i.e. sufficient sample sizes can be obtained to test evolutionary hypotheses, and they have the potential to play a role as indicators of change in a marine environment, and (ii) there is increasing

concern about the conservation status of seabirds because their life-history strategies make them vulnerable to population decline brought about by anthropogenic threats. Seabirds are important indicators of the state of marine ecosystems, and are relatively easy to monitor compared to other marine organisms (Durant et al. 2009). Studying the ecology, demography and population genetics of seabirds is important to build a better understanding of a very threatened group of birds, and a better understanding of the marine ecosystem upon which they depend.

Population genetic structure in seabirds, or the lack thereof, has been shown to result from a number of different historical and contemporary evolutionary forces. The fact that some seabird species show varying patterns of differentiation across their ranges (see Chapter 2) seems to contradict the idea that non-physical barriers to gene-flow, e.g. philopatry, are the primary drivers. All three of the endemic, cold-water adapted species examined in this thesis show little evidence of genetic structure. The strong regional connectivity suggested by the mitochondrial markers indicates that there has been effective regional dispersal over the long-term; ringing- and tracking-data suggest that this has likely been primarily mediated via juvenile dispersal to non-natal colonies. Similar patterns have been reported among populations of closely related seabird species, suggesting a role for phylogeny i.e. evolutionary constraints and shared, inherited life-history characteristics. Overall, an emerging pattern in the literature is that species that have evolved in highly variable and unpredictable ecosystems, such as large upwelling ecosystems, show less phylogeographic structure, retain more genetic diversity (and possibly adaptive potential) and are less prone to local adaptation than species that have evolved in more stable, predictable environments (Akst et al. 2002; Bouzat et al. 2009; Duffie et al. 2009; Taylor et al. 2011a; Ramírez et al. 2013). Under a scenario of increased environmental variability in the form of fluctuating prey availability, selection may favour species with flexible foraging behaviour (foraging effort, diet switching) and breeding behaviour (clutch size, laying dates; i.e phenology and synchronicity), and populations may change quickly due to large fluctuations in N_e under these conditions.

Despite drastic population declines no strong genetic signatures of bottlenecks were detected among African Penguin populations, although lower genetic diversity was observed compared to closely related species. Allendorf (1986) and Spencer et al. (2000) suggest that because population bottlenecks may have little effect on heterozygosity, the number of alleles that remain after a bottleneck may be more important for the survival of populations. It is

likely that insufficient time has passed since the start of their demographic decline for an evolutionary genetic signal of their decline to be detected. The results of this study suggest that ongoing gene-flow has buffered African Penguin populations from the negative genetic effects associated with small and/or declining populations. These results suggest that a pattern of high genetic connectivity is likely to be ongoing. It is clear that further research is required to confirm this hypothesis for Cape Gannets and southern African cormorant species using genetic markers with higher resolution. The focal species of this thesis have different foraging behaviours, and changes in food availability affect them differently. Decreasing food supply seems to impact the African Penguin sooner and more intensely than the flying species, likely due to its limited foraging range (Pichegru et al. 2010b). Certainly it is the African Penguin that has undergone the most recent rapid decline in the ABE compared to any other seabird species (Crawford et al. 2011).

For the most part, mitochondrial and microsatellite data suggest long-term mixing of populations, although Namibian populations of African Penguins are distinctive based on some results. For African Penguins, other results indicate a tighter connectivity between Namibia and the EC than between the WC and either of those two regions, which is contrary to what would be expected if the geographic isolation of regions; i.e. the ~600km distance between regions, represented a barrier to the movement (breeding dispersal) of African Penguins. These results could possibly reflect the growth of Eastern Cape populations of both African Penguins and Cape Gannets following the displacement of their prey. The growth of these populations is thought to be too high to be due to intrinsic population growth alone, the implication being that birds moved to that region despite their propensity to return to their natal- or breeding-sites. Given that (i) adult breeding-site fidelity has been shown to be strong, (ii) breeding adults of all three species forage consistently in similar areas i.e. cultural foraging grounds (although this is uncertain during the non-breeding season), and that (iii) juveniles are known to spend two to three years at sea before settling to breed, the effective dispersal by juveniles is likely the way in which neutral genetic diversity has become distributed across the shared range of these species, minimising population differentiation. This behavioural plasticity represents a critical balancing force between the microevolutionary processes shaping genetic diversity in these species, and is likely a result of adaptation to a highly variable environment, allowing seabird species to track changes in food availability, while maintaining the benefits of breeding colonially (e.g. reduced

predation of chicks, better anti-predator defence, more efficient foraging (Schippers et al. 2011)) and repeatedly returning to breeding sites as adults.

Conservation management implications

Current biodiversity is the product of past evolutionary processes, just as future biodiversity will depend on contemporary evolution (Hendry et al. 2010). A commitment to long-term conservation success requires conservationists to maintain not only those characteristics of seabird species that have evolved in the ABE, and have enabled them to persist until now, but also the adaptive potential for a future in which they are likely to face further environmental change. Conservation strategies need to ensure that the natural distribution, abundance, genetic diversity and ecological niche of these seabird species are preserved. This is a challenge. We should encourage and allow species to exhibit variation, dispersal, evolution, and adaptation in a changing world (Redford et al. 2011a). For a long time, conservation planning emphasised pattern over process; i.e focussed more on ensuring representation of species, rather than conserving genetic diversity and the processes that have generated it (Moritz & Faith 1998; Allendorf & Luikart 2007; Frankham 2010). There are different schools of thought regarding the way in which genetic diversity should be incorporated into conservation planning: one argues for adaptive variation, the other for neutral diversity. These two components reflect different microevolutionary processes (selection / adaptation versus genetic drift), and suggest alternative strategies for conservation. Currently, there is no strong evidence at neutral markers that regional- or colony-level Evolutionary Significant Units (ESUs) exist among contemporary populations of the study species. However, adaptive diversity may reveal a different pattern (see future research section below). Low-diversity mitochondrial lineages, typically disregarded as important from a conservation standpoint, can correspond to recently selected, well-adapted haplotypes that should be managed and preserved (Galtier et al. 2006; Bazin et al. 2006; Nabholz et al. 2009). In this study, there is little evidence for neutral divergence within the three focal species based. Logistically, however, they will have to be managed as at least two MUs, because Namibian and South African management authorities have different conservation priorities and mandates. Connectivity between breeding regions for all three species appears to be high, and conservation efforts should, therefore, focus on maintaining population viability and adaptive genetic diversity by preserving metapopulation structure. Source-sink dynamics may play an important role in allowing these seabird populations to cope with environmental variability over the medium- and long-term and, therefore, it may be crucial to conserve metapopulation

structure. It is important to remember that there is a temporal aspect to this protection: breeding colonies of the focal seabird species can be thought of as a well-connected metapopulation; however, populations of colonially breeding seabirds are not typical metapopulations, and their dynamics may not conform to classic metapopulation theory (Matthiopoulos et al. 2005) e.g. in colonially breeding seabirds, patches (or colonies) exist only during the breeding season and habitat that is unsuitable for breeding may be suitable for other activities, such as foraging. Conservation managers should consider these aspects, before applying classic metapopulation management principles.

The data presented here also have implications for captive management and planning of a reintroduction program for the flagship species of this study, the African Penguin. Despite multiple conservation interventions to alleviate threats to this species (e.g. artificial nests, the release of hand-reared chicks, and fishing closures around colonies), African Penguin numbers continue to decrease (Crawford et al. 2011). It is important to know that the neutral and adaptive genetic diversity of captive populations is similar to that found in the wild, as this may increase the probability of survival of captive bred birds once they are released into their natural environment.

Future research and the importance of adaptive genetic diversity

Overall, this study has provided valuable baseline knowledge for making management decisions and planning future research about the impacts on seabirds of changes in their environment. Further research, however, is still necessary because indirect measures of dispersal from molecular data are often difficult to interpret i.e. different population-level processes may result in similar genetic patterns, which can sometimes only be teased apart by using different classes of molecular markers together with ecological data (Alcaide et al. 2009). The resolution of the mitochondrial and nuclear sequence data employed here can be improved by the use of more rapidly evolving markers (e.g. microsatellites, as in Chapter 4 and 5). Given the possible analytical complications likely to be encountered as a result of duplications in mitochondrial genes e.g. control region (confirmed in sulids) and Numts, it is recommended that a microsatellite or SNP study be carried out on Cape Gannets and Cape Cormorants, which is likely to provide sufficiently fine-scale resolution to test hypotheses regarding gene-flow between colonies and regions. To test more general hypotheses about top-predators in the ABE, similar work could be carried out on other endemic seabirds and marine mammals e.g. Bank Cormorants and Cape Fur Seals (Matthee et al. 2005).

Studies focussing on adaptive genetic diversity, specifically those linked to measures of fitness, will also improve our understanding of the micro-evolutionary forces that are driving the observed population genetic patterns. Studies of the Major Histocompatibility Complex (MHC) in seabirds have had some success in this regard. Genes of the MHC are among the most variable in the vertebrate genome (Radwan et al. 2010; Burri et al. 2010; Ujvari & Belov 2011). MHC genes play a pivotal role in immune-competence, as they code for cell-surface glycoproteins that present specific antigens to the immune system's T-cells, thereby triggering an appropriate immune response. A large body of data supports a model of MHC evolution under positive selection, with polymorphisms at these loci maintained by balancing selection. This selection regime is thought to be mediated via pathogens, mate-choice, or a combination of these. Both coding and non-coding regions of the genome are subject to the effects of neutral evolutionary processes (e.g. gene-flow and genetic drift), however, MHC genes are predominantly subject to selection, reflecting adaptive changes in populations. In the conservation management of declining species it is important to characterise this adaptive genetic variation, as it represents a species' ability to respond to potentially changing environments. Patterns of MHC variation are not necessarily reflected in those of neutral markers. Reduced MHC variability following long-term population decline, especially in combination with strong selective pressure, can have clear repercussions for the overall fitness of the population, as it may significantly limit population-wide responses to novel pathogens.

Further work that investigates selection and mating systems in African Penguins is recommended, as these factors could also influence the observed deviations from HWE. Specifically, heterozygosity-fitness correlations would improve our understanding of the importance of genetic diversity for the fitness of individual birds and would require further input from flipper-banding data and reproductive monitoring at colonies across the range of this species. Improved estimates of gene-flow and dispersal from single nucleotide polymorphism (SNP) data and flipper-banding (or transponder data) may also make it possible to detect the degree and direction of gene-flow among specific colonies. Assessments of neutral genetic markers may not always reflect the patterns of diversity at adaptive loci, and discrepancies are sometimes found between diversity metrics derived from neutral and adaptive markers (e.g. Marsden et al. 2013). Based on this, and that the 'genetic robustness' of a population is difficult to estimate using only a few neutral markers, it is highly recommended that further research be carried out into levels of adaptive genetic

diversity in *Spheniscus* penguins. Through the identification and quantification of genes known to be relevant to the fitness of individuals in the wild, it will become possible to prepare a captive population comprised of African Penguins that are likely to survive and reproduce in the wild.

Unfortunately, molecular techniques and analyses that measure diversity at the individual level across the entire genome (Next-generation sequencing technology (NGS); genome re-sequencing and Restriction site associated DNA (RAD) markers) are expensive and logistically challenging. Even the most extensive studies are only able to sample several score of loci among the many tens of thousands of genes that make up an animal's genome. Managing a species based on the diversity of only a few loci won't necessarily achieve the goal of genome-wide diversity, as it will tend to result in populations with high diversity at the monitored loci, but loss of diversity at others (Ballou et al. 2010). Phenotypic plasticity is fundamental to an animal's ability to deal with environmental change. However, little is known about the nature of plastic responses in wild populations, the genetic basis of such responses, or how natural selection acts these (Nussey et al. 2005a; Pigliucci 2005). The impact of environmental change on animal populations is nonetheless strongly influenced by the ability of individuals to adjust key life-history traits, and considerable interest exists in establishing the degree of plasticity in these traits and how selection acts on them in natural populations (Reed et al. 2006).

Conservation breeding

Conservation genetic principles applied to captive populations can be used for appropriate genetic management through parentage analysis and molecular sexing techniques. This has been successfully implemented for other captive penguin species, with power of discrimination (PD) and probability of exclusion (PE) estimates indicating that microsatellites and single-nucleotide polymorphisms (SNPs) can be powerful tools for individual identification and pedigree determination. It is strongly advised that the captive African Penguin dataset be expanded to include birds in captivity around the world, in order to (i) assess levels of diversity in other captive populations, and (ii) determine their role in future captive breeding programmes. Also, the inclusion of other *Spheniscus* penguins will allow for the identification of hybrids in zoos. Captive populations of penguins are frequently kept in mixed-species exhibits, leading to interbreeding between *Spheniscus* species, and producing fertile hybrids between African and Humboldt, African and Magellanic, and Humboldt and Magellanic (known to occur in the wild) Penguins (Conway 1965; Araya 1983; Thumser &

Karron 1994; Simeone et al. 2009). Hybridization has serious implications for conservation breeding programmes. Molecular data could be used to augment breeding programs to characterise genotypes at some loci known to be important for fitness, and manage the program to maximise retention of valuable alleles. There are two different approaches that are worth considering. First, we could select for animals carrying alleles believed to be especially important, such as variants at the major histocompatibility complex loci (Hedrick 2002; Hughes 1991). Second, we could measure variation at random loci, and then preferentially breed those animals that appear to carry the rarest alleles. In theory, this approach could produce a population with even more gene diversity than was present in the wild population, by creating more equal allele frequencies than existed in the source population. Although these ideas deserve more evaluation, I would caution, as have others (Vrijenhoek and Leberg 1991), that there are some potential drawbacks. First, we know only very few of the many loci that might be critical to individual fitness and population viability. If we select on the basis of those few loci about which we do know something, we are very likely to cause rapid depletion of genetic variability at other loci that may be just as important (Hedrick 2001; Lacy 2000c). This is especially so because the alleles that are advantageous will depend on what environment the animals are in. Thus, many alleles that encode adaptations important in natural environments may be neutral or even deleterious in a specific captive environment. A strategy of preferentially breeding animals that have the rarest alleles, without trying to prejudge which alleles will be most advantageous, has perhaps more merit than attempts to select the animal with superior alleles. However, even this strategy has risks. Initially rare alleles may have been rare for a good reason. Selecting for them may increase frequencies of mutations that were deleterious in the natural populations. I think we are on safer grounds if we use strategies that attempt to minimise the rate at which the populations under our care diverge genetically from what they were before we took control of their breeding. Stopping evolution from causing negative effects in captivity may be a better approach than trying to improve upon the results of prior evolution in wild populations.

Fitness is certainly not equal across African Penguin breeding colonies, with reproductive success higher on the south and east coast compared to Namibia and the west coast of the Western Cape. Investigating the distribution of adaptive genetic diversity in these populations – which may show a different pattern to neutral diversity – will provide important information about prevailing selective regimes across the range of African Penguins. NGS, among other rapidly advancing technologies, may help us to identify important regions of the

genome under selection, and may provide important information for captive-breeding in the future. If supplementation using captive bred African Penguins is likely to be part of a long-term conservation strategy, molecular methods have an important role to play in insuring the successful reintroduction. Validation of the studbook-based pedigree and expansion of this pedigree to include global captive African Penguin populations will increase our confidence in the provenance of released birds. Linking the known pedigree with genetic diversity at neutral and adaptive markers for comparison with wild populations will contribute to the proper genetic management of the global population. It will also ensure that the most suitable individuals possible derived from the captive population can be reintroduced into their natural habitat with minimal impact on wild populations and the best chance of establishing. Also, further research into movements and foraging behaviour of released captive bred birds will provide insight into what environmental cues influence foraging and breeding behaviour and possibly inform the degree to which these are the result of gene-environment interactions.

Conclusion

At the end of the day the field of conservation genetics is rooted in our concerns about the survival of threatened species, and the genetic effects of diminishing population sizes. The pursuit of this knowledge is based on the premise that the more we understand about a species, the better our chances of successfully ensuring its persistence it into the future. Any management intervention that maintains natural processes, and the abundance and biodiversity of life they have generated, and will continue to generate, fulfils the ultimate goal of conservation genetics. Our earth is unique in the known universe in that life evolved here, and the most striking attribute of that life is the staggering diversity that has existed and will most likely exist long after the "Anthropocene". Studying the natural world provides an opportunity to marvel at its wonderful, ancient complexity and I am grateful to have grasped even a small part of it.

"People must feel that the natural world is important and valuable and beautiful and wonderful and an amazement and a pleasure."

David Attenborough

APPENDICES

Appendices for Chapters 2 – 5 are provided on the enclosed CD.

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APPENDICES:
A conservation genetic study of threatened, endemic southern African seabirds

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TABLE OF CONTENTS

CHAPTER 2 APPENDICES:

Appendix 2.1 Results of population genetic studies of seabirds. including their estimated global population size (census size), allozyme and AFLP sample sizes and estimates of population differentiation, mitochondrial target region(s) (mtDNA), sample sizes (N_{mt}) and estimates of genetic diversity (haplotype diversity, *h;* nucleotide diversity, π) and differentiation (global or pairwise range of φ_{ST}), and microsatellite sample sizes (N_{micro}), number of loci (N_{loci}) and results (Heterozygosity (H_O), global and/or pairwise range of F_{ST}), and the threat status based on the IUCN Redlist of threatened species (2013; Least Concern (Lc), Near-threatened (Nt), and Vulnerable (Vu), Endangered (EN) and Critically Endangered (CR)).

Reference	Species	Scientific name	Census size	N_{pops}	N_{AFLP}	global θ^B	N_{mt}	mtDNA	Diversity (*h* |π)	φ_{ST}*	N_{micro}	N_{loci}	H_O	F_{ST}	IUCN
Dearborn et al. (2003)	Great Frigatebird	*Fregata minor*	>10 000 indiv	3	95	0.2667	–	–	–	–	–	–	–	–	Lc
Levin & Parker (2012)	Great Frigatebird	*Fregata minor*	>10 000 indiv	5	–	–	108	cyt b, ND2, COI (1954bp)	0.644 ± 0.051 | 0.00054 ± 0.00048	0.023	114	8	0.65	0.007	Lc
Morris-Pocock (2012)	Great Frigatebird	*Fregata minor*		1	–	–	37	cyt b, dloop	cyt b 0.410 | 0.0005, dloop 0.866 | 0.0032				0.6		
Milot et al. (2008); Rains et al. (2011)	Wandering Albatross complex	*Diomedea exulans & D. spp*	~20 000 indiv	10	344	<0.05	123	dloop		0.806	–	–		–	Vu
Young (2010)	Laysan Albatross	*Phoebastria immutabilis*	> 590 000 pairs	10	–	–	358	dloop	0.989 | 0.045	0.05	417	5		0.01	Nt

Moen (1991)	Atlantic Puffin	*Fratercula arctica*	>10 000 indiv	5		0.0031 (Allozyme F_{ST})	–			–	–	–		–	Lc
Moum & Arnason (2001)	Razorbill	*Alca torda*	> 500 000 pairs	7	–	–	123		| pi % 1.30 (0.93–1.98)	0.042	–	–		–	Lc
Riffault et al. (2005)	Common Murres	*Uria aalge*	>4 mil pairs	22	–	–	–			–	658	6		0.005	Lc
Austin et al. (1994)	Short-Tailed Shearwater	*Puffinus tenuirostris*	> 20 million indiv	11	–	–	–			–	–	–		–	Lc
Morris-Pocock et al. (2008)	Common Murres	*Uria aalge*	> 4 million pairs (over 7 million pairs globally)	29	–	–	575		| pi % Atlantic 0.53 (0.42–0.66), Pacific 0.26 (0.08–1.30)	0.072 (Atlantic) 0.009 (Pacific)	554	4		0.013 * (Atlantic) 0.003 (Pacific)	Lc
Pearce et al. (2006), Huyvaert & Parker (2006), Huyvaert & Anderson (2006)	Waved Albatrosses	*Phoebastria irrorata*	~30 000 indiv	3	–	–	–			–	69	4		< 0.012	CR

Hall et al. (2009)	Marbled Murrelet	*Brachyramphus marmoratus*	> 350 000 indiv	6	–	–	–	–	| pi % 0.70 (0.28–1.04)	0.08		13	0.812	0.025 | 0 to 0.038	EN
Pearce et al. (2002)	Ancient Murrelets	*Synthliboramphus antiquus*	> 1 million indiv	3	–	–	58		|pi % 0.42 (0.40–0.44)	0.00	–	–		–	Lc
Hailer et al (2011); González-Jaramillo & Rocha-Olivares (2011)	Magnificent frigatebirds	*Fregata magnificens*	>10 000 indiv	9	–	–	231	1636bp mtDNA: 531bp (ATPase 6), 550bp (Cyt b) and 555 (NADH2) (NADH3 not reported)	0.817| 0.0026	< 0.2 (non-Galapagos) >0.9(Galapagos)	219	8		<0.05 >0.34 (Galapagos)	Lc
Avise et al. (2000)	Sooty Terns	*Sterna fuscata*	> 20 million invid	5					π % 2.1 (1.8–2.6)	0.38					Lc
Burg & Croxall (2001)	Black-browed Albatross	*Thalassarche melanophris* and *T. impavida*	T melanophris > 1 million indiv; T. impavida > 10 000 indiv	5			73			0.0598 to 0.7408		7		0.0123 to 0.1494 (P < 0.001)	EN

Burg & Croxall (2001)	Grey-headed Albatross	*T. chrysostoma*	> 250 000 indiv	5			50			– 0.1350 to 0.1129		7		0.0059 to 0.0614	Vu
da Silva & Granadeiro (1999)	Cory's shearwater	*Calonectris diomedea*	> 600 000 indiv	8							148	fingerprinting		0.02 to 0.094	Lc
Randi et al. (1989)	Cory's shearwater	*Calonectris diomedea*	> 600 000 indiv	5 (n=145, 36 loci))											Lc
Gomez-Diaz & Gonzalez-Solis (2007)	Mediterranean Cory's Shearwater; Atlantic Cory's Shearwater; Cape Verde Shearwater	*Calonectris diomedea diomedea; C. d. borealis; C. edwardsii*		13; 10; 1											*C. edwardsii* Nt
van Bekkum et al. (2006)	Buller's albatross	*Thalassarche bulleri bulleri*	> 30 000 pairs	4							126	6		−0.0033 to 0.0056	Nt
Abbott & Double (2003a,b)	Shy Albatross	*Thalassarche cauta*	> 50 000 indiv	3			30			0.33 to 0.5	67	6		0.05 to 0.119	Nt

Abbott & Double (2003a,b)	White-capped Albatross	*Thalassarche steadi*	>150 000 indiv	3			29			0.07 to 0.16	70	6		0.007 to 0.016	Nt
Kidd & Friesen (1998a,b)	Black Guillemot	*Cepphus grille*	> 400 000 indiv	7	–	–	65		| π % 0.30 (0.00–0.58)	0.804	–	–		–	Lc
Kidd & Friesen (1998a,b)	Pigeon Guillemot	*Cepphus Columba*	> 400 000 indiv	3	–	–	54		| π % 0.87 (0.47–1.70)	0.562	–	–		–	Lc
Techow et al. (2010)	Northern Giant Petrel	*Macronectes halli*	> 11 000 indiv	9	–	–	51				192	6			Lc
Techow et al. (2010)	Southern Giant Petrel	*Macronectes giganteus*	> 100 000 indiv	11	–	–	74				229	6			Lc
Walsh & Edwards (2005)	Black-footed Albatross	*Phoebastria nigripes*	> 120 000 indiv	4	–	–	140		0.05 (0.00–0.12)	Hawaii vs Japan 0.914					Vu

Patirana et al. (2002)	Red-legged Kittiwakes	*Rissa brevirostris*	> 337 000 indiv		–	–	27			0.17					Vu
Welch et al. (2012); Wiley et al. (2011)	Hawaiian petrels	*P. sandwichensis*	> 6 000 pairs		–	–	322 | 80	cyt b	0.51 to 0.74 | π 0.0031 to 0.0037	0.425	232	18	0.57 to 0.62	0.019 *	Vu

Appendix 2.2: Bank *Phalacrocorax neglectus,* Crowned *Microcarbo coronatus* and Reed Cormorant *M. africanus* samples included in this study.

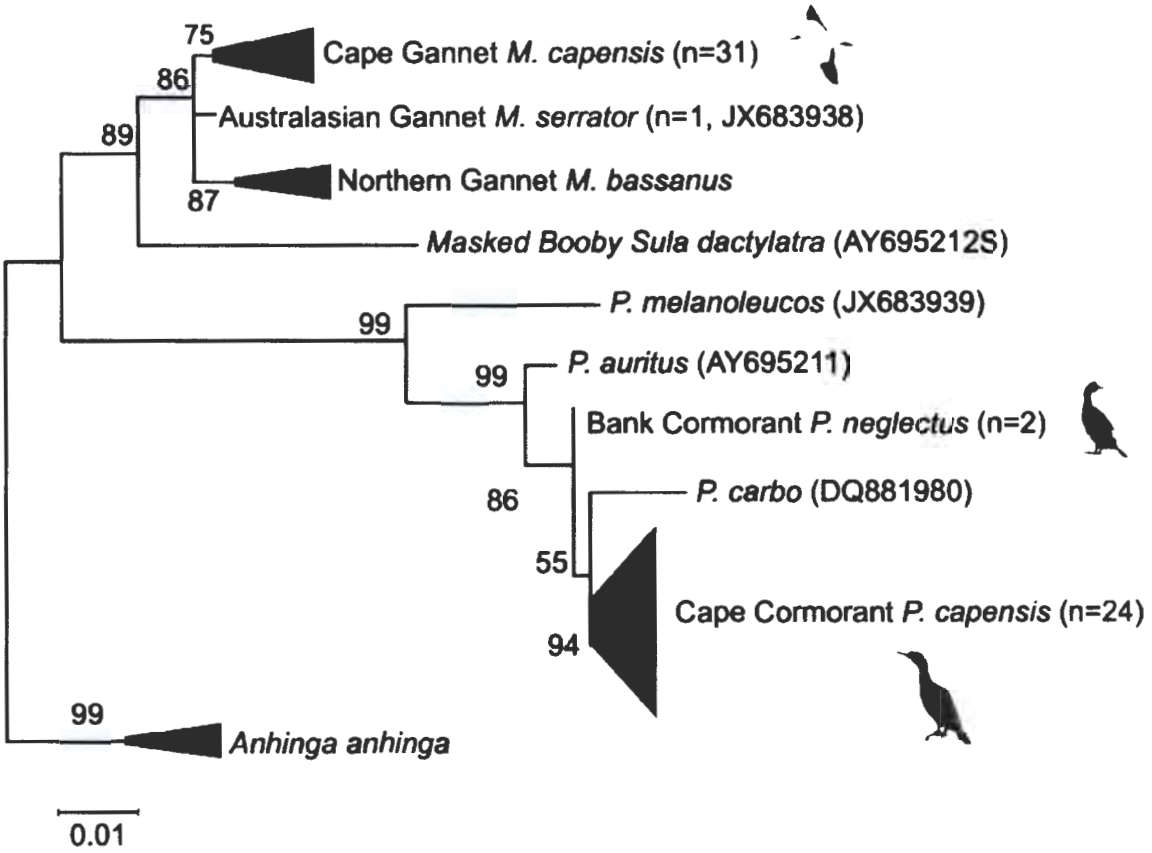
Sample code	Date	Species	Sample type	Locality	12S	cyt b	ATP6	ND3	COI
CR56	14-Oct-10	Crowned Cormorant	blood	Malgas Island	x		X	x	
CR57	14-Oct-10	Crowned Cormorant	blood	Malgas Island				x	
CR62	14-Oct-10	Crowned Cormorant	blood	Malgas Island	x	X			
CR63	15-Oct-10	Crowned Cormorant	blood	Malgas Island	x	x			
CR61	15-Oct-10	Crowned Cormorant	blood	Malgas Island	x				
CR58	14-Oct-10	Crowned Cormorant	blood	Malgas Island			X		
CR71	01-Nov-10	Crowned Cormorant	blood	Jutten Island	x		X		
BC46	16-Nov-09	Bank Cormorant	blood	Robben Island	x		X	x	
BC45	16-Nov-09	Bank Cormorant	blood	Robben Island	x		X		
BC48	16-Nov-09	Bank Cormorant	blood	Robben Island	x				
BC47	16-Nov-09	Bank Cormorant	blood	Robben Island	x		X		x
BC44	16-Nov-09	Bank Cormorant	blood	Robben Island			X		x
BC1SAN		Bank Cormorant	blood	SANCCOB			X		
BC3SAN		Bank Cormorant	blood	SANCCOB			X		
BCMIB1	24-Jan-02	Bank Cormorant	feathers	Mercury Island					
BCMIB2	24-Jan-02	Bank Cormorant	feathers	Mercury Island				x	
BCMIB3	24-Jan-02	Bank Cormorant	feathers	Mercury Island				x	
BCMIB4	24-Jan-02	Bank Cormorant	feathers	Mercury Island		X		x	
BCMIB5	24-Jan-02	Bank Cormorant	feathers	Mercury Island				x	
BCMIB6	24-Jan-02	Bank Cormorant	feathers	Mercury Island					
BCMIB7	24-Jan-02	Bank Cormorant	feathers	Mercury Island					
BCMIB8	24-Jan-02	Bank Cormorant	feathers	Mercury Island					
BCMIB9	24-Jan-02	Bank Cormorant	feathers	Mercury Island					
BCMIB10	24-Jan-02	Bank Cormorant	feathers	Mercury Island					x
BCMIB11	24-Jan-02	Bank Cormorant	feathers	Mercury Island					x
BCMIB12	24-Jan-02	Bank Cormorant	feathers	Mercury Island					
BCMIB13	24-Jan-02	Bank Cormorant	feathers	Mercury Island					
BCMIB14	24-Jan-02	Bank Cormorant	feathers	Mercury Island					
BCBICH1	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island	x		X	x	
BCBICH2	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island	x		X	x	

BCBICH3	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island			X	x	
BCBICH4	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island			X		
BCBICH5	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island			X		x
BCBICH6	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island			X		
BCBICH7	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island			X		
BCBICH8	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island			X		
BCBICH9	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island			X	x	
BCBICH10	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island			X		
BCBICH11	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island					
BCBICH12	26-Jan-03	Bank Cormorant	feathers	Ichaboe Island					
RC2	28-May-09	Reed Cormorant	feathers	Century city WC	x		X		
CSM3	09-Oct-05	Crozet Shag	feather	Sealers Beach, Marion Island	x	X	X		
CSM4	09-Oct-05	Crozet Shag	feather	Sealers Beach, Marion Island	x	X	X		
CSM1	09-Jan-05	Crozet Shag	feather	Sealers Beach, Marion Island	x	X	X		
CSM2	17-Jan-05	Crozet Shag	feather	Mixed Pickle Bay, Marion Island	x	X	X		
CSM5	09-Jan-05	Crozet Shag	feather	Sealers Beach, Marion Island	x	x	X		
KS7	05-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island		X	x		
KS1	05-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island	x	X	x		
KS8	17-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island					
KS3	17-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island	x	x	x		
KS10	17-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island					
KS9	15-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island					
KS4	18-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island					
KS6	21-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island			x		
KS2	23-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island					
KS5	23-Jan-06	Kerguelen Shag	blood	Pointe Suzanne, Kerguelen Island			X		
CS1	27-Feb-95	Crozet Shag	blood	Pointe Basse, Crozet Island			X		
CS2	27-Feb-95	Crozet Shag	blood	Pointe Basse, Crozet Island			X		
CS3	27-Feb-95	Crozet Shag	blood	Pointe Basse, Crozet Island	x		X		
CS4	27-Feb-95	Crozet Shag	blood	Pointe Basse, Crozet Island			X		
CS5	27-Feb-95	Crozet Shag	blood	Pointe Basse, Crozet Island			X		
CS6	27-Feb-95	Crozet Shag	blood	Pointe Basse, Crozet Island					

Appendix 2.3: Outgroup taxa for the ATPase-6 phylogenetic tree (Figure 2.6): Grey Crowned Crane Balearica regulorum (FJ769841) as an outgroup, Cape Cormorant P. capensis (AY009350), Japanese Cormorant P. capillatus (AB233986 and AY009355), Great Cormorant P. carbo (AY009347), Pelagic Cormorant P. pelagicus (AY009361), Rock Shag or Magellanic Cormorant P. magellanicus (AY009359), Bank Cormorant P. neglectus (GU445906), P. bougainvillii (AY009354), Campbell Island Shag P. campbelli (AY009349), Bronze or Stewart Island Shag P. chalconotus (GU071054 and AY009368), Bounty Island Shag P. ranfurlyi (GU445908), Spotted Shag P. punctatus (AY009367), Pitt or Chatham Island Shag P. featherstoni (AY009363), Brandt's Cormorant P. penicillatus (AY009348), P. fuscescens (GU445905), P. varius (AY009362), P. nigrogularis (AF410794), Little Black Cormorant P. sulcirostris (AY009356), Brazilian or Olivaceous or Neotropic Cormorant P. brasilianus (AY009360), South Georgia Shag P. georgianus (GU445909), Auckland Island Shag P. colensoi (GU445907), Macquarie Island Shag P. purpurascens (AY009358), Red-faced Cormorant or Violet Shag P. urile (AY009364), Falkland Blue-eyed Shag P. albiventer (AY009366), Chatham Island Shag P. onslowi (AY009351), Flightless or Galapagos Cormorant P. harrisi (GQ205457), Double-crested Cormorant P. auritus (AY009352), European Shag P. aristotelis (AY009353), Red-legged Cormorant P. gaimardi (AY009365) and Little Pied Cormorant P. melanoleucos (Microcarbo melanoleucos (AY009357).

Appendix 2.4: BOLD and Genbank COI sequences employed as outgropu taxa (Figure 2.7): *P. carbo* (GU572016, GU572015, GU571539, DQ433900, JF499154, JF499153, JF499152, DQ433898, DQ433899, JF499151, JF499150, DQ433078 and JF499149), *P. aristotelis* (GU571538), *P. atriceps* (JN801909, FJ028005 and FJ028006), *P. melanoleucos* (JQ175767, BROMB72107 and BROMB72307), *P. auritus* (AY666385, DQ433896, DQ433077 and AY666386), *P. brasilianus* (JQ175766, FJ028008, DQ433897, FJ028007 and FJ028009), *P. pelagicus* (DQ433901, DQ433909, DQ433904, GQ482357, GQ482356, DQ433903, JN801352, JN801351, DQ433908, DQ433902, DQ433906, DQ433907 and DQ433905), *P. penicillatus* (DQ433910, DQ433079, DQ433911, DQ433913 and DQ433912), *P. chalconotus* (GU071054) and *P. urile* (JN801355, JN801353, JN801354, JN801356 , DQ433080 and JN801357).

Appendix 2.5 Maximum likelihood (ML) phylogenetic tree based on the nuclear Beta-fibrinogen gene from sequences available on GenBank for Anhingas, Sulids and Phalacrocoracids (n=70, 514bp). M. bassanus (n=4, AY695213, EU739445, EF552786, DQ881997), A. anhinga (n=4, AY695210, EF552751, EU739364, DQ881941)



CHAPTER 3 APPENDICES

Appendix 3.1 (a to c) Samples of each species collected for this study and the target gene region amplified for each sample of (a) Cape Gannet, (b) African Penguin, and (c) Cape Cormorant [WC=Western Cape Province; EC=Eastern Cape Province, NAM=Mamibia]

Code (LNG)	Date	Ring number	Locality	Blood / Feathers	REGION	*NADH3* (N=94)	*ATP6* (N=28)	*COI* (N=23)	*ND2* (N=20)	*cyt b* (N=15)	*BFIB* (N=31)	*GAPDH* (N=24)
5	22-Oct-08	GA36907	Malgas Island	B	WC	x				x		x
4	22-Oct-08	9-36066	Malgas Island	B	WC	x						
6	22-Oct-08	GA37641	Malgas Island	B	WC	x	x					
61	22-Oct-08	GA43389	Malgas Island	B	WC	x	x	x				x
58	22-Oct-08	GA21988	Malgas Island	B	WC	x					x	
1	22-Oct-08	no ring	Malgas Island	B	WC	x						
2	22-Oct-08	9-44997	Malgas Island	B	WC	x						
3	22-Oct-08	9-99533	Malgas Island	B	WC	x					x	
26	22-Oct-08	GA13614	Malgas Island	B	WC	x						
27	22-Oct-08	GA04395	Malgas Island	B	WC	x					x	
89	22-Oct-08	9-67176	Malgas Island	B	WC	x	x			x		
30	22-Oct-08	GA36774	Malgas Island	B	WC			x				
88	22-Oct-08	GA43000	Malgas Island	B	WC	x	x			x		
86	22-Oct-08	GA43331	Malgas Island	B	WC	x	x					
63	22-Oct-08	GA39311	Malgas Island	B	WC		x					x
60	22-Oct-08	GA36797	Malgas Island	B	WC	x		x				
31	22-Oct-08	GA20533	Malgas Island	B	WC						x	
87	22-Oct-08	GA12686	Malgas Island	B	WC	x	x					
62	22-Oct-08	GA21978	Malgas Island	B	WC	x						
25	22-Oct-08	GA37763	Malgas Island	B	WC	x						
59	22-Oct-08	GA38200	Malgas Island	B	WC	x						

29	22-Oct-08	GG0234	Malgas Island	B	WC	x						
57	22-Oct-08	GA42993	Malgas Island	B	WC	x	x	x				x
56	22-Oct-08	GA37073	Malgas Island	B	WC	x						
81	27-Jan-09	9A29056	Bird Island, PE	B	EC							x
124	27-Jan-09	920167	Bird Island, PE	B	EC	x			x	x		
125	27-Jan-09	980836	Bird Island, PE	B	EC				x			
126	27-Jan-09	9A19085	Bird Island, PE	B	EC	x			x		x	
127	27-Jan-09	9A32385	Bird Island, PE	B	EC	x			x			
128	27-Jan-09	9A41482	Bird Island, PE	B	EC	x			x			
129	27-Jan-09	930510	Bird Island, PE	B	EC	x						
24	27-Jan-09	chick of GAN 12	Bird Island, PE	B	EC	x						
46	28-Jan-09	974577	Bird Island, PE	B	EC		x					x
130	28-Jan-09	9A33081	Bird Island, PE	B	EC	x						
40	28-Jan-09	953991	Bird Island, PE	B	EC		x				x	x
131	28-Jan-09	945284	Bird Island, PE	B	EC	x			x		x	
76	28-Jan-09	chick of GAN 20	Bird Island, PE	B	EC	x						
80	28-Jan-09	939247	Bird Island, PE	B	EC	x				x	x	
39	28-Jan-09	chick of GAN 22	Bird Island, PE	B	EC	x						
132	28-Jan-09	978566	Bird Island, PE	B	EC	x			x			
16	28-Jan-09	chick of GAN 26	Bird Island, PE	B	EC	x						
15	28-Jan-09	chick of GAN 30	Bird Island, PE	B	EC	x						
77	29-Jan-09	9A02326	Bird Island, PE	B	EC	x						
78	29-Jan-09	971705	Bird Island, PE	B	EC	x	x					x
23	29-Jan-09	945258	Bird Island, PE	B	EC						x	
79	29-Jan-09	chick of GAN 36	Bird Island, PE	B	EC	x						
147	29-Jan-09	9A10387	Bird Island, PE	B	EC						x	
17	29-Jan-09	962887	Bird Island, PE	B	EC	x						
172	01-Nov-08	no ring	Bird Island, PE	B	EC			x				
47	28-Jan-09	975976	Bird Island, PE	B	EC			x				

13	29-Jan-09	9A22472	Bird Island, PE	B	EC	x		x				
14	30-Jan-09	979110	Bird Island, PE	B	EC	x						
84	24-Feb-09	9A31454	Bird Island, Lamberts Bay	B	WC	x						
50	24-Feb-09	9A44060	Bird Island, Lamberts Bay	B	WC	x						x
54	24-Feb-09	9A14254	Bird Island, Lamberts Bay	B	WC							x
37	24-Feb-09	9A15047	Bird Island, Lamberts Bay	B	WC						x	
7	24-Feb-09	9A44589	Bird Island, Lamberts Bay	B	WC	x	x					
9	24-Feb-09	966361	Bird Island, Lamberts Bay	B	WC	x	x					
55	24-Feb-09	9A14738	Bird Island, Lamberts Bay	B	WC	x						
8	24-Feb-09	9A38329	Bird Island, Lamberts Bay	B	WC	x	x					
85	24-Feb-09	9A13119	Bird Island, Lamberts Bay	B	WC	x		x				
83	24-Feb-09	9A13716	Bird Island, Lamberts Bay	B	WC	x						
22	24-Feb-09	9A36546	Bird Island, Lamberts Bay	B	WC						x	
21	24-Feb-09	9A28301	Bird Island, Lamberts Bay	B	WC	x			x			
12	24-Feb-09	9A21627	Bird Island, Lamberts Bay	B	WC						x	
10	24-Feb-09	9A36046	Bird Island, Lamberts Bay	B	WC	x			x			
52	24-Feb-09	9A20934	Bird Island, Lamberts Bay	B	WC	x						
11	24-Feb-09	9A36127	Bird Island, Lamberts Bay	B	WC	x			x			
82	24-Feb-09	9A39753	Bird Island, Lamberts Bay	B	WC	x						
38	24-Feb-09	9A32590	Bird Island, Lamberts Bay	B	WC						x	
20	24-Feb-09	9A13345	Bird Island, Lamberts Bay	B	WC	x						
19	24-Feb-09	9A55577	Bird Island, Lamberts Bay	B	WC	x						
18	24-Feb-09	9A36216	Bird Island, Lamberts Bay	B	WC	x						
100	23-Feb-10	no ring	Ichaboe	B	NAM				x	x		
101	23-Feb-10	no ring	Ichaboe	B	NAM			x	x	x		
102	23-Feb-10	no ring	Ichaboe	B	NAM			x	x	x	x	
103	23-Feb-10	no ring	Ichaboe	B	NAM				x	x	x	
104	23-Feb-10	no ring	Ichaboe	B	NAM				x		x	
70	23-Feb-10	no ring	Ichaboe	B	NAM	x						

105	23-Feb-10	no ring	Ichaboe	B	NAM	x			x			
106	23-Feb-10	no ring	Ichaboe	B	NAM	x			x			
107	23-Feb-10	no ring	Ichaboe	B	NAM	x			x		x	
108	23-Feb-10	no ring	Ichaboe	B	NAM	x						
109	23-Feb-10	no ring	Ichaboe	B	NAM	x						
139	23-Feb-10	no ring	Ichaboe	B	NAM		x					x
110	23-Feb-10	no ring	Ichaboe	B	NAM	x		x				
72	23-Feb-10	no ring	Ichaboe	B	NAM		x					x
73	23-Feb-10	no ring	Ichaboe	B	NAM			x				
74	23-Feb-10	no ring	Ichaboe	B	NAM	x						
111	23-Feb-10	no ring	Ichaboe	B	NAM	x		x				x
133	23-Feb-10	no ring	Ichaboe	B	NAM	x		x			x	
134	23-Feb-10	no ring	Ichaboe	B	NAM						x	
140	23-Feb-10	no ring	Ichaboe	B	NAM			x				
90	23-Feb-10	no ring	Ichaboe	B	NAM	x						
91	23-Feb-10	no ring	Ichaboe	B	NAM	x						
92	23-Feb-10	no ring	Ichaboe	B	NAM	x						
93	23-Feb-10	no ring	Ichaboe	B	NAM	x	x					x
94	23-Feb-10	no ring	Ichaboe	B	NAM	x						
64	26-Feb-10	no ring	Mercury Island	B	NAM	x	x					
95	26-Feb-10	no ring	Mercury Island	B	NAM	x						
96	26-Feb-10	no ring	Mercury Island	B	NAM	x						
65	26-Feb-10	no ring	Mercury Island	B	NAM	x	x					
98	26-Feb-10	no ring	Mercury Island	B	NAM	x						
112	26-Feb-10	no ring	Mercury Island	B	NAM				x		x	
116	26-Feb-10	no ring	Mercury Island	B	NAM	x		x				
117	26-Feb-10	no ring	Mercury Island	B	NAM	x	x					x
66	26-Feb-10	no ring	Mercury Island	B	NAM	x	x					
67	26-Feb-10	no ring	Mercury Island	B	NAM	x		x				

