PALAEOECOLOGICAL CHANGES IN POPULATIONS OF ANTARCTIC ICE-DEPENDENT PREDATORS AND THEIR ENVIRONMENTAL DRIVERS

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DECLARATION OF ORIGINALITY

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Jane Younger

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AUTHORITY OF ACCESS

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ABBREVIATIONS

bp	base pairs
BP	Before physics (i.e. before 1950)
ca.	circa
CytB	mitochondrial gene cytochrome *b*
EAWS	emperor penguin genetic population spanning East Antarctica and the Weddell Sea
EBSP	extended Bayesian skyline plot
ESS	effective sample size
F_{ST}	F-statistics
gDNA	genomic DNA
H_e	expected heterozygosity
HPD	highest posterior density interval
HVR	mitochondrial hypervariable region
kya	thousand years ago
Kyr	thousand years
LGM	last glacial maximum
MCMC	Markov chain Monte Carlo
mya	million years ago
Myr	million years
N_e	effective population size
N_{ef}	female effective population size
RADSeq	restriction-site-associated DNA sequencing
SD	standard deviation
SNPs	single nucleotide polymorphisms
Tau	generation time
T_{MRCA}	time to most recent common ancestor

TIME PERIODS REFERRED TO IN TEXT

Pleistocene	2.58 mya to 11.7 kya
Last glacial maximum (LGM)	26–19.5 kya
Glacial-interglacial transition	19.5–11.7 kya
Holocene	11.7 kya to present
Anthropocene	1610 onwards

TYPES OF ICE REFERRED TO IN TEXT

Sea ice	The frozen sea water that surrounds Antarctica. Sea ice undergoes an annual cycle of growth and retreat, with a maximum areal extent of approximately 20 million km^2 (19 September 2014 extent).
Fast ice	The stable region of sea ice adjacent to the continent, held fast by coastal features and icebergs.
Pack ice	Sea ice that drifts with the currents offshore.
Glacial ice	Also referred to as glacier. Blanket term used to describe a mass of ice that moves over the land. Ice sheets are a type of glacial ice (see below). Alpine glaciers are rivers of ice that flow down from mountain ranges.
Ice sheet	Ice sheets are formed when snow falls but does not melt, so is gradually compressed over thousands of years, becoming denser and thicker. The Antarctic ice sheets cover approximately 14 million km^2 and are divided into the East and West Antarctic ice sheets. At its thickest point the East Antarctic Ice Sheet is approximately 4,800 m thick.
Ice shelf	Ice shelves are sections of glacial ice that are floating on the ocean. They are formed by the outflow of ice from the ice sheets into the ocean. They may be hundreds of metres thick and hundreds of kilometres across. Icebergs form when bits of ice shelves break away.

STATEMENT OF ETHICAL CONDUCT

This reseach made use of existing genetic samples wherever possible in order to minimise disturbance to the Antarctic ecosystem. Therefore, the majority of the genetic material included was collected by other researchers in the past. Where additional sampling was required, genetic material was only taken from already deceased animals; no live animals were handled for this research. The collection of tissue from deceased penguins in Antarctica was conducted under permits from the Australian Antarctic Division, issued following independent ethical review. Sample collections were made under AAS project #4088 and IPEV project #1091.

STATEMENTS OF CO-AUTHORSHIP FOR PUBLISHED CHAPTERS

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Candidate, author 8 and author 11 contributed to sample collections, either by carrying out fieldwork or organising logistics.

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ABSTRACT

The Southern Ocean is undergoing rapid physical and biological changes that are likely to have profound implications for Antarctic fauna. One such change is a projected decline in the extent of the Antarctic sea ice field by the end of the century, and an associated shortening of the sea ice season. Fauna that are dependent on Antarctic sea ice for breeding or foraging habitat are likely to be vulnerable to climate change. The Weddell seal (*Leptonychotes weddellii*) and emperor penguin (*Aptenodytes forsteri*) both use Antarctic sea ice as a breeding platform, while the Adélie penguin (*Pygoscelis adeliae*) breeds on ice-free ground, but forages largely within the sea ice zone. In order to develop successful conservation plans for such at-risk taxa, an understanding of the likely impacts of climate change is essential. As the changes currently underway in the Southern Ocean represent a long-term, environmental regime shift (as opposed to a short-term fluctuation), it is prudent to consider the responses of species to similar climate regime shifts in the past. By examining changes in the abundance and distribution of taxa over tens of thousands of years and placing these changes into context with palaeoclimate records, valuable insights into species' long-term responses to environmental change can be gained. When considered in combination with contemporary ecology, information on historical trends can give a more complete picture of species' likely responses to climate change in the future. For this thesis, changes in the abundance and distribution of Weddell seals, emperor penguins and Adélie penguins over millennia were examined using mitochondrial DNA and genome-wide SNPs. Genetic diversity and contemporary population structure were also assessed, in order to provide context for the historical findings and to better understand the resilience of these predators to environmental change.