118	26-Feb-10	no ring	Mercury Island	B	NAM	x						
119	26-Feb-10	no ring	Mercury Island	B	NAM	x	x	x	x			x
120	26-Feb-10	no ring	Mercury Island	B	NAM			x				
121	26-Feb-10	no ring	Mercury Island	B	NAM						x	
122	26-Feb-10	no ring	Mercury Island	B	NAM	x					x	
123	26-Feb-10	no ring	Mercury Island	B	NAM	x						
136	26-Feb-10	no ring	Mercury Island	B	NAM	x		x		x		
144	26-Feb-10	no ring	Mercury Island	B	NAM					x		
68	26-Feb-10	no ring	Mercury Island	B	NAM	x						
137	26-Feb-10	no ring	Mercury Island	B	NAM	x	x					x
69	26-Feb-10	no ring	Mercury Island	B	NAM	x						
138	26-Feb-10	no ring	Mercury Island	B	NAM	x						
154	24-Jan-03	no ring	Mercury Island	F	NAM		x					x
160	26-Jan-03	no ring	Ichaboe	F	NAM						x	x
161	26-Jan-03	no ring	Ichaboe	F	NAM						x	x
163	26-Jan-03	no ring	Ichaboe	F	NAM			x				
164	28-Jan-03	no ring	Possession	F	NAM	x	x				x	x
165	28-Jan-03	no ring	Possession	F	NAM	x	x				x	x
166	28-Jan-03	no ring	Possession	F	NAM	x	x				x	
167	28-Jan-03	no ring	Possession	F	NAM	x				x		
168	28-Jan-03	no ring	Possession	F	NAM			x		x		
169	28-Jan-03	no ring	Possession	F	NAM		x			x	x	x
170	28-Jan-03	no ring	Possession	F	NAM					x	x	x
171	28-Jan-03	no ring	Possession	F	NAM			x			x	x

Appendix 3.1 (b) African Penguin samples sequenced for this study

Sample Code (NUP)	Date	Locality	Breeding Region	*NADH3* (N=124)	*ATPase 6* (N=130)	*COI* (N=38)	*BFIB* (N=31)	*GAPDH* (N=15)
31	04-Feb-09	Bird Island, PE	EC	x	x			
48	30-Jun-09	Bird Island, PE	EC	x			x	
50	30-Jun-09	Bird Island, PE	EC	x				
59	30-Jun-09	Bird Island, PE	EC	x				
130	30-Jun-09	Bird Island, PE	EC		x			
155	30-Jun-09	Bird Island, PE	EC	x	x			
156	30-Jun-09	Bird Island, PE	EC	x	x			
157	30-Jun-09	Bird Island, PE	EC	x	x			
158	30-Jun-09	Bird Island, PE	EC	x	x			
274	01-Mar-11	Bird Island, PE	EC	x		x	x	
275	01-Mar-11	Bird Island, PE	EC	x			x	x
276	01-Mar-11	Bird Island, PE	EC	x		x	x	
277	01-Mar-11	Bird Island, PE	EC	x				
283	01-Mar-11	Bird Island, PE	EC		x			
284	01-Mar-11	Bird Island, PE	EC		x			
285	01-Mar-11	Bird Island, PE	EC		x	x		
286	01-Mar-11	Bird Island, PE	EC		x			
287	01-Mar-11	Bird Island, PE	EC		x			
309	01-Mar-11	Bird Island, PE	EC	x				
220	Jun-10	Boulders Beach	WC	x	x		x	x
221	Jun-10	Boulders Beach	WC	x	x		x	
222	Jun-10	Boulders Beach	WC	x				
223	Jun-10	Boulders Beach	WC	x	x		x	
224	Jun-10	Boulders Beach	WC	x	x			
225	Jun-10	Boulders Beach	WC		x		x	x
234	Jun-10	Boulders Beach	WC			x		
26	31-Mar-09	Dassen	WC	x	x			
27	30-Mar-09	Dassen	WC	x	x			
28	25-Mar-09	Dassen	WC	x	x			
29	25-Mar-09	Dassen	WC	x	x			
30	30-Mar-09	Dassen	WC	x	x			
38	10-Jun-09	Dassen	WC		x			
39	09-Jun-09	Dassen	WC		x			
40	09-Jun-09	Dassen	WC	x	x		x	x
41	10-Jun-09	Dassen	WC		x			
236	25-Mar-09	Dassen	WC	x	x			
237	31-Mar-09	Dassen	WC			x		
240	25-Mar-09	Dassen	WC	x	x	x		
247	09-Jun-09	Dassen	WC	x	x	x	x	x

85	05-Aug-10	Dyer	WC	x	x	x		
86	05-Aug-10	Dyer	WC	x	x			
87	05-Aug-10	Dyer	WC	x	x			
88	05-Aug-10	Dyer	WC	x	x			
89	05-Aug-10	Dyer	WC	x	x			
90	05-Aug-10	Dyer	WC	x	x			
91	05-Aug-10	Dyer	WC	x	x			
92	05-Aug-10	Dyer	WC	x	x			
21	22-Apr-09	Halifax	NAM	x	x	x		
22	22-Apr-09	Halifax	NAM	x		x		
23	22-Apr-09	Halifax	NAM	x		x		
24	22-Apr-09	Halifax	NAM	x		x		
25	22-Apr-09	Halifax	NAM	x				
185	22-Apr-09	Halifax	NAM	x	x			
187	22-Apr-09	Halifax	NAM	x	x			
188	22-Apr-09	Halifax	NAM	x	x	x		
189	22-Apr-09	Halifax	NAM	x	x			
191	22-Apr-09	Halifax	NAM	x	x			
196	22-Apr-09	Halifax	NAM	x	x			
208	22-Apr-09	Halifax	NAM	x	x			
211	22-Apr-09	Halifax	NAM	x	x			
214	22-Apr-09	Halifax	NAM	x	x			
177	02-Mar-10	Halifax Island, Namibia	NAM	x	x	x		
178	02-Mar-10	Halifax Island, Namibia	NAM	x	x			
179	02-Mar-10	Halifax Island, Namibia	NAM	x	x			
180	02-Mar-10	Halifax Island, Namibia	NAM	x	x		x	
181	02-Mar-10	Halifax Island, Namibia	NAM	x	x	x		
182	02-Mar-10	Halifax Island, Namibia	NAM	x	x			
19	22-Apr-09	Ichaboe	NAM			x		
20	22-Apr-09	Ichaboe	NAM	x	x	x		
171	24-Feb-10	Ichaboe	NAM	x	x		x	
172	24-Feb-10	Ichaboe	NAM	x	x	x		
173	24-Feb-10	Ichaboe	NAM	x	x			
174	24-Feb-10	Ichaboe	NAM	x	x			
175	24-Feb-10	Ichaboe	NAM	x	x			
176	24-Feb-10	Ichaboe	NAM	x	x	x		
204	24-Feb-10	Ichaboe	NAM	x	x		x	
205	24-Feb-10	Ichaboe	NAM	x	x	x		
206	24-Feb-10	Ichaboe	NAM	x	x			
261	24-Feb-10	Ichaboe	NAM			x	x	
262	24-Feb-10	Ichaboe	NAM			x	x	x
148	28-Jun-10	Jutten	WC	x	x			
149	28-Jun-10	Jutten	WC	x	x			
150	28-Jun-10	Jutten	WC	x	x			

151	28-Jun-10	Jutten	WC	x	x			
152	28-Jun-10	Jutten	WC	x	x			
153	28-Jun-10	Jutten	WC	x	x			
154	28-Jun-10	Jutten	WC	x	x			
163	28-Jun-10	Jutten	WC			x		
6	22-Apr-09	Mercury	NAM	x	x			
7	22-Apr-09	Mercury	NAM	x	x			
8	22-Apr-09	Mercury	NAM	x	x			
9	22-Apr-09	Mercury	NAM	x	x			
73	25-Feb-10	Mercury	NAM	x	x		x	x
74	25-Feb-10	Mercury	NAM	x	x			
75	25-Feb-10	Mercury	NAM	x	x	x	x	
76	25-Feb-10	Mercury	NAM	x	x			
77	25-Feb-10	Mercury	NAM	x	x			
78	25-Feb-10	Mercury	NAM	x	x			
268	25-Feb-10	Mercury	NAM				x	x
269	25-Feb-10	Mercury	NAM				x	x
300	25-Feb-10	Mercury	NAM	x	x			
301	25-Feb-10	Mercury	NAM	x	x		x	
302	25-Feb-10	Mercury	NAM	x	x	x		
303	25-Feb-10	Mercury	NAM	x	x			
304	25-Feb-10	Mercury	NAM	x	x			
305	25-Feb-10	Mercury	NAM	x	x			
306	25-Feb-10	Mercury	NAM		x		x	x
307	25-Feb-10	Mercury	NAM		x	x		
308	25-Feb-10	Mercury	NAM		x			
311	25-Feb-10	Mercury	NAM				x	
11	22-Apr-09	Possession	NAM	x	x			
12	22-Apr-09	Possession	NAM	x	x			
62	22-Apr-09	Possession	NAM	x	x			
63	22-Apr-09	Possession	NAM	x	x			
64	22-Apr-09	Possession	NAM	x	x			
33	13-Aug-09	Robben Island	WC	x	x		x	
35	13-Aug-09	Robben Island	WC	x	x			
36	13-Aug-09	Robben Island	WC	x	x		x	
37	13-Aug-09	Robben Island	WC		x			
226	13-Aug-09	Robben Island	WC		x		x	x
228	13-Aug-09	Robben Island	WC	x	x		x	
229	13-Aug-09	Robben Island	WC	x	x			
2	25-Jan-09	St Croix	EC		x			
5	25-Jan-09	St Croix	EC	x				
65	Jun-09	St Croix	EC	x	x			
66	Jun-09	St Croix	EC	x	x	x		
105	25-Jan-09	St Croix	EC	x				

107	25-Jan-09	St Croix	EC	x		x		
109	25-Jan-09	St Croix	EC		x		x	x
110	25-Jan-09	St Croix	EC		x	x		
111	25-Jan-09	St Croix	EC		x		x	x
113	25-Jan-09	St Croix	EC		x			
114	25-Jan-09	St Croix	EC		x		x	x
115	25-Jan-09	St Croix	EC	x				
116	25-Jan-09	St Croix	EC	x			x	
118	Jun-09	St Croix	EC	x	x			
119	Jun-09	St Croix	EC	x	x			
120	Jun-09	St Croix	EC	x	x			
121	Jun-09	St Croix	EC	x	x	x		
122	Jun-09	St Croix	EC	x	x	x		
123	Jun-09	St Croix	EC	x	x			
124	Jun-09	St Croix	EC	x	x	x		
125	Jun-09	St Croix	EC	x	x			
126	Jun-09	St Croix	EC	x	x			
127	Jun-09	St Croix	EC	x	x			
128	Jun-09	St Croix	EC	x	x			
159	Jun-09	St Croix	EC		x			
54	20-Jul-09	Stony Point	WC	x	x			
55	20-Jul-09	Stony Point	WC	x	x	x		
56	20-Jul-09	Stony Point	WC		x			
70	22-May-09	Stony Point	WC	x	x			
71	22-May-09	Stony Point	WC	x	x			
72	22-May-09	Stony Point	WC	x	x	x		
131	22-May-09	Stony Point	WC	x	x	x		
132	22-May-09	Stony Point	WC		x			
135	22-May-09	Stony Point	WC			x		
136	20-Jul-09	Stony Point	WC	x	x	x		
138	20-Jul-09	Stony Point	WC			x		
141	20-Jul-09	Stony Point	WC	x	x		x	x

Appendix 3.1 (c) Cape Cormorants sequenced for this study (BRW: Bird Rock, Walvis Bay; ICH: Ichaboe Island; ROB: Robben Island; MAL: Malgas Island; JUT: Jutten Island; DYE: Dyer Island; NAM: Namibia; WC: Western Cape)

Sample Code	Date	Blood / Feathers	Colony	Region	ATPase6 (N=47)	Cyt b (N=41)	NADH3 (N=72)	CO1 (N=11)	BFIB (N=23)	GAPDH (N=5)
CCBR1	22-Jan-03	F	BRW	NAM	x		x			
CCBR2	22-Jan-03	F	BRW	NAM	x	x	x		x	x
CCBR3	22-Jan-03	F	BRW	NAM	x	x	x		x	
CCBR4	22-Jan-03	F	BRW	NAM	x	x	x		x	
CCBR5	22-Jan-03	F	BRW	NAM	x	x	x			
CCICH1	26-Jan-03	F	ICH	NAM	x		x	x	x	
CCICH10	26-Jan-03	F	ICH	NAM	x	x	x		x	x
CCICH11	26-Jan-03	F	ICH	NAM		x	x			
CCICH12	26-Jan-03	F	ICH	NAM		x	x			
CCICH13	26-Jan-03	F	ICH	NAM		x	x			
CCICH14	26-Jan-03	F	ICH	NAM		x	x			
CCICH15	26-Jan-03	F	ICH	NAM		x	x			
CCICH16	26-Jan-03	F	ICH	NAM			x			
CCICH17	26-Jan-03	F	ICH	NAM		x	x			
CCICH18	26-Jan-03	F	ICH	NAM		x	x			
CCICH2	26-Jan-03	F	ICH	NAM	x		x	x	x	
CCICH3	26-Jan-03	F	ICH	NAM	x		x	x		
CCICH4	26-Jan-03	F	ICH	NAM	x		x	x	x	
CCICH5	26-Jan-03	F	ICH	NAM	x	x	x			
CCICH6	26-Jan-03	F	ICH	NAM	x		x			
CCICH7	26-Jan-03	F	ICH	NAM	x	x	x			
CCICH8	26-Jan-03	F	ICH	NAM	x		x			
CCICH9	26-Jan-03	F	ICH	NAM	x		x			
CAP1	10-12-2009	B	ROB	WC	x	x	x			
CAP10	10-12-2009	B	ROB	WC			x		x	
CAP2	10-12-2009	B	ROB	WC	x	x	x			
CAP3	11-12-2009	B	ROB	WC	x	x	x			
CAP4	11-12-2009	B	ROB	WC	x					
CAP5	11-12-2009	B	ROB	WC	x	x	x			
CAP6	11-12-2009	B	ROB	WC		x	x			
CAP7	11-12-2009	B	ROB	WC		x	x			
CAP8	12-12-2009	B	ROB	WC		x	x		x	
CAP9	11-12-2009	B	ROB	WC			x		x	
CC1	23-Oct-09	B	MAL	WC	x		x			
CC10	26-Oct-09	B	MAL	WC	x	x	x		x	
CC11	26-Oct-09	B	MAL	WC		x	x			
CC12	28-Oct-09	B	MAL	WC		x	x			
CC2	24-Oct-09	B	MAL	WC	x		x			
CC3	25-Oct-09	B	MAL	WC	x	x	x	x	x	x

CC4	25-Oct-09	B	MAL	WC	x		x	x	x	x
CC5	28-Oct-09	B	MAL	WC	x		x	x		
CC6	27-Oct-09	B	MAL	WC	x		x			
CC64	17-Oct-10	B	MAL	WC		x				
CC67	18-Oct-10	B	MAL	WC		x				
CC7	27-Oct-09	B	MAL	WC	x		x		x	
CC8	26-Oct-09	B	MAL	WC	x		x		x	
CC82	01-Nov-10	B	JUT	WC		x	x			
CC86	03-Nov-10	B	JUT	WC	x					
CC9	26-Oct-09	B	MAL	WC	x	x	x		x	
CCJ72	28-Oct-10	B	JUT	WC		x	x		x	
CCJ73	28-Oct-10	B	JUT	WC	x	x	x			
CCJ79	31-Oct-10	B	JUT	WC			x		x	
CCJ95	03-Nov-10	B	JUT	WC			x			
CCML1dy	22-11-2008	F	DYE	WC		x				
CCML1Mal	08-10-2008	F	MAL	WC		x	x			
CCML2Mal	08-10-2008	F	MAL	WC			x			
DCC100	18-Nov-10	F	DYE	WC	x					
DCC101	19-Nov-10	F	DYE	WC	x					
DCC102	20-Nov-10	F	DYE	WC	x	x			x	
DCC105	23-Nov-10	F	DYE	WC	x					
DCC106	23-Nov-10	F	DYE	WC	x					
DCC107	24-Nov-10	F	DYE	WC		x				
DCC109	26-Nov-10	F	DYE	WC		x				
DCC111	27-Nov-10	F	DYE	WC		x	x			
DCC112	28-Nov-10	F	DYE	WC			x			
DCC113	28-Nov-10	F	DYE	WC			x			
DCC114	29-Nov-10	F	DYE	WC	x					
DCC115	30-Nov-10	F	DYE	WC	x					
DCC116	29-Nov-10	F	DYE	WC	x					
DCC117	30-Nov-10	F	DYE	WC	x					
DCC118	30-Nov-10	F	DYE	WC	x	x				
DCC13	26-Nov-09	F	DYE	WC			x		x	x
DCC14	26-Nov-09	F	DYE	WC			x			
DCC15	26-Nov-09	F	DYE	WC			x			
DCC16	27-Nov-09	F	DYE	WC		x	x			
DCC17	28-Nov-09	F	DYE	WC			x	x		
DCC18	28-Nov-09	F	DYE	WC			x	x		
DCC19	29-Nov-09	F	DYE	WC			x	x		
DCC20	29-Nov-09	F	DYE	WC			x		x	
DCC21	30-Nov-09	F	DYE	WC			x	x		
DCC22	30-Nov-09	F	DYE	WC			x			
DCC23	30-Nov-09	F	DYE	WC			x			
DCC35	20-Dec-09	F	DYE	WC			x			
DCC36	20-Dec-09	F	DYE	WC			x			

DCC37	22-Dec-09	F	DYE	WC			x			
DCC38	22-Dec-09	F	DYE	WC			x			
DCC39	24-Dec-09	F	DYE	WC		x	x			
DCC41	28-Dec-09	F	DYE	WC		x				
JCC76	29-Oct-10	B	JUT	WC	x					
JCC78	30-Oct-10	B	JUT	WC	x					
JCC83	03-Nov-10	B	JUT	WC	x				x	
JCC88	03-Nov-10	B	JUT	WC	x		x			
JCC90	03-Nov-10	B	JUT	WC					x	
JCC92	03-Nov-10	B	JUT	WC	x	x				
JCC93	03-Nov-10	B	JUT	WC			x			

Appendix 3.2 (a to c) The models selected for each gene region in each of the focal species in (a) MEGA 5.1, (b) jmodeltest and (c) MrModeltest.

(a)	Gene region	N	length (bp)	MEGA Model	BIC	AICc	- lnL
Cape Gannet	ATPase6	28	669	HKY	2372.98	1926.569	906.108
	ATPase6 with outgroups (4)	32	669	HKY	2665.27	2147.799	1008.698
	ATPase6 with outgroups (8)	36	624	HKY+G+I	4034.022	3433.056	1641.273
	BFIB	31	559	T92	2121.702	1648.772	763.167
	BFIB with outgroups (5)	36	559	T92	2491.113	1930.075	893.782
	BFIB with outgroups (9)	40	559	HKY	2800.633	2152.052	994.728
	COI	26	668	HKY	2484.092	2073.016	983.343
	COI with outgroups (15)	41	668	HKY	3123.368	2441.796	1137.643
	cytb	15	834	HKY	2526.253	2295.949	1116.895
	cytb with outgroup (1)	16	834	HKY	2638.02	2390.728	1162.28
	cytb with outgroups (8)	25	794	HKY+G	3949.89	3539.621	1717.671
	GAPDH	25	419	JC	1661.864	1321.23	613.399
	GAPDH with outgroups (2)	27	419	K2	2078.644	1681.226	786.358
	ND2	20	555	HKY	1866.954	1567.363	724.526
	ND2 with outgroup (1)	21	555	HKY	1951.841	1635.537	774.606
	ND3	94	400	HKY	3115.063	1503.913	561.997
	ND3 with outgroups (2)	96	352	HKY	3285.061	1635.998	624.018
African Penguin	ATPase6	130	672	HKY	4854.089	2408.055	942.243
	ATPase6+2OG	132	672	HKY	5038.983	2551.416	1009.911
	ATPase6+19OG	149	672	HKY+G	6386.678	3534.228	1466.21
	BFIBI7+OG	35	575	T92	2361.772	1816.487	839.003
	COI	38	688	HKY	2699.034	2070.299	957.919
	COI+2OG (2 Aps from Genbank)	42	688	HKY	2856.724	2154.158	991.825
	COI+41OG	79	605	HKY	3784.011	2389.905	1035.419
	COI+56OG	94	443	HKY	3607.736	1977.101	798.684

	COI+37OG	75	673	TN93+G	4360.71	3010.774	1351.919
	COI+105OG	143	518	HKY+G	5463.212	2812.176	1116.96
	GAPDH	15	369	Invariable			
	GAPDH+OG	16	358	JC	1448.991	1255.523	598.613
	ND3	124	358	HKY	3687.244	1523.567	511.373
	ND3+1OG	125	358	HKY	3752.583	1569.517	532.337
	ND3+17OG	140	358	HKY	4432.469	1956.629	695.724
	ATPase6 + NADH3	106	1030	Partitioned			
Cape Cormorant	ATPase 6	47	682	HKY	2777.299	1982.228	895.828
	ATPase 6 + 1 outgroup	48	682	HKY	3228.354	2414.502	1109.96
	ATPase 6 + all outgroups	89	525	HKY+G+I	8306.603	6723.9	3180.242
	ATPase 6 phylogeny	113	567	TN93+G	8945.182	6870.35	3205.35
	BFIB	24	567	invariable, except one ambiguous site			
	BFIB Phylogeny	70	514	T92	3938.549	2761.552	1241.224
	COI	11	925	invariable, except at one site			
	COI + all outgroups truncated (phylogeny)	65	650	HKY+G+I	5949.835	4800.867	2267.008
	COI + all outgroups (phylogeny)	71	693	HKY+G	7399.456	6126.387	2918.786
	cytb	41	864	HKY	3205.957	2502.914	1168.26
	cytb+1og (P chalconotus)	42	864	HKY	3788.248	3066.216	1447.906
	cytb + 8og	50	774	HKY+G	5623.67	4750.73	2273.09
	GAPDH	5	416	invariable			
	NADH3	71	393	HKY	2535.005	1358.671	535.593
	NADH3 + 1 outgroup (P chalconotus)	72	393	HKY	2797.852	1603.037	655.766
	NADH3 + 2 outgroups (P auritus some data missing)	73	394	HKY	3050.413	1818.83	759.641
	NADH3 + Bank and Crowned Cormorants + outgroups	89	389	HKY+I	3883.874	2345.734	989.909

(b)	Gene region	jModeltest					
		Model (AICc)	AICc	- lnL	Model (BIC)	BIC	- lnL
Cape Gannet	ATPase6	TPM3uf	1938.8618	904.6181	F81	2187.845	908.5074
	ATPase6 with outgroups (4)	HKY	2164.0018	1008.655	HKY	2446.693	1008.655
	ATPase6 with outgroups (8)	TrN+I	3442.0708	1634.377	TrN+I	3757.822	1634.377
	BFIB	F81	1667.0175	762.3633	F81	1923.274	762.3633
	BFIB with outgroups (5)	HKY	1955.7038	892.385	HKY	2252.905	892.385
	BFIB with outgroups (9)	TVM	2198.672	998.8815	TPM2uf	2531.178	1003.054
	COI	TPM2uf	2082.9189	981.4268	HKY	2317.901	983.3345
	COI with outgroups (15)	HKY+G	2465.7508	1135.315	HKY	2821.603	1137.622
	cytb	F81	2300.42	1117.973	F81	2444.46	1117.973
	cytb with outgroup (1)	HKY	2395.5689	1162.295	HKY	2553.282	1162.295
	cytb with outgroups (8)	TIM3+G	3540.394	1711.024	TPM3uf+ G	3785.585	1712.511
	GAPDH	F81	1321.2806	602.4141	F81	1512.76	602.4141
	GAPDH with outgroups (2)	TPM3uf	1689.0309	775.7094	K80	1905.061	786.3582
	ND2	HKY	1576.1141	742.5297	HKY	1750.456	742.5297
	ND2 with outgroup (1)	HKY	1644.987	774.6112	HKY	1827.257	774.6112
	ND3	HKY	1851.3314	562.0294	HKY	2262.437	562.0294
	ND3 with outgroups (2)	HKY	1973.2248	551.657	HKY	2240.858	551.657
African Penguin	ATPase6	HKY	2745.4984	942.2749	HKY	3590.238	942.2749
	ATPase6+2OG	HKY	2902.4225	1009.848	HKY	3751.425	1009.848
	ATPase6+19OG	HKY+G	4024.3521	1465.495	HKY+G	4890.578	1465.495
	BFIBI7+OG	TPM3uf	1839.2861	835.8606	F81	2133.433	841.1363
	COI	HKY	2092.1178	957.9407	HKY	2425.517	957.9407
	COI+2OG (2 Aps from Genbank)	HKY	2180.5949	991.8482	HKY	2545.602	991.8482
	COI+41OG	TIM3	2506.1457	1031.331	HKY	3095.27	1035.217
	COI+56OG	HKY+G	2263.8886	794.8407	HKY+G	2753.553	794.8407
	COI+37OG	TrN+G	3098.6861	1349.262	TrN+G	3701.333	1349.262

	COI+105OG	TrN+G	3537.5403	1107.008	TrN+G	4026.509	1107.008
	GAPDH						
	GAPDH+OG	TrN	1259.8156	591.124	JC	1374.551	598.6133
	ND3	JC	2659.1751	536.1821	F81	2491.468	513.6075
	ND3+1OG	JC	2742.6696	556.8027	F81	2543.401	533.6938
	ND3+17OG	K80	4004.1859	21.5545	TPM2uf	3053.366	694.5876
	ATPase6 + NADH3						
Cape Cormorant	ATPase 6	F81	2015.0414	896.9576	F81	2413.793	896.9576
	ATPase 6 + 1 outgroup	TPM3uf	2448.8972	1108.438	HKY	2859.125	1109.836
	ATPase 6 + all outgroups	HKY+I+G	6872.6964	3156.962	HKY+I+G	7453.863	3156.962
	ATPase 6 phylogeny	TrN+G	7153.8507	3188.8	TrN+G	7835.883	3188.8
	BFIB						
	BFIB Phylogeny	TPM2uf	2869.9335	1236.313	TPM2uf	3365.263	1236.313
	COI						
	COI + all outgroups truncated (phylogeny)	TPM2uf+G	4824.7299	2243.239	TPM2uf+G	5354.392	2243.239
	COI + all outgroups (phylogeny)						
	cytb	HKY	2522.8899	1168.279	F81	2904.453	1171.621
	cytb+1og (P chalconotus)	TPM1uf	3080.7745	1443.521	TPM1uf	3475.299	1443.521
	cytb + 8og	TPM2uf+G	4769.6975	2264.526	TPM2uf+G	5220.815	2264.526
	GAPDH						
	NADH3	F81	1527.0735	537.8379	F81	1929.931	537.8379
	NADH3 + 1 outgroup (P chalconotus)	HKY	1777.5862	655.5492	HKY	2183.275	655.5492
	NADH3 + 2 outgroups (P auritus some data missing)	TPM2uf	1993.974	756.3886	HKY	2403.15	759.325
	NADH3 + Bank and Crowned Cormorants + outgroups	HKY+I	2669.8541	987.6734	HKY+I	3066.682	987.6734

(c)	Gene region			MrModeltest			
		Model (hLRTs)	- lnL	K	Model (AIC)	AIC	K
Cape Gannet	ATPase6	F81	908.5074	3	HKY	1820.205	4
	ATPase6 with outgroups (4)	HKY	1008.6553	4	HKY	1008.655	4
	ATPase6 with outgroups (8)	HKY+G	1637	5	HKY+I	1635.86	5
	BFIB	F81	762.3633	3	F81	762.3633	3
	BFIB with outgroups (5)	HKY	892.3847	4	HKY	1792.769	4
	BFIB with outgroups (9)	HKY	1003.6059	4	GTR	2013.431	8
	COI	HKY	983.3345	4	HKY	1974.669	4
	COI with outgroups (15)	HKY	1137.6213	4	HKY+I	########	5
	cytb	F81	1117.9731	3	HKY	2241.789	4
	cytb with outgroup (1)	HKY	1162.2732	4	HKY	2332.546	4
	cytb with outgroups (8)	GTR+G	1708.642	9	GTR+I	3435.032	9
	GAPDH	F81	602.4141	3	F81	1210.828	3
	GAPDH with outgroups (2)	HKY	777.3772	4	HKY	1562.754	4
	ND2	HKY	742.5189	4	HKY	742.5189	4
	ND2 with outgroup (1)	HKY	774.5828	4	HKY	774.5828	4
	ND3	HKY	561.9934	4	HKY	1131.987	4
	ND3 with outgroups (2)	HKY	623.9233	4	HKY	1255.847	4
African Penguin	ATPase6	HKY	942.2166	4	HKY	1892.433	4
	ATPase6+2OG	HKY	1009.848	4	GTR	2027.507	8
	ATPase6+19OG	HKY+G	1465.4852	5	HKY+I	2940.949	5
	BFIBI7+OG	F81	841.1363	3	GTR	1684.228	8
	COI	HKY	957.9161	4	HKY	1923.832	4
	COI+2OG (2 Aps from Genbank)	HKY	991.814	4	HKY	1991.628	4
	COI+41OG	HKY	1036.2128	4	GTR	2078.186	8
	COI+56OG	HKY+G	794.8397	5	HKY+I	1599.364	5
	COI+37OG	GTR+G	1350.1685	9	GTR+I	2718.249	9
	COI+105OG	GTR+G	1110.672	9	GTR+I	2237.647	9

	GAPDH						
	GAPDH+OG						
	ND3	F81	513.6075	3	HKY	1030.709	4
	ND3+1OG	F81	533.6937	3	HKY	1072.632	4
	ND3+17OG						
	ATPase6 + NADH3	Partitioned					
Cape Cormorant	ATPase 6	F81	896.9576	3	HKY	1799.648	4
	ATPase 6 + 1 outgroup	HKY	1109.8359	4	HKY	2227.672	4
	ATPase 6 + all outgroups	GTR+I+G	3160.5537	10	HKY+I+G	6339.592	6
	ATPase 6 phylogeny	GTR+G	3191.6392	9	GTR+I+G	6398.785	10
	BFIB						
	BFIB Phylogeny						
	COI						
	COI + all outgroups truncated (phylogeny)	GTR+G	2236.6941	9	GTR+I+G	4488.09	10
	COI + all outgroups (phylogeny)	GTR+I+G	2417.5107	10	GTR+I+G	4855.022	10
	cytb	HKY	1168.2578	4	HKY	2344.516	4
	cytb+1og (P chalconotus)	HKY	1447.722	4	GTR	2900.93	8
	cytb + 8og	GTR+G	2261.4492	9	GTR+G	4540.898	9
	GAPDH						
	NADH3	F81	537.838	3	HKY	1079.18	4
	NADH3 + 1 outgroup (P chalconotus)	HKY	655.5492	4	HKY	1319.098	4
	NADH3 + 2 outgroups	HKY	759.325	4	HKY+I	1525.995	5
	NADH3 outgroups	GTR+G	984.5521	9	GTR+I	1987.001	9

Appendix 3.3 The forward and reverse primers (5' – 3') used to amplify nuclear and mitochondrial target regions in the three study species. The source publications for primers are shown (primers developed during the study are cited as Nupen, unpublished).

Species	Target Region*	Primer name	Primer sequence	Source**
African Penguin, Cape Gannet and Cape Cormorant	BFIBI7	FIBB7I7L2	5'-TGGGAGGTGAAGCAGCTAAGAAAAACAA-3'	1 (Nupen, unpublished)
		PHDBFIB7R	5'-TGTCCATGCAGTTCCCTTGATGTAGCC-3'	
African Penguin, Cape Gannet and Cape Cormorant	GAPDH	L890	5'-ACCTTTAATGCGGGTGCTGGCATTGC-3'	2
		H950	5'-CATCAAGTCCACAACACGGTTGCTGTA-3'	2
African Penguin, Cape Gannet and Cape Cormorant	ATPase6	A8PWL	5'-CCTGAACCTGACCATGAAC-3'	3
		CO3HMH	5'-CATGGGCTGGGGTCGACTATGTG-3'	3
African Penguin, Cape Gannet and Cape Cormorant	COI	EM5287	5'-CACATCAATGAGCTTGCAACTC-3'	4
		R722	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	4
		L-tyr	5'-TGTAAAAAGGWCTACAGCCTAACGC-3'	5
		COI907aH2	5'-GTRGCNGAYGTRAARTATGCTCG-3'	5
Cape Gannet Cape Cormorant	Cyt b	B3	5'-GGACGAGGCTTTTACTACGGCTC-3'	6
		B6	5'-GTCTTCAGTTTTTGGTTTACAAGAC-3'	6
Cape Gannet African Penguin	ND2	H6313	5'-ACTCTTRTTTAAGGCTTTGAAGGC-3'	7
		L5758	5'-GGAGGATGAATGGGACTTAACCAGAC-3'	7
African Penguin, Cape Gannet and Cape Cormorant	ND3	H11151	5'-GATTTGTTGAGCCGAAATCAAC-3'	8
		L10775	5'-GACTTCCAATCTTTAAAATCTGG-3'	8
African Penguin	Control Region	HDbox	5'-CTGACCGAGGAACCAGAGGCGC-3'	9
		tRNA-Glu	5'-CCTGCTTGGCTTTTYTCCAAGACC-3'	9

* Abbreviations: BFIBI7 (Beta-fibronogen intron 7), GAPDH (Glyceraldehyde 3-phosphate dehydrogenase), ATPase6 (Adenosine triphosphate synthase F0 subunit 6), COI (Cytochrome c oxidase subunit 1), Cyt b (cytochrome b), ND2 (NADH dehydrogenase 2), ND3 (NADH dehydrogenase 3).
** Source publications 1:(Prychitko & Moore 1997) 2:(Friesen et al. 1997) 3: (Eberhard & Bermingham 2004) 4: (Bouzat et al. 2009) 5: (Tavares & Baker 2008) 6: (Morris-Pocock 2012) 7: (Johnson & Sorenson 1998) 8: (Brumfield & Edwards 2007) 9: (Roeder et al. 2002)

Appendix 3.4 Annealing temperature (T_A) and the corresponding annealing time (in seconds) for PCR profiles for all primer pairs. Reaction volumes were the same for all PCRs (25µl), and contained 1_U *Taq*, 0.5µM each primer, 200µM each dNTP and 1X reaction buffer. Optimal magnesium chloride concentrations are also given for each primer pair. All PCR profiles had an initial 3 minute denaturing phase and a final extension phase of 5 minutes. All PCRs were cycled 35 to 40 times.

Target Region*	Primer name	T_A (°C)	[$MgCl_2$]
B-FIB-I7	FIBB7I7L2 / PHDBFIB7R	59 (60s)	2.5mM
GAPDH	L890 H950	60 (60s)	2.5mM
ATPase 6	A8PWL CO3HMH	54 (40s)	1.5mM
COI	EM5287 R722	55 (30s)	2.5mM
	L-tyr COI907aH2	50 (45s)	2.5mM
Cytochrome b	B3 B6	49 (45s)	2.5mM
ND2	H6313 L5758	56 (30s)	1.5mM
NADH3	H11151 L10775	54 (40s)	1.5mM

* See Appendix 3.3 for abbreviations.

Appendix 3.5 (a) Population genetic diversity indices based on the NADH3 sequence data (400bp) subdivided into Cape Gannet breeding regions (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

	NAM	WC	EC	TOTAL
Haplotype diversity (h)	0.20571	0.05405	0.10526	0.14322 ± 0.049
Nucleotide diversity (π)	0.00053	0.00014	0.0005	0.00037 ± 0.00013
N	37	37	20	94
Number of haplotypes	4	2	3	5

Appendix 3.5 (b) Population genetic diversity indices based on the NADH3 sequence data (400bp) subdivided into Cape Gannet breeding colonies (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

	Mercury (NAM)	Ichaboe (NAM)	Possession (NAM)	Lamberts Bay (WC)	Malgas (WC)	Bird Island (EC)	TOTAL
Haplotype diversity (h)	0.21569 ± 0.124	0.25714 ± 0.142	0	0.125 ± 0.106	0	0.19474 ± 0.115	0.14322 ± 0.049
Nucleotide diversity (π)	0.00056	0.00067	0	0.00031	0	0.0005	0.00037 ± 0.00013
N	18	15	4	16	21	20	94
Number of haplotypes	3	3	1	2	1	3	5

Appendix 3.6 (a) Population genetic diversity indices based on the ATPase 6 sequence data (669bp) subdivided into Cape Gannet breeding regions (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

	NAM	WC	EC	TOTAL
Haplotype diversity (h)	0.58242 ± 0.092	0.61818 ± 0.104	0.667 ± 0.314	0.57672 ± 0.055
Nucleotide diversity (π)	0.00122	0.0013	0.001	0.0012
N	14	11	3	28
Number of haplotypes	3	3	2	4

Appendix 3.6 (b) Population genetic diversity indices based on the ATPase 6 sequence data (669bp) subdivided into Cape Gannet breeding colonies (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

	Mercury (NAM)	Ichaboe (NAM)	Possession (NAM)	Lamberts Bay (WC)	Malgas (WC)	Bird Island (EC)	TOTAL
Haplotype diversity (h)	0.57143 ± 0.119	1 ± 0.272	0.5 ± 0.265	0.66667 ± 0.314	0.67857 ± 0.122	0.66667 ± 0.314	0.57672 ± 0.055
Nucleotide diversity (π)	0.00085	0.00299	0.00075	0.001	0.00155	0.001	0.0012
N	7	3	4	3	8	3	28
Number of haplotypes	2	3	2	2	3	2	4

Appendix 3.7 Pairwise comparisons of genetic structure using various metrics at (a and b) the regional scale and (c and d) colony level based on the ATPase 6 dataset for Cape Gannets (669bp).

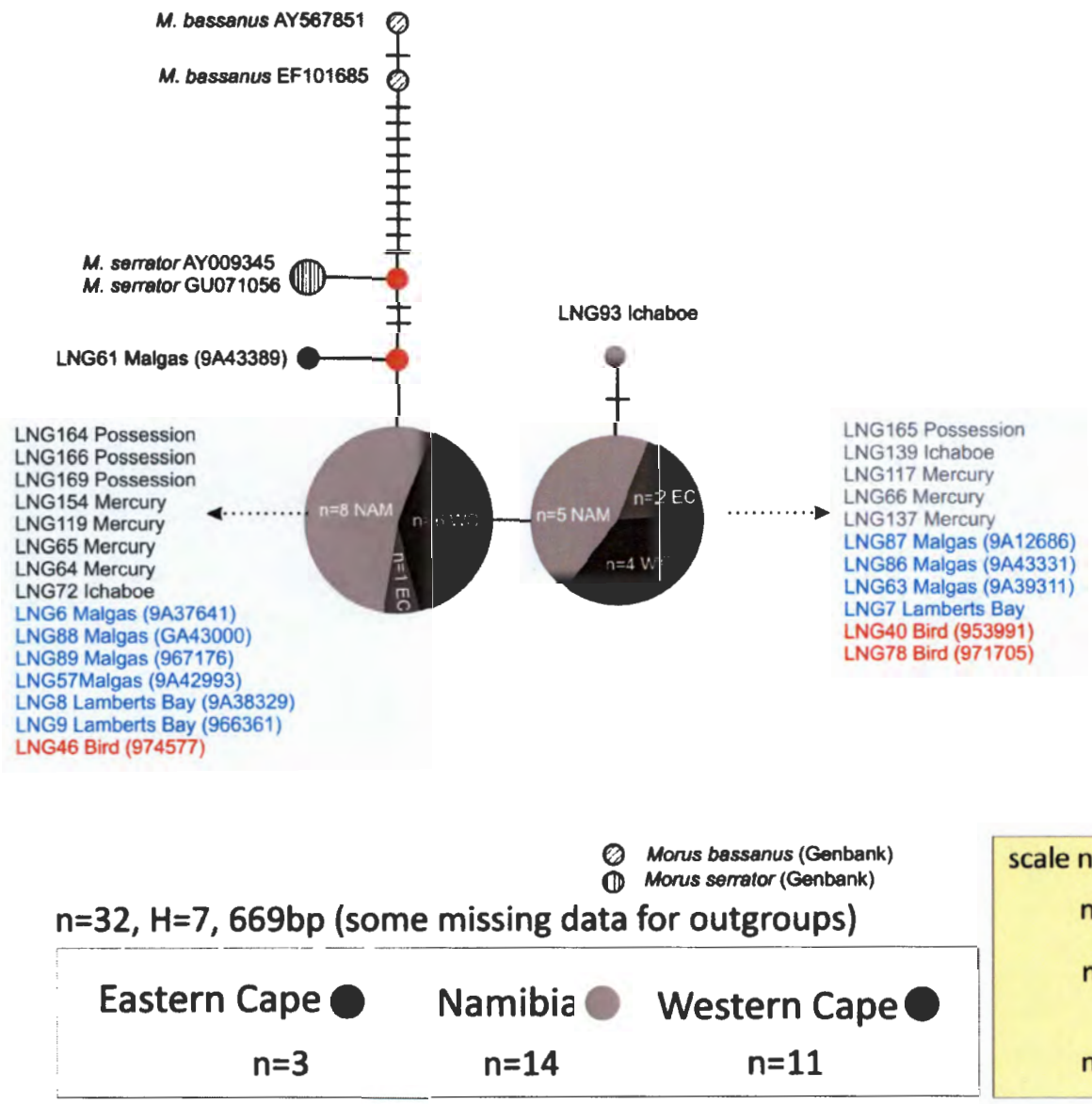
(a) ATPase6 (669bp)		φst		
		NAM	WC	EC
Gst	Namibia (NAM)		-0.0469	- 0.10989
	Western Cape (WC)	-0.03509		-0.05833
	Eastern Cape (EC)	0.01625	-0.00108	

(b)		*kxy*		
		NAM	WC	EC
Dxy	Namibia (NAM)		0.805	0.667
	Western Cape (WC)	0.0012		0.727
	Eastern Cape (EC)	0.001	0.00109	

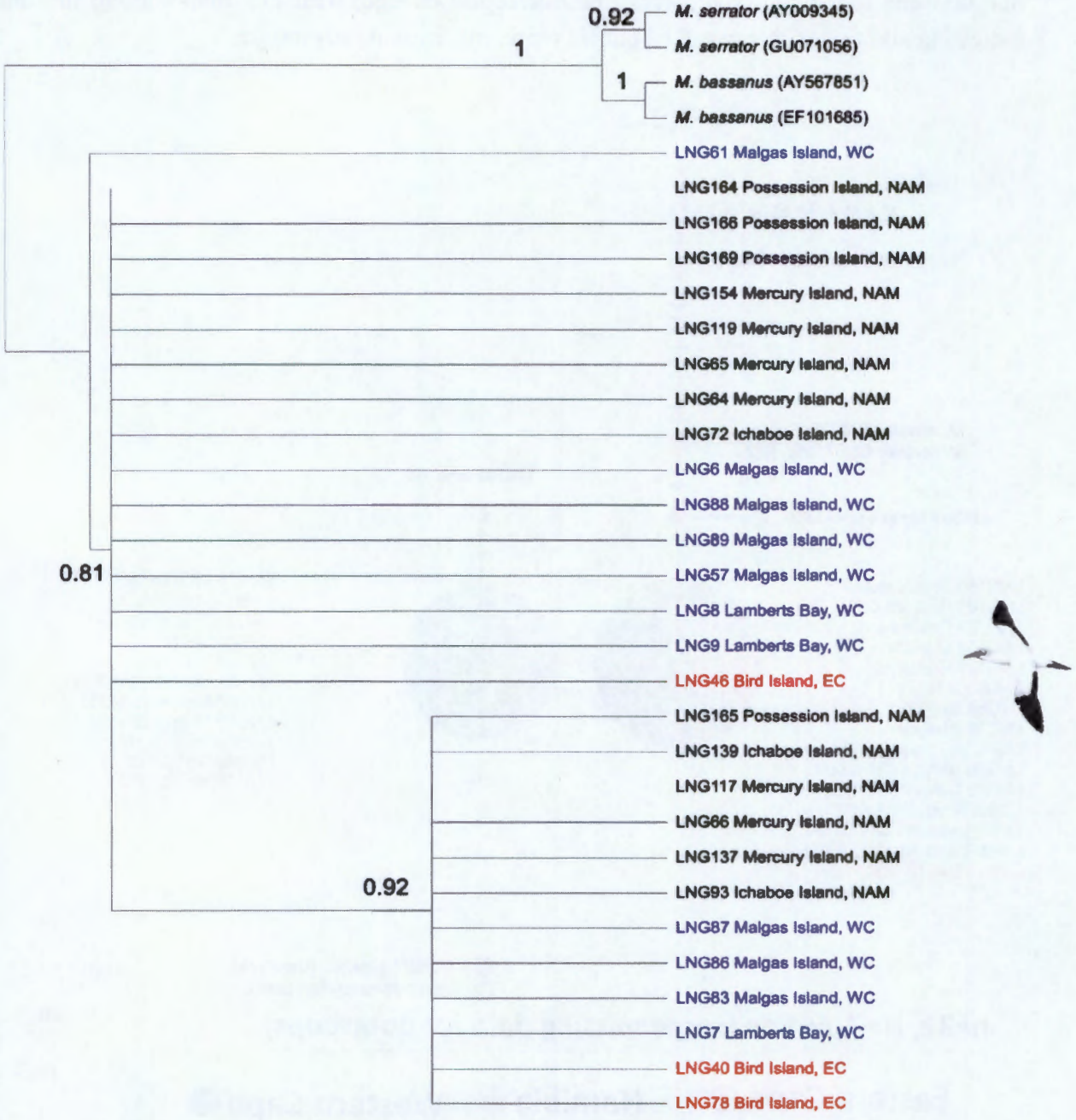
(c)			πst				
		Ichaboe (NAM)	Mercury (Namibia)	Possession (NAM)	Lamberts Bay (WC)	Malgas (WC)	Bird Island (EC)
Gst	Ichaboe (NAM)		-0.03125	0	0	0	-0.02941
	Mercury (NAM)	-0.02242		0	-0.04167	0	-0.02703
	Possession (NAM)	0.03319	0.03516		0	0	0
	Lamberts Bay (WC)	-0.0095	-0.02091	0.02736		0	0
	Malgas (WC)	0.02191	0.0162	1	0.0005		0
	Bird Island (EC)	-0.02032	-0.01977	0.03646	-0.01068	0.01367	

(d)			kxy				
		Ichaboe (NAM)	Mercury (Namibia)	Possession (NAM)	Lamberts Bay (WC)	Malgas (WC)	Bird Island (EC)
Dxy	Ichaboe (NAM)		1.19	1.25	1.222	1.458	1.111
	Mercury (NAM)	0.00178		0.464	0.476	0.732	0.524
	Possession (NAM)	0.00187	0.00069		0.417	0.688	0.583
	Lamberts Bay (WC)	0.00183	0.00071	0.00062		0.708	0.556
	Malgas (WC)	0.00218	0.00109	0.00103	0.00106		0.792
	Bird Island (EC)	0.00166	0.00078	0.00087	0.00083	0.00118	

Appendix 3.8 Cape Gannet haplotype network based on the ATPase 6 target region (669bp). Numbers in brackets are accession numbers for sequences extracted from Genbank or ring numbers (SAFRING) for ringed individuals sampled for the present study. The size of the circles represents the number of individuals that possess a particular haplotype and the black line between haplotypes represents one nucleotide change, with tick marks along the line indicating additional changes. Red circles represent "missing haplotypes".



Appendix 3.9 Bayesian Phylogenetic tree based on the ATPase 6 sequence data for Cape Gannets (669bp). Numbers at the nodes are Bayesian posterior probabilities. Collection localities (colonies and regions) are indicated for each sample: Western Cape (WC, blue), Eastern Cape (EC, red) and Namibia (NAM, black).



Appendix 3.10 (a) Population genetic diversity indices based on the COI sequence data (668bp) subdivided into Cape Gannet breeding regions (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

	NAM	WC	EC	TOTAL
Haplotype diversity (h)	0.79048	0.78571	1	0.82769
Nucleotide diversity (π)	0.00223	0.00192	0.0039	0.0027
N	15	8	3	26
Number of haplotypes	6	4	3	10

Appendix 3.10 (b) Population genetic diversity indices based on the COI sequence data (668bp) subdivided into Cape Gannet breeding colonies (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

	Mercury (NAM)	Ichaboe (NAM)	Possession (NAM)	Lamberts Bay (WC)	Malgas (WC)	Bird Island (EC)	TOTAL
Haplotype diversity (h)	0.7	0.89286	1	-	0.71429	1	0.81667
Nucleotide diversity (π)	0.0012	0.00315	0.0015	-	0.00128	0.00399	0.00263
N	5	8	2	1	7	3	26
Number of haplotypes	3	5	2	1	3	3	10

Appendix 3.11 Pairwise comparisons of genetic structure using various metrics at (a and b) the regional scale and (c and d) colony level based on the COI dataset for Cape Gannets (668bp).

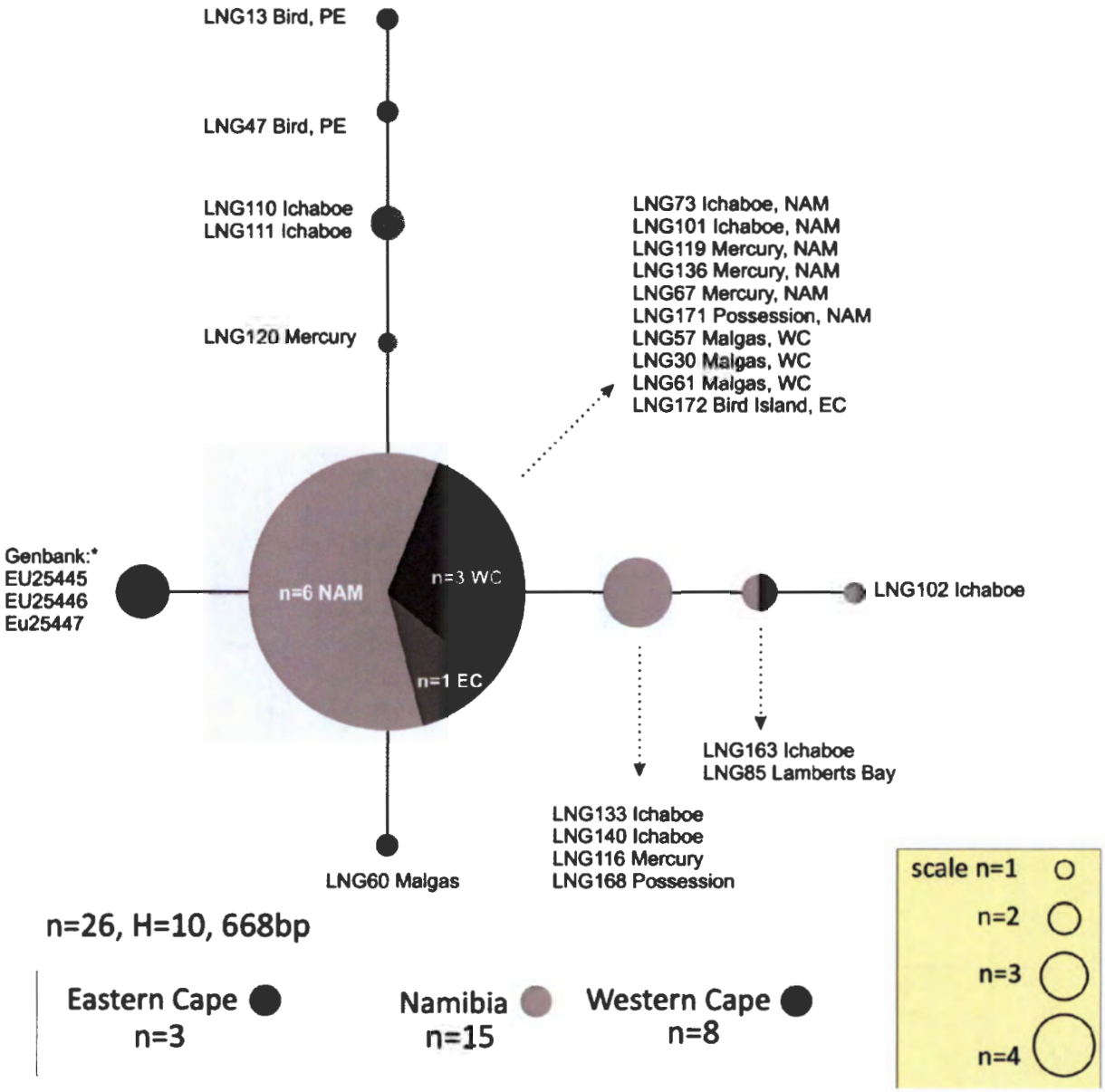
(a) COI (668bp)		φ_{ST}		
		NAM	WC	EC
G_{ST}	Namibia (NAM)		0.106	0.265
	Western Cape (WC)	0.03405		0.359
	Eastern Cape (EC)	0.03366	0.01505	

(b)		*kxy*		
		NAM	WC	EC
Dxy	Namibia (NAM)		1.55	2.822
	Western Cape (WC)	0.00232		3.083
	Eastern Cape (EC)	0.00422	0.00462	

(c)		φ_{ST}				
		Ichaboe (NAM)	Mercury (Namibia)	Possession (NAM)	Malgas (WC)	Bird Island (EC)
G_{ST}	Ichaboe (NAM)		0.01453	-0.12987	0.23853	0.21526
	Mercury (NAM)	0.00635		-0.28571	0.14706	0.2973
	Possession (NAM)	-0.01266	-0.10831		0.13333	0.35294
	Malgas (WC)	0.05231	0.02619	0.01754		0.39344
	Bird Island (EC)	0.01075	-0.02094	-0.0772		

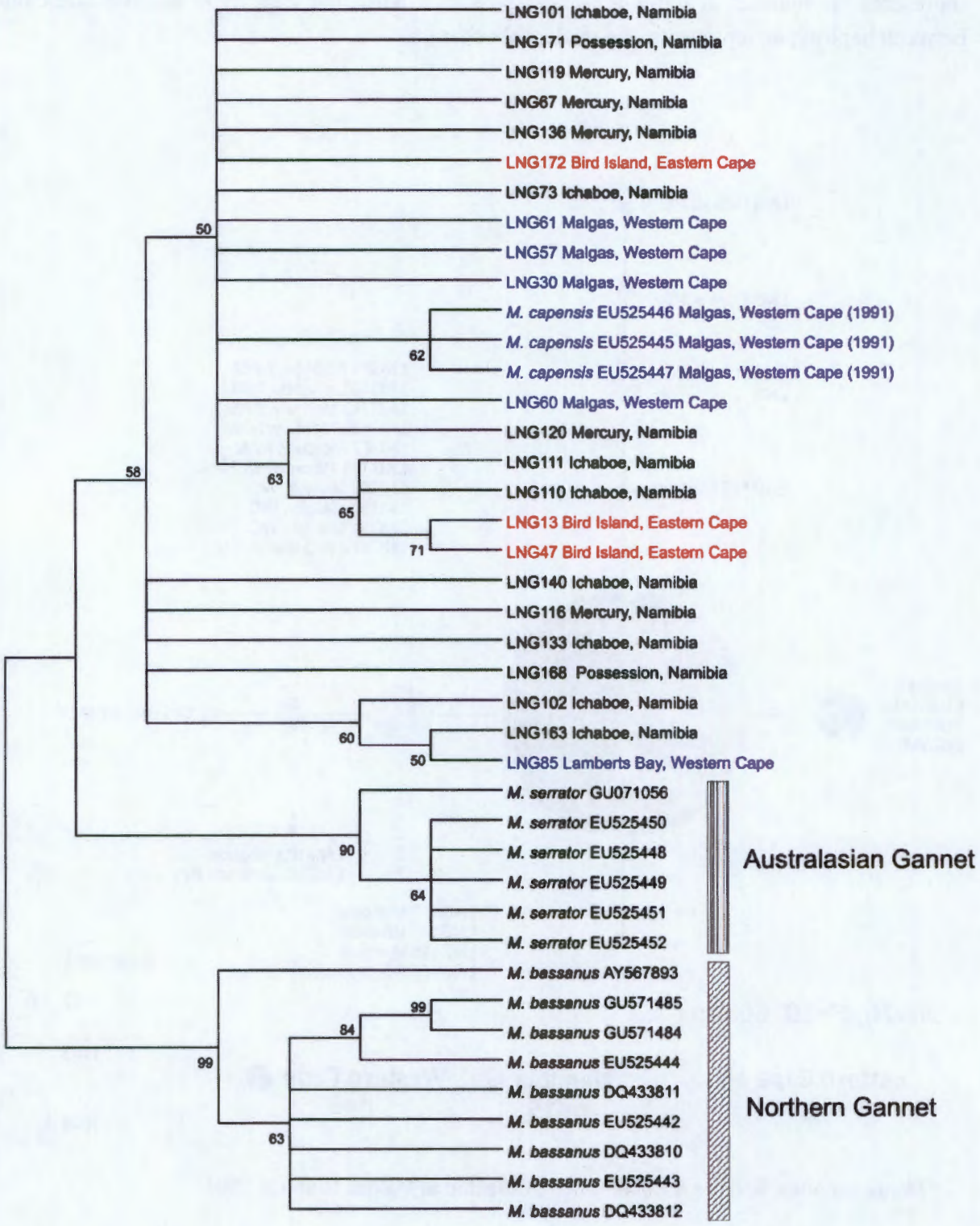
(d) COI (bp)		*kxy*					
		Ichaboe (NAM)	Mercury (NAM)	Possession (NAM)	Lamberts Bay (WC)	Malgas (WC)	Bird Island (EC)
Dxy	Ichaboe (NAM)		1.475	1.375	1.875	1.946	3.042
	Mercury (NAM)	0.00221		0.7	2	0.971	2.467
	Possession (NAM)	0.00206	0.00105		1.5	1.071	2.833
	Lamberts Bay (WC)	0.00281	0.00299	0.00225		2.571	4.3
	Malgas (WC)	0.00291	0.00145	0.0016	0.00385		2.905
	Bird Island (EC)	0.00455	0.00369	0.00424	0.00649	0.00435	

Appendix 3.12 The relationships between the ten haplotypes identified in the Cytochrome Oxidase I (COI) sequence dataset (n=26, 668bp), and their frequencies in each Cape Gannet breeding region (Eastern Cape, Namibia and Western Cape). The size of the circles represents the number of individuals that possess a particular haplotype and the black line between haplotypes represents one nucleotide change.



**Morus capensis* Tavares & Baker (2008), collected at Malgas Island in 1991

Appendix 3.13 Maximum likelihood phylogenetic tree based on the COI gene region for three gannet species (n=41, 668bp). Numbers at the nodes are bootstrap support (1000 replicates)



Appendix 3.14 (a) Population genetic diversity indices based on the NADH2 sequence data (555bp) subdivided into Cape Gannet breeding regions (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

	NAM	WC	EC	TOTAL
Haplotype diversity (h)	0.53333	0	0	0.28421 ± 0.128
Nucleotide diversity (π)	0.00108	0	0	0.00054
N	10	3	7	20
Number of haplotypes	4	1	1	4

Appendix 3.14 (b) Population genetic diversity indices based on the NADH2 sequence data (555bp) subdivided into Cape Gannet breeding colonies (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

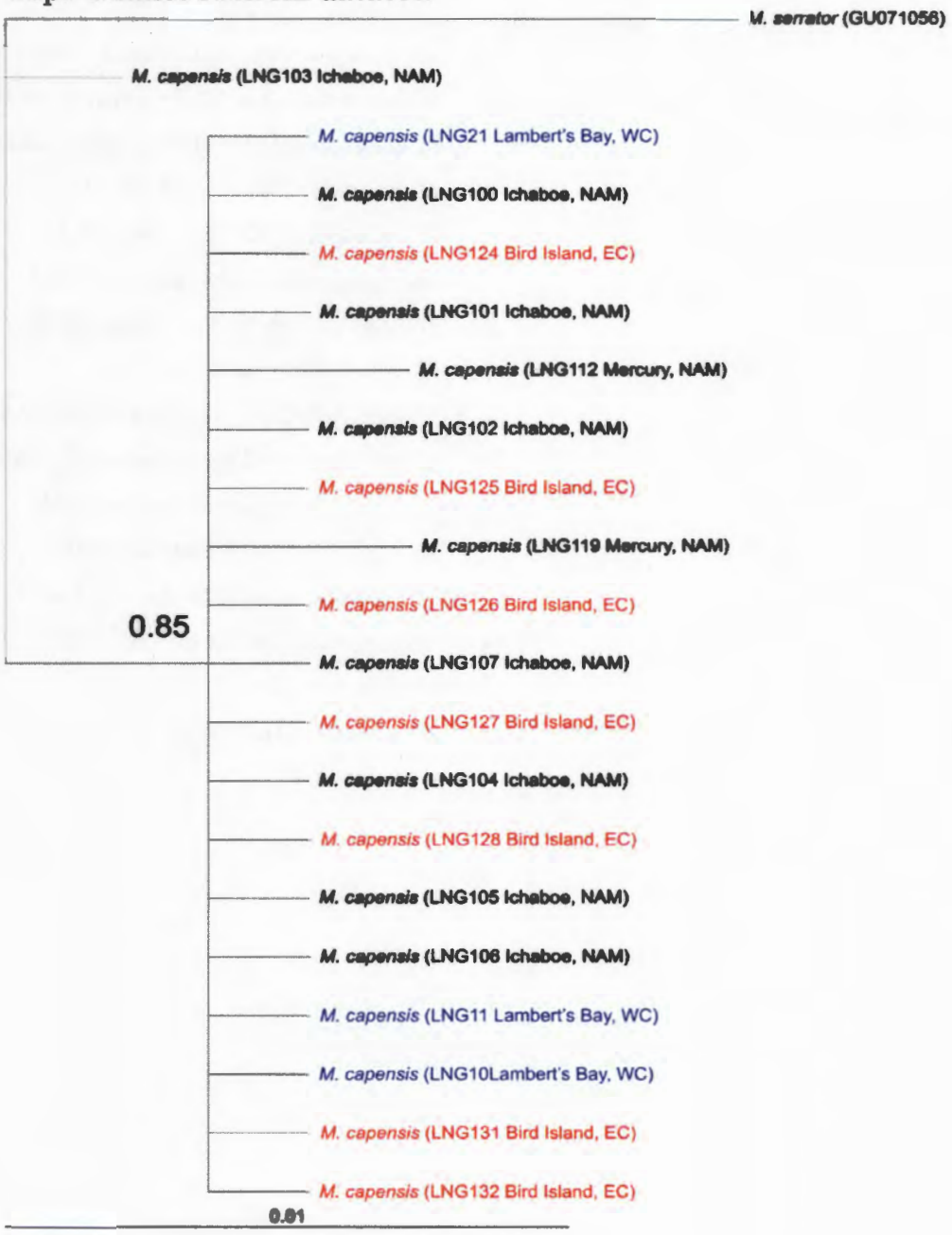
	Mercury (NAM)	Ichaboe (NAM)	Possession (NAM)	Lamberts Bay (WC)	Malgas (WC)	Bird Island (EC)	TOTAL
Haplotype diversity (h)	1	0.25	-	0	-	0	0.28421
Nucleotide diversity (π)	0.0036	0.00045	-	0	-	0	0.00054
N	2	8	0	3	0	7	20
Number of haplotypes	2	2	0	1	0	1	4

Appendix 3.15. Pairwise comparisons of genetic structure using various metrics at (a and b) the regional scale based on the NADH2 dataset for Cape Gannets (555bp).

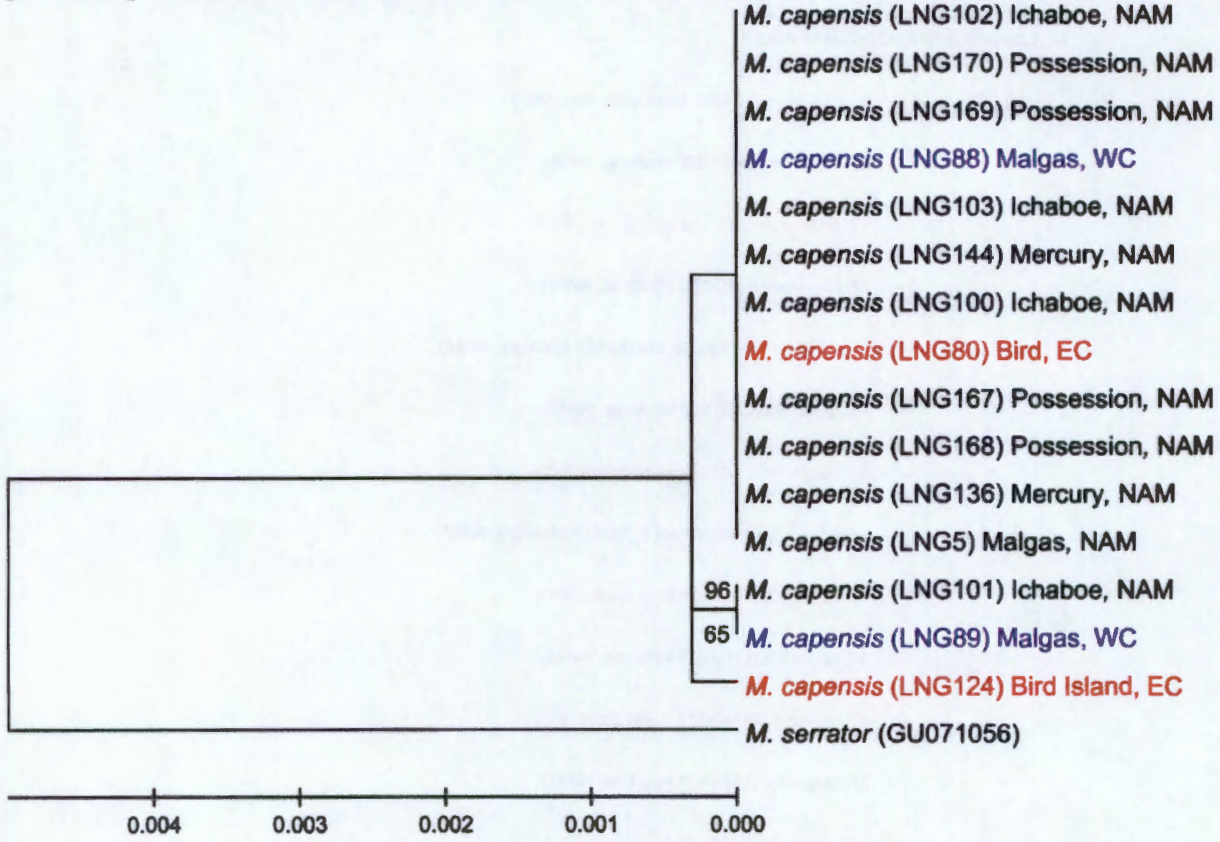
(a) NADH2 (555bp)		φ_{ST}		
		NAM	**WC**	**EC**
G_{ST}	**Namibia (NAM)**		0	0
	Western Cape (WC)	0.05683		0
	Eastern Cape (EC)	0.05066	1	

(b)		*kxy*		
		NAM	**WC**	**EC**
Dxy	**Namibia (NAM)**		0.3	0.3
	Western Cape (WC)	0.00054		0
	Eastern Cape (EC)	0.00054	0	

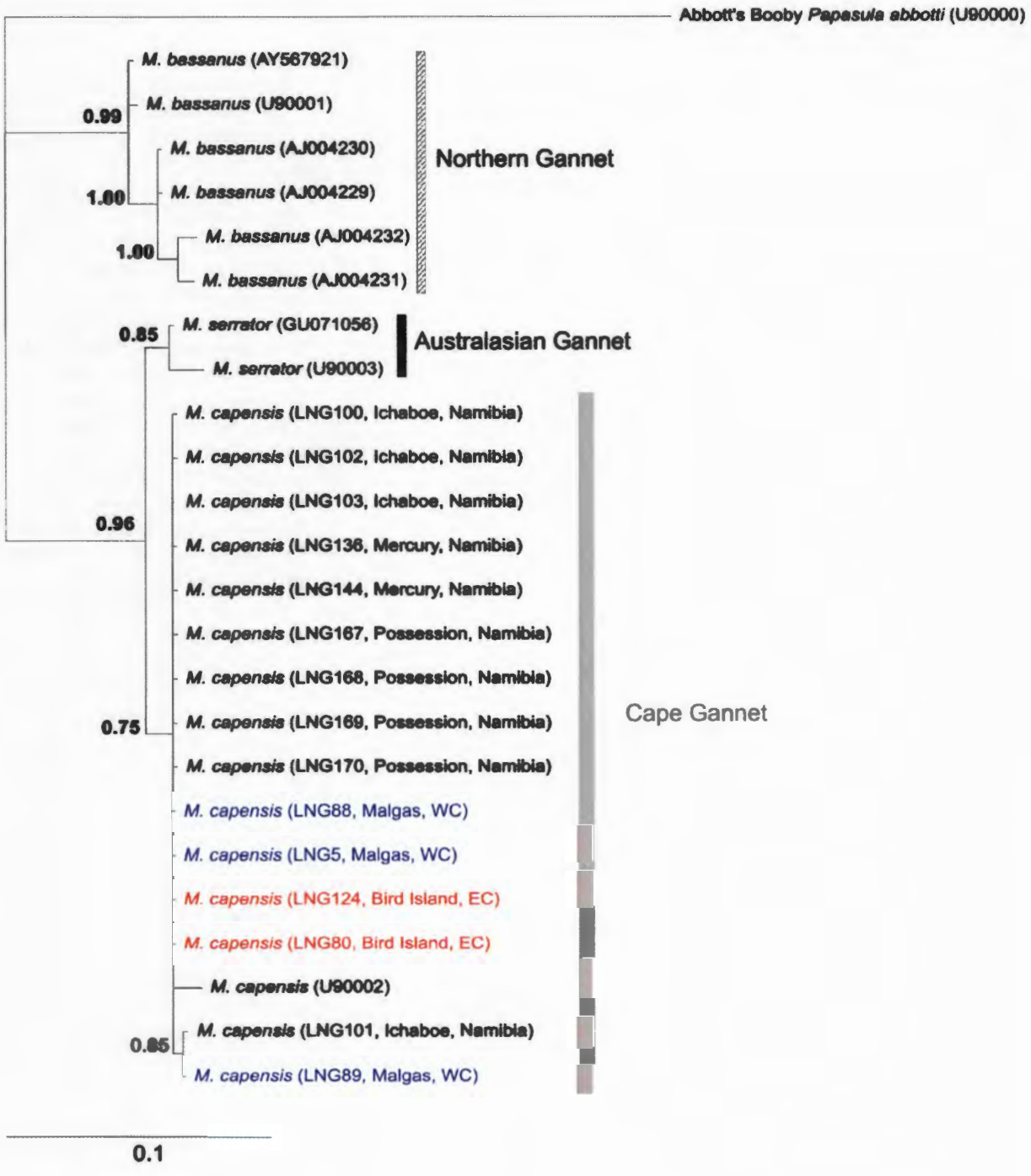
Appendix 3.16 Bayesian 50% majority rule bootstrap consensus tree based on the Cape Gannet NADH2 dataset.



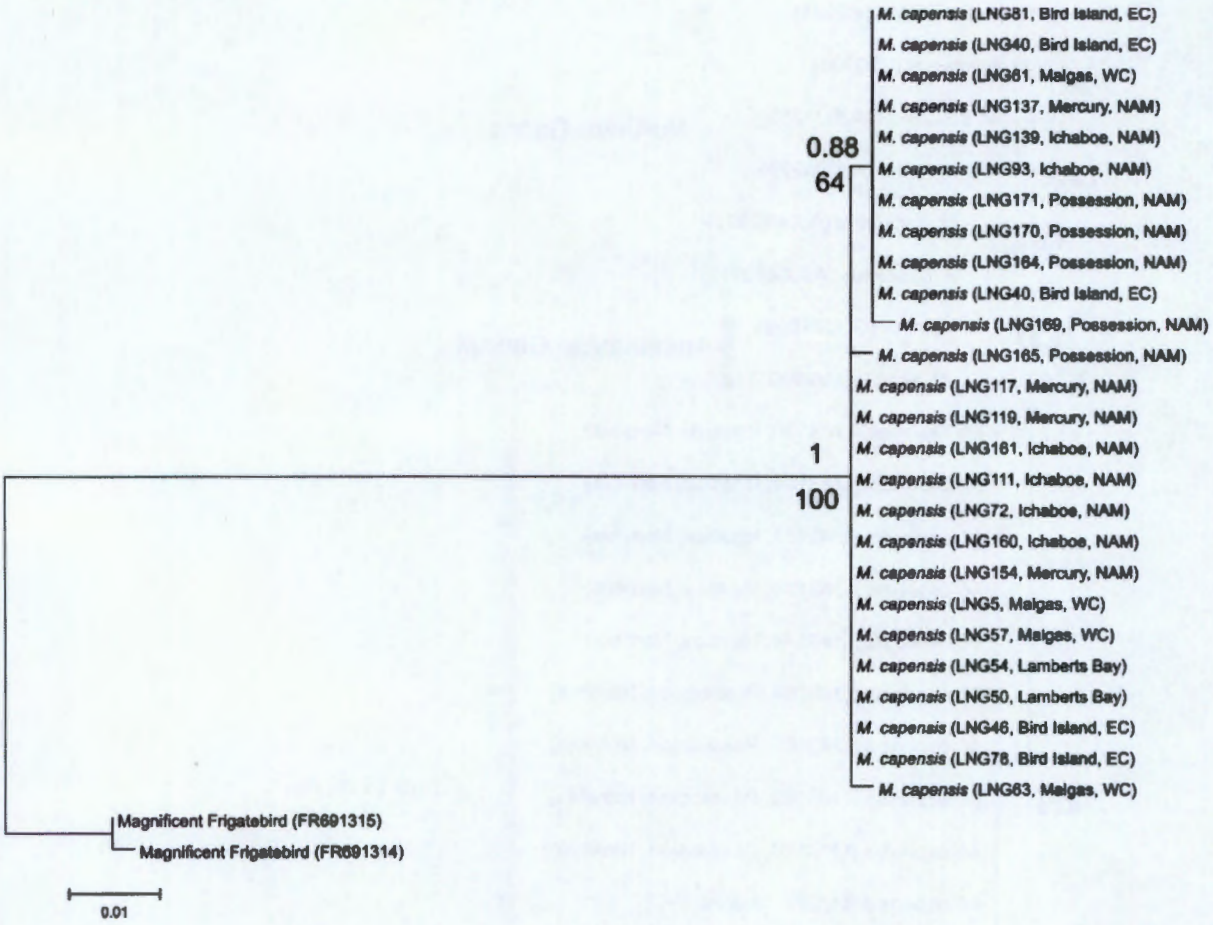
Appendix 3.17 Maximum likelihood and Bayesian phylogenetic tree based on the cytochrome b dataset (HKY model, 834bp). Numbers above branches at nodes are Bayesian posterior probabilities and below the branches are bootstrap values (2000 replicates).



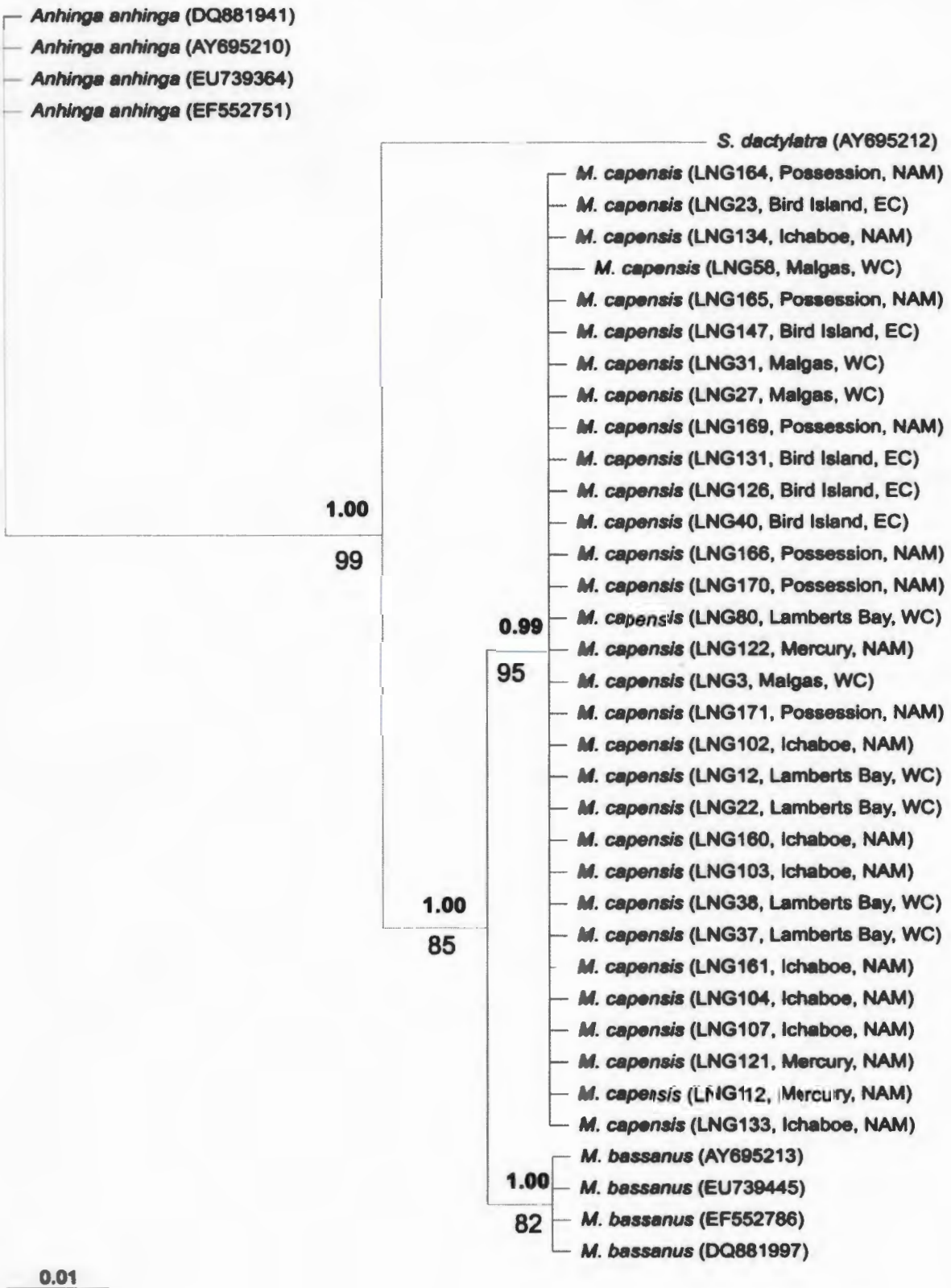
Appendix 3.18 Bayesian phylogenetic tree based on *Morus* cytochrome b sequences (794bp, HKY+G model) rooted with Abbott's Booby *Papasula abbotti*. Numbers at the nodes are Bayesian posterior probabilities.



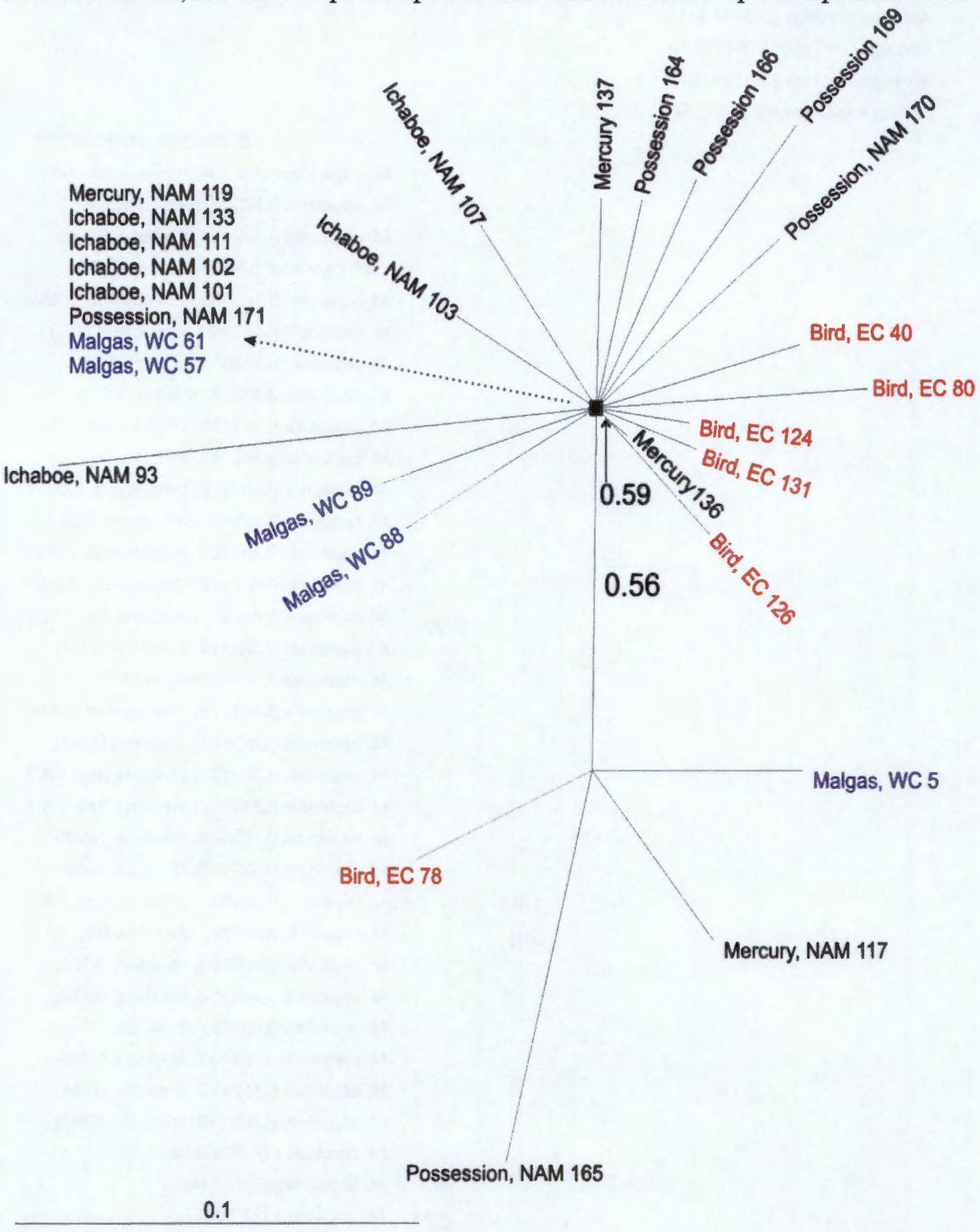
Appendix 3.19 Maximum likelihood and Bayesian phylogenetic tree (Kimura 2-parameter model) based on of the GAPDH dataset for Cape Gannets (419bp, n=27). Numbers above nodes are Bayesian posterior probabilities and below nodes are ML bootstrap values (1000 bootstrap replicates).



Appendix 3.20 Maximum likelihood and Bayesian phylogenetic tree (2 million generations) based on the BFIB intron 7 target region. Bold numbers at the nodes are posterior probabilities calculated during the two MCMC runs and numbers below the nodes are ML bootstrap values (1000 replicates).



Appendix 3.21 The unrooted, strict consensus Bayesian phylogenetic tree based on the combined (concatenated), partitioned Cape Gannet sequence dataset that includes individuals for which three or more of the 7 gene regions were successfully sequenced (n=28, 4104bp). Samples from Namibian colonies are shown in black, Eastern Cape samples in red and Western Cape samples in blue.



Appendix 3.22 (a) Population genetic diversity indices, including haplotype diversity $h \pm$ SD, based on the NADH3 sequence data (358bp) for the whole dataset and subdivided into African Penguin breeding regions (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

	NAM	WC	EC	TOTAL
Haplotype diversity (h)	0.153 ± 0.067	0.228 ± 0.084	0.176 ± 0.088	0.182 ± 0.046
Nucleotide diversity (π)	0.00043	0.00066	0.00051	0.00053
N	50	41	33	124
Number of haplotypes	3	4	4	6

Appendix 3.22 (b) Population genetic diversity indices, including haplotype diversity ($h \pm$ SD), based on the NADH3 sequence data (358bp) for the whole dataset and subdivided into African Penguin breeding colonies (NAM=Namibia, WC=Western Cape, EC=Eastern Cape)

	Mercury (NAM)	Ichaboe (NAM)	Possession (NAM)	Halifax (NAM)	Boulders Beach (WC)	Robben Island (WC)	Dassen Island (WC)	Jutten Island (WC)	Stoney Point (WC)	Dyer Island (WC)	St Croix Island (EC)	Bird Island (EC)	TOTAL
Haplotype diversity (h)	0.233 ± 0.126	0	0	0.195 ± 0.115	0	0	0.417 ± 0.191	0	0.524 ± 0.209	0.25 ± 0.180	0.105 ± 0.092	0.275 ± 0.148	0.182 ± 0.046
Nucleotide diversity (π)	0.00065	0	0	0.00056	0	0	0.00124	0	0.0016	0.0007	0.00029	0.0008	0.00053
N	16	10	4	20	5	5	9	7	7	8	19	14	124
Number of haplotypes	2	1	1	3	1	1	3	1	3	2	2	3	6

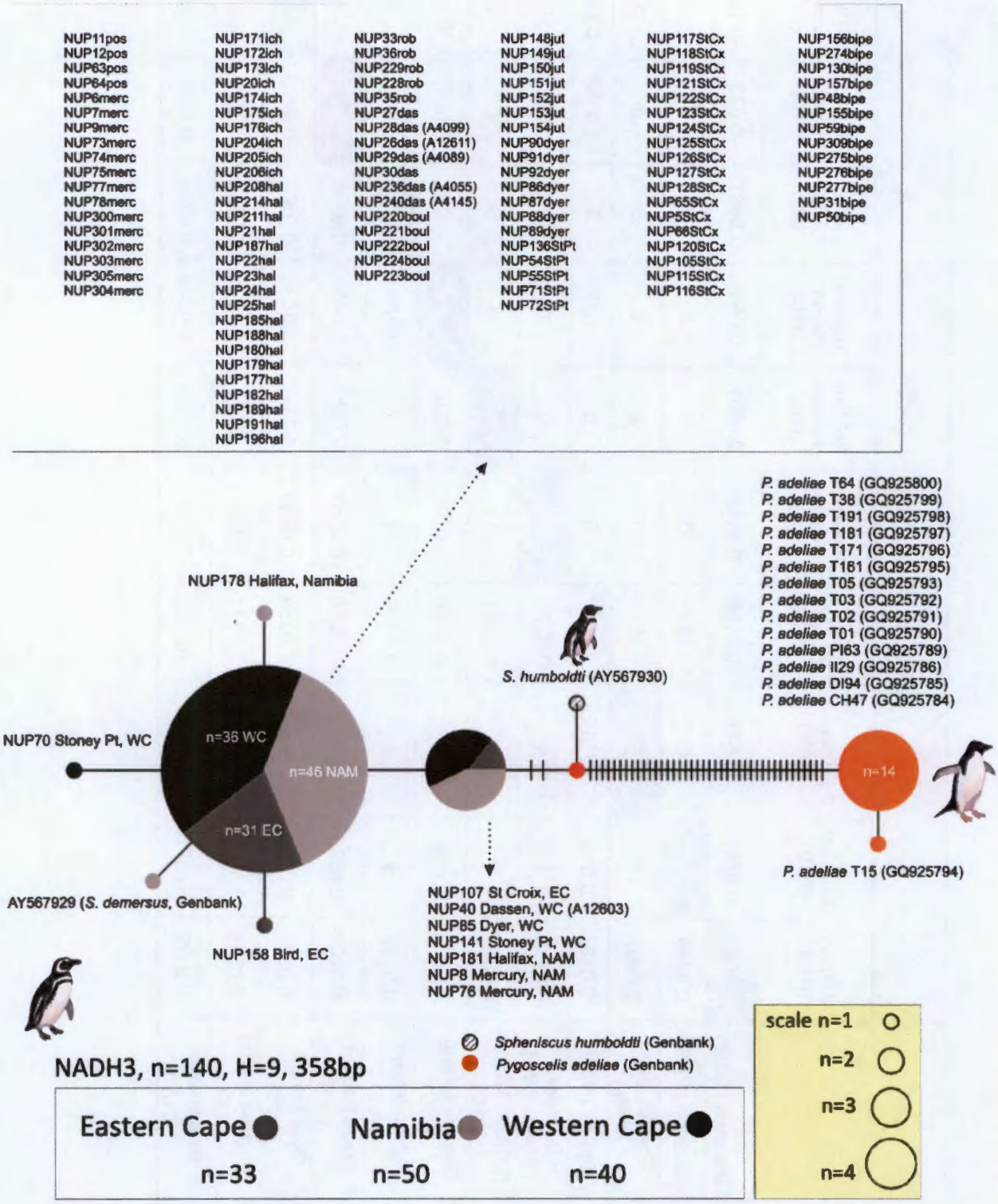
Appendix 3.23 Pairwise comparisons of genetic structure using various metrics at (a and b) the regional scale and (c) colony level based on the NADH3 dataset for African Penguins (358bp).