Two mitochondrial markers (the control region and cytochome *b*) were sequenced for extant colonies and radiocarbon dated sub-fossil remains of the three species. In total, 250 emperor penguins from nine colonies spanning the Ross Sea, Weddell Sea and East Antarctica, 96 Weddell seals from seven sites in East Antarctica, and 56 Adélie penguins from six colonies in East Antarctica, were sequenced. The East Antarctic Adélie penguin sequences were supplemented with existing datasets of 36 penguins from the Scotia Arc and 49 penguins from the Ross Sea, to allow for circum-Antarctic comparisons. Using mitochondrial DNA sequences, the population trends of all three species in East Antarctica during and since the last glacial maximum (LGM, 26 – 19.5 kya) were reconstructed using coalescent Bayesian skyline methods. Phylogenies were also constructed for emperor and Adélie penguins from around the continent in order to identify genetic lineages associated with past refugia.

It was hypothesised that the sea ice-breeding Weddell seal and emperor penguin may have prospered during the glacial period, due to an increase in breeding habitat and reduced competition from other, less cold-tolerant predators, whereas Adélie penguins were likely to have been reduced in number. However, findings indicated that both Adélie and emperor penguin populations were smaller during the last glacial period than they are today, and populations expanded in East Antarctica (135-fold and 5.7-fold, respectively) during the glacial-interglacial transition and the Holocene. The timing and magnitude of the population expansions were different for the two penguin species, and appear to have had different environmental drivers. In the case of the Adélie penguin, expansion began *ca*. 14 kya, suggesting that deglaciation, and the associated increase in ice-free ground suitable for nesting, was the likely driver. Meanwhile, the emperor penguin expansion in East Antarctica occurred 4,000 years later, coincident with reductions in sea ice that may have led to more favourable foraging conditions. The phylogenetic analyses indicated that both penguin species were restricted to refugia during the LGM. In the case of the Adélie penguin, there was evidence for two refugia, while for emperor penguins there were probably three refugia. Results strongly suggested that both species had a refuge in the Ross Sea, possibly associated with a polynya in the region which may have provided an oasis within the sea ice field. The location of other refugia were unable to be identified based on mitochondrial data analysis.

Despite the similar ecological niches and overlapping distributions of emperor penguins and Weddell seals, findings indicated the two meso-predators responded very differently to historical climate change. While emperor penguin numbers increased rapidly in the Holocene, the size of Weddell seal populations was unchanged. Emperor penguins appear to possess a greater capacity to adapt to environmental change than Weddell seals. Emperor penguins prospered during the Holocene warming while Weddell seals did not, most likely due to a higher dispersal ability (and hence gene flow among colonies), higher evolutionary rate and fine-scale differences in their preferred foraging locations. The vastly different climate change responses of two ecologically equivalent predators suggests that differing adaptive capacities and/or fine-scale niche differences play a major role in species' climate change responses. Meanwhile, the similarity in the responses of the ice-breeding emperor penguin and rock-breeding Adélie penguin to climate change was also surprising. Overall, the climate change responses of species are complex and may prove difficult to predict.

The degree of dispersal among breeding colonies is an important component of understanding a species' likely climate change response. This is because dispersal facilitates range shifts, and increases adaptive capacity by facilitating gene flow among breeding sites, thus replenishing the gene pool with new, potentially adaptive alleles. Delimiting breeding populations is also crucial for conservation planning, so that populations can be managed at an ecologically relevant spatial scale.

A total of 114 individuals from eight emperor penguin colonies from the Ross Sea, Weddell Sea and East Antarctica were genotyped using 20,005 genome-wide SNPs generated using restriction-site-associated DNA sequencing (RADSeq). Their genetic population structure was then assessed using STRUCTURE analysis, principal components analysis, and analyses of pairwise F_{ST}, revealing a total of six extant breeding populations among the eight emperor penguin colonies sampled. There was indications of ongoing gene flow between colonies located up to 550 km apart, providing evidence against strong philopatry in emperor penguins. This finding has important implications for forecasting studies for the species, which have previously considered each colony as an isolated unit.