(a) NADH3 (358bp)		φst		
		NAM	**WC**	**EC**
Gst	**Namibia (NAM)**		-0.01384	- 0.00710
	Western Cape (WC)	-0.00669		-0.01089
	Eastern Cape (EC)	- 0.00714	-0.0078	

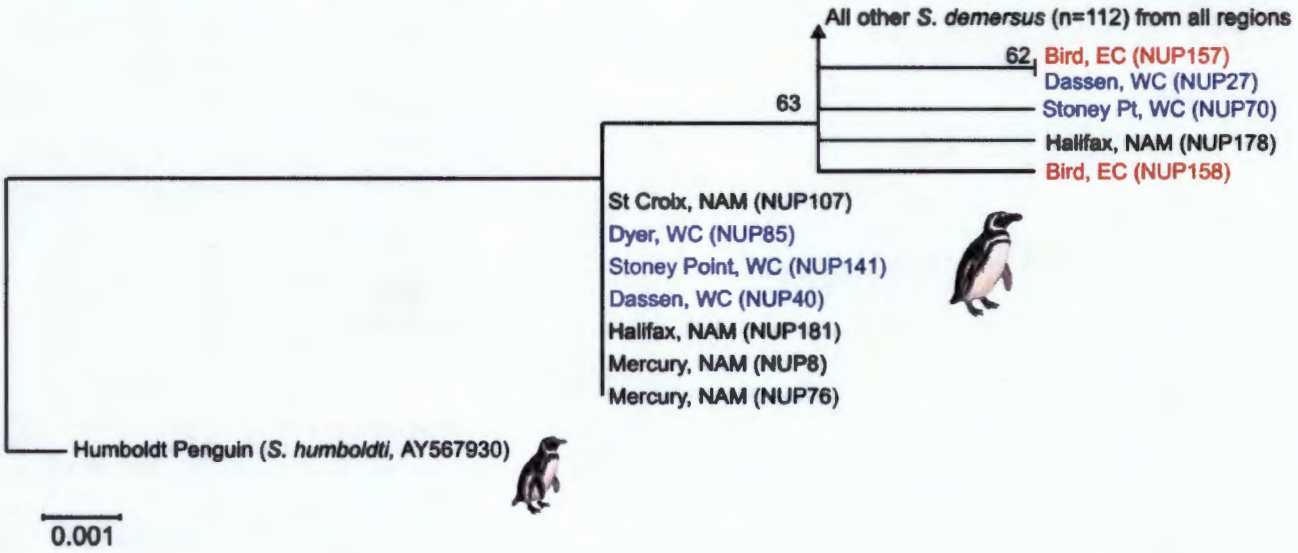
(b)		*kxy*		
		NAM	**WC**	**EC**
Dxy	**Namibia (NAM)**		0.193	0.167
	Western Cape (WC)	0.00054		0.207
	Eastern Cape (EC)	0.00047	0.00058	

(c)		φ_{ST}											
		Mercury (NAM)	**Ichaboe (NAM)**	**Possession (NAM)**	**Halifax (NAM)**	**Boulders Beach (WC)**	**Robben Island (WC)**	**Dassen Island (WC)**	**Jutten Island (WC)**	**Stoney Point (WC)**	**Dyer Island (WC)**	**St Croix Island (EC)**	**Bird Island (EC)**
G_{ST}	**Mercury (NAM)**		**0.0667**	**0.0667**	-0.0196	**0.0667**	**0.0667**	-0.0609	**0.0667**	-0.073	-0.1048	-0.0293	**0.0311**
	Ichaboe (NAM)	0.0288		0	0	0	0	0	0	0	0	0	0
	Possession (NAM)	0.0407	**1**		0	0	0	0	0	0	0	0	0
	Halifax (NAM)	-0.0181	0.013	0.0365		0	0	-0.0357	0	-0.0385	-0.0588	-0.0357	0
	Boulders Beach (WC)	0.0338	**1**	**1**	0.0271		0	0	0	0	0	0	0
	Robben Island (WC)	0.0338	**1**	**1**	0.0271	**1**		0	0	0	0	0	0
	Dassen Island (WC)	-0.0273	0.034	0.0328	-0.0126	0.0293	0.0293		0	-0.0667	-0.087	-0.0444	-0.0455
	Jutten Island (WC)	0.0287	**1**	**1**	0.0179	**1**	**1**	0.0293		0	0	0	0
	Stoney Point (WC)	-0.0208	0.0525	0.0388	-0.0015	0.0387	0.0387	-0.0452	0.0435		-0.0952	-0.0465	0
	Dyer Island (WC)	-0.0389	0.0007	0.0103	-0.0238	0.0043	0.0043	-0.0424	0.0003	-0.0405		-0.08	0
	St Croix Island (EC)	-0.0146	0.0037	0.0312	-0.0177	0.021	0.021	-0.0025	0.0103	0.0125	-0.0306		0
	Bird Island (EC)	-0.0079	0.0175	0.0326	-0.0123	0.0251	0.0251	-0.0279	0.0189	-0.0084	-0.0182	-0.0049	

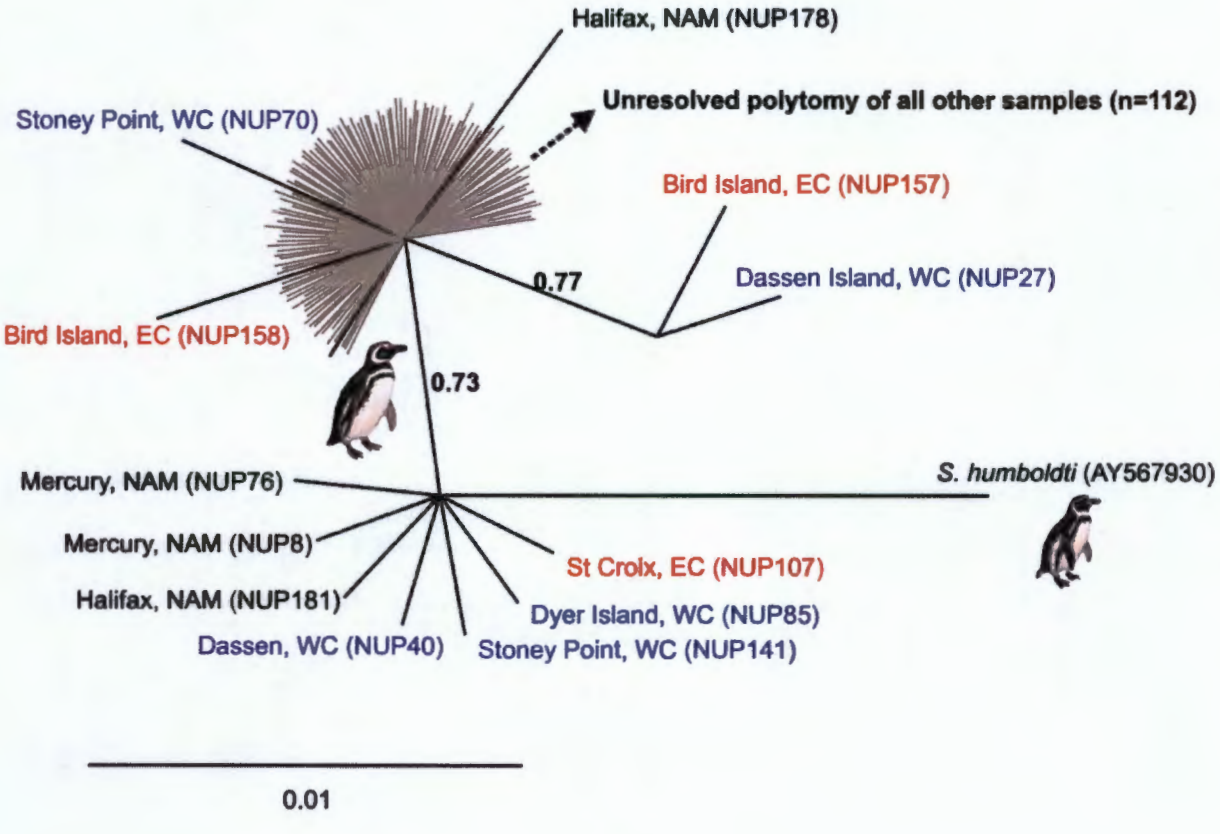
Appendix 3.24 The relationships among the five African Penguin NADH3 haplotypes (n=124, 358bp) and those of other penguin taxa. The size of the circle represents the number of individuals with a particular haplotype and connecting lines represent one nucleotide change (tick marks along connecting lines represent additional changes). Numbers in brackets are flipper-band numbers (SAFRING) or accession numbers for sequences extracted from Genbank.



Appendix 3.25 ML phylogenetic tree based on the African Penguin NADH3 data (n=124, 358bp) and rooted with the Humboldt Penguin. Samples from Namibia are shown in black, those from the Western Cape are in blue, and Eastern Cape samples are shown in red.



Appendix 3.26 Unrooted Bayesian phylogenetic tree based on the African Penguin NADH3 dataset (n=124, 358bp), including one Humboldt Penguin sequence. The numbers in bold are Bayesian posterior probabilities and the sampling colony, and region, is indicated (Western Cape (WC) samples in blue, Eastern Cape (EC) samples in red and Namibia (NAM) samples in black), along with the sample number.



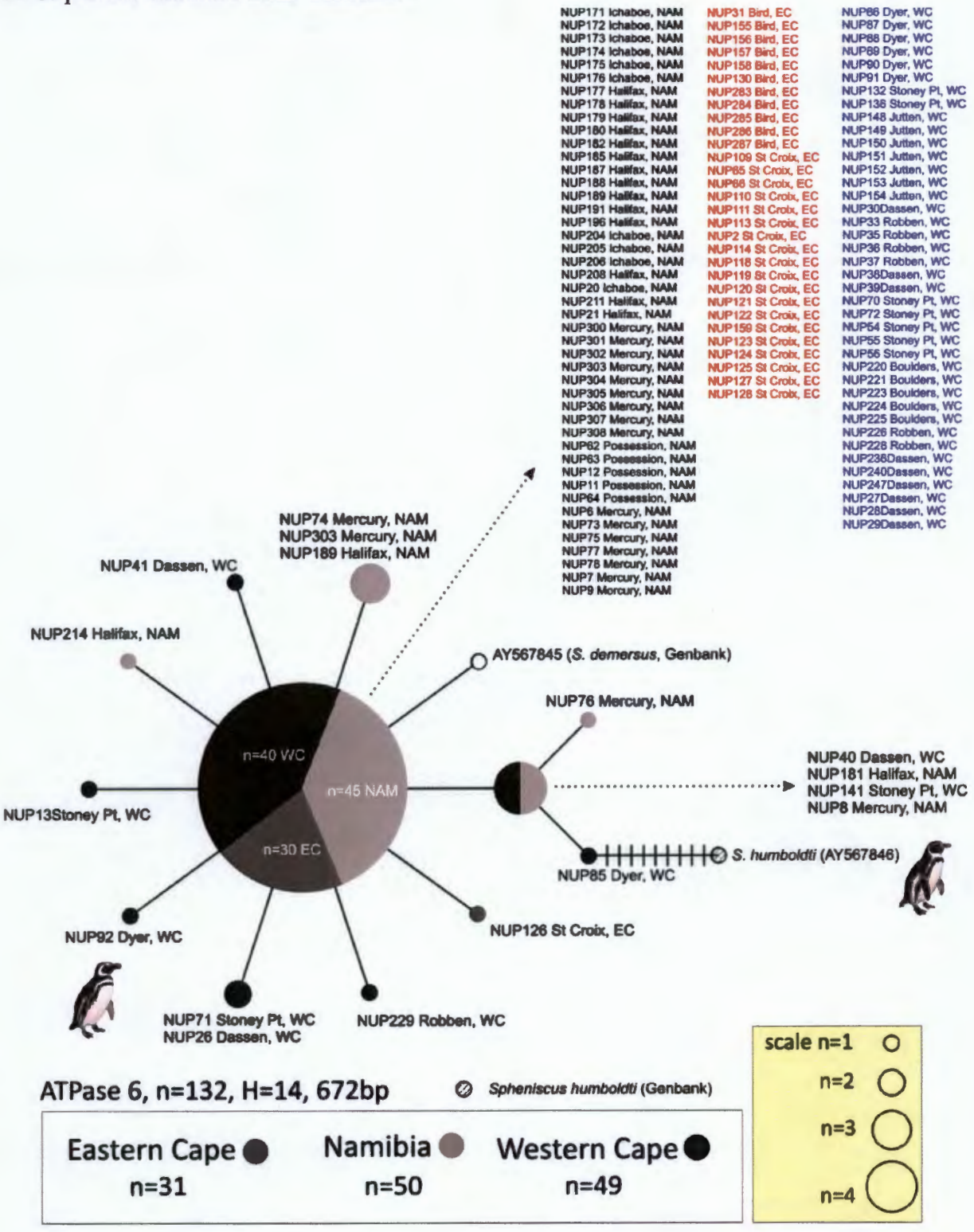
Appendix 3.27 (a) Population genetic diversity indices, including haplotype diversity h ± SD, based on the ATPase6 sequence data (672bp) for the whole dataset and subdivided into African Penguin breeding regions (NAM=Namibia, WC=Western Cape, EC=Eastern Cape).

	NAM	WC	EC	TOTAL
Haplotype diversity (h)	0.26 ± 0.08	0.335 ± 0.087	0.065 ± 0.059	0.244 ± 0.050
Nucleotide diversity (π)	0.00046	0.0006	0.0001	0.00043
N	50	49	31	130
Number of haplotypes	5	8	2	12

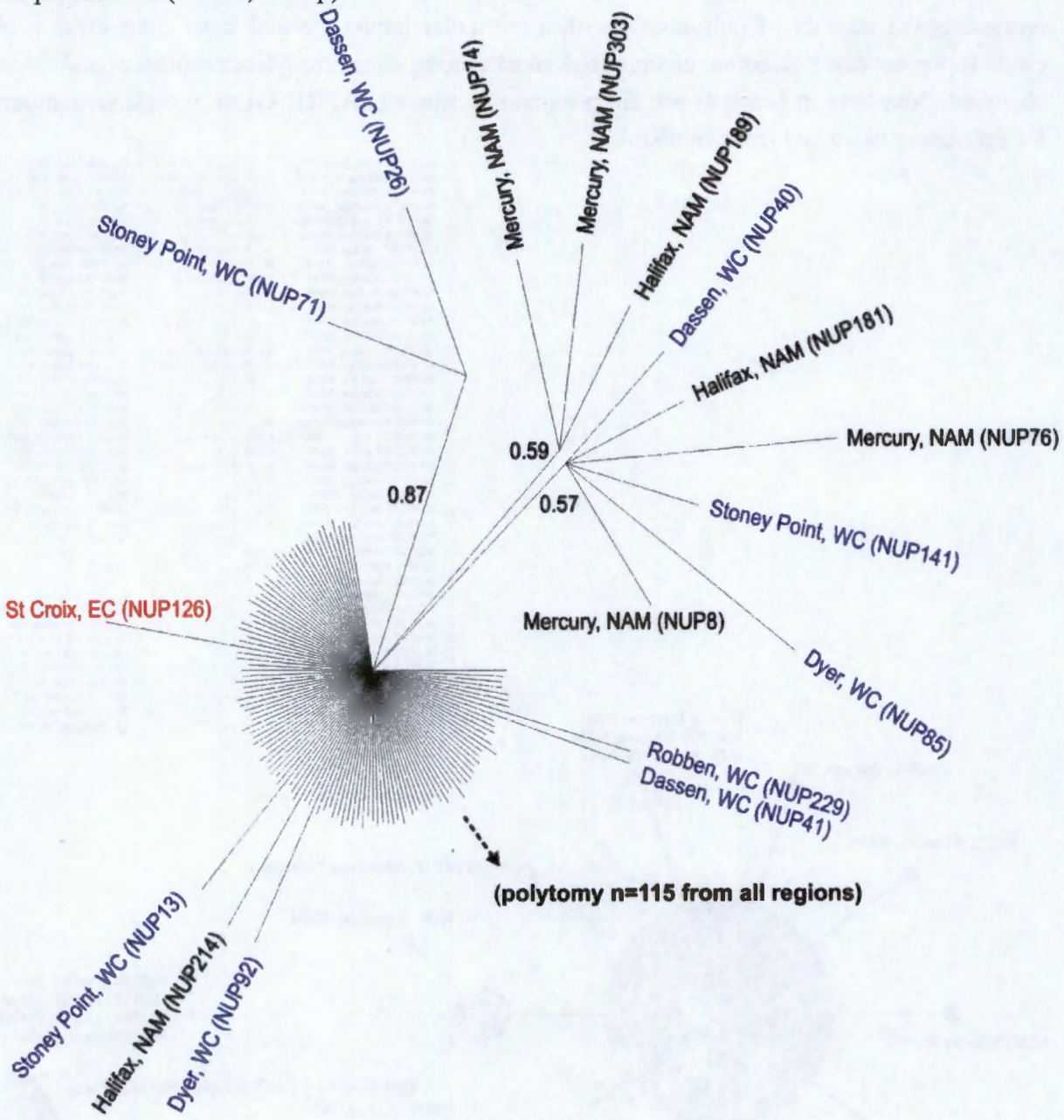
Appendix 3.27 (b) Population genetic diversity indices, including haplotype diversity (h ± SD), based on the ATPase6 sequence data (672bp) for the whole dataset and subdivided into African Penguin breeding colonies (NAM=Namibia, WC=Western Cape, EC=Eastern Cape)

	Mercury (NAM)	Ichaboe (NAM)	Possession (NAM)	Halifax (NAM)	Boulders Beach (WC)	Robben Island (WC)	Dassen Island (WC)	Jutten Island (WC)	Stoney Point (WC)	Dyer Island (WC)	St Croix Island (EC)	Bird Island (EC)	TOTAL
Haplotype diversity (h)	0.38 ± 0.134	0	0	0.35 ± 0.148	0	0.286 ± 0.196	0.455 ± 0.170	0	0.533 ± 0.180	0.464 ± 0.200	0.1 ± 0.088	0	0.244 ± 0.050
Nucleotide diversity (π)	0.00075	0	0	0.00056	0	0.00043	0.00074	0	0.00089	0.00112	0.00015	0	0.00043
N	19	10	5	16	5	7	12	7	10	8	20	11	130
Number of haplotypes	4	1`	1	4	1	2	4	1	4	3	2	1	12

Appendix 3.28 The relationships among the five African Penguin ATPase6 haplotypes (n=130, 672bp), including one Humboldt Penguin outgroup sequence. The size of the circle represents the number of individuals with a particular haplotype and lines connecting each circle represent one nucleotide change (tick marks along connecting lines represent additional changes). Numbers in brackets are flipper-band numbers (SAFRING) or accession numbers for sequences extracted from Genbank.



Appendix 3.29 Unrooted Bayesian phylogenetic tree based on the African Penguin ATPase 6 sequence data (n=130, 672bp).



Appendix 3.30 (a) Population genetic diversity indices, including haplotype diversity $h \pm$ SD, based on the COI sequence data (688bp) for the whole dataset and subdivided into African Penguin breeding regions (NAM=Namibia, WC=Western Cape, EC=Eastern Cape)

	NAM	WC	EC	TOTAL
Haplotype diversity (h)	0.484 ± 0.138	0.182 ± 0.144	0.222 ± 0.166	0.33 ± 0.094
Nucleotide diversity (π)	0.00079	0.00026	0.00032	0.00051
N	18	11	9	38
Number of haplotypes	5	2	2	5

Appendix 3.30 (b) Population genetic diversity indices, including haplotype diversity ($h \pm$ SD), based on the COI sequence data (688bp) for the whole dataset and subdivided into African Penguin breeding colonies, although some of the breeding colonies are pooled due to low sample sizes (NAM=Namibia, WC=Western Cape, EC=Eastern Cape)

	Mercury & Ichaboe Islands (NAM)	Halifax Island (NAM)	WC (sample sizes small)	Bird Island (EC)	TOTAL
Haplotype diversity (h)	0.491 ± 0.175	0.524 ± 0.209	0.182 ± 0.144	0.222 ± 0.166	0.33 ± 0.094
Nucleotide diversity (π)	0.00079	0.00083	0.00026	0.00032	0.00051
N	11	7	11	9	38
Number of haplotypes	4	3	2	2	5

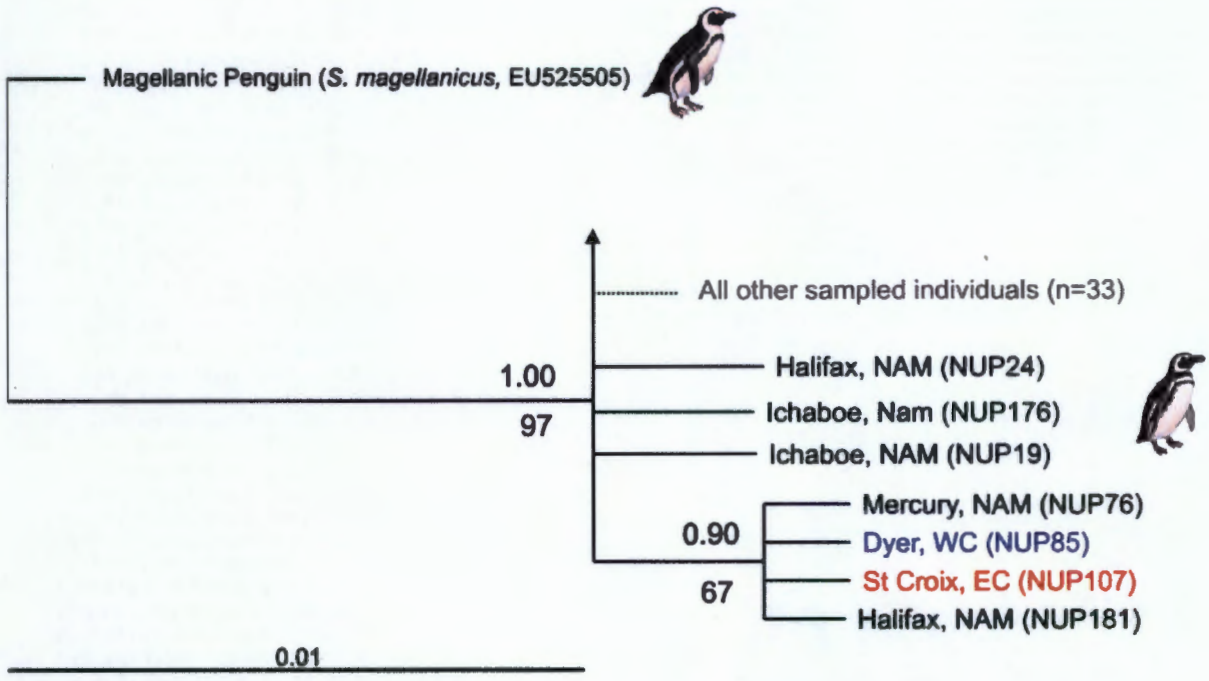
Appendix 3.31 (a) Population genetic diversity indices, including haplotype diversity h ± SD, based on the NADH3 sequence data (393bp) for the whole dataset and subdivided into Cape Cormorant breeding regions (NAM=Namibia, WC=Western Cape).

	NAM	WC	TOTAL
Haplotype diversity (h)	0.522 ± 0.033	0.52 ± 0.032	0.515 ± 0.021
Nucleotide diversity (π)	0.00133	0.00137	0.00134
N	23	48	71
Number of haplotypes	2	3	3

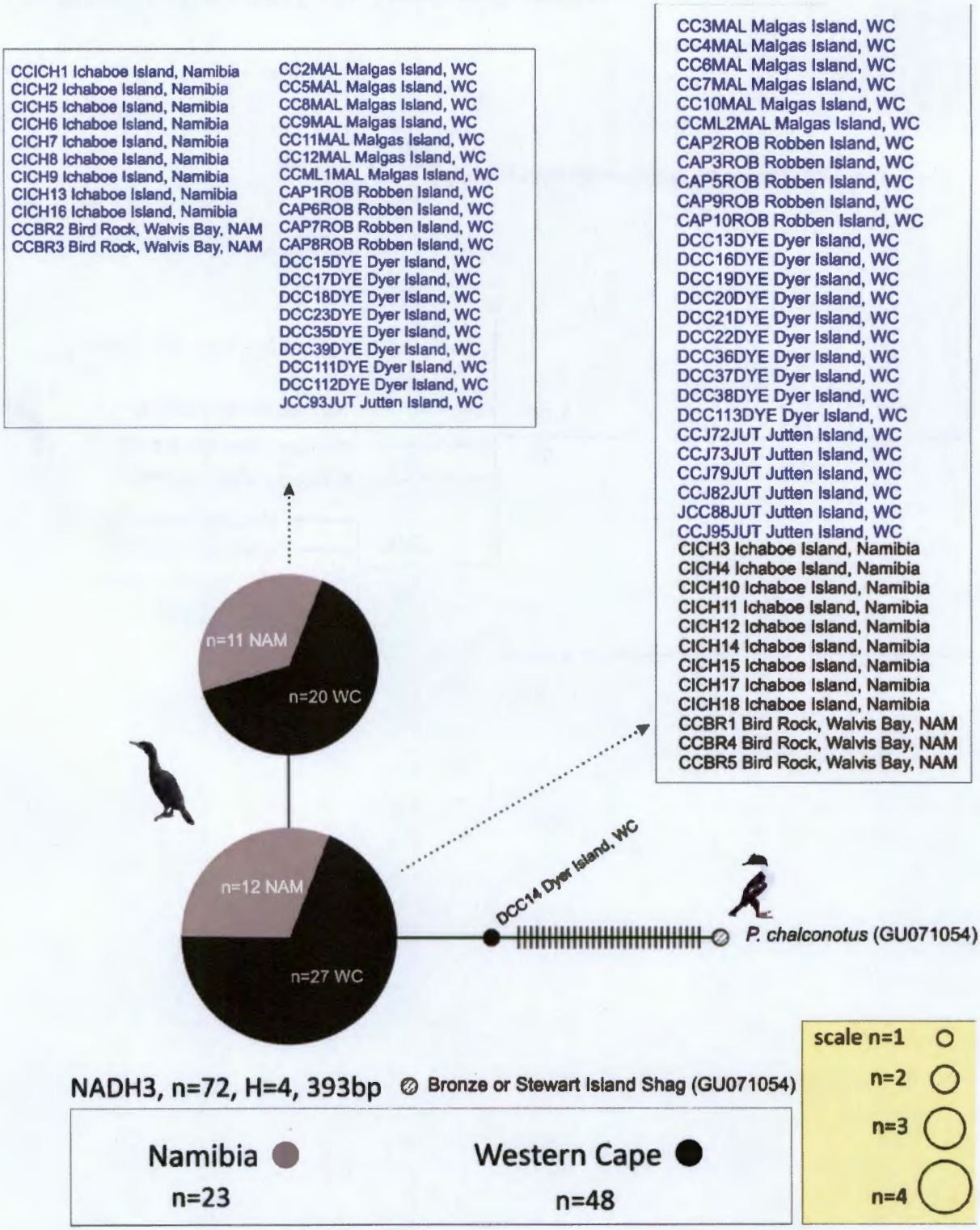
Appendix 3.31 (b) Population genetic diversity indices, including haplotype diversity (h ± SD), based on the NADH3 sequence data (393bp) for the whole dataset and subdivided into Cape Cormorant breeding colonies (NAM=Namibia, WC=Western Cape).

	Ichaboe Island (NAM)	Bird Rock (NAM)	Jutten Island (WC)	Robben Island (WC)	Malgas Island (WC)	Dyer Island (WC)	TOTAL
Haplotype diversity (h)	0.529 ± 0.04	0.6 ± 0.175	0.286 ± 0.196	0.556 ± 0.090	0.538 ± 0.06	0.573 ± 0.061	0.515 ± 0.021
Nucleotide diversity (π)	0.00135	0.00153	0.00073	0.00141	0.00137	0.00158	0.00134
N	18	5	7	9	13	19	71
Number of haplotypes	2	2	2	2	2	3	3

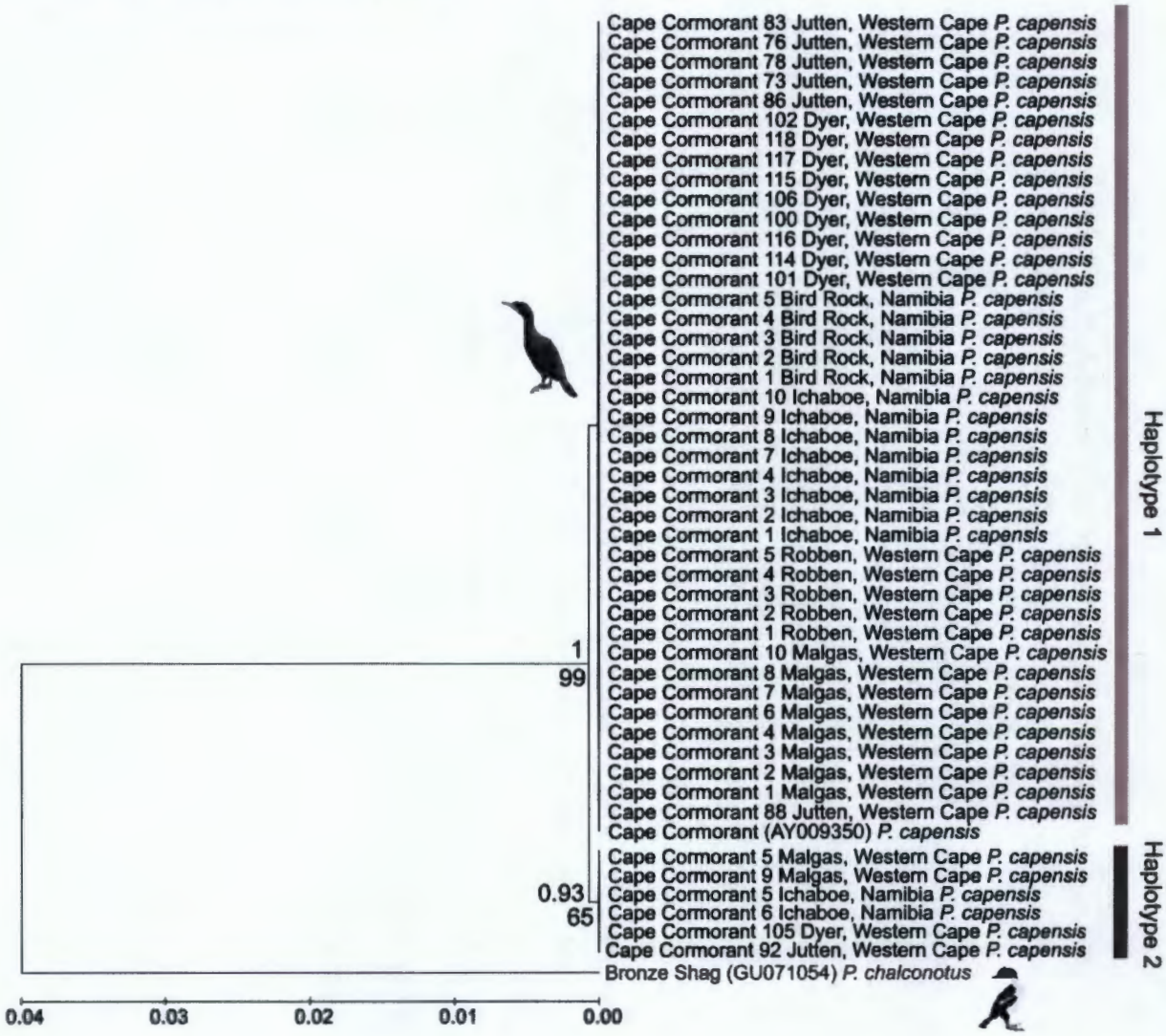
Appendix 3.32 Maximum likelihood and Bayesian Phylogenetic tree based on the African Penguin COI samples (n=40, 688bp, including two African Penguin COI sequences from Genbank) rooted on a Magellanic Penguin sequence. Sampling localities are indicated and numbers in brackets are sample codes or Genbank Accession numbers. Numbers at the nodes are bootstrap values (1000 replicates, below branch) and Bayesian posterior probabilities (above).



Appendix 3.33 The relationships among the Cape Cormorant NADH3 haplotypes (n=71, 393bp), including one outgroup taxon. The size of the circle represents the number of individuals with a particular haplotype and connecting lines represent one nucleotide change (tick marks along connecting lines represent additional changes). Numbers in brackets are accession numbers for sequences extracted from Genbank.



Appendix 3.36 ATPase 6 gene tree for Cape Cormorants and one outgroup Phalacrocorax species (n=48, 682bp). Numbers at nodes are Bayesian posterior probabilities (above) and Maximum likelihood bootstrap values (below, 1000 replicates).



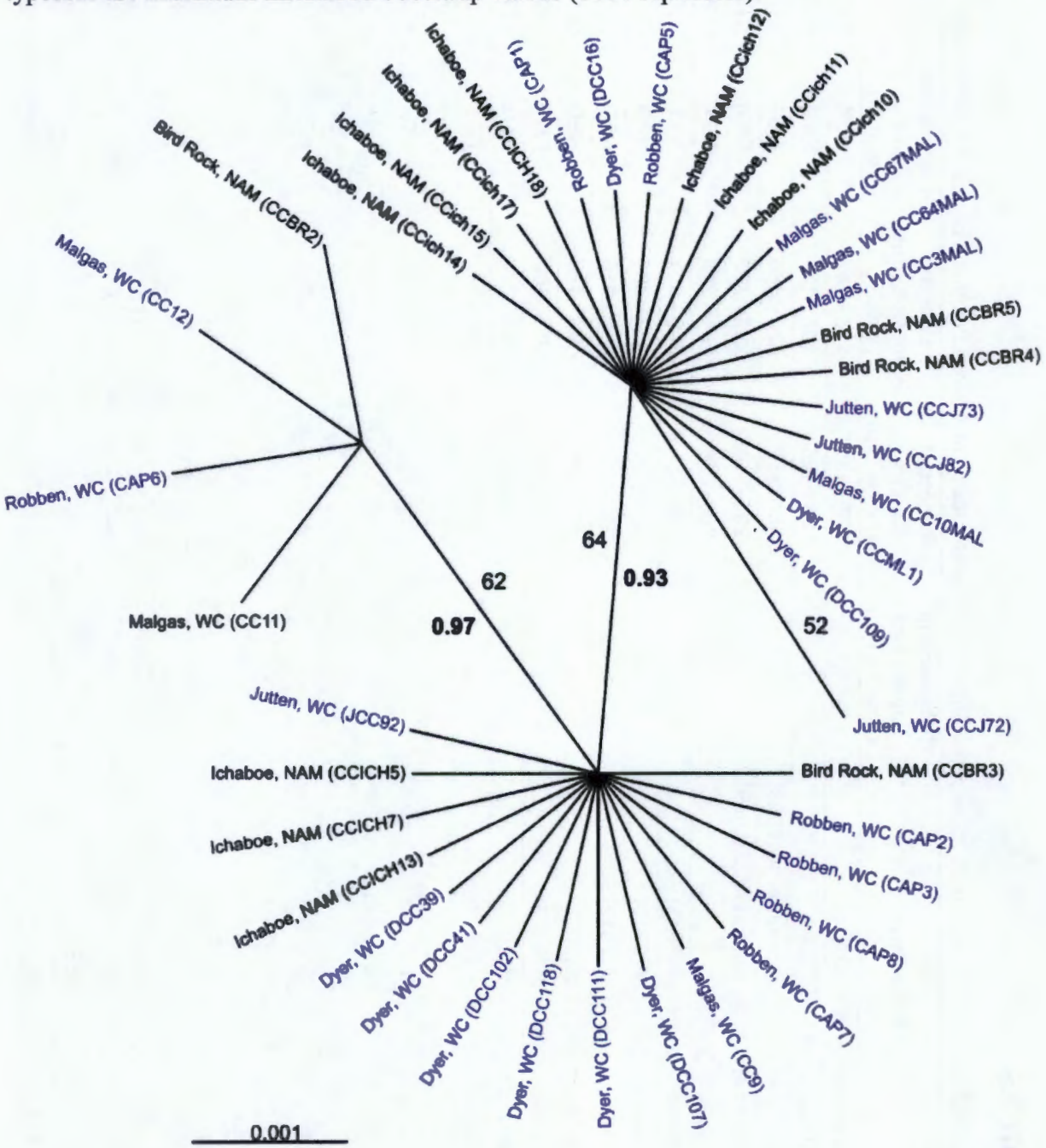
Appendix 3.37 (a) Population genetic diversity indices, including haplotype diversity h ± SD, based on the cytochrome b sequence data (864bp) for the whole dataset and subdivided into Cape Cormorant breeding regions (NAM=Namibia, WC=Western Cape).

	NAM	WC	TOTAL
Haplotype diversity (h)	0.538 ± 0.115	0.647 ± 0.052	0.615 ± 0.043
Nucleotide diversity (π)	0.00074	0.00092	0.00086
N	14	27	41
Number of haplotypes	3	4	4

Appendix 3.37 (b) Population genetic diversity indices, including haplotype diversity (h ± SD), based on the cytochrome b sequence data (864bp) for the whole dataset and subdivided into Cape Cormorant breeding colonies (NAM=Namibia, WC=Western Cape).

	Ichaboe Island (NAM)	Bird Rock (NAM)	Jutten Island (WC)	Robben Island (WC)	Malgas Island (WC)	Dyer Island (WC)	TOTAL
Haplotype diversity (h)	0.467 ± 0.132	0.833 ± 0.222	0.833 ± 0.222	0.667 ± 0.160	0.667 ± 0.160	0.5 ± 0.128	0.615 ± 0.043
Nucleotide diversity (π)	0.00054	0.00135	0.00116	0.00088	0.00121	0.00058	0.00086
N	10	4	4	7	7	9	41
Number of haplotypes	2	3	3	3	3	2	4

Appendix 3.38 Unrooted phylogenetic tree based on the Cape Cormorant cytochrome b dataset (n=41, 864bp). Numbers in bold are Bayesian posterior probabilities and plain typeface are maximum likelihood bootstrap values (1000 replicates).



CHAPTER 4 APPENDICES

Appendix 4.1 Source references and details associated with 25 microsatellite loci previously tested in African Penguins. Bold loci were used in this study.

Locus name	Genbank Accession #	Reference	Polymorphic among test samples (y/n)	Reported Length Variation (bp)	Length Variation (this study)	Fluorescent label (F-primer)	Repeat Motif
Sh1Ca9	**AF540006**	**Schlosser *et al* (2003)**	**y**	**128 - 139**	**118 - 142**	**HEX**	**T5(GT)17**
Sh2Ca21	**AF540011**	**Schlosser *et al* (2003)**	**y**	**106 - 128**	**106 - 130**	**FAM**	**(CA)11**
G2-2	**-**	**Akst *et al* (2003), Bouzat *et al* (2009)**	**y**	**367–387**	**370 - 386**	**FAM**	**(GT)13**
B3-2	**-**	**Akst *et al* (2003), Bouzat *et al* (2009)**	**y**	**292–314**	**297 - 309**	**FAM**	**(GT)12**
G3-6	**-**	**Akst *et al* (2003)**	**y**	**258–308**	**261 - 279**	**HEX**	**(TG)10**
PNN01	**JX494403**	**Labuschagne *et al* (2013)**	**y**	**309-321**	**309 - 321**	**VIC**	**(GGAT)7**
PNN03	**JX494404**	**Labuschagne *et al* (2013)**	**y**	**362-374**	**362 - 386**	**NED**	**(TAC)12**
PNN06	**JX494406**	**Labuschagne *et al* (2013)**	**y**	**127-139**	**292 - 316**	**NED**	**(TCTA)10**
PNN08	**JX494408**	**Labuschagne *et al* (2013)**	**y**	**127-139**	**123 - 143**	**NED**	**(ATAG)9**
PNN09	**JX494409**	**Labuschagne *et al* (2013)**	**y**	**356-376**	**356 -400**	**FAM**	**(GATA)12**
PNN12	**JX494410**	**Labuschagne *et al* (2013)**	**y**	**244-256**	**246 - 256**	**FAM**	**(GT)10**
PNN05	**JX494405**	**Labuschagne *et al* (2013)**	**y**	**245-247**	**245 - 247**	**PET**	**(TG)10**
TP500	AF289545	Roeder *et al* (2002)	n	106 - 126	104 - 106	FAM	(CA)14
AM12	AF131242	Roeder *et al* (2002)	n	140 - 154	132 - 142	HEX	(CA)13
H2-6*	-	Akst *et al* (2003), Bouzat et al (2009)	n	282–296	289 - 291	HEX	(TG)15
RM6	AF289547	Roeder *et al* (2002)	n	168 - 180	N/A	FAM	(CA)10
G3-11	-	Akst *et al* (2003)	n	300–306	307	HEX	(TG)12
M1-11*	-	Akst *et al* (2003), Bouzat et al (2009)	N/A	128	N/A	-	(TG)7 CGTGC(GT)3
AM3	AF131241	Roeder *et al* (2002)	N/A	173 - 176	N/A	-	(A)8(n)5(TA)4
Sh1Ca12	AF540007	Schlosser *et al* (2003)	y	123–147	N/A	-	(T)7(CTA)(T)4
Sh1Ca16	AF540008	Schlosser *et al* (2003)	y	98–116	N/A	-	(CCCT)2(CT)1(CA)20
Sh1Ca17	AF540009	Schlosser *et al* (2003)	y	105 - 121	N/A	-	(CA)15TATGCAA(CA)4
Sh2Ca22	AF540012	Schlosser *et al* (2003)	y	95 - 129	N/A	-	(CA)17A2(CA)5
Sh2Ca12	AF540010	Schlosser *et al* (2003)	y	99–111	N/A	-	T3(CA)14A3
PNN07	JX494407	Labuschagne *et al* (2013)	y	355-363	N/A	VIC	(CT)11

Appendix 4.2 Forward and reverse primer sequences, and the respective PCR conditions, for 25 microsatellite loci that have been tested in African Penguins. Those in bold type were employed in the present study.

Locus name	Forward Primer (5' - 3')	Reverse Primer (5' - 3')	Annealing temperature	[MgCl2] (mM)
Sh1Ca9	**AGCAGATGTGGGGGTTGTAG**	**AAGTCAGTGTTATGCCAAGATACG**	**56°C**	**2**
Sh2Ca21	**AAAATAAAGCCTATACACAACAGG**	**GTGCACTTAATGGGGTGTATG**	**53°C**	**2**
G2-2	**ATGACATATTGATTGGC**	**CTGCCTGAACTAAGCTTTGTC**	**50°C**	**2**
B3-2	**GGTGGTTATAGATGCACGAC**	**CAGTGCCCAGGAATCCAGTT**	**50°C**	**2**
G3-6	**TCTTAAGGTCTTGCACAC**	**CAGCTCAGTAACTGCAGGCA**	**54°C**	**1**
PNN01	**ATGATGAGAGGGATGAATGGAC**	**GAGTACACCTGCGCCAGAC**	**50 - 60°C**	**1**
PNN03	**ACAAACTTCCCACCTGACTGTT**	**GCTCCTATTTCACGACTCATCC**	**50 - 60°C**	**1**
PNN06	**TCAGAAAGGAACTGTGTAGAGGC**	**TCCTGAGTAACACTTGTGGGTG**	**50 - 60°C**	**1**
PNN08	**GGAAATGCCACTGAAAACCTAA**	**GATAGATGGGGAACTGGAAACA**	**50 - 60°C**	**1**
PNN09	**CTGAGCAGACAAACTGGTAAAA**	**TCAACTCGTCTTTGCTTACAAC**	**50 - 60°C**	**1**
PNN12	**TGGAGGTGTTATGTTTAGCAT**	**TTCAGTGGCTGTATTTGCTG**	**50 - 60°C**	**1**
PNN05	**CAGTGACAGGCAAGGGTCTTAT**	**TGAGTAAGCAATGAGTTGGCAC**	**50 - 60°C**	**1**
TP500	GGGACACAGGCAGCCACG	GGGAGTGGTATGGCTGGGTT	62°C	1.5
AM12	AAAAACCCAACACAACAAAC	CCCAAGAAGAGATTTGTGAG	55°C	1.5
H2-6*	GTACATGCCTTCTGATATGG	CATTCCTCTAGAGATTGC	50°C	2
RM6	CAGGAGGCTTTGAGACAAGA	CTGTTTACATCCGATGCAGG	57°C	1.5
G3-11	ATGATTCAGGGCAGGTGGA	CAGAAGCTTCAGGAAGGGCA	55°C	1
M1-11*	CTCGTTCTAGCCTTCTGTTCC	AGCCCAGTCTTCACAACTGC	53°C	1
AM3	AGGAAAGAAGTAACTGAAGCAG	CATCTTCCCACAGAAGAAAC	55°C	1.5
Sh1Ca12	GCACACTGTGAGCTTGCAC	CTTGGGCTCTCAAATACCC	60	2
Sh1Ca16	GTAGGGCAGCAGCACACC	TCTCCTGGAAAGCAGGAATCC	60	2
Sh1Ca17	GCCCTCAGTGGTTGCACA	GGTGGTCAAAACCCCTCTTT	60	2
Sh2Ca22	CAGCTGGCAGTGAGTCTGAG	GGACAGCTACCAGATAGAGTGC	58	2
Sh2Ca12	TCAGTGTACGAGCCAGAAGG	CTAGGATCCCGGCTTTTGTC	60	2
PNN07	GAGAGATGTTCATAGCACGCAG	CTACCTTCTTCTTGGTTCTGGC	50 - 60°C	1

Appendix 4.3 The observed heterozygosity (Ho) and number of alleles (Na) among 24 wild Humboldt Penguins, 20 Magellanic Penguins and 20 captive African Penguins based on genotype data from Schlosser (2003, Pers. Comm). The results of the present study for the two loci in common with that research are reported in the last column.

	Humboldt Penguin		Magellanic Penguin		African Penguin		Present study (African Penguin)	
	Na	Ho	Na	Ho	Na	Ho	Na	Ho
Sh1Ca9	4	0.500	6	**0.950**	11	0.450	10	0.83
Sh2Ca22	*9*	0.583	4	**0.800**	5	0.500	6	0.67
Sh2Ca21	*9*	**0.667**	6	0.650	8	0.600		
Sh2Ca12	6	**0.708**	7	0.471	11	0.526		
Sh1Ca16	*9*	0.708	8	**0.737**	5	0.444		
Sh1Ca12	*11*	**0.875**	2	0.300	3	0.300		
Sh1Ca17	9	**0.875**	9	0.850	10	0.650		

Appendix 4.4 Regional null allele frequency estimates (GENEPOP option 8, sub-option 1). Only non-zero results are shown (all other population-locus combinations had null allele frequency estimates of zero). Values in bold indicate those population-locus combinations that significantly deviated from HWE. Asterisk indicates population-locus combinations that show evidence of heterozygote deficit.

Locus	Population	Frequency estimate
G2-2	Namibia	0.0456
SH1CA9	Namibia	**0.0421***
	Western Cape	0.0212
SH2CA21	Namibia	0.0049
	Western Cape	0.0202
B3-2	Western Cape	**0.1008***
G3-6	Eastern Cape	**0**
	Namibia	0.0183
	Western Cape	**0**
PNN01	Eastern Cape	0.0907
	Namibia	0.0457
	Western Cape	0.0436
PNN03	Namibia	**0.0768**
	Western Cape	**0.0443**
PNN06	Eastern Cape	0.0508
	Namibia	0.0095
PNN08	Eastern Cape	0.0212
	Western Cape	0.0182
PNN09	Eastern Cape	0
	Namibia	**0.1262***
	Western Cape	0.0028
PNN12	Eastern Cape	0.0449

	Namibia	**0.1926**
	Western Cape	0.1043
PNN05	Western Cape	0.1125

Appendix 4.5 Locus pairs in each breeding region for which significant (P<0.05) correlations were detected while testing for LD.

Region	Locus 1	Locus 2	P-value	Standard Error
Western Cape	SH1CA9	PNN12	0.00018	0.0002
Western Cape	SH2CA21	PNN09	0.00467	0.0034
Western Cape	G2-2	G3-6	0.00849	0.0035
Namibia	B3-2	PNN08	0.00932	0.0008
Western Cape	PNN01	PNN12	0.01074	0.0018
Eastern Cape	SH1CA9	SH2CA21	0.02373	0.0128
Namibia	SH2CA21	PNN01	0.02537	0.0047
Namibia	SH1CA9	B3-2	0.03532	0.0032
Namibia	SH2CA21	PNN05	0.03770	0.0017
Eastern Cape	G3-6	PNN12	0.03776	0.0044
Eastern Cape	SH2CA21	G3-6	0.04403	0.0073
Eastern Cape	SH1CA9	PNN03	0.04414	0.0186
Namibia	B3-2	PNN09	0.04471	0.0020
Eastern Cape	B3-2	PNN06	0.04500	0.0028

Appendix 4.6 The number of alleles detected at each locus across all samples (n=189) in the present study

Locus	Number of alleles
PNN05	2
PNN01	4
PNN12	5
B3-2	6
PNN06	6
PNN08	6
G2-2	7
PNN09	7
G3-6	9
PNN03	9
SH2CA21	11
SH1CA9	17

Appendix 4.7 Colony-level null allele frequency estimates (GENEPOP option 8, sub-option 1). Only non-zero results are shown (all other population-locus combinations had null allele frequency estimates of zero). Values in bold indicate those population-locus combinations that significantly deviated from HWE. Asterisk indicates colony-locus combinations that show evidence of heterozygote deficiency.

Locus	Population	Frequency
G22	Halifax Island, Namibia	0.0891*
	Mercury Island, Namibia	0.0614
SH1CA9	Ichaboe Island, Namibia	0.0773*
	Mercury Island, Namibia	0.0211
	Dassen Island, Western Cape	0.0615*
SH2CA21	Ichaboe Island, Namibia	**0**
	Halifax Island, Namibia	0.0489
	Robben Island, Western Cape	0.1161
	Dassen Island, Western Cape	**0.0081**
B32	Possession Island, Namibia	monomorphic
	Stony Point, Western Cape	0.0871
	Dassen Island, Western Cape	**0.1729***
G36	Bird Island, Eastern Cape	0.0363
	Ichaboe Island, Namibia	0.0421
	Mercury Island, Namibia	0.0394
	Boulders Beach, Western Cape	0.0755
PNN01	St Croix Island, Eastern Cape	0.1451*
	Ichaboe Island, Namibia	0.0379
	Halifax Island, Namibia	0.1402
	Boulders Beach, Western Cape	0.0952
	Dassen Island, Western Cape	0.0616
	Dyer Island, Western Cape	0.0918
PNN03	Ichaboe Island, Namibia	0.1082
	Mercury Island, Namibia	**0.17**
	Stony Point, Western Cape	0.092
	Boulders Beach, Western Cape	0.1891
	Dassen Island, Western Cape	0.0187
PNN06	St Croix Island, Eastern Cape	0.0323
	Bird Island, Eastern Cape	0.0686
	Mercury Island, Namibia	**0.0447***
	Stony Point, Western Cape	0.1263*
PNN08	St Croix Island, Eastern Cape	0.046
	Possession Island, Namibia	0.3333
	Mercury Island, Namibia	**0.0054**
	Stony Point, Western Cape	0.0788
	Dyer Island, Western Cape	0.0685
PNN09	St Croix Island, Eastern Cape	0.0322
	Ichaboe Island, Namibia	0.063
	Halifax Island, Namibia	**0.1859***
	Mercury Island, Namibia	0.0965*

	Dassen Island, Western Cape	0.0323
	Dyer Island, Western Cape	0.0073
PNN12	St Croix Island, Eastern Cape	0.0465
	Halifax Island, Namibia	0.2122
	Mercury Island, Namibia	0.401
	Stony Point, Western Cape	0.1102
	Dassen Island, Western Cape	0.2073
	Dyer Island, Western Cape	0.1377
PNN05	Ichaboe Island, Namibia	monomorphic
	Possession Island, Namibia	monomorphic
	Halifax Island, Namibia	monomorphic
	Stony Point, Western Cape	monomorphic
	Jutten Island, Western Cape	monomorphic
	Dassen Island, Western Cape	**0.2***

Appendix 4.8 Heterozygosity at each of the 12 microsatellite loci employed with populations defined as (a) breeding regions and (b) breeding colonies.

(a)

Locus	Total expected heterozygosity (H_T)	Mean expected heterozygosity across populations (H_E)	Mean observed heterozygosity across populations (H_O)	Unbiased Heterozygosity (UH_E or Nei's gene diversity, *h*)
G2-2	0.707	0.700	0.729	0.706
SH1CA9	0.858	0.848	0.859	0.855
SH2CA21	0.707	0.704	0.699	0.709
B3-2	0.213	0.212	0.192	0.214
G3-6	0.700	0.698	0.683	0.704
PNN01	0.688	0.679	0.670	0.685
PNN03	0.601	0.598	0.575	0.603
PNN06	0.705	0.698	0.687	0.704
PNN08	0.710	0.707	0.713	0.713
PNN09	0.731	0.726	0.684	0.732
PNN12	0.636	0.634	0.599	0.641
PNN05	0.072	0.072	0.067	0.073

(b)

Locus	Total expected heterozygosity (H_T)	Mean expected heterozygosity across populations (H_E)	Mean observed heterozygosity across populations (H_O)	Unbiased Heterozygosity (UH_E or Nei's gene diversity, *h*)
G2-2	0.693	0.669	0.746	0.710
SH1CA9	0.862	0.811	0.872	0.859
SH2CA21	0.688	0.654	0.685	0.689
B3-2	0.187	0.181	0.168	0.187
G3-6	0.691	0.661	0.650	0.702
PNN01	0.684	0.633	0.650	0.667

PNN03	0.598	0.577	0.590	0.615
PNN06	0.699	0.649	0.725	0.690
PNN08	0.705	0.669	0.698	0.708
PNN09	0.718	0.686	0.688	0.723
PNN12	0.637	0.584	0.611	0.626
PNN05	0.072	0.069	0.068	0.071

Appendix 4.9 The total (H_T), mean expected (H_E) and mean observed (H_O) heterozygosities for each locus when populations are defines as (a) breeding regions and (b) breeding colonies. Mean hierarchical F-statistics are in bold, and are given for each locus.

(a) Regional level	G2-2	SH1CA9	SH2CA21	B3-2	G3-6	PNN01	PNN03	PNN06	PNN08	PNN09	PNN12	PNN05	Mean	SE
H_T	0.707	0.858	0.707	0.213	0.700	0.690	0.601	0.705	0.710	0.731	0.636	0.072		
Mean H_E	0.700	0.848	0.704	0.212	0.698	0.680	0.598	0.698	0.707	0.726	0.634	0.072		
Mean H_O	0.729	0.859	0.699	0.192	0.683	0.670	0.575	0.687	0.713	0.684	0.599	0.067		
F_{IS}	-0.041	-0.013	0.006	0.094	0.022	0.016	0.038	0.016	-0.008	0.058	0.055	0.073	**0.026**	**0.011**
F_{IT}	-0.031	-0.001	0.011	0.097	0.025	0.029	0.043	0.026	-0.005	0.065	0.058	0.077	**0.033**	**0.011**
F_{ST}	0.010	0.012	0.005	0.004	0.003	0.014	0.006	0.010	0.003	0.007	0.004	0.005	**0.007**	**0.001**
(b) Colony level														
H_T	0.693	0.862	0.688	0.187	0.691	0.685	0.598	0.699	0.705	0.718	0.637	0.072		
Mean H_E	0.669	0.811	0.654	0.181	0.661	0.634	0.577	0.649	0.669	0.686	0.584	0.069		
Mean H_O	0.746	0.872	0.685	0.168	0.650	0.650	0.590	0.725	0.698	0.688	0.611	0.068		
F_{IS}	-0.114	-0.075	-0.046	0.070	0.016	-0.025	-0.022	-0.117	-0.042	-0.003	-0.046	0.014	**-0.033**	**0.016**
F_{IT}	-0.075	-0.011	0.004	0.103	0.060	0.051	0.013	-0.037	0.011	0.042	0.041	0.055	**0.021**	**0.014**
F_{ST}	0.035	0.060	0.048	0.035	0.044	0.075	0.034	0.072	0.051	0.045	0.083	0.041	**0.052**	**0.005**

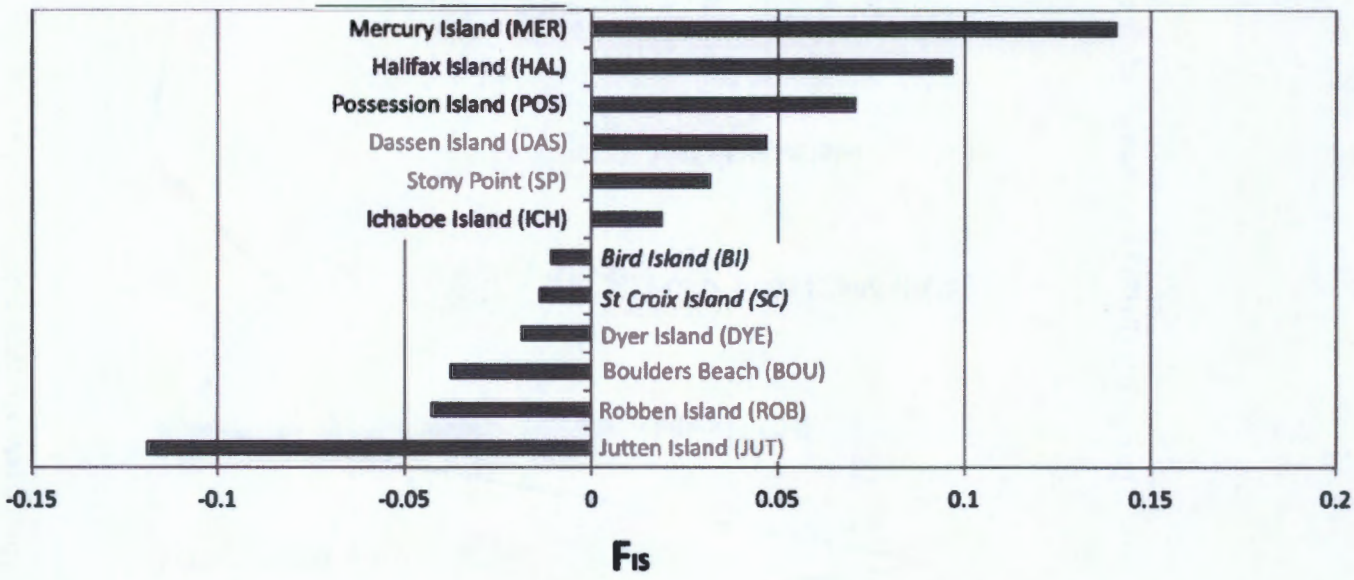
Appendix 4.10 Allelic richness overall (across the breeding range of African Penguins), and allelic richness per locus and breeding region calculated in FSTAT (based on a minimum sample size of 30 diploid individuals and corrected for sample size).

Locus	Eastern Cape	Namibia	Western Cape	OVERALL
G2-2	5.636	5.553	5.089	5.447
SH1CA9	12.317	12.582	13.854	13.303
SH2CA2	8.285	8.151	9.148	8.594
B3-2	2.974	2.966	4.554	3.755
G3-6	5.885	6.381	6.377	6.182
PNN01	3.99	3.999	3.915	3.968
PNN03	7.048	7.26	6.976	7.114
PNN06	5.052	3.991	3.971	4.309
PNN08	4.991	5.818	5.93	5.729
PNN09	5.674	5.587	5.766	5.73
PNN12	4.954	5	4.849	4.816
PNN05	1.976	1.823	1.98	1.929

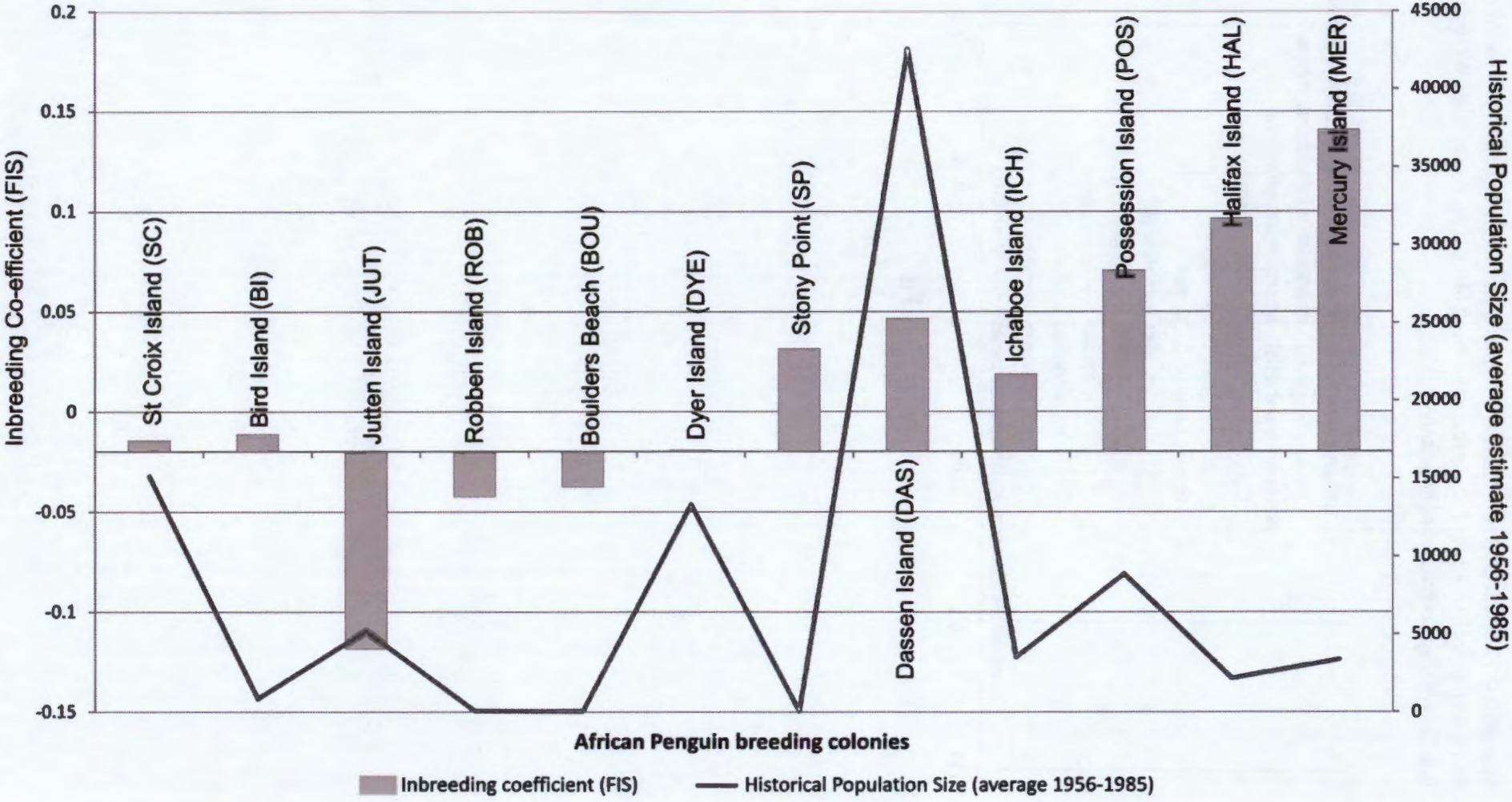
Appendix 4.11 Colony-level F_{IS} estimates based on 10 000 bootstrap replicates executed in GENETIX.

Population	F_{IS}	R_{IS}
EASTERN CAPE	-0.024	**0.021**
St Croix Island	-0.014	0.054
Bird Island	-0.011	-0.026
NAMIBIA	0.056	**0.107**
Ichaboe Island	0.019	-0.006
Possession Island	0.071	0.124
Halifax Island	0.097	0.107
Mercury Island	0.141	0.199
WESTERN CAPE	0.032	**-0.014**
Stony Point	0.032	-0.046
Jutten Island	-0.119	-0.063
Boulders Beach	-0.038	-0.202
Robben Island	-0.043	-0.099
Dassen Island	0.048	-0.002
Dyer Island	-0.019	0.048

Appendix 4.12 Inbreeding co-efficients calculated for each breeding colony based on 12 microsatellite loci. Colonies in Namibia are in bold black type, those in the Western Cape are in grey and Eastern Cape colonies are italicised.



Appendix 4.13 The inbreeding coefficient (F_{IS}, left-hand vertical axis), and historical population size (secondary (RHS) axis, average estimate from 1956 – 1985), calculated for each of the African Penguin breeding colonies.



Appendix 3.34 (a) Population genetic diversity indices, including haplotype diversity h ± SD, based on the ATPase 6 sequence data (682bp) for the whole dataset and subdivided into Cape Cormorant breeding regions (NAM=Namibia, WC=Western Cape).

	NAM	WC	TOTAL
Haplotype diversity (h)	0.248 ± 0.131	0.226 ± 0.088	0.228 ± 0.073
Nucleotide diversity (π)	0.00036	0.00033	0.00033
N	15	32	47
Number of haplotypes	2	2	2

Appendix 3.34 (b) Population genetic diversity indices, including haplotype diversity (h ± SD), based on the ATPase 6 sequence data (682bp) for the whole dataset and subdivided into Cape Cormorant breeding colonies (NAM=Namibia, WC=Western Cape).

	Ichaboe Island (NAM)	Bird Rock (NAM)	Jutten Island (WC)	Robben Island (WC)	Malgas Island (WC)	Dyer Island (WC)	TOTAL
Haplotype diversity (h)	0.356 ± 0.159	0	0.286 ± 0.196	0	0.356 ± 0.159	0.2 ± 0.154	0.228 ± 0.073
Nucleotide diversity (π)	0.00052	0	0.00042	0	0.00052	0.00029	0.00033
N	10	5	7	5	10	10	47
Number of haplotypes	2	1	2	1	2	2	2

Appendix 3.35 Pairwise comparisons of genetic structure based on four commonly reported metrics calculated at the regional scale ((a) and (b)) and colony level ((c) and (d)) based on the ATPase 6 dataset for Cape Cormorants (682bp).

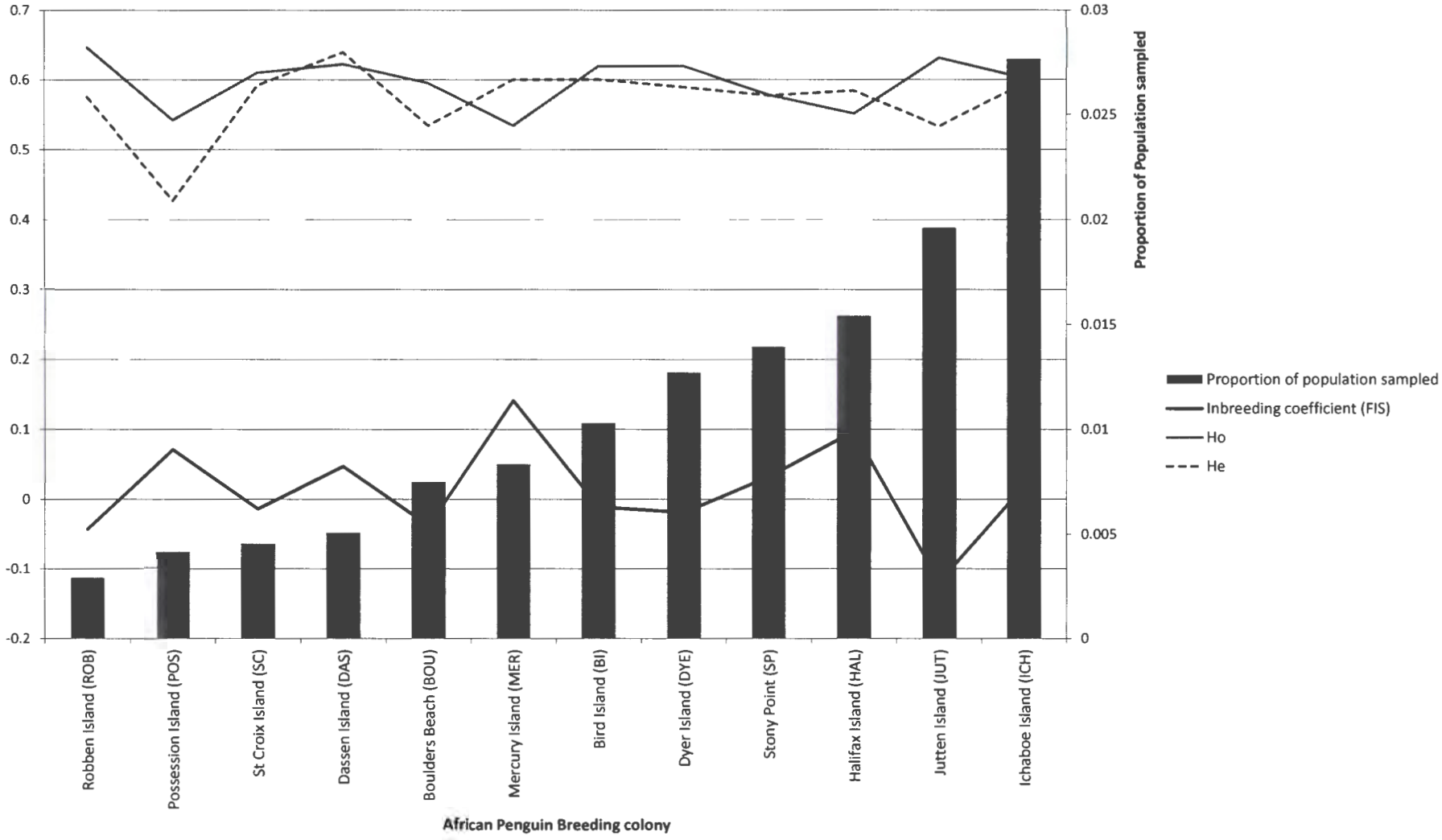
(a)		φ_{ST}	
		NAM	**WC**
G_{ST}	**Namibia (NAM)**		
	Western Cape (WC)		
	Eastern Cape (EC)		

(b)		*kxy*	
		NAM	**WC**
Dxy	**Namibia (NAM)**		
	Western Cape (WC)		
	Eastern Cape (EC)		

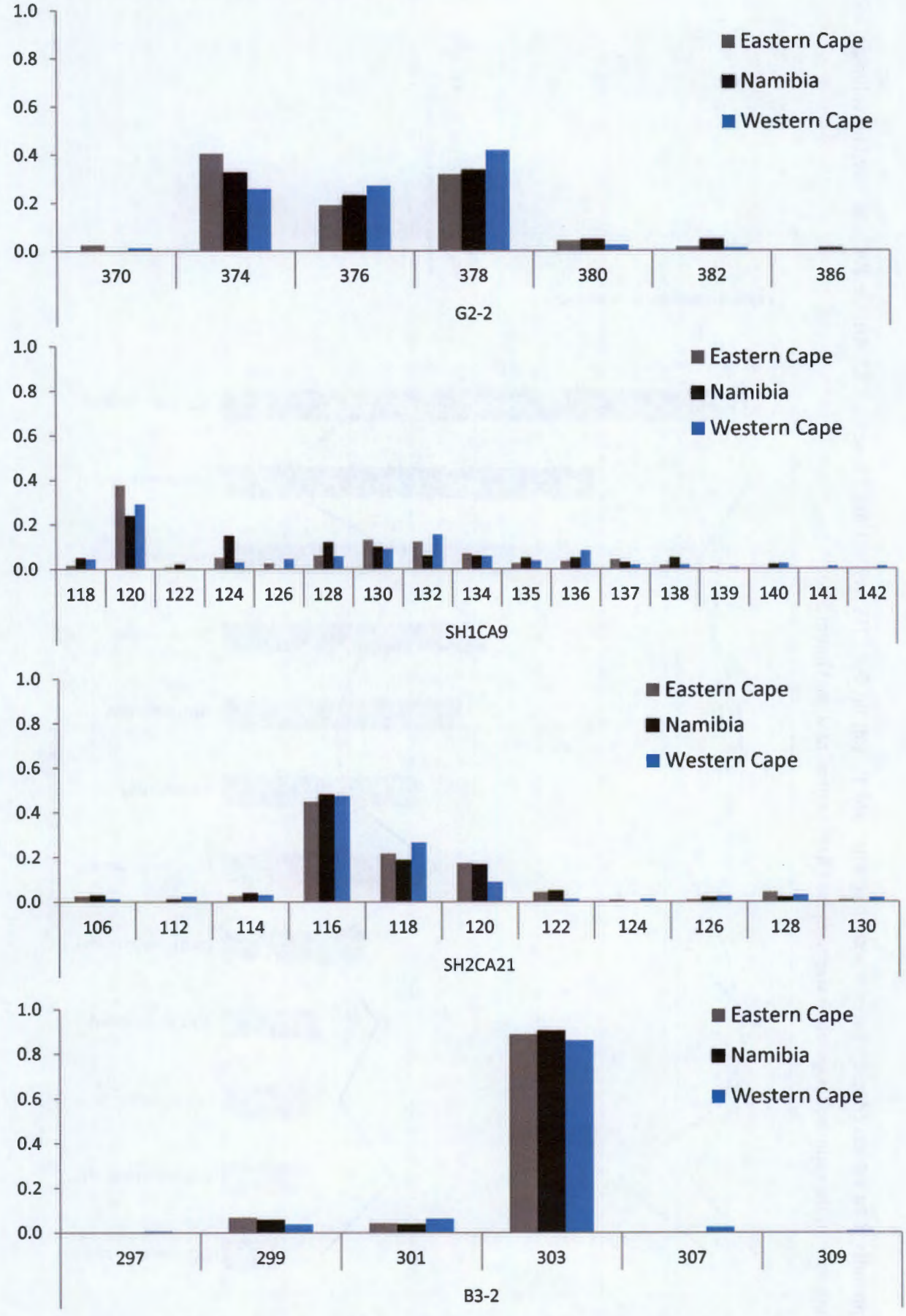
(c)		φ_{ST}					
		Ichaboe Island (NAM)	**Bird Rock (NAM)**	**Malgas Island (WC)**	**Robben Island (WC)**	**Jutten Island (WC)**	**Dyer Island (WC)**
G_{ST}	**Ichaboe Island (NAM)**		0.1111	-0.1111	0.1111	-0.1222	-0.0684
	Bird Rock (NAM)	0.0476		0.1111	0	0	0
	Malgas Island (WC)	-0.0526	0.0476		0.1111	-0.1222	-0.0684
	Robben Island (WC)	0.0476	1	0.0476		0	0
	Jutten Island (WC)	-0.0525	0.0024	-0.0525	0.0024		-0.1333
	Dyer Island (WC)	-0.0331	0.0083	-0.0331	0.0083	-0.0604	

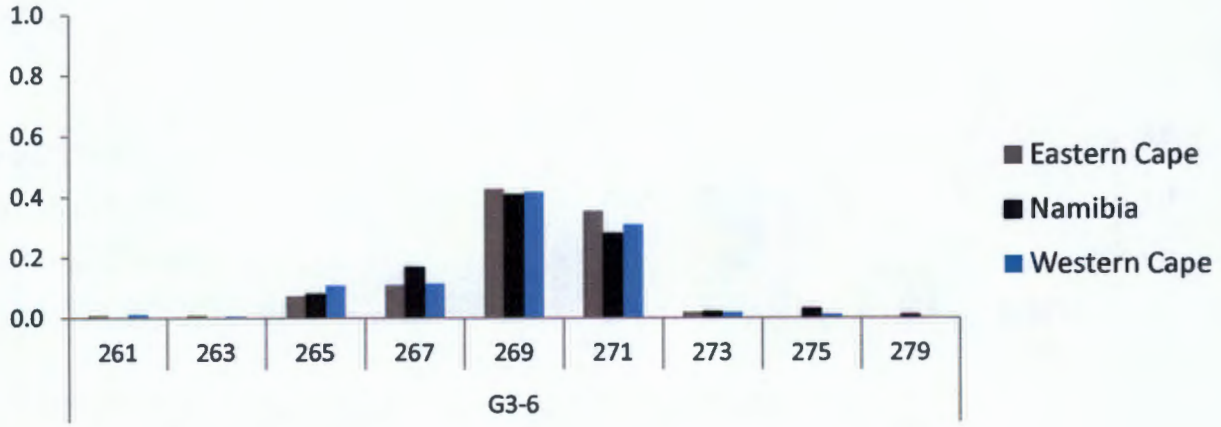
(d)		Average number of Nucleotide differences between populations (Kxy)					
		Ichaboe Island (NAM)	**Bird Rock (NAM)**	**Malgas Island (WC)**	**Robben Island (WC)**	**Jutten Island (WC)**	**Dyer Island (WC)**
Dxy	**Ichaboe Island (NAM)**		0.2	0.32	0.2	0.2857	0.26
	Bird Rock (NAM)	0.0003		0.2	0	0.1429	0.1
	Malgas Island (WC)	0.0005	0.0003		0.2	0.2857	0.26
	Robben Island (WC)	0.0003	0	0.0003		0.1429	0.1
	Jutten Island (WC)	0.0004	0.0002	0.0004	0.0002		0.2143
	Dyer Island (WC)	0.0004	0.0001	0.0004	0.0001	0.0003	

Appendix 4.14 Observed and expected heterozygosity, and F_{IS} (all on the LHS vertical axis) based on 12 African Penguin breeding colonies; and the proportion of the estimated population size sampled (RHS vertical axis, maximum 3%).



Appendix 4.14 Regional allele frequencies at each locus

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279
G3-6
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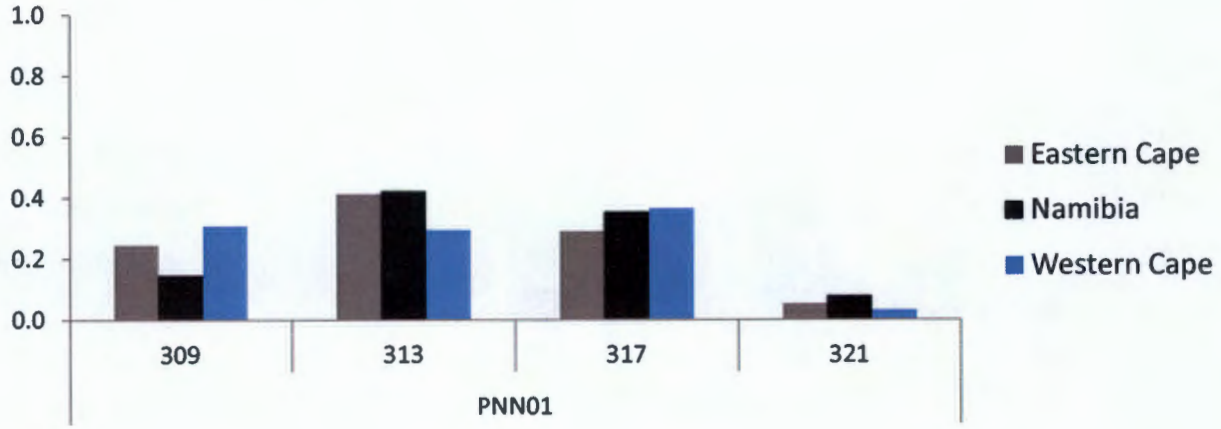
Namibia
Western Cape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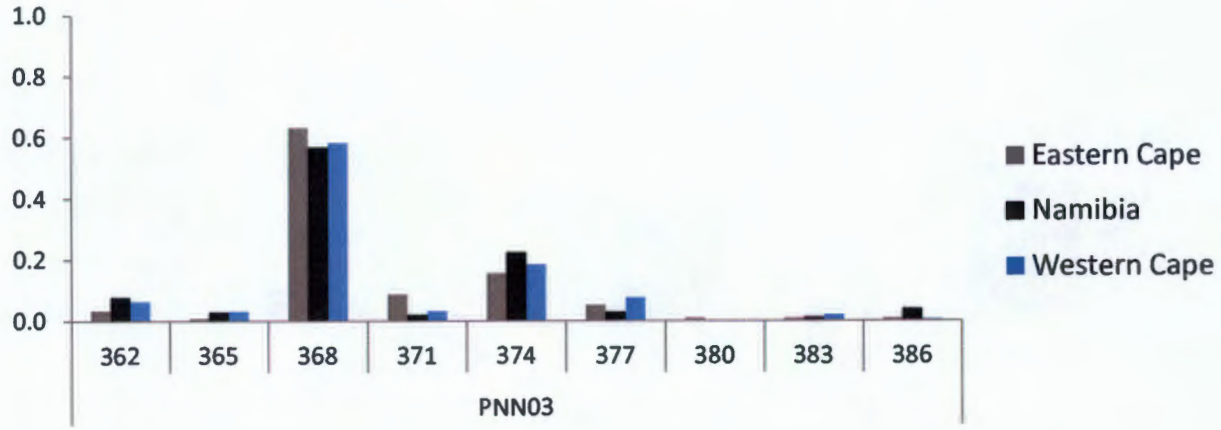
Western Cape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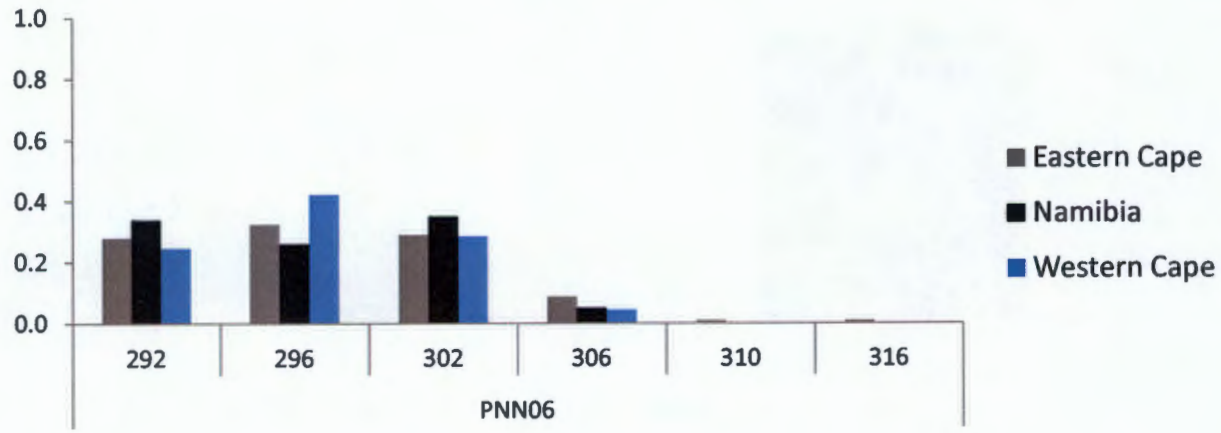
Namibia
Western Cape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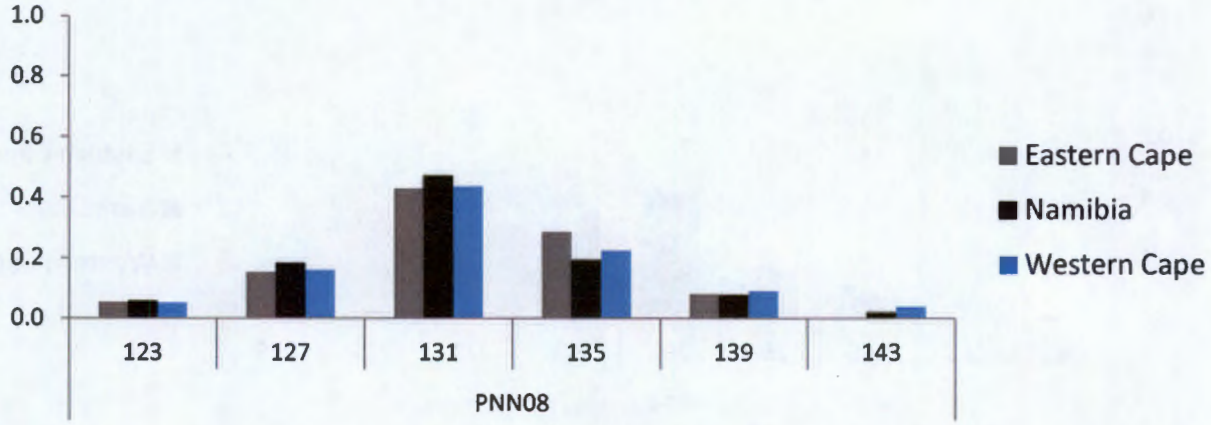
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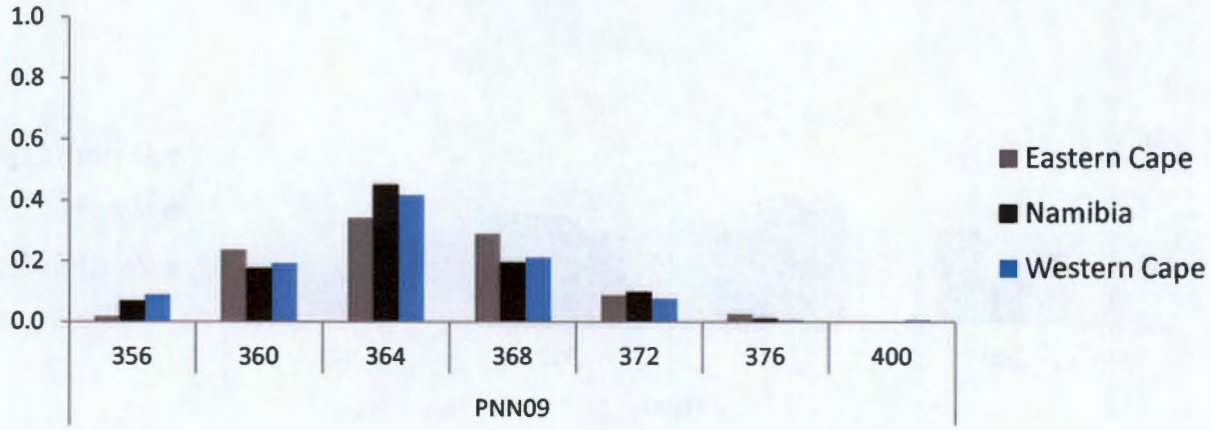
Western Cape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400
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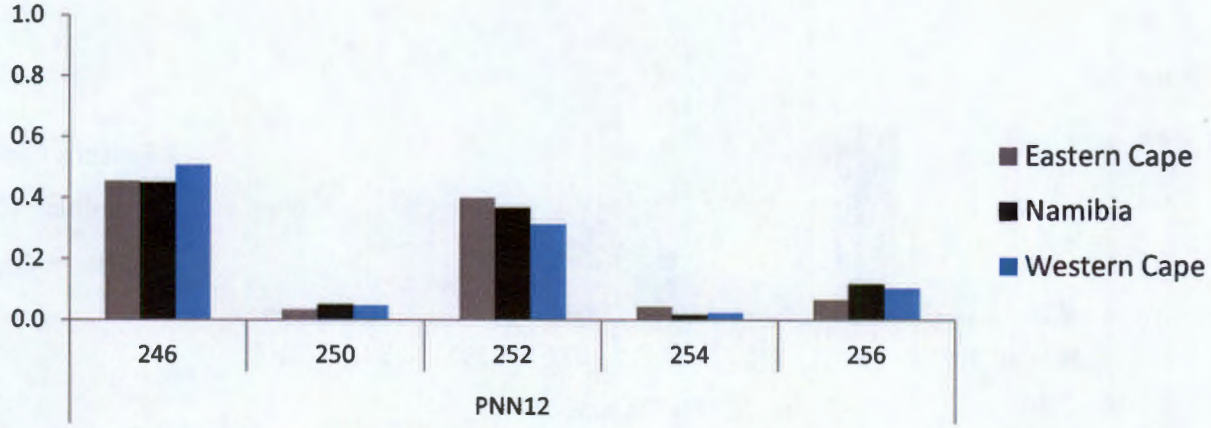
Eastern Cape
Namibia
Western Cape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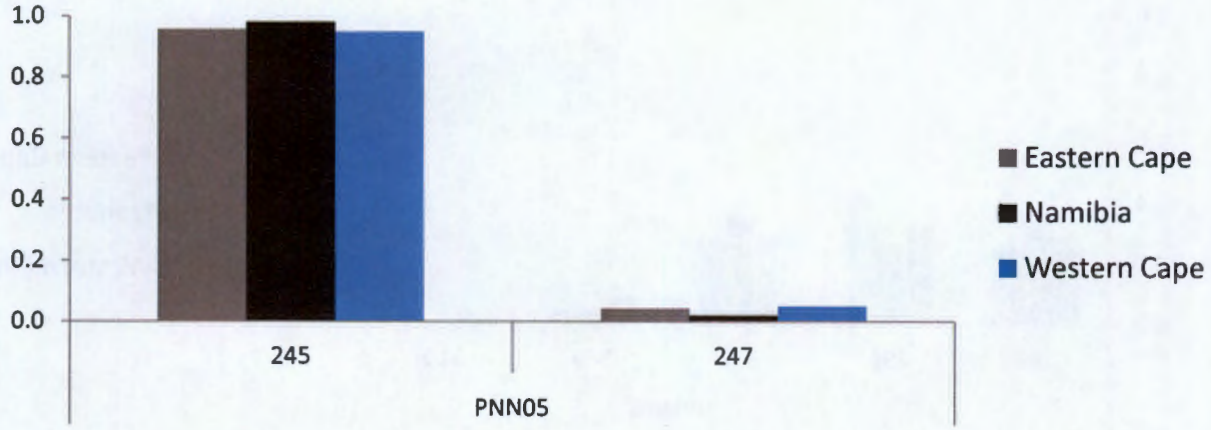
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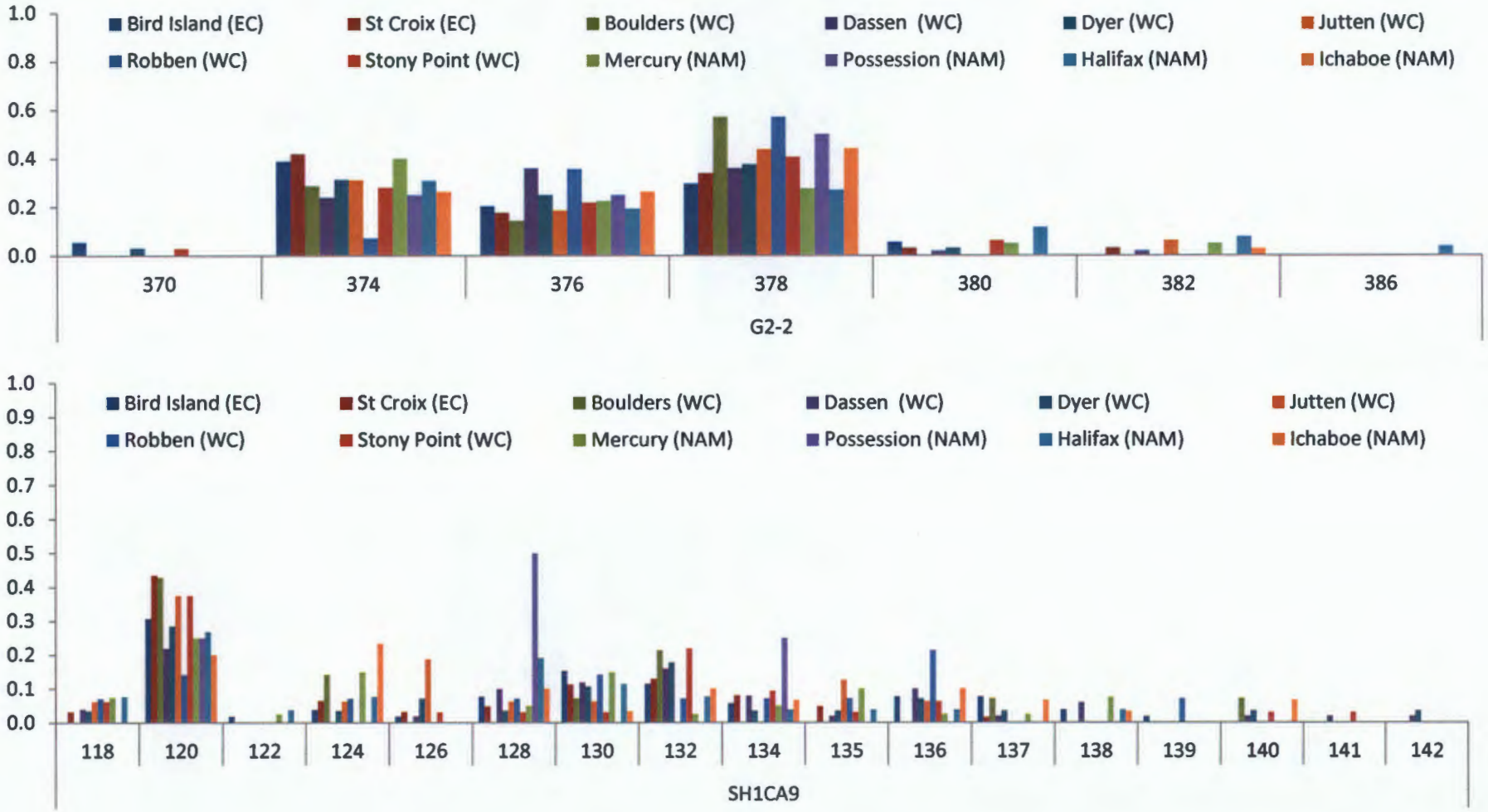
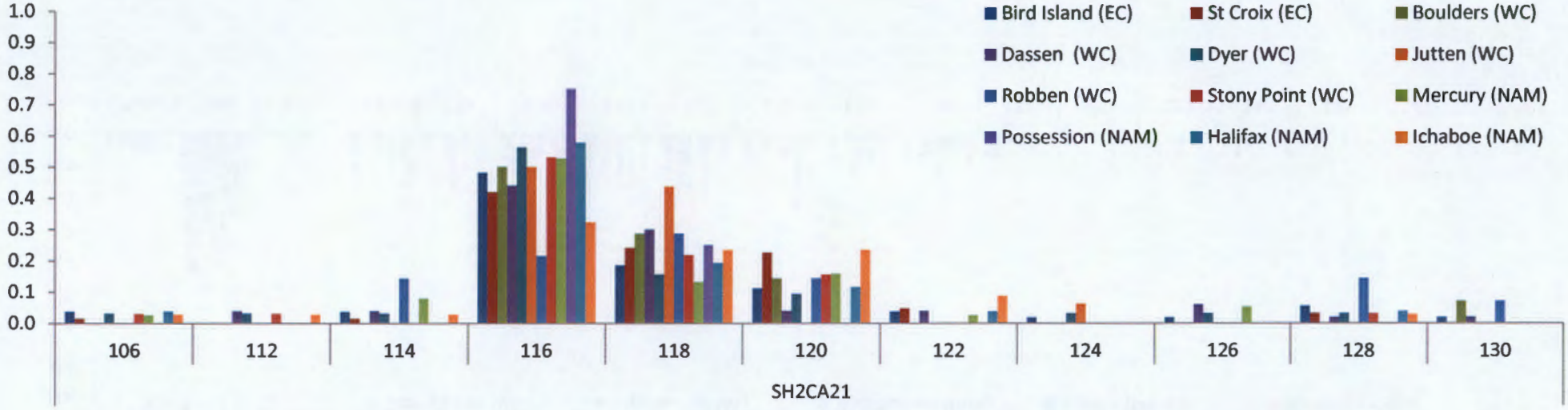
Namibia
Western Cape