The demographic histories of emperor penguin breeding populations were assessed over the past 50,000 years using RAD-Seq loci in combination with mitochondrial DNA sequences, via the coalescent Bayesian skyline method. This approach allowed for comparisons of the timing and magnitude of population expansions in different locations. The genetic evidence strongly indicated that there was an emperor penguin refuge at Cape Roget in the Ross Sea. This colony, unlike all other emperor penguin populations in this study, did not experience a population bottleneck during the LGM, and also had significantly high genetic diversity consistent with an older, ancestral population. The Cape Roget colony is proximate to the proposed location of an LGM polynya offshore from the Adare Peninsula that could have supported a refuge population of emperor penguins, by providing foraging access amidst the extensive sea ice field. This region in the north-western Ross Sea may have been an important penguin breeding habitat for at least 50,000 years and should therefore be considered in any future management for conservation.

While decadal monitoring studies provide invaluable data on the short-term environmental sensitivities of predator populations, given the long-term nature of projected climate change it is also prudent to consider the climate-driven responses of populations over longer time scales. This study suggests that interspecific differences, even for sympatric species with similar ecological niches, are likely to affect the future climate change responses of Southern Ocean marine predators and should be considered in future conservation plans. The findings also highlight the importance of protecting productive foraging grounds proximate to breeding locations, as well as the potential role of polynyas as future Southern Ocean refugia.