Appendix 4.15 Colony-level allele frequencies at each locus

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St Croix (EC)
Boulders (WC)
Dassen (WC)
Dyer (WC)
Jutten (WC)
Robben (WC)
Stony Point (WC)
Mercury (NAM)
Possession (NAM)
Halifax (NAM)
Ichaboe (NAM)
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126
128
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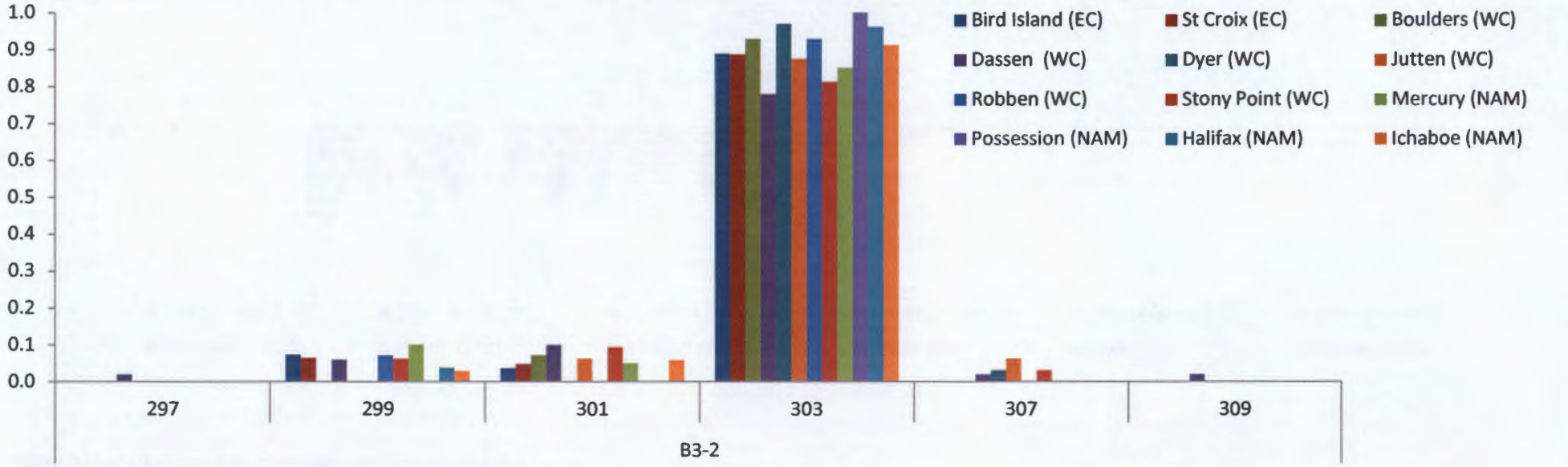
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St Croix (EC)
Boulders (WC)
Dassen (WC)
Dyer (WC)
Jutten (WC)
Robben (WC)
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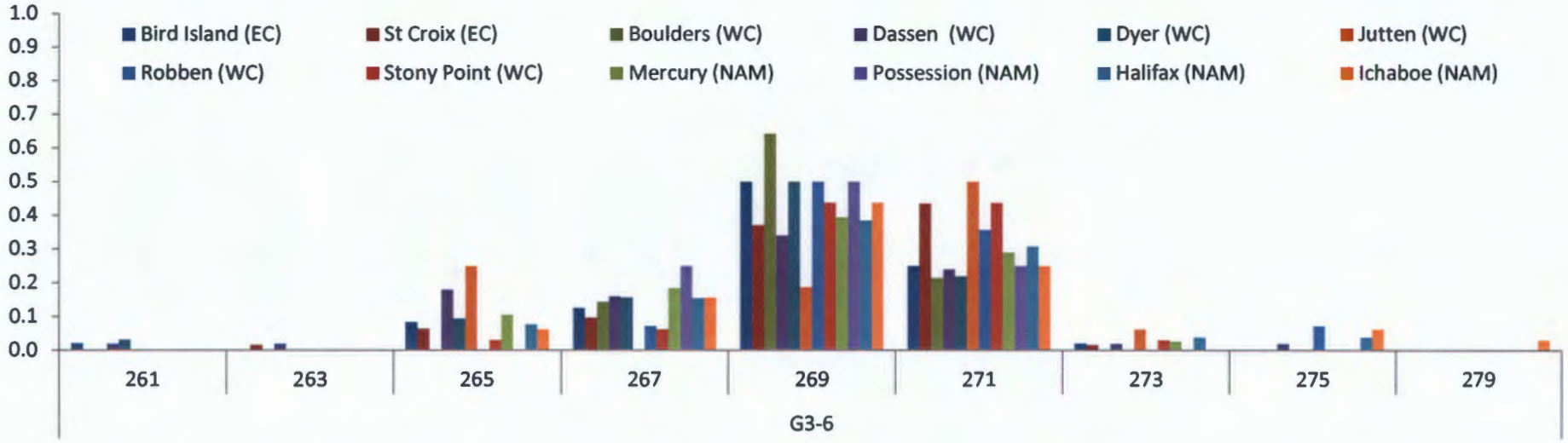
B3-2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St Croix (EC)
Boulders (WC)
Dassen (WC)
Dyer (WC)
Jutten (WC)
Robben (WC)
Stony Point (WC)
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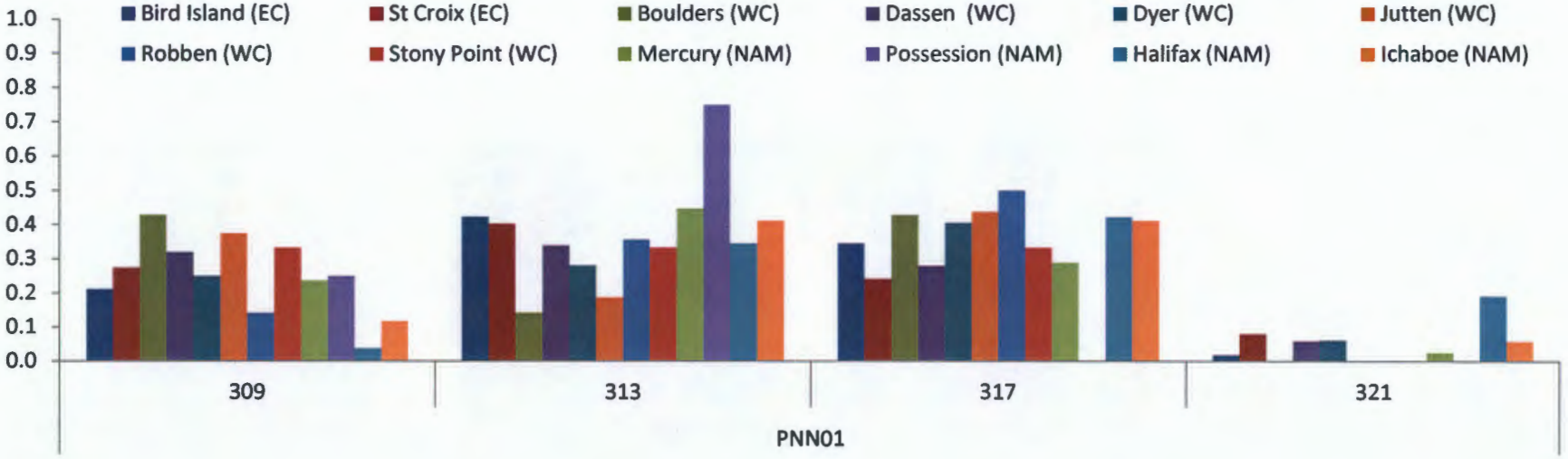
G3-6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St Croix (EC)
Boulders (WC)
Dassen (WC)
Dyer (WC)
Jutten (WC)
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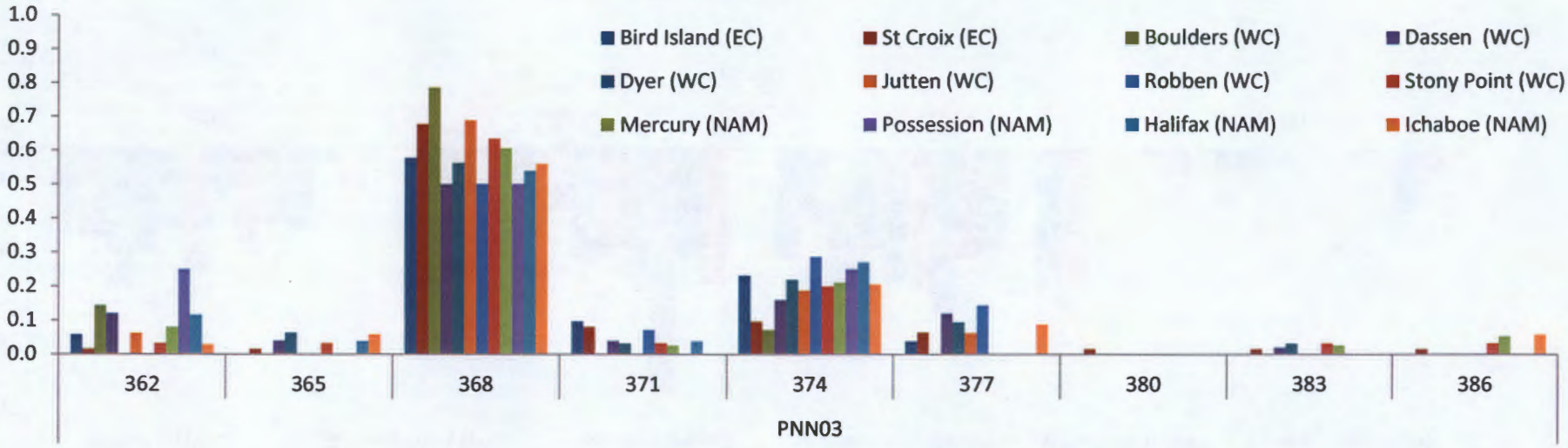
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Robben (WC)
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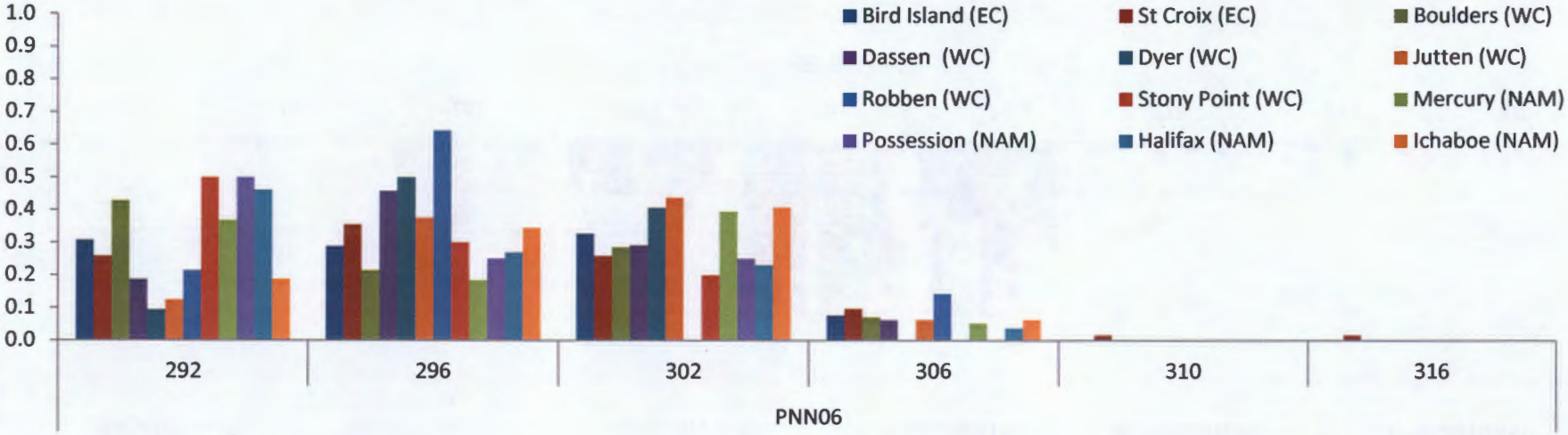
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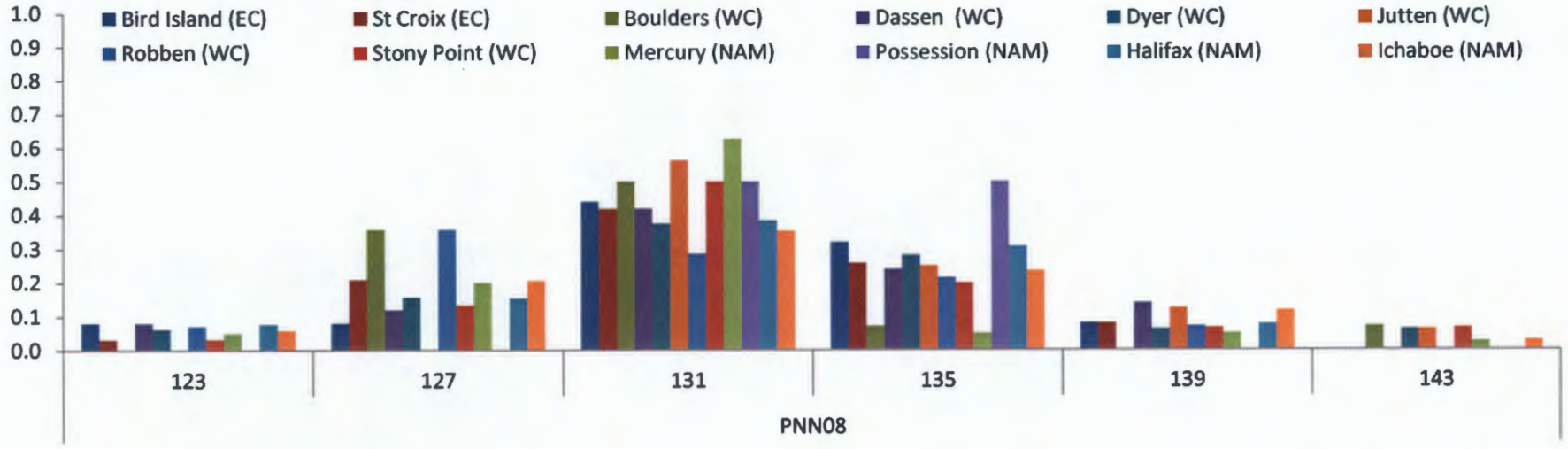
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Possession (NAM)
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Ichaboe (NAM)
1.0
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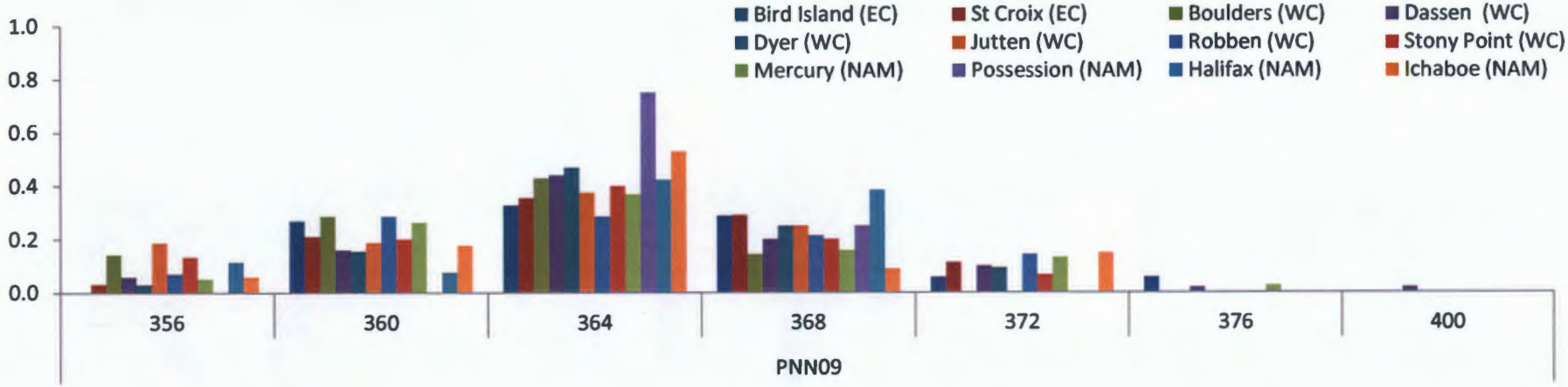
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Possession (NAM)
Halifax (NAM)
Ichaboe (NAM)
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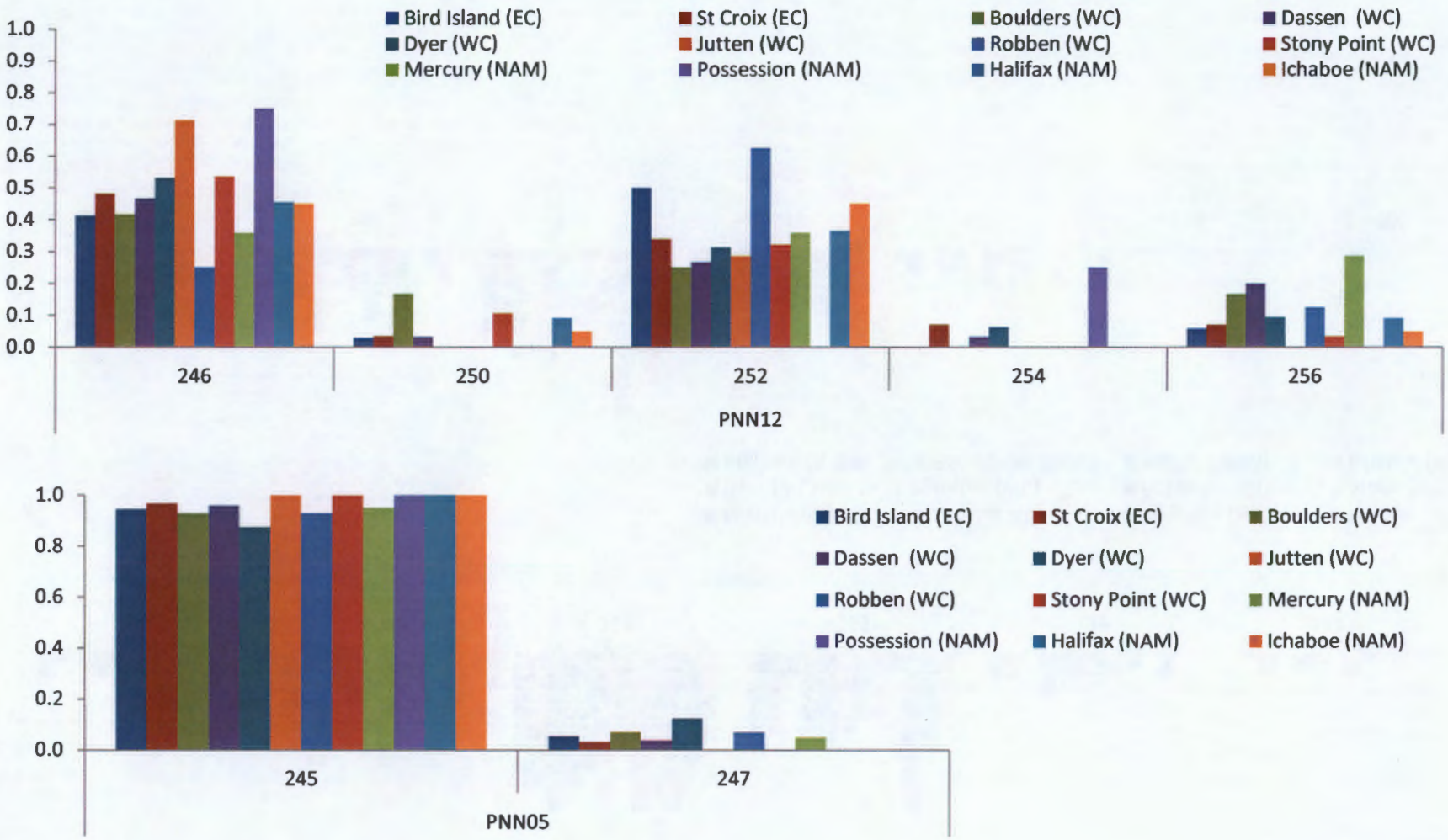
PNN09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Dyer (WC)
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Robben (WC)
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Mercury (NAM)
Possession (NAM)
Halifax (NAM)
Ichaboe (NAM)
1.0
0.9
0.8
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0.6
0.5
0.4
0.3
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246
250
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254
256
PNN12
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0.6
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0.2
0.0
245
247
PNN05

Appendix 4.16 Regional Pairwise Population D_{EST} values (below the diagonal) and probabilities (above the diagonal, based on 9 999 permutations.

	EC	NAM	WC
Eastern Cape (EC)	-	0.365	0.073
Namibia (NAM)	0.001	-	0.072
Western Cape (WC)	0.005	0.005	-

Appendix 4.17 Regional-scale measures of population differentiation per locus, and overall, (GENALEX, G-statistics option), including G_{ST}, Nei's standardized G_{ST} (G'_{STN}), Hedrick's standardized G_{ST} (G'_{STH}), Hedrick's further standardized G_{ST} (G''_{ST}, for a small number of populations) and Jost's estimate of differentiation (D_{EST}). Probabilities are ascertained by 9999 permutations of 999 pairwise population permutations and 1000 bootstrap replicates were carried out to calculate standard errors.

Statistic	Loci												Total	Probability (Total)
	G2-2	SH1CA9	SH2CA21	B3-2	G3-6	PNN01	PNN03	PNN06	PNN08	PNN09	PNN12	PNN05		
F_{IS}	-0.041	-0.013	0.006	0.094	0.022	0.014	0.038	0.016	-0.008	0.058	0.055	0.073	**0.016**	**0.999**
F_{ST}	0.010	0.012	0.005	0.004	0.003	0.013	0.006	0.010	0.003	0.007	0.004	0.005	**0.007**	**0.218**
G_{IS}	-0.033	-0.005	0.015	0.102	0.030	0.022	0.046	0.024	0.000	0.067	0.067	0.081	**0.025**	**0.999**
G_{ST}	0.005	0.006	-0.001	-0.002	-0.003	0.008	0.000	0.004	-0.002	0.002	-0.005	-0.001	**0.001**	**0.229**
P (G_{ST})	0.127	0.037	0.492	0.521	0.675	0.060	0.451	0.137	0.650	0.320	0.701	0.530		
G'_{STN}	0.007	0.009	-0.001	-0.004	-0.004	0.011	0.000	0.007	-0.003	0.002	-0.007	-0.001	**0.002**	**0.229**
G'_{STH}	0.021	0.062	-0.004	-0.003	-0.012	0.033	0.000	0.020	-0.011	0.008	-0.017	-0.001	**0.005**	**0.229**
G''_{ST}	0.024	0.065	-0.004	-0.005	-0.014	0.036	0.000	0.022	-0.012	0.009	-0.019	-0.002	**0.006**	**0.229**
D_{EST}	0.017	0.056	-0.003	-0.001	-0.010	0.025	0.000	0.016	-0.009	0.006	-0.012	0.000	**0.003**	**0.229**
P (D_{EST})	0.125	0.037	0.492	0.520	0.675	0.061	0.451	0.135	0.648	0.320	0.703	0.530		

Appendix 4.18. Regional-scale effective number of alleles (Ne), corrected Ne, measures of heterozygosity (Ho, Hs and Ht), corrected total expected heterozygosity (cHt), corrected sub-population heterozygosity (cH_S) and the maximum value for G_{ST} (G_{STmax}) for each locus and overall calculated in GENALEX Version 6.5 (G-statistics option). Corrected values are used to estimate standardized fixation indices and Jost's estimate of differentiation (D_{EST}). Probabilities are ascertained by 9999 permutations of 9999 pairwise population permutations and 10000 bootstrap replicates were carried out to calculate standard errors.

Locus	N	N_a	N_{eA}	cN_e	H_O	H_S	H_T	cH_S	cH_T	G_{STmax}
G2-2	189	6.000	3.345	3.335	0.729	0.700	0.707	0.706	0.709	0.218
SH1CA9	184	14.333	6.816	6.590	0.859	0.848	0.858	0.855	0.861	0.101
SH2CA21	188	10.000	3.378	3.373	0.699	0.704	0.707	0.710	0.709	0.214
B3-2	189	4.000	1.271	1.269	0.192	0.212	0.213	0.214	0.213	0.710
G3-6	184	7.333	3.326	3.315	0.683	0.698	0.700	0.704	0.703	0.219
PNN01	186	4.000	3.116	3.115	0.670	0.679	0.688	0.685	0.690	0.235
PNN03	186	8.333	2.493	2.485	0.575	0.598	0.601	0.603	0.603	0.305
PNN06	184	4.667	3.323	3.309	0.687	0.698	0.705	0.704	0.707	0.219
PNN08	186	5.667	3.422	3.418	0.713	0.707	0.710	0.713	0.712	0.211
PNN09	186	6.333	3.652	3.648	0.684	0.726	0.731	0.732	0.734	0.196
PNN12	137	5.000	2.731	2.729	0.599	0.634	0.636	0.642	0.639	0.271
PNN05	189	2.000	1.078	1.078	0.067	0.072	0.072	0.073	0.073	0.895
Total	182.333	6.472	3.163	3.139	0.596	0.606	0.611	0.612	0.613	0.297
SE		0.941	0.411	0.396	0.067	0.065	0.066	0.066	0.066	0.057

Appendix 4.19 Regional Pairwise Population G''_{ST} values (below the diagonal) and probabilities (above the diagonal, based on 9 999 permutations).

G''_{ST}	EC	NAM	WC
Eastern Cape (EC)	-	0.365	0.072
Namibia (NAM)	0.002	-	0.072
Western Cape (WC)	0.007	0.009	-

Appendix 4.20. Colony-level measures of population differentiation for each locus, and for the total dataset. Estimates include G_{ST}, Nei's standardized G_{ST} (G'_{STN}), Hedrick's standardized G_{ST} (G'_{STH}), Hedrick's further standardized G_{ST} (G''_{ST}, for a small number of populations) and Jost's estimate of differentiation (D_{EST}). Values are based on 10 loci and 11 colonies (Possession Island and 3 individuals with missing data are excluded; 184 individuals). Probabilities are ascertained by 9999 permutations of 999 pairwise population permutations and 1000 bootstrap replicates were carried out to calculate standard errors.

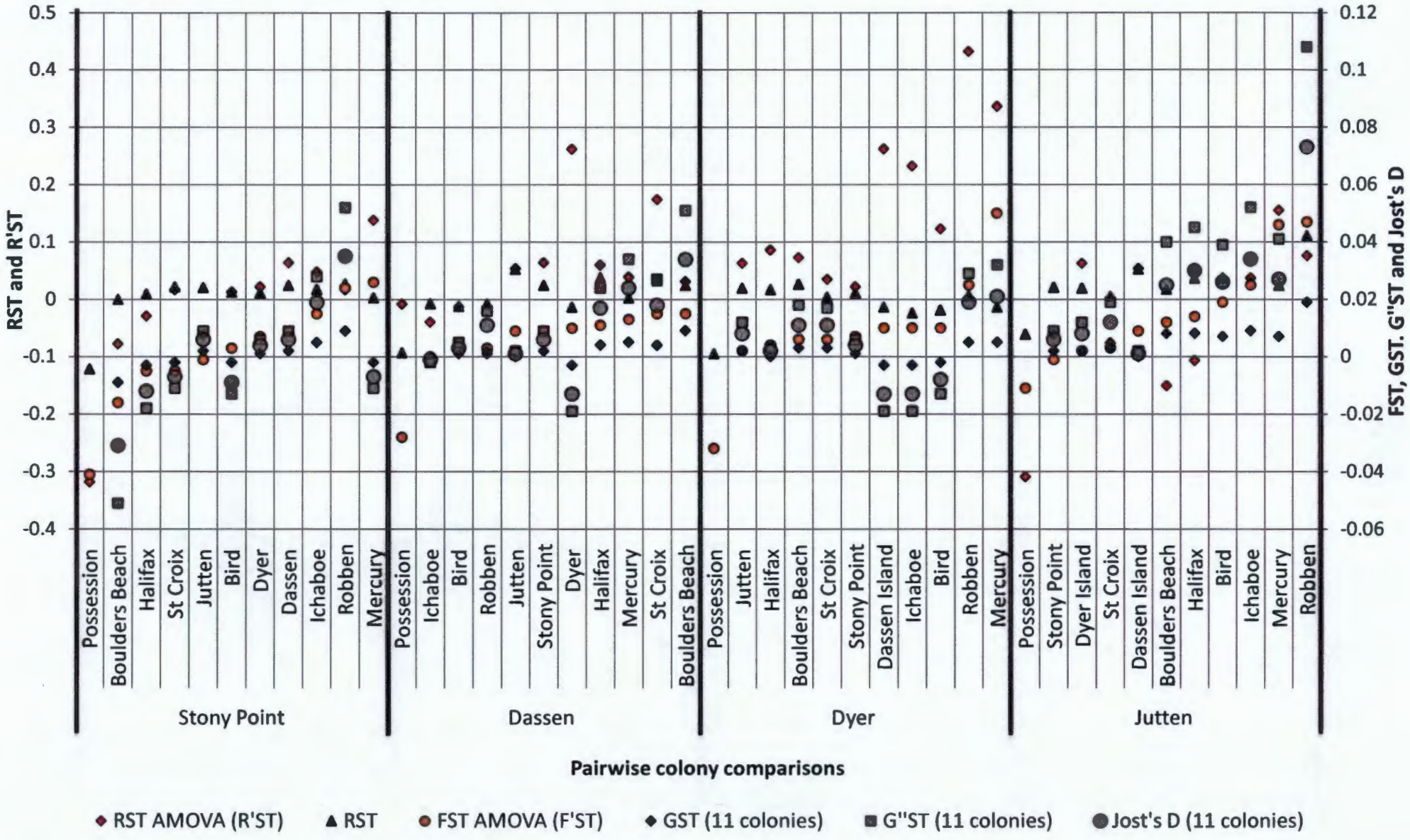
Statistic	Loci										Total	Probability (Total)
	G2-2	SH1CA9	SH2CA21	B3-2	G3-6	PNN01	PNN03	PNN06	PNN08	PNN09		
F_{IS}	-0.075	-0.039	-0.033	0.077	-0.005	-0.011	0.035	-0.075	-0.112	0.013	**-0.031**	**0.981**
F_{ST}	0.037	0.039	0.040	0.028	0.043	0.043	0.030	0.071	0.040	0.028	**0.041**	**0.199**
P (F_{ST})	0.382	0.334	0.363	0.572	0.314	0.277	0.588	0.010	0.341	0.761	**0.199**	
G_{IS}	-0.036	0.002	0.006	0.116	0.035	0.028	0.074	-0.035	-0.073	0.053	**0.009**	**0.981**
G_{ST}	0.003	0.004	0.005	-0.012	0.007	0.008	-0.008	0.038	0.008	-0.009	**0.006**	**0.182**
P (G_{ST})	0.387	0.331	0.364	0.566	0.321	0.277	0.600	0.007	0.292	0.764	**0.182**	
G'_{STN}	0.004	0.004	0.006	-0.013	0.007	0.008	-0.008	0.042	0.008	-0.010	**0.006**	**0.182**
G'_{STH}	0.011	0.029	0.018	-0.015	0.023	0.025	-0.020	0.126	0.028	-0.039	**0.018**	**0.185**
G''_{ST}	0.012	0.030	0.019	-0.016	0.024	0.026	-0.021	0.129	0.029	-0.040	**0.018**	**0.185**
D_{EST}	0.008	0.026	0.013	-0.003	0.017	0.018	-0.012	0.091	0.021	-0.029	**0.012**	**0.186**
P (D_{EST})	0.389	0.326	0.365	0.559	0.326	0.280	0.594	0.008	0.292	0.764	**0.186**	

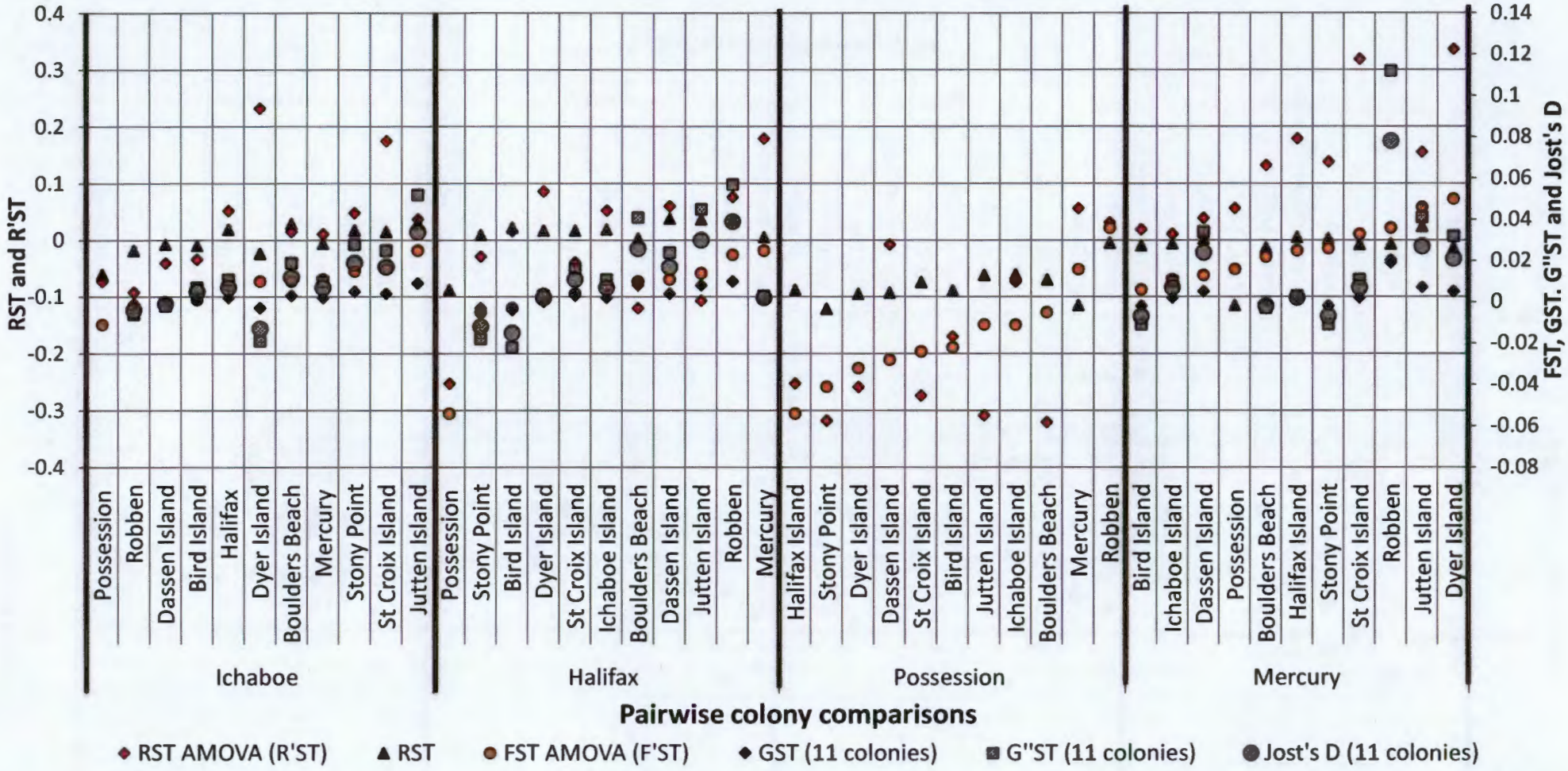
Appendix 4.21 Colony-scale effective number of alleles (N_{Ae}), corrected Ne (cN_a; by rarefaction), measures of heterozygosity (H_O, H_S and Ht), corrected total expected heterozygosity (cH_T), corrected sub-population heterozygosity (cH_S) and the maximum value for G_{ST} (G_{STmax}) for each locus and overall calculated in GENALEX Version 6.5 (G-statistics option). Corrected values are used to estimate standardized fixation indices and Jost's estimate of differentiation (D_{EST}). Probabilities are ascertained by 9999 permutations of 9999 pairwise population permutations and 10000 bootstrap replicates were carried out to calculate standard errors.