MAP OF ANTARCTICA
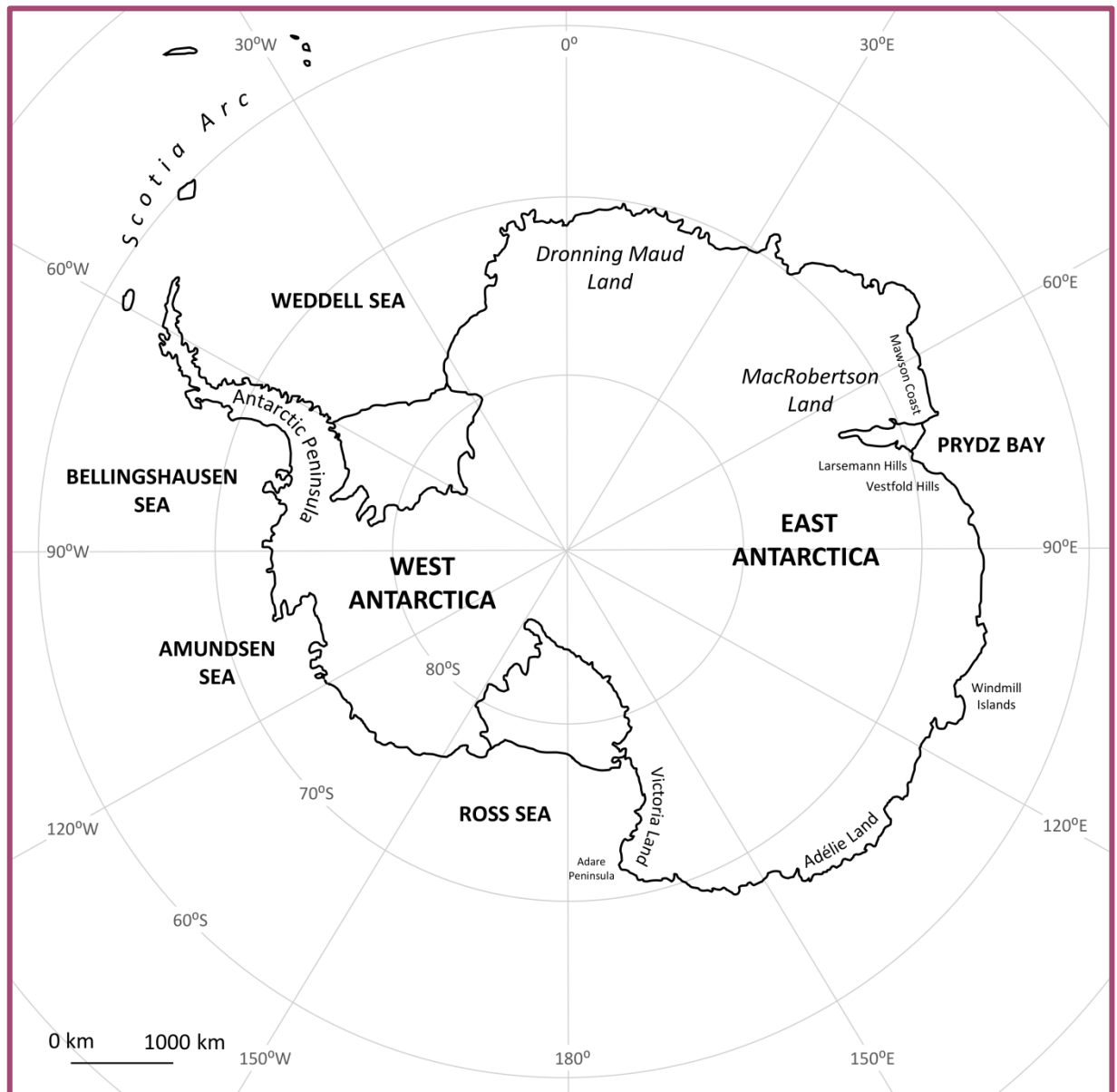
CHAPTER 1

A REVIEW OF PALAEOECOLOGICAL CHANGES IN SOUTHERN OCEAN MARINE PREDATOR POPULATIONS AND THEIR ENVIRONMENTAL DRIVERS

1.1 Introduction

Southern Ocean marine ecosystems have undergone rapid physical changes in recent decades, including increases in air and ocean temperatures, changes in the extent and seasonality of sea ice, decreasing salinity, and poleward shifts of oceanographic fronts (Böning *et al.*, 2008, Bracegirdle *et al.*, 2008, Sokolov & Rintoul, 2009, Stammerjohn *et al.*, 2012, Turner *et al.*, 2009, 2014). In turn, the changes in the physical environment are driving change in marine biota at all trophic levels, including fish, cephalopods and zooplankton such as Antarctic krill (*Euphausia superba*), all of which are important prey species for higher-order predators such as seabirds and marine mammals (Constable *et al.*, 2014). Further physical changes are projected by climate models into the future (Collins *et al.*, 2013a). Collectively, these changes are likely to have profound implications for Southern Ocean marine predators, with effects varying by both species and location, and expected to impact both breeding habitat and marine foraging grounds (Constable *et al.*, 2014, Smetacek & Nicol, 2005).

The ongoing physical and biological changes in the Southern Ocean are the result of a marked shift in the Earth's environmental state (Lewis & Maslin, 2015). This shift is being considered as the dawn of a new epoch, termed the Anthropocene, that is characterised by changes to land, oceans, the atmosphere and evolutionary pressures (Lewis & Maslin, 2015). The extinction rate during this new regime is estimated at 100 to 1,000 times greater than in previous epochs and is likely to result in a sixth mass extinction (Barnosky *et al.*, 2011). In order to understand how species might respond to the unprecedented environmental change predicted for the Anthropocene, we must consider not only their recent population trends over decadal timescales, but also how they responded to similar long-term environmental regime shifts in past epochs.

Evidence of species' histories can be found in various forms such as fossil remains and genetic data. With the right tools this evidence can be used to build a picture of a species' past ecology, known as palaeoecology, over periods of hundreds to millions of years (Seddon *et al.*, 2014). Such palaeoecological evidence provides long-term insight into how species have coped with environmental change in the past. Reconstructing historical trends in abundance and distribution can provide clues about how specific environmental changes affected populations, thereby increasing our understanding of species' environmental niches. If a species was robust to past climate perturbations we might expect them to have good resilience to future change, depending on the direction and pace of the environmental shift. When combined with information on extant population structure and abundance, information on the past processes that shaped population characteristics may enable predictions of how species will respond as habitat availability and quality

changes, and help us to assess the risk of local or widespread extinction (Hoelzel, 2010, Seddon *et al.*, 2014). By comparing trends for different species we can begin to understand which are the most important of these environmental factors.

Predators have an integral role in regulating ecosystems via top-down processes (Beschta & Ripple, 2009, Ritchie *et al.*, 2012); hence how they might respond to environmental change is particularly important. For predators to prosper, productive foraging grounds must exist within reach of suitable breeding habitat. However, what constitutes suitable marine foraging grounds or terrestrial breeding sites varies by species due to differences in their foraging modes and abilities, as well as the required physical characteristics of their breeding sites. Both marine and terrestrial conditions are projected to change across the Southern Ocean, and predator populations in both the sub-Antarctic and Antarctic are likely to be affected. Potential mechanisms for these impacts include, but are not limited to: the loss of breeding habitat in cases where predators use sea ice as a breeding platform; the impact of changing sea ice seasonality on the timing of breeding; the effects of increasing temperatures for species that are highly cold adapted; and changes in prey abundance, distribution and accessibility. Different populations of the same species are likely to experience different impact mechanisms due to regional variation in environmental trends.

Here, historical changes in populations of Antarctic and sub-Antarctic marine predators, specifically seabirds and mammals, are reviewed, compared and placed into context with past environmental changes. I focus on predators with breeding sites that are distributed in and around mainland Antarctica, its offshore islands and the sub-Antarctic islands, because these species utilise the Southern Ocean for both breeding and foraging. Migratory species that periodically reside in the Southern Ocean, including cetaceans, are excluded. In order to restrict this synthesis to purely environmental driven responses, only changes in predator populations that occurred prior to anthropogenic impacts, defined here as 1610 to coincide with the proposed inception of the Anthropocene (Lewis & Maslin, 2015), are considered.

1.2 An overview of Southern Ocean palaeoclimate

The Pleistocene geologic period (2.58 mya to 11.7 kya) was characterized by large-scale global climate oscillations, varying from cold periods of glaciation to warmer interglacial periods (Figure 1.1) (Jouzel *et al.*, 2007). The last glacial maximum (LGM, 26 – 19.5 kya) marks the most recent time of maximum ice sheet extent in Antarctica (Clark *et al.*, 2009). The Southern Ocean during the LGM was a very different environment than it is now. Air temperatures were *ca.* 13°C colder than the present day (Jouzel *et al.*, 2007) and glaciation was extensive, with most of the continental shelf covered by

either marine-based ice sheets or thick, perennial sea ice (Anderson *et al.*, 2002, Bentley *et al.*, 2014, Gersonde *et al.*, 2005). Many sub-Antarctic islands were glaciated (Hodgson *et al.*, 2014) and surrounded by sea ice in the winter (Gersonde *et al.*, 2005). Oceanographic fronts, which are the boundaries between two water masses that tend to be associated with high prey abundance due to both enhanced local primary production and transport of prey to the front by currents (Bost *et al.*, 2009), were displaced northwards by around five degrees of latitude (Figure 1.2) (Gersonde *et al.*, 2005).
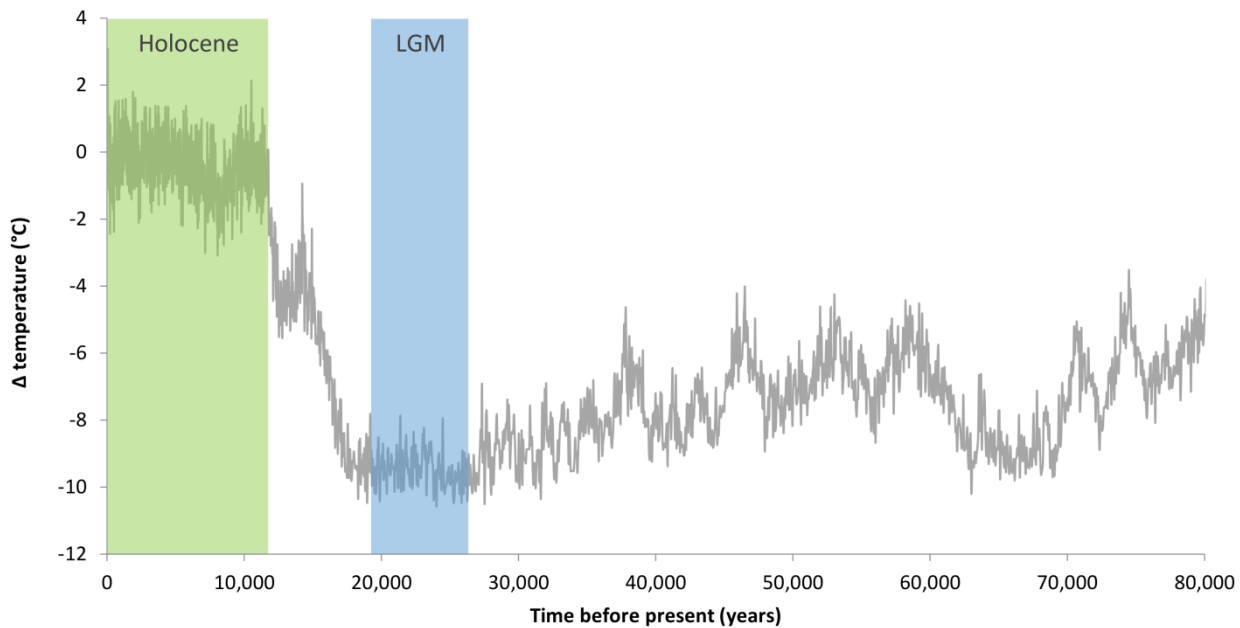
Figure 1.1. The Antarctic temperature anomaly (the difference from the average of the last 1000 years) over the past 80,000 years. Temperatures are as estimated from the EPICA Dome C ice core (Jouzel *et al*., 2007), with the last glacial maximum (LGM) indicated by blue shading and the Holocene by green shading.