Locus	N	N_a	N_{Ae}	cN_a	H_O	H_S	H_T	cH_S	cH_T	G_{STmax}
G2-2	184	4.545	3.166	3.060	0.724	0.673	0.699	0.699	0.701	0.281
SH1CA9	179	10.636	6.180	5.766	0.858	0.827	0.860	0.860	0.863	0.129
SH2CA21	184	6.818	3.290	3.113	0.701	0.679	0.707	0.706	0.709	0.275
B3-2	184	3.000	1.261	1.244	0.181	0.196	0.202	0.205	0.202	0.779
G3-6	181	5.273	3.086	2.979	0.667	0.664	0.694	0.692	0.696	0.288
PNN01	184	3.636	2.935	2.912	0.664	0.657	0.686	0.683	0.689	0.296
PNN03	184	5.636	2.429	2.341	0.553	0.573	0.590	0.597	0.593	0.380
PNN06	182	3.909	2.938	2.868	0.700	0.651	0.701	0.676	0.703	0.303
PNN08	183	5.182	3.297	3.191	0.764	0.687	0.715	0.712	0.717	0.269
PNN09	184	5.000	3.549	3.498	0.705	0.714	0.734	0.744	0.737	0.238
Total	**182.900**	**5.364**	**3.213**	**3.097**	**0.652**	**0.632**	**0.659**	**0.657**	**0.661**	**0.321**
Standard Error		**0.679**	**0.388**	**0.356**	**0.058**	**0.052**	**0.055**	**0.054**	**0.055**	**0.053**

Appendix 4.22 Plot of pairwise population comparisons of various colony-level fixation indices, standardized fixation indices and a pure differentiation index (Jost's D). Matrices of the data and the associated probabilities that are not discussed in the text are given in the Appendix (Appendice 4.23 to 4.xxx).

RST and R'ST
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4
0.5
St Croix
Possession
Stony Point
Boulders Beach
Jutten Island
Halifax
Bird
Dyer Island
Dassen Island
Ichaboe
Robben
Mercury
Bird
Possession
Halifax
Ichaboe
Dassen Island
Stony Point
Mercury
St Croix Island
Dyer Island
Boulders Beach
Robben
Jutten Island
Robben
Ichaboe
Dassen
Bird
Halifax
Stony Point
Boulders Beach
Dyer
St Croix
Mercury
Possession
Jutten
Boulders Beach
Stony Point
Possession
St Croix
Dyer
Ichaboe
Bird
Halifax
Jutten
Dassen
Mercury
Robben
Pairwise colony comparisons
-0.11
-0.06
-0.01
0.04
0.09
0.14
FST, GST. G''ST and Jost's D
RST AMOVA (R'ST)
RST
FST AMOVA (F'ST)
GST (11 colonies)
G''ST (11 colonies)
Jost's D (11 colonies)


RST and R'ST
FST, GST. G''ST and Jost's D
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
Possession
Boulders Beach
Halifax
St Croix
Jutten
Bird
Dyer
Dassen
Ichaboe
Robben
Mercury
Stony Point
Possession
Ichaboe
Bird
Robben
Jutten
Stony Point
Dyer
Halifax
Mercury
St Croix
Boulders Beach
Dassen
Possession
Jutten
Halifax
Boulders Beach
St Croix
Stony Point
Dassen Island
Ichaboe
Bird
Robben
Mercury
Dyer
Possession
Stony Point
Dyer Island
St Croix
Dassen Island
Boulders Beach
Halifax
Bird
Ichaboe
Mercury
Robben
Jutten
Pairwise colony comparisons
RST AMOVA (R'ST)
RST
FST AMOVA (F'ST)
GST (11 colonies)
G''ST (11 colonies)
Jost's D (11 colonies)


RST and R'ST
FST, GST. G''ST and Jost's D
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
Possession
Robben
Dassen Island
Bird Island
Halifax
Dyer Island
Boulders Beach
Mercury
Stony Point
St Croix Island
Jutten Island
Ichaboe
Possession
Stony Point
Bird Island
Dyer Island
St Croix Island
Ichaboe Island
Boulders Beach
Dassen Island
Jutten Island
Robben
Mercury
Halifax
Halifax Island
Stony Point
Dyer Island
Dassen Island
St Croix Island
Bird Island
Jutten Island
Ichaboe Island
Boulders Beach
Mercury
Robben
Possession
Bird Island
Ichaboe Island
Dassen Island
Possession
Boulders Beach
Halifax Island
Stony Point
St Croix Island
Robben
Jutten Island
Dyer Island
Mercury
Pairwise colony comparisons
RST AMOVA (R'ST)
RST
FST AMOVA (F'ST)
GST (11 colonies)
G''ST (11 colonies)
Jost's D (11 colonies)

Appendix 4.23 Colony-level pairwise population matrix of G_{ST} values based on 11 colonies (Possession Island excluded) and 10 loci (PNN05 and PNN12 omitted due to missing data, as were three individuals with missing genotype data, n=184). G_{ST} values are below the diagonal. Probabilities based on permutation are shown above diagonal. Significant values are in bold type.

	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	HAL	ICH
Bird Island (BI)	-	0.575	0.245	0.353	0.745	0.127	0.101	0.699	0.710	0.816	0.356
St Croix Island (SC)	-0.001	-	0.277	**0.036**	0.180	0.225	**0.050**	0.705	0.234	0.224	0.094
Boulders Beach (BOU)	0.004	0.003	-	0.075	0.291	0.174	0.141	0.925	0.475	0.178	0.280
Dassen Island (DAS)	0.001	**0.004**	0.009	-	0.856	0.464	0.302	0.311	**0.044**	0.152	0.502
Dyer Island (DYE)	-0.002	0.003	0.003	-0.003	-	0.341	0.221	0.357	0.088	0.418	0.790
Jutten Island (JUT)	0.007	0.003	0.008	0.000	0.002	-	**0.034**	0.355	0.139	0.156	0.087
Robben Island (ROB)	0.009	**0.011**	0.013	0.002	0.005	**0.019**	-	0.122	**0.010**	0.119	0.495
Stony Point (SP)	-0.002	-0.002	-0.009	0.002	0.001	0.002	0.009	-	0.638	0.711	0.158
Mercury Island (MER)	-0.002	0.002	0.000	**0.005**	0.005	0.007	**0.019**	-0.002	-	0.424	0.306
Halifax Island (HAL)	-0.004	0.003	0.008	0.004	0.000	0.008	0.01	-0.003	0.001	-	0.339
Ichaboe Island (ICH)	0.001	0.004	0.003	0.000	-0.003	0.009	-0.001	0.005	0.002	0.002	-

Appendix 4.24 Colony-level pairwise population matrix of G'_{ST} (Nei's standardized G_{ST}) values based on 11 colonies (Possession Island excluded) and 10 loci (PNN05 and PNN12 omitted due to missing data, as were three individuals with missing genotype data, n=184). G'_{ST} values are below the diagonal. Probabilities based on permutation are shown above diagonal. Significant values are in bold type.

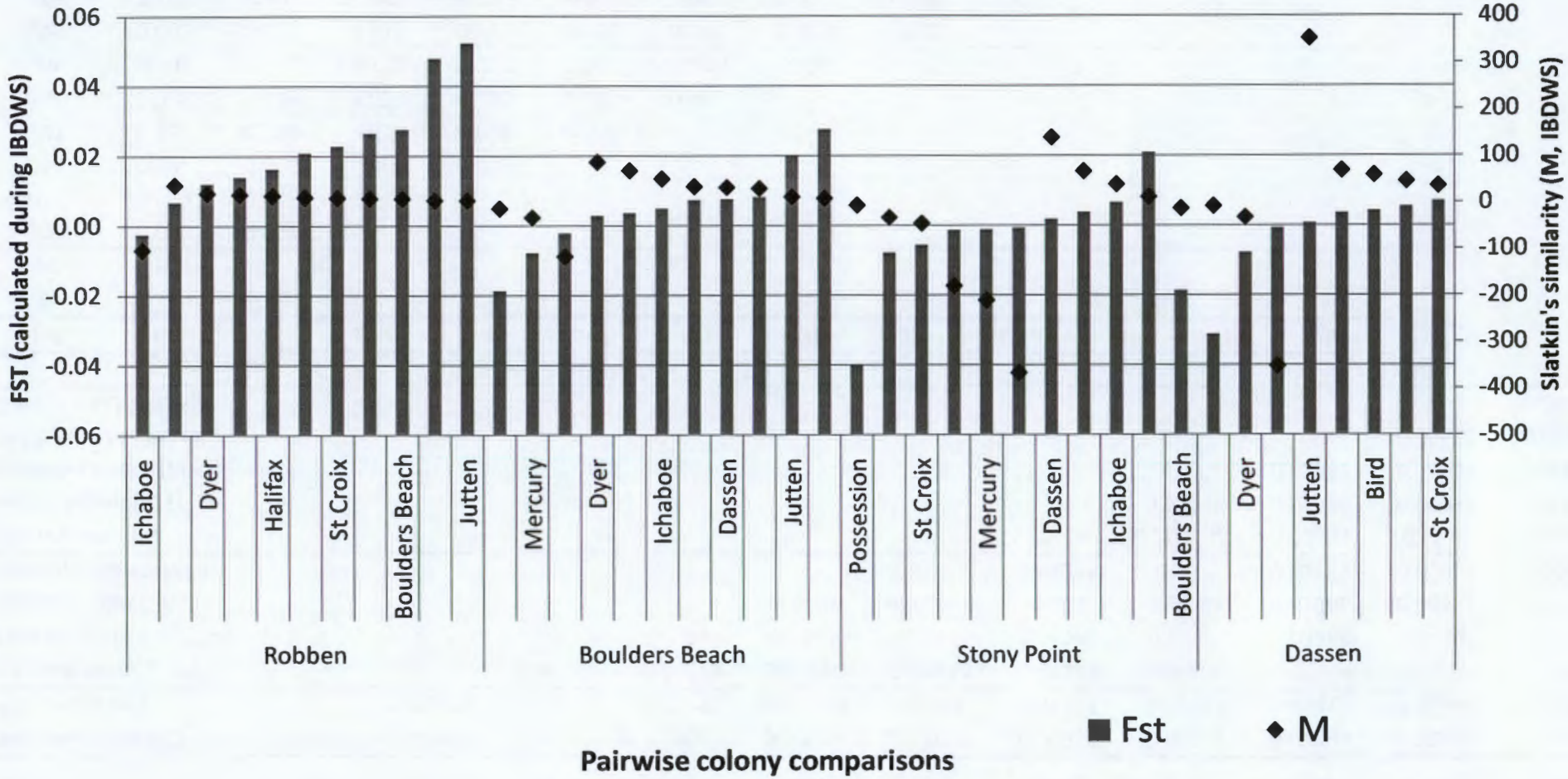
	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	HAL	ICH
Bird Island (BI)	-	0.575	0.245	0.353	0.745	0.127	0.101	0.699	0.710	0.816	0.356
St Croix Island (SC)	-0.001	-	0.277	**0.036**	0.180	0.225	**0.050**	0.705	0.234	0.224	0.094
Boulders Beach (BOU)	0.009	0.007	-	0.075	0.291	0.174	0.141	0.925	0.475	0.178	0.280
Dassen Island (DAS)	0.002	**0.009**	0.018	-	0.856	0.464	0.302	0.311	**0.044**	0.152	0.502
Dyer Island (DYE)	-0.004	0.006	0.007	-0.006	-	0.341	0.221	0.357	0.088	0.418	0.790
Jutten Island (JUT)	0.014	0.007	0.015	0.001	0.004	-	**0.034**	0.355	0.139	0.156	0.087
Robben Island (ROB)	0.017	**0.022**	0.025	0.005	0.010	**0.037**	-	0.122	**0.010**	0.119	0.495
Stony Point (SP)	-0.005	-0.004	-0.019	0.003	0.002	0.003	0.018	-	0.638	0.711	0.158
Mercury Island (MER)	-0.004	0.004	-0.001	**0.011**	0.011	0.014	**0.037**	-0.004	-	0.424	0.306
Halifax Island (HAL)	-0.007	0.006	0.015	0.008	0.001	0.016	0.019	-0.006	0.001	-	0.339
Ichaboe Island (ICH)	0.002	0.009	0.007	0.000	-0.007	0.018	-0.002	0.009	0.003	0.004	-

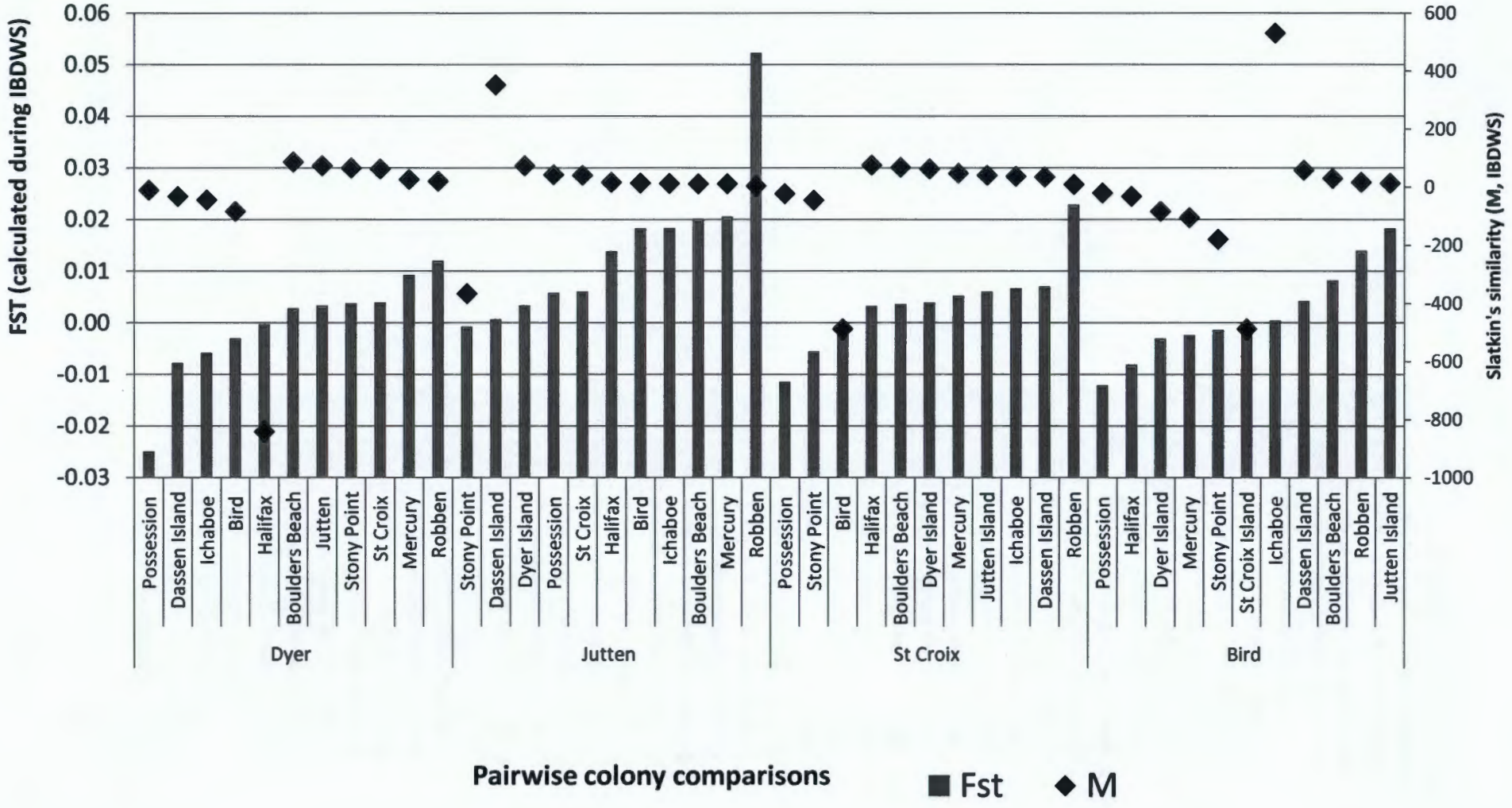
Appendix 4.25 Colony-level population pairwise (a) D-statistics (where D =-ln(1-Fst) and (b) linearized F_{ST}-values calculated in GENETIX. Negative values are in red type

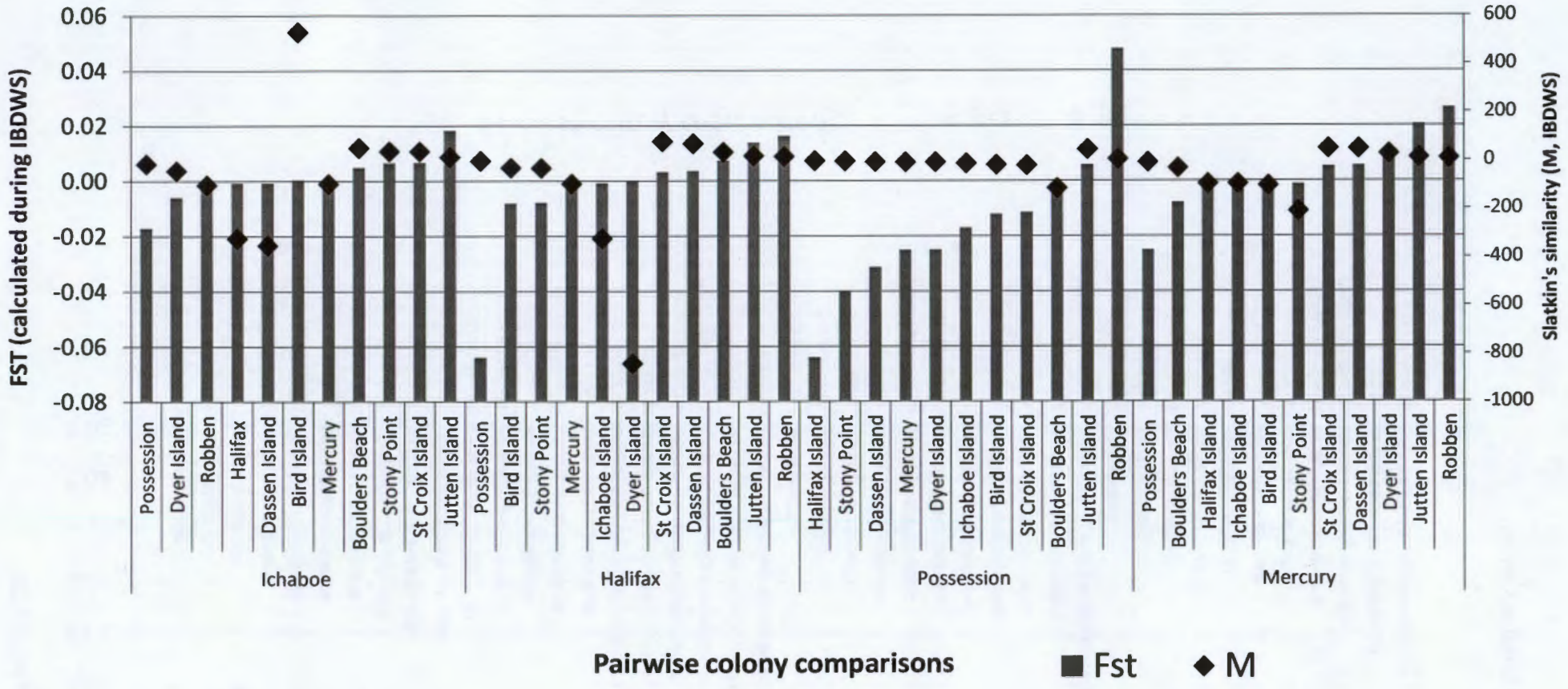
(a)	BI	ICH	POS	HAL	MER	SP	JUT	BOU	ROB	DAS	DYE
St Croix Island (SC)	-0.00046	0.00654	-0.02263	0.0032	0.00514	-0.00551	0.00613	0.00364	0.02394	0.00698	0.004
Bird Island (BI)	-	0.00046	-0.02772	-0.00791	-0.00235	-0.00147	0.01736	0.00797	0.01415	0.00426	-0.00311
Ichaboe Island (ICH)		-	-0.02732	-0.00072	0.00296	0.00653	0.01822	0.00506	-0.00264	-0.00068	-0.00584
Possession Island (POS)			-	-0.06983	-0.04175	-0.0472	0.00568	-0.00295	0.03984	-0.04988	-0.03081
Halifax Island (HAL)				-	-0.0026	-0.00776	0.014	0.00741	0.01606	0.00385	-0.00036
Mercury Island (MER)					-	-0.00106	0.01935	-0.00663	0.02799	0.00491	0.00905
Stony Point (SP)						-	-0.00063	-0.01838	0.02181	0.0018	0.00375
Jutten Island (JUT)							-	0.02039	0.05476	0.00031	0.00334
Boulders Beach (BOU)								-	0.02817	0.00847	0.00286
Robben Island (ROB)									-	0.00682	0.01317
Dassen Island (DAS)										-	-0.00772

(b)	BI	SC	BOU	DAS	DYE	JUT	ROB	SP	MER	POS	HAL	ICH
BI	-											
SC	0.006	-										
BOU	0.006	0.003	-									
DAS	0.000	0.015	0.011	-								
DYE	0.008	0.006	0.006	0.008	-							
JUT	0.017	0.004	0.015	0.006	0.003	-						
ROB	0.009	0.029	0.024	0.000	0.025	0.051	-					
SP	0.001	-0.006	-0.020	0.007	0.005	-0.003	0.020	-				
MER	0.002	0.033	0.015	0.010	0.050	0.042	0.029	0.022	-			
POS	-0.036	-0.025	-0.006	-0.041	-0.034	0.000	0.032	-0.058	-0.012	-		
HAL	-0.006	0.003	0.006	0.008	0.003	0.012	0.018	-0.009	0.020	-0.074	-	
ICH	-0.001	0.016	0.007	-0.003	0.008	0.023	-0.008	0.012	0.007	-0.027	0.004	-

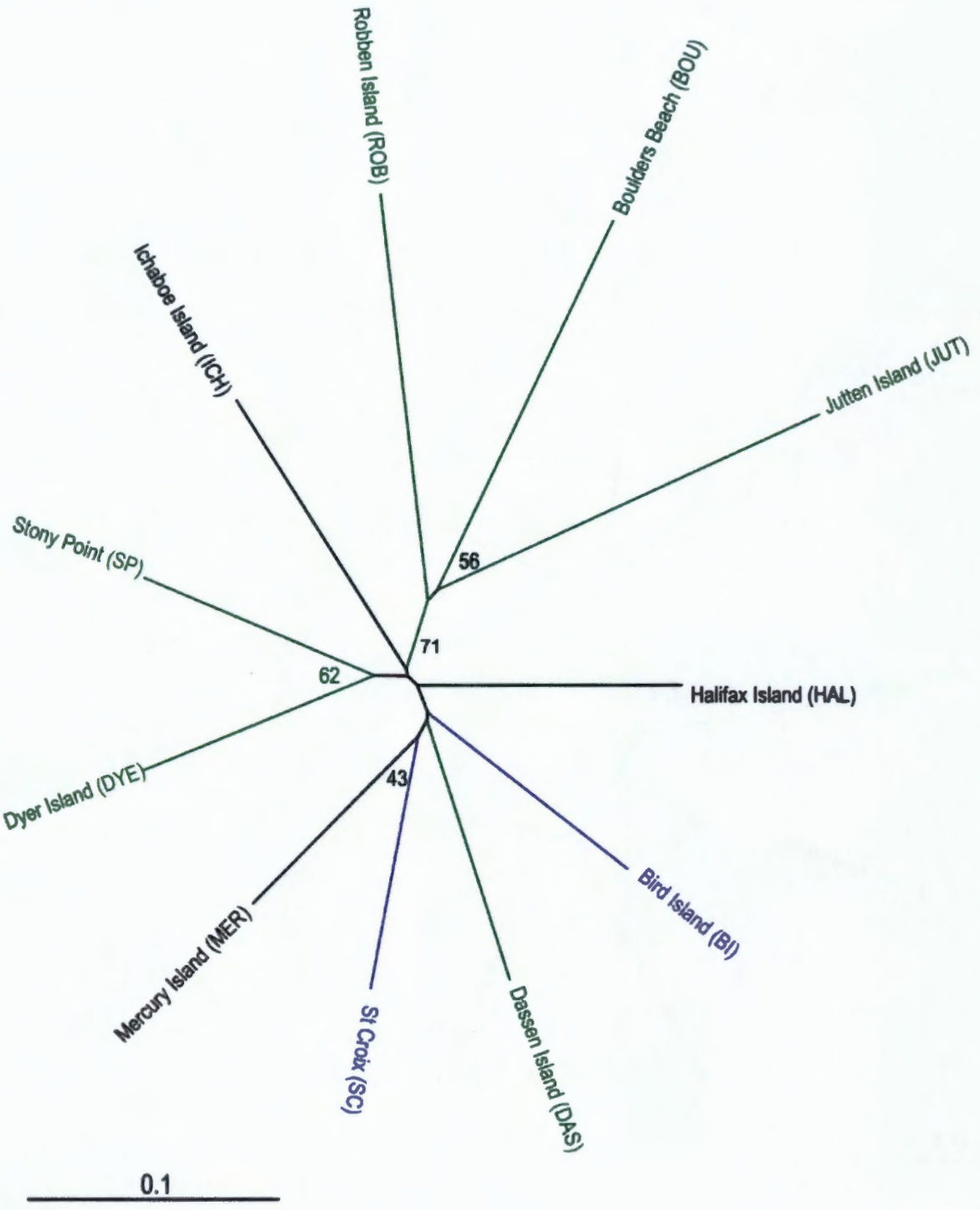
Figure 4.26 Pairwise colony F_{ST} and Slatkin's similarity index (M) calculated during the isolation by distance analysis (Jensen et al. 2005). Grey bars represent F_{ST} values (left hand vertical axix) and black diamonds represent genetic similarity (negative values are more dissimilar).



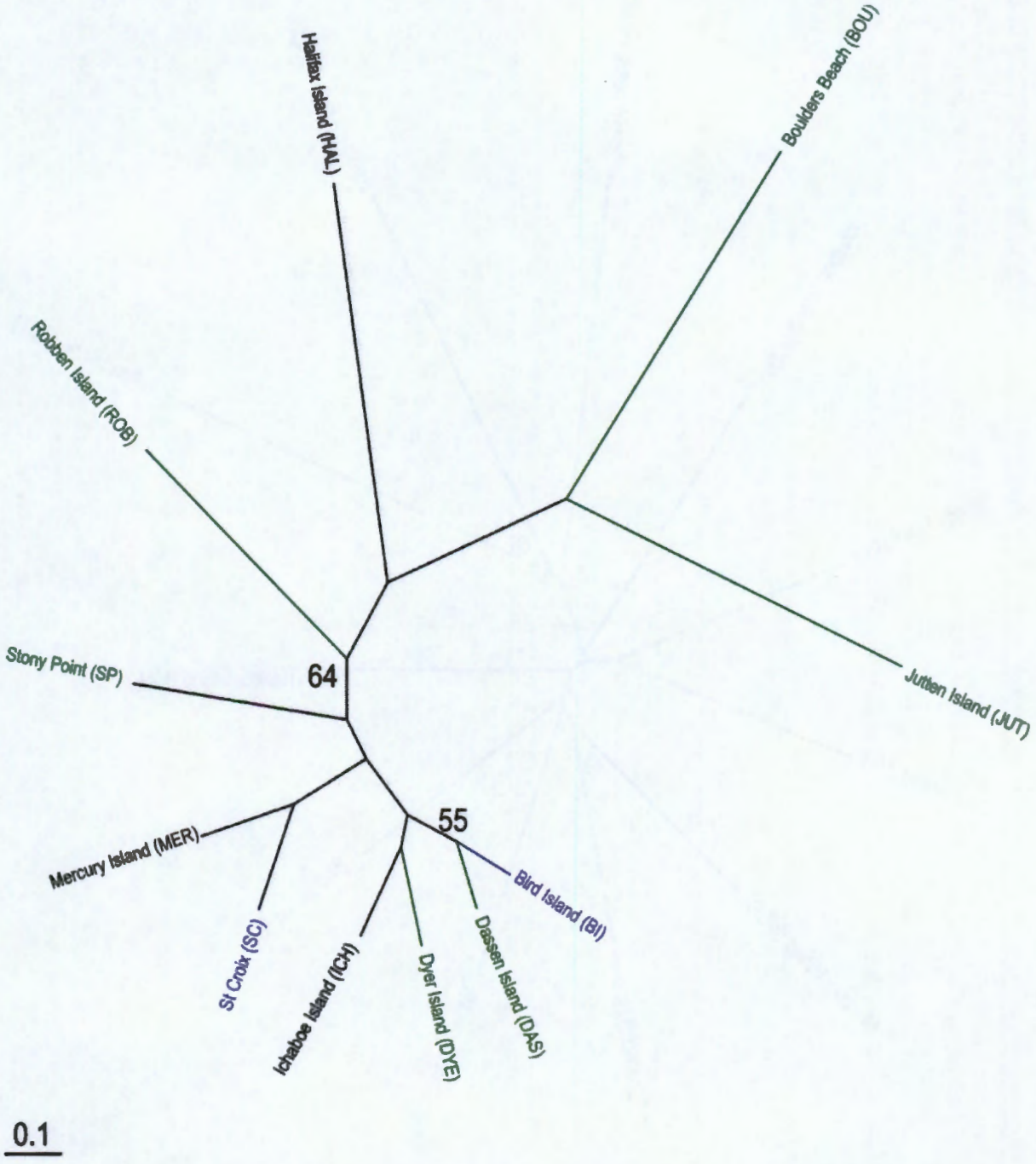

FST (calculated during IBDWS)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
600
400
200
0
-200
-400
-600
-800
-1000
Slatkin's similarity (M, IBDWS)
Possession
Dassen Island
Ichaboe
Bird
Halifax
Boulders Beach
Jutten
Stony Point
St Croix
Mercury
Robben
Stony Point
Dassen Island
Dyer Island
Possession
St Croix
Halifax
Bird
Ichaboe
Boulders Beach
Mercury
Robben
Possession
Stony Point
Bird
Halifax
Boulders Beach
Dyer Island
Mercury
Jutten Island
Ichaboe
Dassen Island
Robben
Possession
Halifax
Dyer Island
Mercury
Stony Point
St Croix Island
Ichaboe
Dassen Island
Boulders Beach
Robben
Jutten Island
Dyer
Jutten
St Croix
Bird
Pairwise colony comparisons
Fst
M


FST (calculated during IBDWS)
Slatkin's similarity (M, IBDWS)
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
600
400
200
0
-200
-400
-600
-800
-1000
Possession
Dyer Island
Robben
Halifax
Dassen Island
Bird Island
Mercury
Boulders Beach
Stony Point
St Croix Island
Jutten Island
Possession
Bird Island
Stony Point
Mercury
Ichaboe Island
Dyer Island
St Croix Island
Dassen Island
Boulders Beach
Jutten Island
Robben
Halifax Island
Stony Point
Dassen Island
Mercury
Dyer Island
Ichaboe Island
Bird Island
St Croix Island
Boulders Beach
Jutten Island
Robben
Possession
Boulders Beach
Halifax Island
Ichaboe Island
Bird Island
Stony Point
St Croix Island
Dassen Island
Dyer Island
Jutten Island
Robben
Ichaboe
Halifax
Possession
Mercury
Pairwise colony comparisons
Fst
M

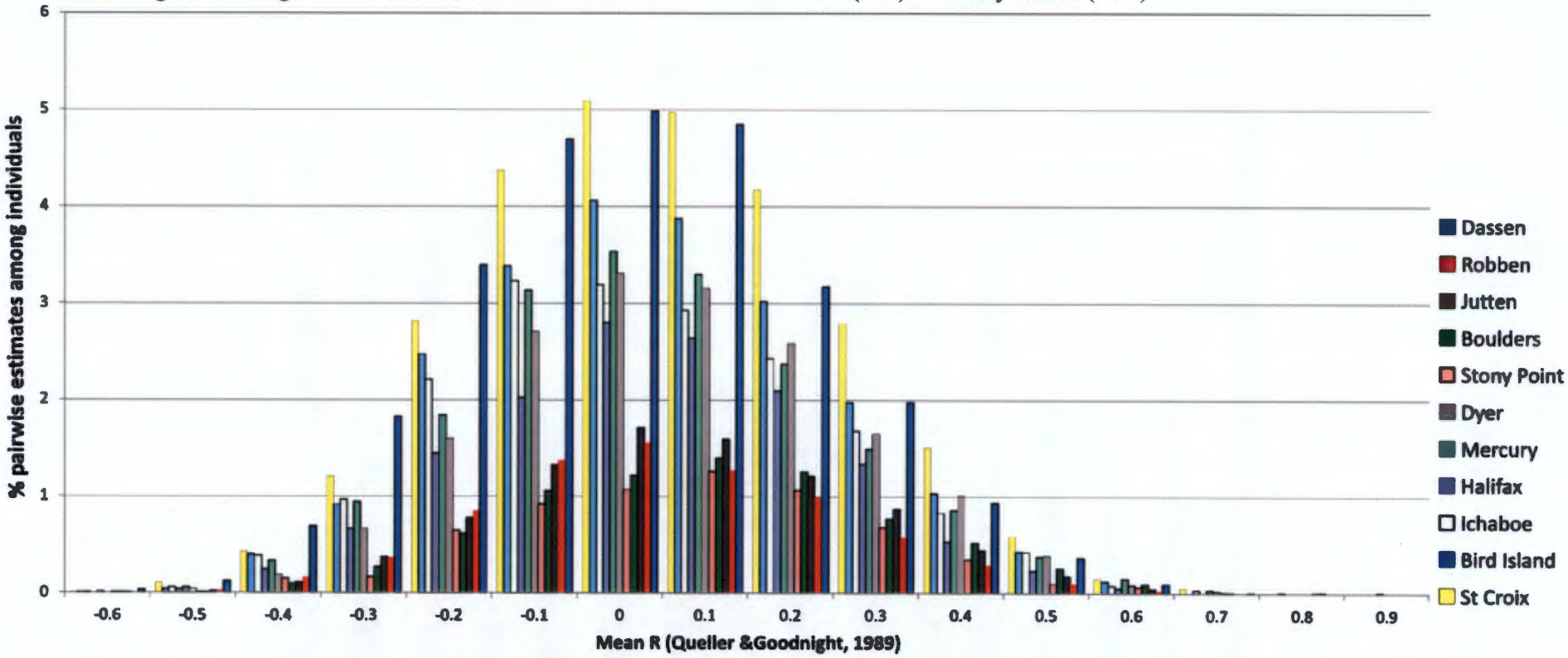
Appendix 4.27 Unrooted UPGMA dendrogram of African Penguin colonies from all three breeding regions (Namibia in black, Western Cape in green and Eastern Cape in blue), based on Cavalli-Sforza & Edward's chord distance (D_{CE}) calculated from a reduced dataset of 174 individuals and 11 loci - and excluding Possession Island - to eliminate missing data. Numbers at nodes are bootstrap values based on 1000 bootstrap replicates.



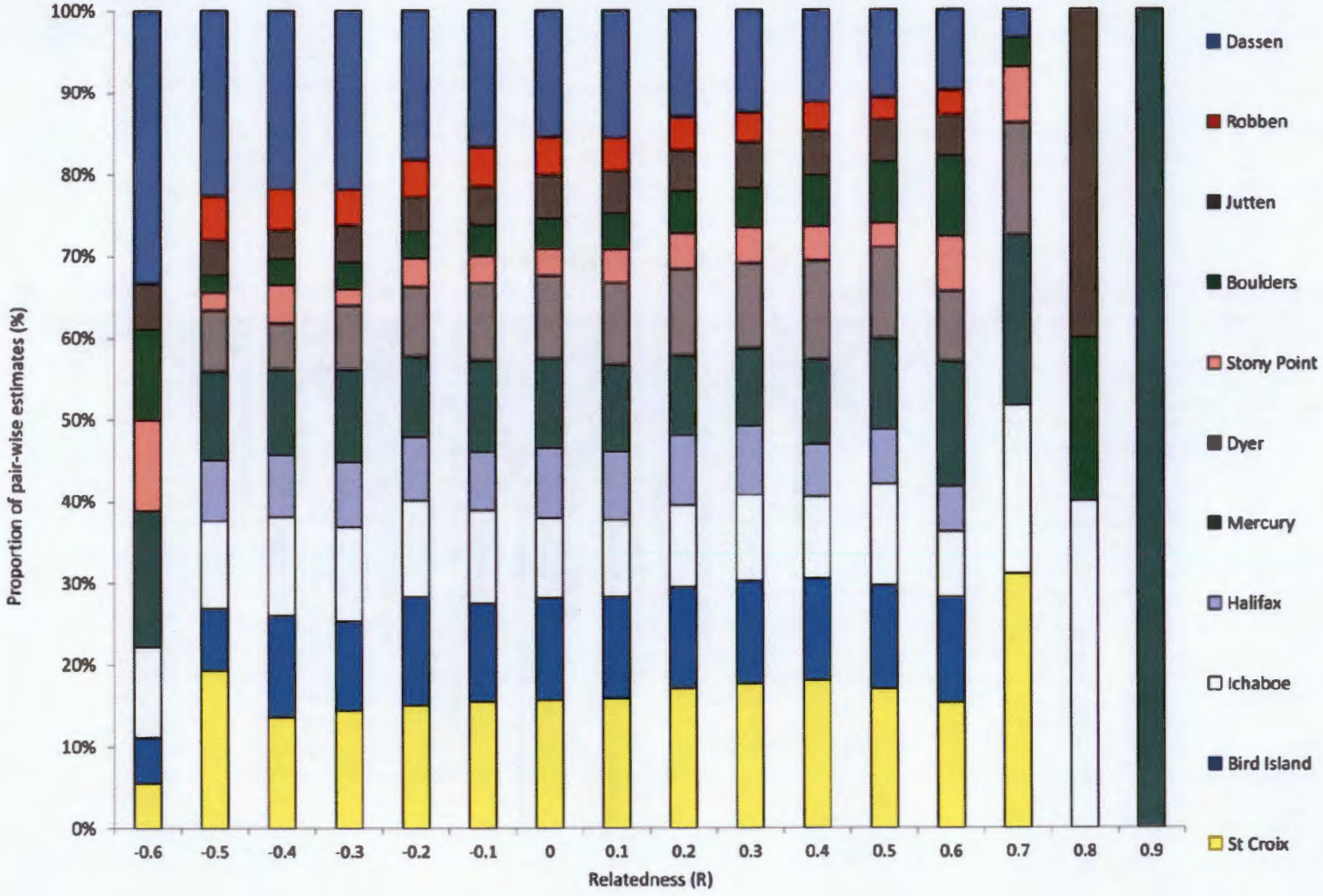
Appendix 4.28 Unrooted UPGMA dendrogram of African Penguin colonies from all three breeding regions (Namibia in black, Western Cape in green and Eastern Cape in blue), based on Goldstein et al.'s (1995) delta μ^2, calculated from a reduced dataset of 174 individuals and 11 loci (to eliminate missing data) and excluding Possession Island. Numbers at nodes are bootstrap values based on 1000 bootstrap replicates.



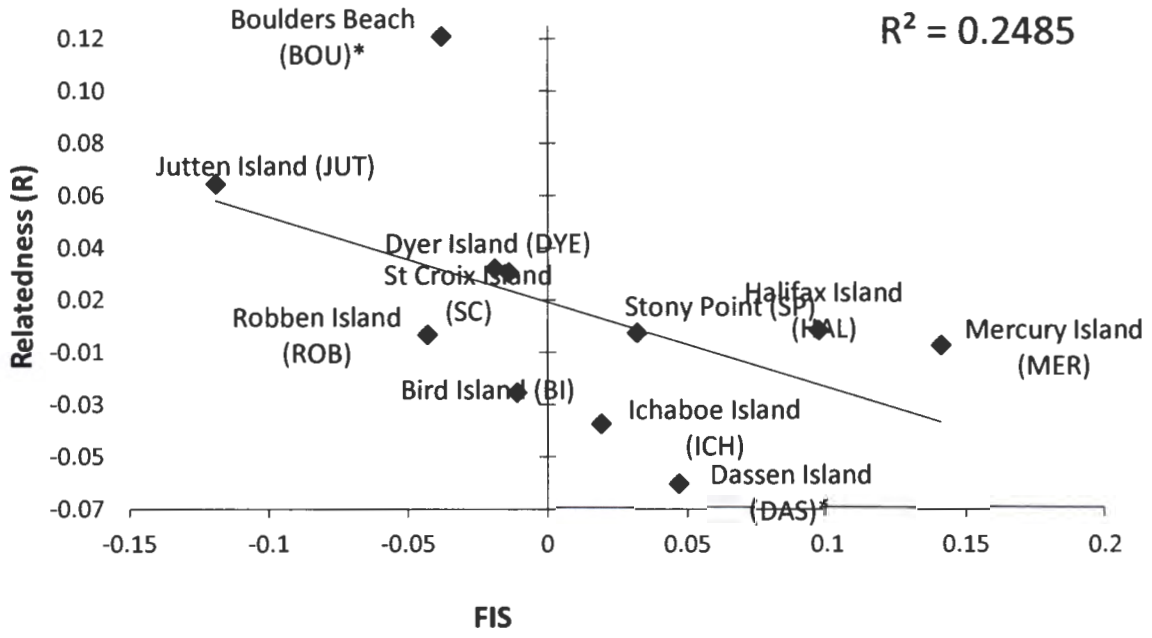
Appendix 4.29 Histogram showing the percentage of pairwise estimates of Queller and Goodnight's (1989) relatedness index (R) for individuals collected at 11 African Penguin breeding colonies that fall into 0.1 interval "bins" from unrelated (-0.6) to closely related (>0.6).



Appendix 4.30 The proportion of individuals from each of 11 African Penguin breeding colonies that fall into each relatedness category.



Appendix 4.xxx Negative relationship between F_{IS} and Relatedness. Significant relatedness values marked with an asterisk.