The dynamics of Antarctic sea ice are highly complex and can be described in terms of areal extent, concentration, thickness, formation rate and seasonality. Many of these variables are difficult to measure for contemporary sea ice, and reconstructing historical sea ice conditions is an ongoing challenge (Collins et al., 2013b, de Vernal et al., 2013). Fossils of diatom taxa that live in association with sea ice, and have particularly intense blooms at the ice edge, are preserved in marine sediments and can provide an excellent proxy for historical sea ice limits (Allen et al., 2011, Gersonde & Zielinski, 2000). Diatom records are commonly used in Antarctic sea ice reconstructions, often coupled with other evidence such as lithology (Allen et al., 2011), radiolarians (Gersonde et al., 2005), or, more recently, highly branched isoprenoid biomarkers (Collins et al., 2013b). Diatom records indicate that at its maximum extent during the last glacial period the circum-Antarctic winter

sea ice field was approximately double its present area, extending to about 50°S (Figure 1.2) (Allen et al., 2011, Collins et al., 2012, Gersonde et al., 2005). The extent of summer sea ice during the last glacial period is poorly constrained and an area of ongoing study (Collins et al., 2013b), however, the data that are available suggest a northerly expansion to 55°S in some locations (Figure 1.2) (Allen et al., 2011, Collins et al., 2012, Gersonde et al., 2005). Interestingly, the timing of the maximum extent of sea ice pre-dates the LGM (Allen et al., 2011), with maximum winter and summer extents occuring at 25–23.5 kya and 31–23.5 kya, respectively (Collins et al., 2012).

During the LGM, biological productivity south of the Polar Front was greatly reduced (Hillenbrand & Cortese, 2006), with estimates placing primary production at between two and five times less than current values (Kohfeld *et al.*, 2005). This estimate is inferred from export production records in sediment cores, where export production is the portion of primary production that accumulates in sediments (Kohfeld *et al.*, 2005). The low primary productivity during the LGM is likely to have had a regulatory effect on all trophic levels in the Southern Ocean food web by severely limiting the food resources that were available (Thatje *et al.*, 2008).

While overall biological productivity of the Southern Ocean was much reduced during the LGM, polynyas may have provided oases of productivity (Thatje *et al.*, 2008) and access points for predators amidst the extensive sea ice field. Polynyas are areas of open water within the sea ice field that are associated with enhanced primary production, due to an earlier spring melting of sea ice and a resultant earlier start in photosynthetic primary production (Martin, 2001). Coastal polynyas are formed by the outflow of katabatic winds that push sea ice away from the coastline, whereas open-ocean polynyas are generally formed by upwelling of warm, deep water associated with submarine geological formations such as seamounts (Comiso & Gordon, 1987, Holland, 2001, Martin, 2001, Thatje *et al.*, 2008). Sediment cores suggest the existence of LGM polynyas in several locations in the Weddell and Ross Seas (Figure 1.2) (Brambati *et al.*, 2002, Mackensen *et al.*, 1994, Smith *et al.*, 2010, Sprenk *et al.*, 2014, Thatje *et al.*, 2008). Polynyas are thought to have been more widespread during the LGM due to a heightened intensity of katabatic winds (Smith *et al.*, 2010, Sprenk *et al.*, 2014), however the sediment core record around Antarctica is sparse, so the locations of other LGM polynyas are currently unknown.
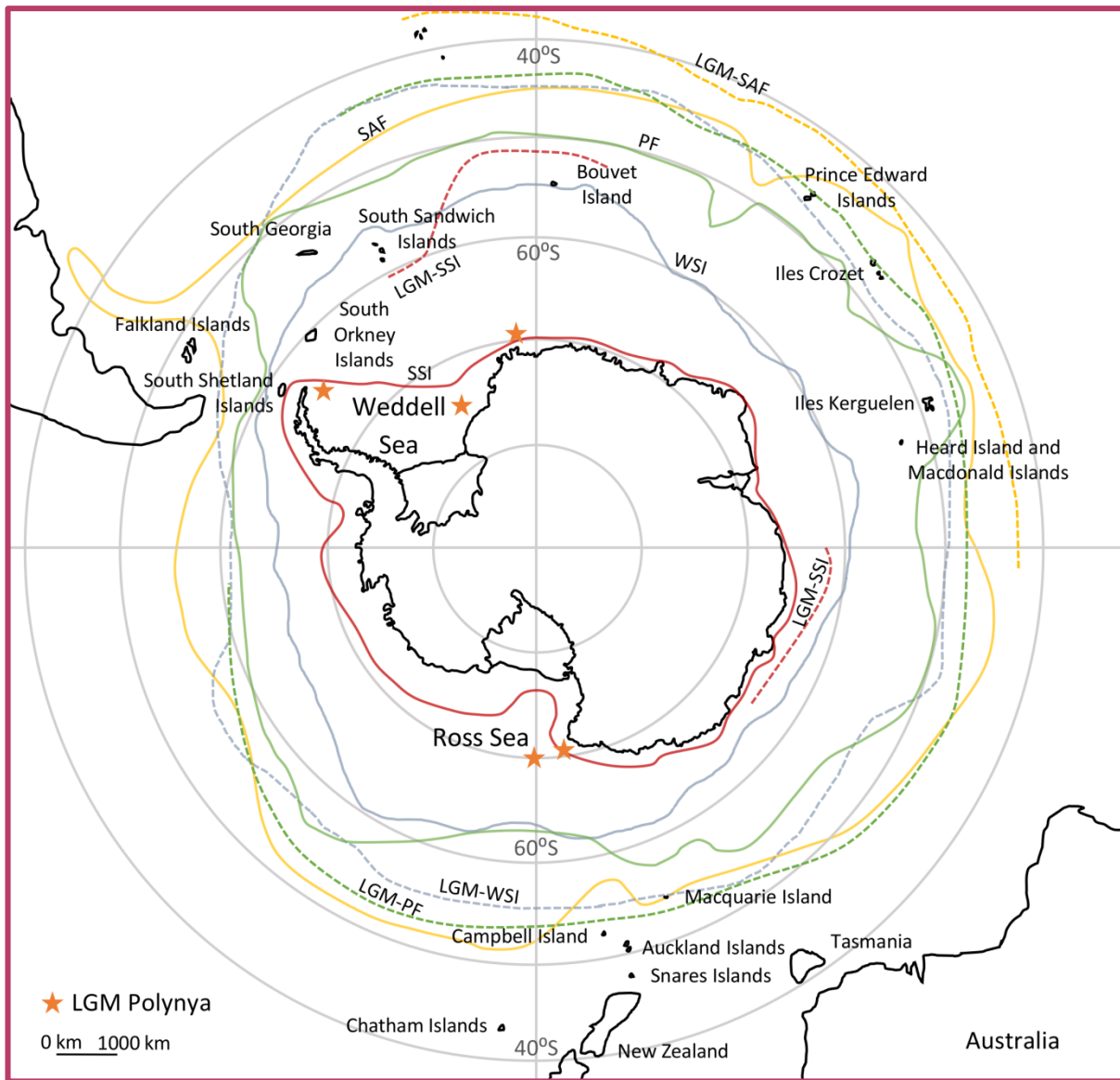
Figure 1.2. Map of the Antarctic and sub-Antarctic with key physical features. The approximate positions of both modern and last glacial maximum (LGM) sea ice extents and oceanographic fronts are indicated, after (Gersonde *et al.*, 2005). The summer sea ice extents (SSI) are indicated by red lines, the winter sea ice extents (WSI) by blue lines, the polar front (PF) by green lines, and the sub-Antarctic front (SAF) by yellow lines. Solid lines indicate modern features, dashed lines are the inferred locations of LGM features. The locations of LGM polynyas are indicated by orange stars (Smith *et al.*, 2010, Sprenk *et al.*, 2014).

Following the LGM, warming temperatures saw the Southern Ocean shift away from glacial conditions and into the warm Holocene (11.7 kya to present, Figure 1.1). The transition to the Holocene was characterised by retreating ice sheets, rising sea levels, increased seasonality of a decreasing sea ice cover, and increasing primary productivity (Anderson *et al.*, 2009, Barbara *et al.*, 2010, Bentley *et al.*, 2014, Crosta *et al.*, 2004, 2008, Denis *et al.*, 2009b, Gersonde *et al.*, 2005, Kohfeld *et al.*, 2005). These environmental responses to climatic changes happened gradually and asynchronously across the Southern Ocean. Throughout the Holocene there have been less dramatic fluctuations in local climate, including shifts in sea level, sea ice cover and some minor readvancement of glaciers.