CHAPTER 5 APPENDICES

Appendix 5.1 Samples of captive African Penguins included in this study

Institution	Name	Studbook number	Sample number	Age in 2013	sex
SANCCOB	Dyer	T257	PNN01	30	m
	Bailey	T258	PNN02	16	f
	Flipper	T256	PNN03	10	m
	Flo	T261	PNN04	15	f
	Milo	T251	PNN05	4	m
	Marigold	T255	PNN06	6	f
	Robben	T334	PNN07	4	f
	Izzy	T333	PNN08	18	m
	Peta	T246	PNN09	5	f
	Cedric	T252	PNN10	6	m
	Edgar	T253	PNN11	5	m
	Jaeger	T336	PNN12	2	?
	Lambert	T260	PNN13	12	f
	Columbine	T254	PNN14	6	f
	Jill	T259	PNN15	15	f
	Cuddles	T335	PNN16	2	m
Two Oceans	Aerial	T29	PNN20	dead	?
	Dorris	T19	PNN35	18	F
	Zukiswe	T310	PNN37	4	f
	Alan	T247	PNN38	11	f
	Chuck	T20	PNN39	18	m
	Tasmyn	T27	PNN40	15	m
	Faraday	T18	PNN41	18	m
	Diesel	T116	PNN42	11	f
	Belinda	T21	PNN43	18	f
	Gaia	T23	PNN44	15	f
	George	T22	PNN45	18	m
	Neptune	T24	PNN46	16	m
NZG	Gizmo	T322	PNN47	2	m
	Sushi	T325	PNN50	2	m
	Coral	T326	PNN51	2	f
	Bubbles	T321	PNN62	2	m
	Jasmine	T137	PNN64	11	f
	Samson	T264	PNN65	12	f
	Cargo	T42	PNN66	13	m
	Pepi	T40	PNN 67	12	m
	Jj	T52	PNN68	21	m
	Flipper	T265	PNN69	7	m
	Adamant	T41	PNN70	13	f
	Skewer	T139	PNN71	12	f
	Stompie	T266	PNN73	7	f
	Pingu	T138	PNN74	12	m
	Kabousie	T271	PNN75	3	m
	Denver	T270	PNN76	3	m
	Fairy	T51	PNN77	25	f
	Vanilla	T46	PNN78	17	f

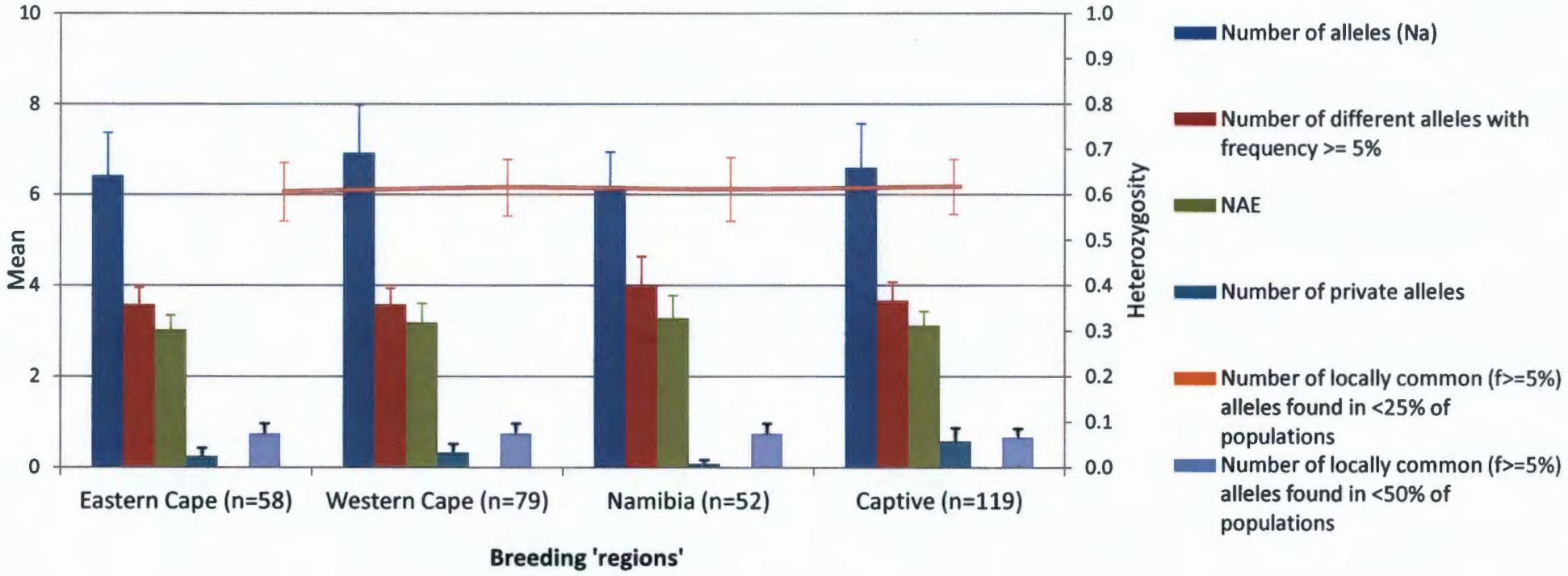
	Fundi	T45	PNN80	15	m
	Nobert	T37	PNN81	15	f
	Humphrey	T57	PNN82	22	f
	Kempston	T64	PNN83	20	m
	Mojito	T323	PNN84	2	f
	Guppy	T55	PNN85	25	m
	Stranding	T48	PNN87	20	f
	Denny	T328	PNN88	2	f
	Seymour	T329	PNN89	2	f
	Sheldon	T324	PNN93	2	f
	Gem	T444	PNN94	2	m
	Treasure	T441	PNN96	2	m
	Pumi	T446	PNN102	2	?
	Zanzi	T445	PNN105	2	?
	Foster	T443	PNN113	2	f
	Squishy	T327	PNN117	2	f
	Angel	T330	PNN120	2	?
	Tristan	T442	PNN122	2	?
	Popsicle	T439	PNN181	2	?
uShaka	Snoop-dog	T235	PNN17	5	m
	Tweedle dee	T5	PNN18	33	?
	Alladin	T217	PNN123	8	F
	Anchovy	T1	PNN124	14	F
	Arnette	T218	PNN125	5	F
	Beethoven	T219	PNN126	6	F
	Basil	T14	PNN127	13	F
	Bentley	T211	PNN128	9	F
	Clover	T15	PNN129	13	F
	Cockroach	T3	PNN130	15	F
	Coral	T175	PNN131	11	F
	Cupid	T4	PNN132	16	M
	Daquiri	T213	PNN133	10	M
	Elmer	T17	PNN134	13	M
	Juno	T7	PNN135	16	F
	Labamba	T224	PNN136	3	F
	Larnie	T348	PNN137	3	M
	Marilyn	T228	PNN138	6	F
	Miracle	T229	PNN139	6	f
	Peanut	T9	PNN140	14	f
	Rascal	T11	PNN141	14	f
	Rolo	T12	PNN142	13	f
	Ruff	T13	PNN143	13	f
	Tinkerbell	T242	PNN145	4	m
	Timberland	T243	PNN146	4	m
	Tony	T176	PNN147	9	m
	Zeus	T245	PNN148	6	f
	Ariel	T110	PNN149	12	f
	Corsa	T220	PNN151	9	m
	Chubbychecker	T222	PNN152	6	m
	Laduma	T226	PNN154	3	f
	Leroy	T349	PNN155	3	m

	Levi	T225	PNN156	3	f
	Lexy	T350	PNN157	3	m
	Linkin	T351	PNN158	3	m
	Lionel	T227	PNN159	3	m
	Lucky	T249	PNN160	3	f
	Luigi	T352	PNN161	3	f
	Malaika	T231	PNN162	6	f
	Nelson	T353	PNN163	2	m
	Nemo	T214	PNN164	9	f
	Nonu	T354	PNN165	2	m
	Nuttie	T355	PNN166	2	f
	Ocean	T215	PNN167	8	m
	Oenone	T8	PNN168	16	f
	Puck	T10	PNN169	14	f
	Scooby Doo	T233	PNN170	5	f
	Socrates	T236	PNN172	5	m
	Sugar	T237	PNN173	5	m
	Tamia	T238	PNN174	4	f
	Teddy	T239	PNN175	4	f
	Tito J	T240	PNN176	4	m
	Tina	T241	PNN177	4	f
	Tori	T244	PNN178	4	m

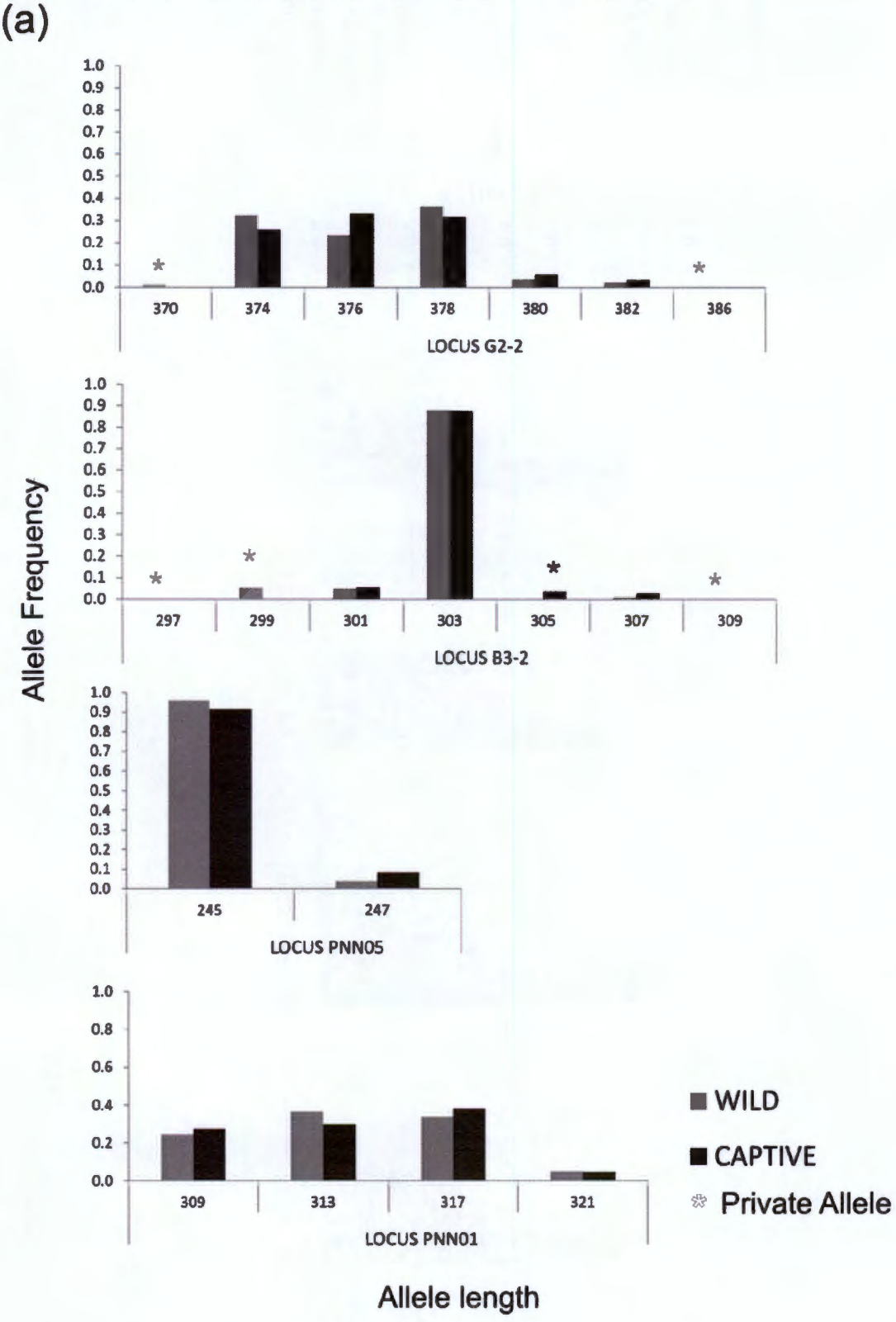
Appendix 5.2 List of 22 captive birds that possess private alleles i.e. alleles not present in the wild populations (populations defined as breeding colonies and captive institutions).

Name	Study code	Studbook number	Institution	Age in 2013
Milo	PNN05	T251	SANCCOB	4
Marigold	PNN06	T255	SANCCOB	6
Robben	PNN07	T334	SANCCOB	4
Izzy	PNN08	T333	SANCCOB	18
Peta	PNN09	T246	SANCCOB	5
Edgar	PNN11	T253	SANCCOB	5
Jaeger	PNN12	T336	SANCCOB	2
Lambert	PNN13	T260	SANCCOB	12
Columbine	PNN14	T254	SANCCOB	6
Jill	PNN15	T259	SANCCOB	15
Cuddles	PNN16	T335	SANCCOB	2
Aerial	PNN20	T29	Two Oceans	?
Zukiswe	PNN37	T310	Two Oceans	4
Chuck	PNN39	T20	Two Oceans	18
Zanzi	PNN105	T445	NZG	2
Foster	PNN113	T443	NZG	2
Snoop Dog	PNN17	T235	uShaka	5
Tweedle-Dee	PNN18	T5	uShaka	33
Bentley	PNN128	T211	uShaka	9
Chubby Checker	PNN152	T222	uShaka	6
Linkin	PNN158	T351	uShaka	3
Tina	PNN177	T241	uShaka	4

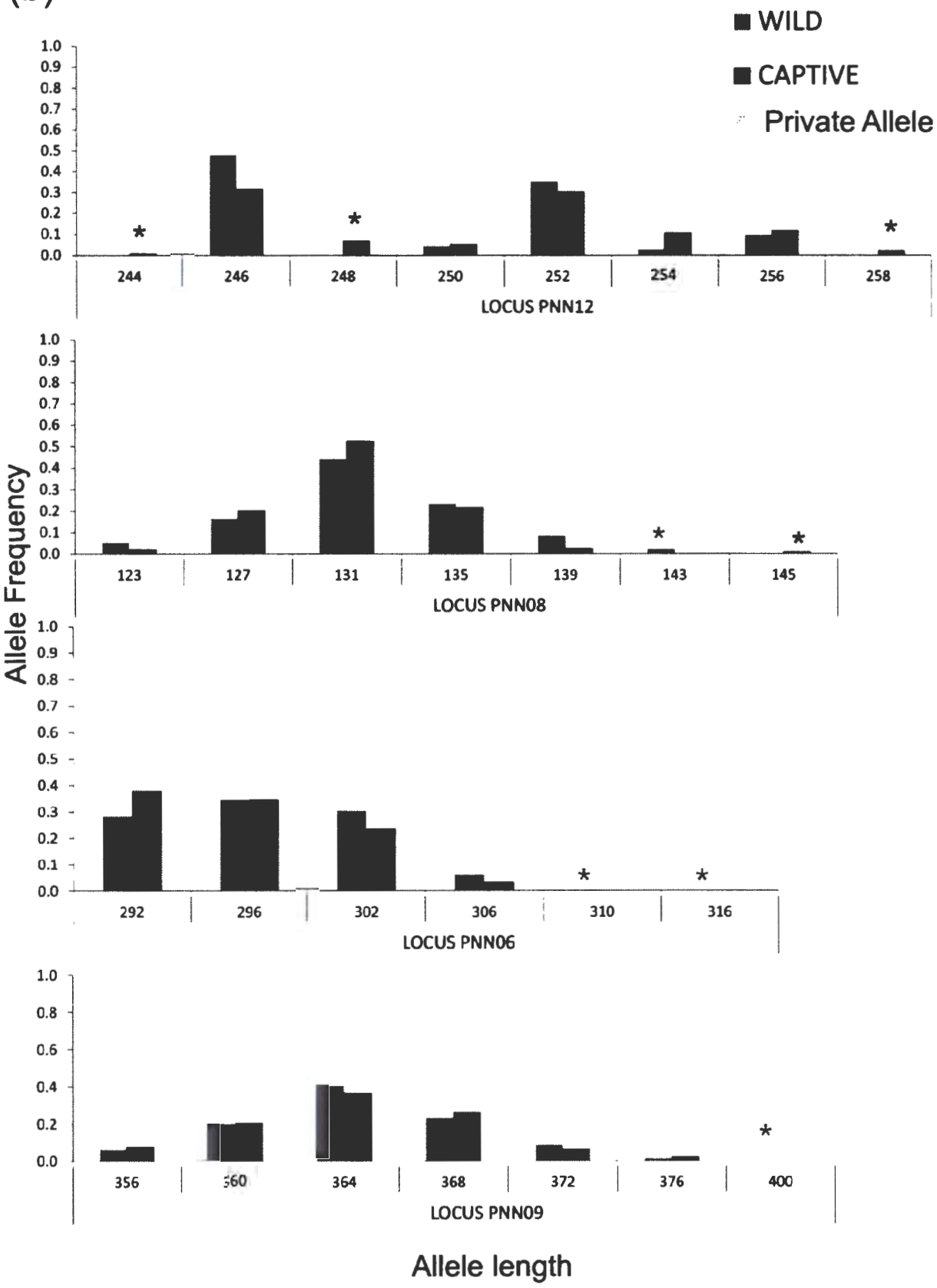
Appendix 5.3 Regioanl allelic patterns observed among the four African Pengun breeding regions (including all captive birds).

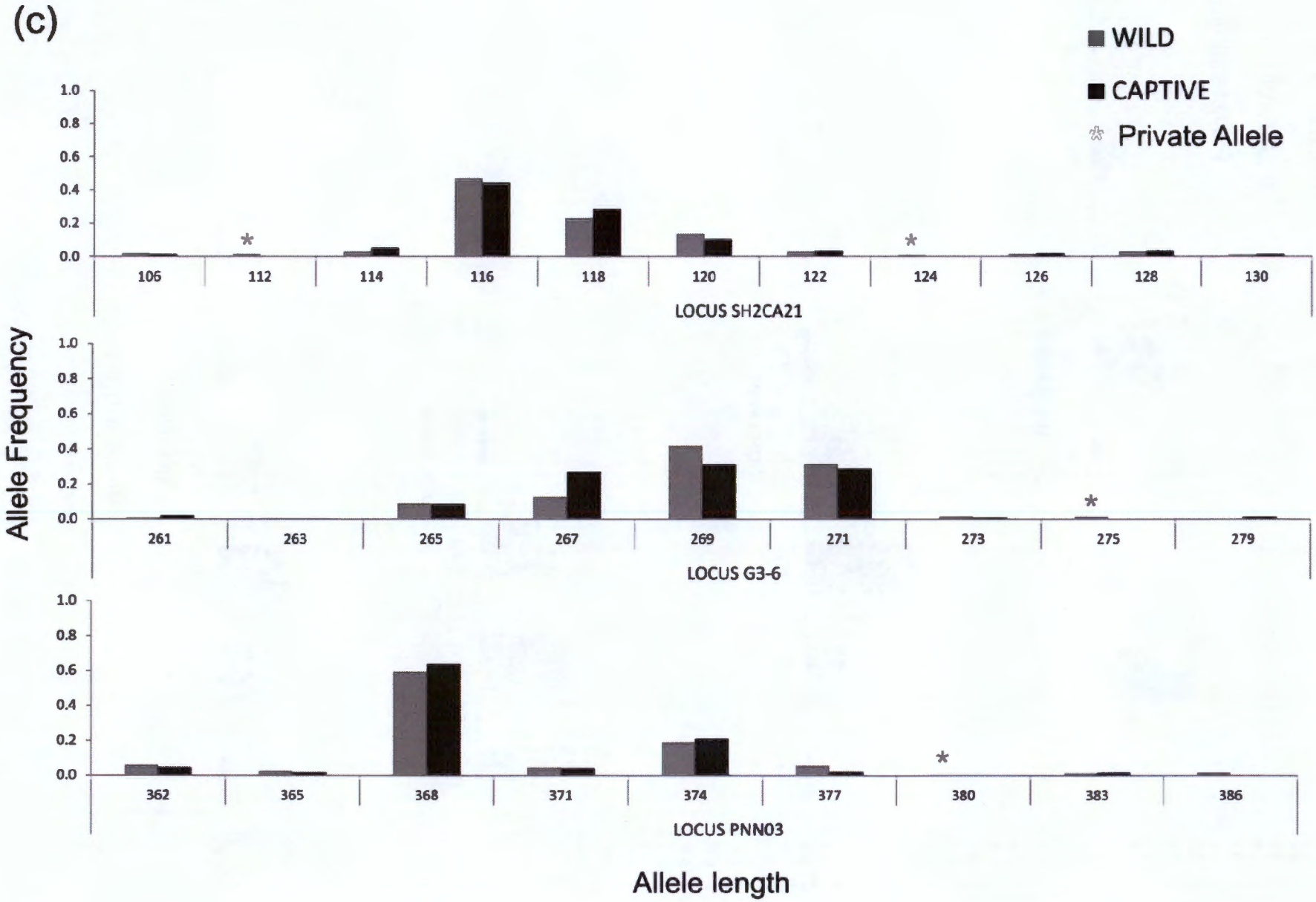


Appendix 5.4 Allele frequency histograms based on 119 captive African Penguins and 189 wild African Penguins: (a) Loci G2-2, PNN01, B3-2, PNN05; (b) loci PNN06, PNN09, PNN08, PNN12; (c) loci Sh2Ca21, G3-6, PNN03; and (d) locus Sh1Ca9. Wild birds are represented by grey bars and captive birds by black bars. Alleles that are private to either the wild or the captive populations are marked with an asterisk with the same colour as the population it represents.

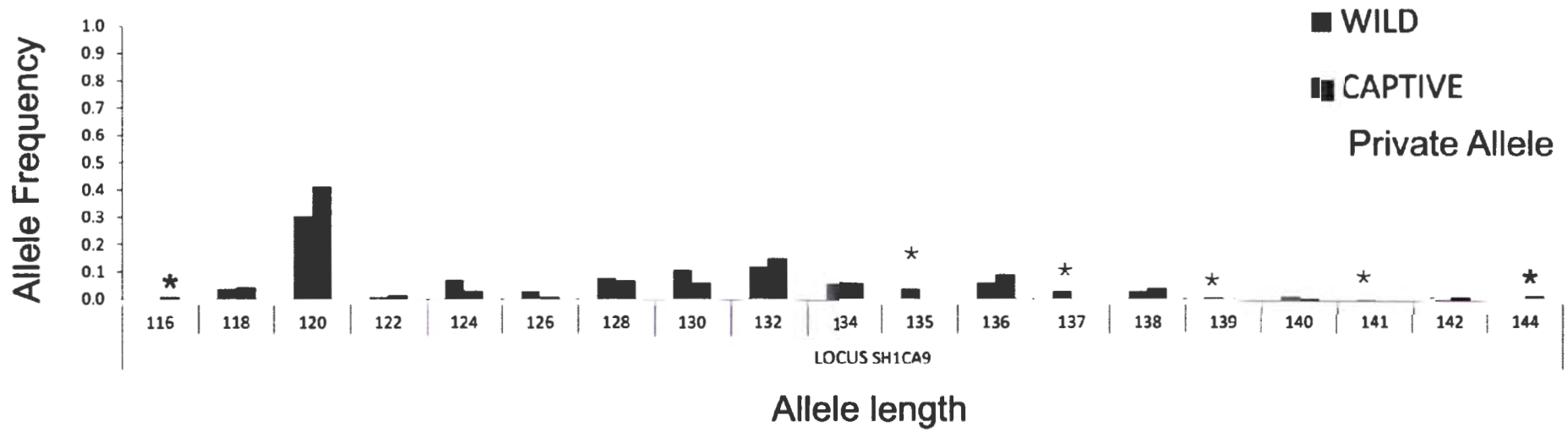


(b)

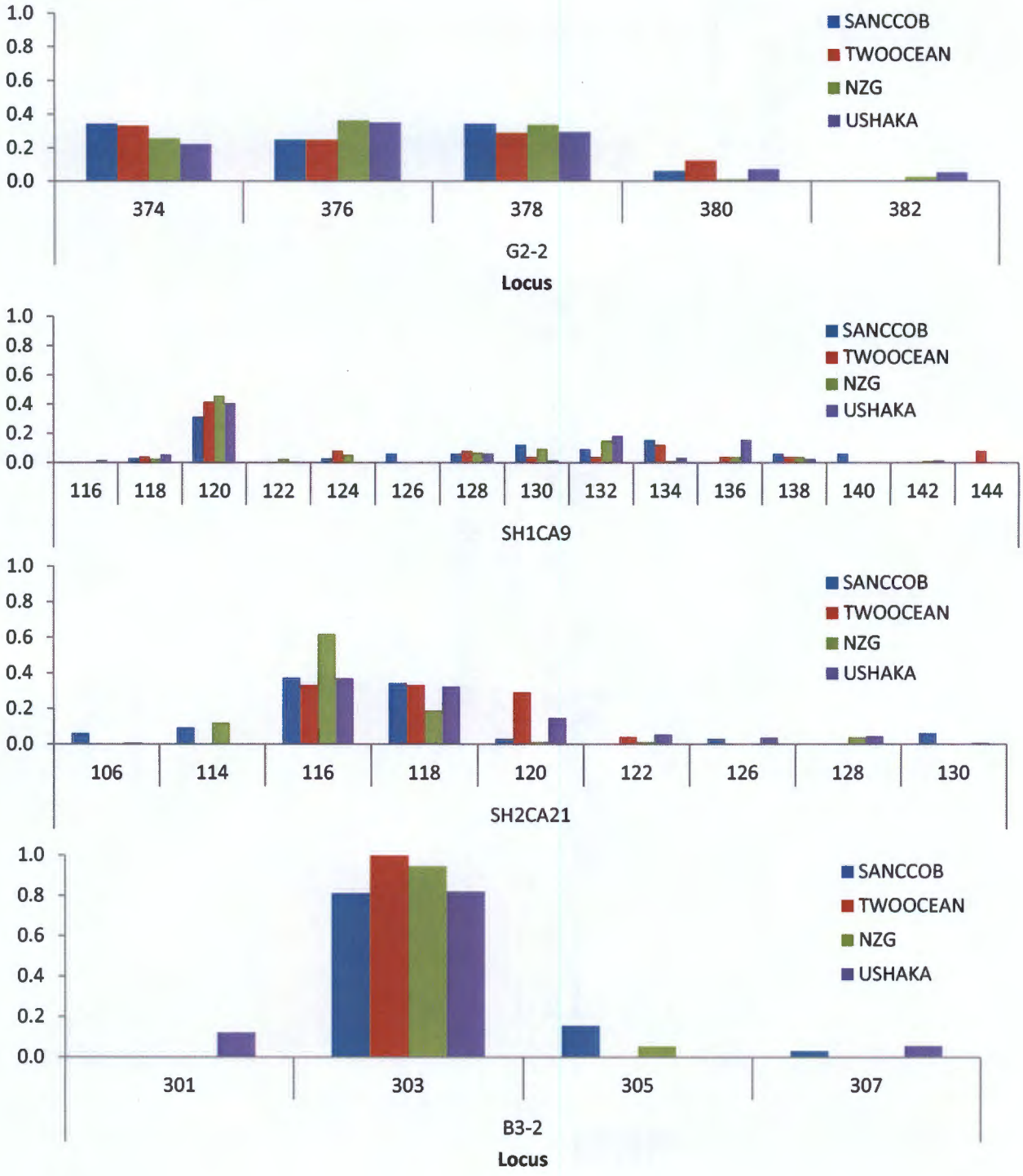

WILD
CAPTIVE
Private Allele
Allele Frequency
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
244
246
248
250
252
254
256
258
LOCUS PNN12
123
127
131
135
139
143
145
LOCUS PNN08
292
296
302
306
310
316
LOCUS PNN06
356
360
364
368
372
376
400
LOCUS PNN09
Allele length

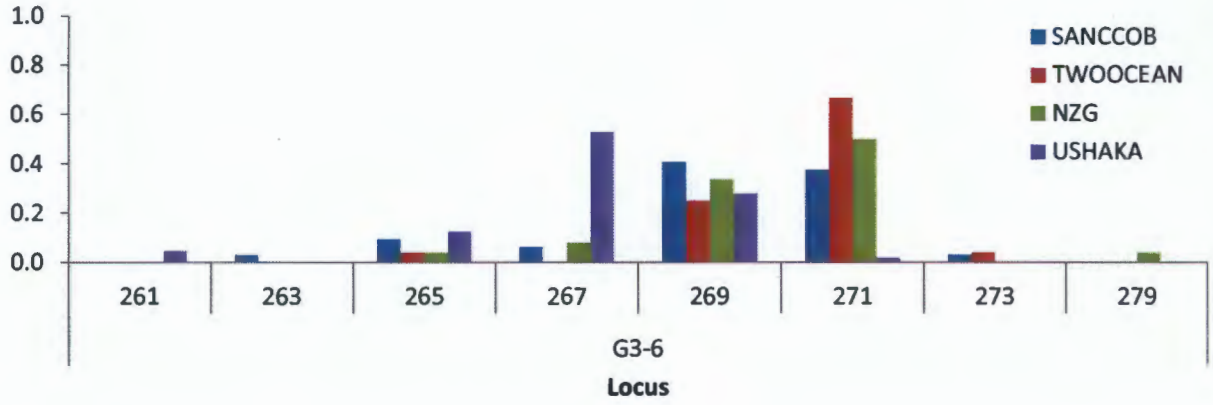

(c)
WILD
CAPTIVE
Private Allele
Allele Frequency
1.0
0.8
0.6
0.4
0.2
0.0
106
112
114
116
118
120
122
124
126
128
130
LOCUS SH2CA21
261
263
265
267
269
271
273
275
279
LOCUS G3-6
362
365
368
371
374
377
380
383
386
LOCUS PNN03
Allele length

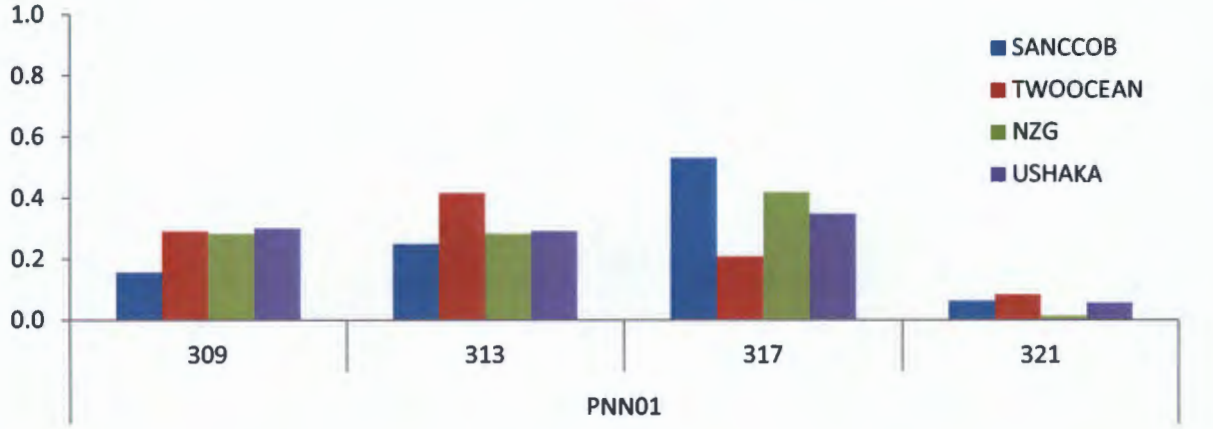
(d)

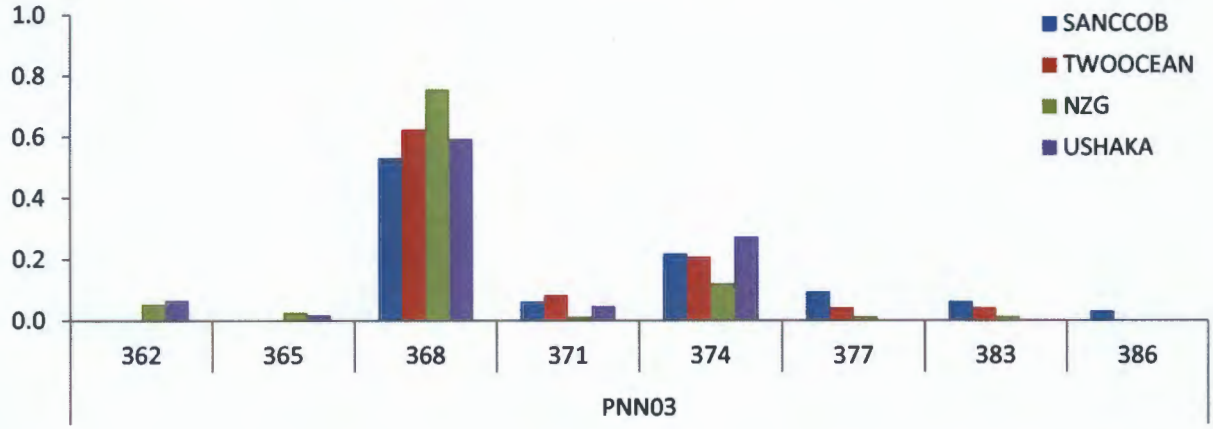

Allele Frequency
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
WILD
CAPTIVE
Private Allele
116
118
120
122
124
126
128
130
132
134
135
136
137
138
139
140
141
142
144
LOCUS SH1CA9
Allele length

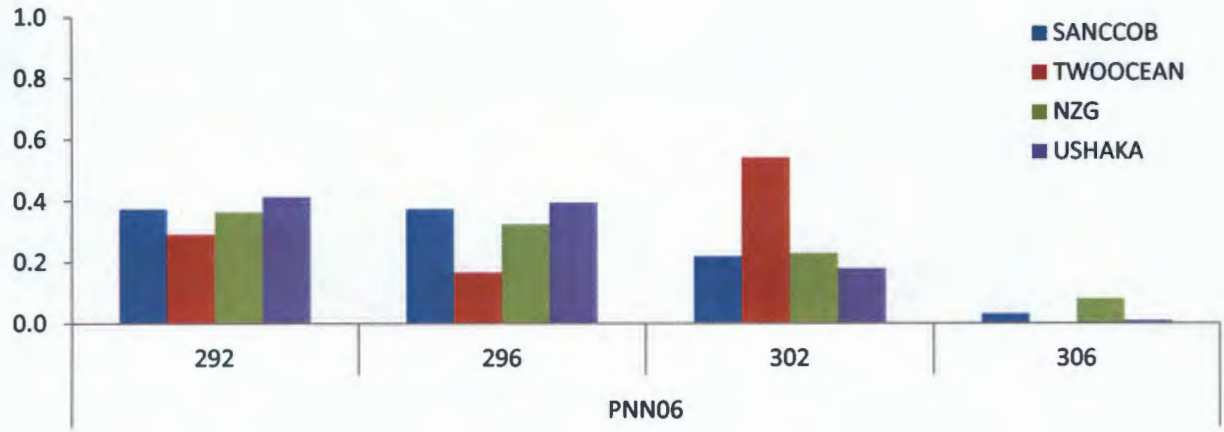
Appendix 5.5 Allele frequency histograms for 12 loci among 119 captive African Penguins across four institutions

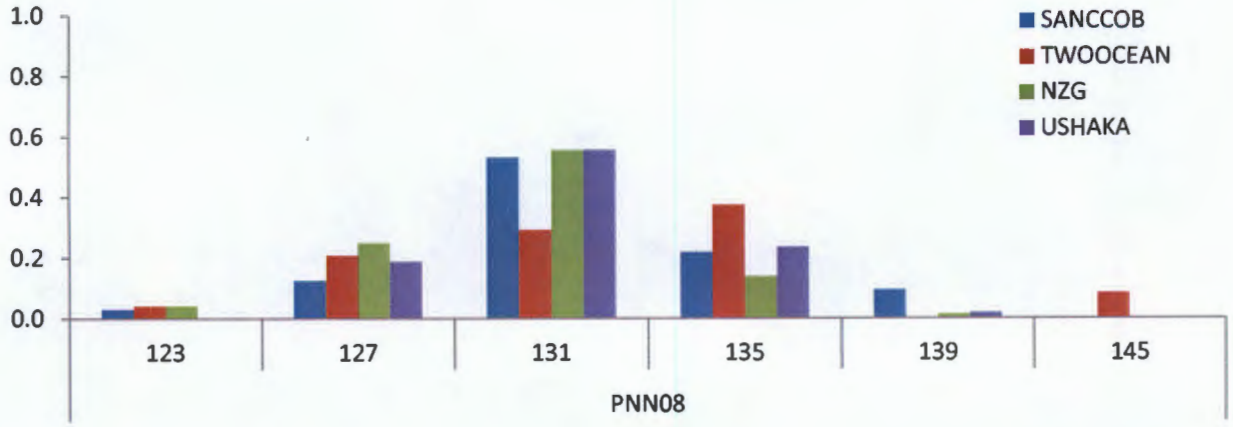


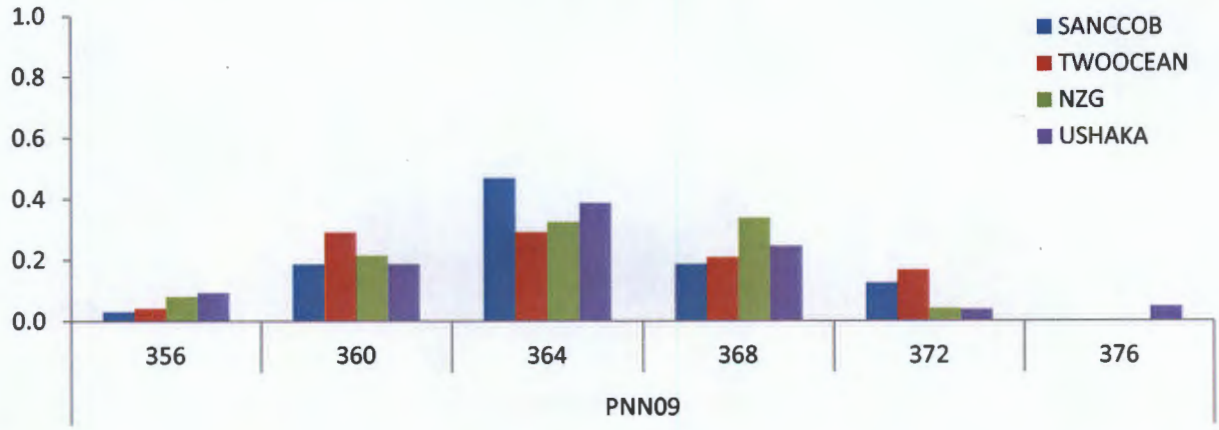

1.0
0.8
0.6
0.4
0.2
0.0
SANCCOB
TWOOCEAN
NZG
USHAKA
261
263
265
267
269
271
273
279
G3-6
Locus

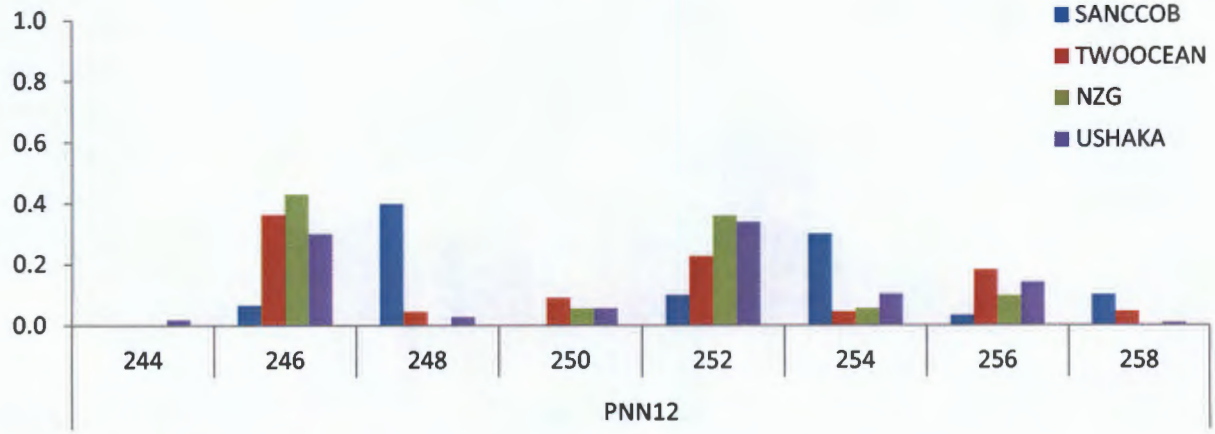

1.0
0.8
0.6
0.4
0.2
0.0
SANCCOB
TWOOCEAN
NZG
USHAKA
309
313
317
321
PNN01

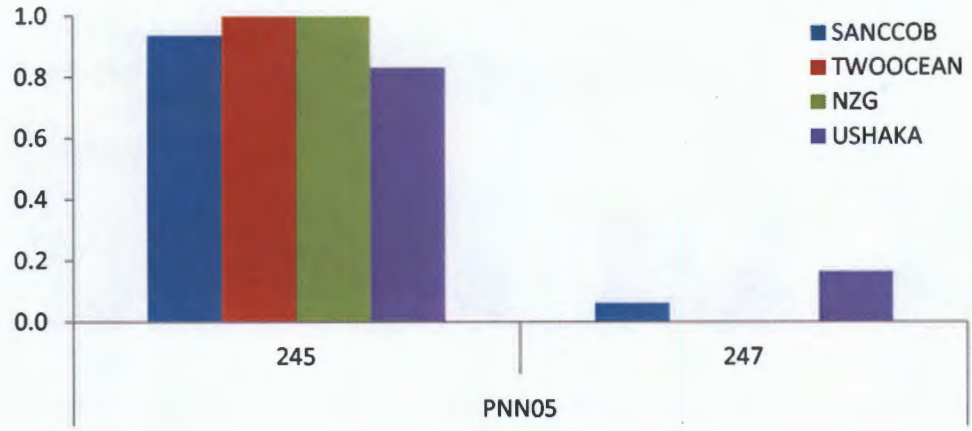

1.0
0.8
0.6
0.4
0.2
0.0
SANCCOB
TWOOCEAN
NZG
USHAKA
362
365
368
371
374
377
383
386
PNN03


1.0
0.8
0.6
0.4
0.2
0.0
SANCCOB
TWOOCEAN
NZG
USHAKA
292
296
302
306
PNN06


1.0
0.8
0.6
0.4
0.2
0.0
SANCCOB
TWOOCEAN
NZG
USHAKA
123
127
131
135
139
145
PNN08


1.0
0.8
0.6
0.4
0.2
0.0
SANCCOB
TWOOCEAN
NZG
USHAKA
356
360
364
368
372
376
PNN09


1.0
0.8
0.6
0.4
0.2
0.0
SANCCOB
TWOOCEAN
NZG
USHAKA
244
246
248
250
252
254
256
258
PNN12


1.0
0.8
0.6
0.4
0.2
0.0
SANCCOB
TWOOCEAN
NZG
USHAKA
245
247
PNN05

Appendix 5.6 The ten most closely related pairs of individuals among the captive African Penguins sampled. Results for each of the three measures of relatedness are presented: Ritland's estimator (RI), Lynch & Ritland's estimator (LRM) and Queller & Goodnight's mean estimator (QGM). Individuals are colour-coded by captive institutions: Grey = Ushaka, yellow = SANCCOB, blue=Two Oceans and white=NZG. Samples in bold are either siblings or a parent-offspring pair, and the family number (from Figure 5.2) is given after the sample names.

Ritland (1996)				Lynch & Ritland (1999)				Queller & Goodnight (1989)			
Individual 1	Studbook	Individual 2	Studbook	Individual 1	Studbook	Individual 2	Studbook	Individual 1	Studbook	Individual 2	Studbook
Rascal (7)	T11	**Teddy (7)**	T239	**Ruff (1)**	T13	**Puck (1)**	T10	**Ruff (1)**	T13	**Puck (1)**	T10
Clover (11)	T15	**Nuttie (11)**	T355	Skewer	T139	Alladin*	T217	Popsicle	T439	Alladin*	T217
Peanut (7)	T9	**Rascal (7)**	T11	**Tony (1)**	T176	**Laduma (1)**	T226	**Zeus (1)**	T245	**Laduma (1)**	T226
Cuddles	T335	Zanzi**	T445	**Malaika (7)**	T231	**Nonu (7)**	T354	Basil$^{\Delta}$	T14	Bentley$^{\Delta}$	T211
Basil$^{\Delta}$	T14	Bentley$^{\Delta}$	T211	Tinkerbell	T242	Socrates	T236	**Coral (12)**	T326	**Pumi (12)**	T446
Clover (11)	T15	**Lexy (11)**	T350	Popsicle	T439	Alladin*	T217	Sheldon	T324	Alladin*	T217
Zanzi	T445	Foster	T443	Sheldon	T324	Alladin*	T217	Bubbles	T321	Ariel	T110
Jill	T259	Zanzi**	T445	**Denver (12)**	T270	**Nobert (12)**	T37	Marilyn	T228	Tito J	T240
Jill	T259	Cuddles	T335	**Zukiswe (3)**	T310	**Chuck (3)**	T20	Tinkerbell	T242	Socrates	T236
Zukiswe (3)	T310	**Chuck (3)**	T20	Timberland	T243	Ariel	T110	Timberland	T243	Ariel	T110

*missing data for 6 loci (50%)

** missing data for 2 loci

$^{\Delta}$ Have never bred in captivity (wild parents)

Tinkerbell and Socrates are in in families 1 and 10 respectively, but appear to be possible siblings. Similar for Zanzi and Foster (family 12 and 6). Similary Ariel and Timerbland might connect families 10 and 1, and Marilynn and Tito J, families 11 and 10.

Appendix 5.7 Examples of STRUCTURE triangle plots for K=3 and K=4 for the analysis based on 119 captive African Penguins from 4 South African captive institutions. The colour of the dots represents their "collection locality" (Red=SANCCOB [population 1], Green= TWOOCEANS [population 2], Blue=NZG [population 3] and Yellow =Ushaka [population 4]).