1.3 Palaeoecology of the Southern Ocean

Evidence of palaeoecological processes can be found in a species' physical remains, including bone, tissue, eggshell or guano. Such evidence can be analysed using a combination of genetic, radioisotopic and geochemical methods, in order to study changes in distribution and abundance over thousands of years, identify locations of refugia and assess dietary changes. When combined with palaeoclimatic evidence, such as ice core and sediment records, it is possible to identify potential environmental drivers of population changes. In combination, these varied approaches can build a compelling picture of the past responses of Southern Ocean predators to environmental change.

Genetic studies using coalescent modelling allow for the estimation of changes in population size through time. In coalescent Bayesian skyline analysis, sequence data from multiple individuals in a population are used to co-estimate genealogies and the effective population size at different points in time (Drummond *et al.*, 2005, Pybus *et al.*, 2000). If calibrating information such as ancient DNA, (see de Bruyn *et al.* (2011) for a comprehensive review), fossils or a known genetic mutation rate is available, the timings of these changes in abundance can be estimated and correlated with palaeoclimate records. A related method is the pairwise sequentially Markovian coalescent (PSMC), which is used to estimate past population sizes using whole genome data from a single individual (Li & Durbin, 2011). This method is effective for estimating population sizes over long time scales (i.e. 100 kya to 10 mya), but is less accurate over shorter time scales compared to Bayesian coalescent methods, making these approaches complementary (Sheehan *et al.*, 2013).

Genetic data may also identify past refugia (Hewitt, 1996, 2000). Phylogenetic analyses can reveal genetically distinct lineages that may have arisen as a result of past isolation in refugia (Hewitt, 1996, 2000). Again, if the analyses can be calibrated, the timings of these events can be determined and

the environmental drivers of isolation in refugia may be thus identified. Telltale patterns of genetic diversity may also be used to pinpoint the locations of past refugia; refugia are typically characterised by a pattern of clinal variation, decreasing with distance from the refuge, arising from founder effects as new areas are colonized following the expansion from the refuge (Hewitt, 1996, 2000).

Radiocarbon dating of organic remains, which can survive for thousands of years in the cold, dry Antarctic environment where microbial degradation is inhibited, can provide valuable palaeoecological data. The occupation history of the snow petrel (*Pagodroma nivea*) has been investigated by radiocarbon dating of solidified stomach oil, called mumiyo, which accumulates among rocks in breeding colonies (Hiller *et al.*, 1988). Dating of these deposits can provide a record of occupation at a given site. Radiocarbon dating of excavated penguin guano sediments, called ornithogenic soils, that contain guano, eggshell fragments and bones can likewise be used to investigate occupation history (Baroni & Orombelli, 1994a, Huang *et al.*, 2009, Millar *et al.*, 2012, Stonehouse, 1970b). Such studies may be supplemented with geochemical analyses of typical bio-elements found in penguin guano, which provide a proxy for the abundance of penguins (Sun *et al.*, 2000) and stable isotopic analyses of eggshells and guano can provide an understanding of diet through time (Emslie *et al.*, 2013). Finally, radiocarbon dated remains found outside a species' current range can provide clues to their distributions under different climatic regimes (Hall *et al.*, 2006).

1.4 Ice-free breeding species – the sub-Antarctic

The sub-Antarctic islands are populated by breeding colonies of many predators, including pinnipeds, penguins, petrels and albatrosses. Of these species, palaeoecological data are currently available for king penguins (*Aptenodytes patagonicus*) and Southern elephant seals (*Mirounga leonina*). Both species congregate in breeding colonies established on coastal ice-free ground; the king penguin breeds exclusively on sub-Antarctic islands (Figure 1.3a) (Bost *et al.*, 2013), while the Southern elephant seal breeds mostly in the sub-Antarctic, with colonies also located in South America and on the Antarctic continent at Palmer Station on the Antarctic Peninsula and in the Windmill Islands in East Antarctica (Figure 1.3b) (Heimark & Heimark, 1986, Lewis *et al.*, 2006, Murray, 1981). Both are meso-predators with wide foraging ranges, extending to the edge of the pack ice in the case of the king penguin (Bost *et al.*, 2004, Moore *et al.*, 1999) and well into the sea ice zone in the case of the elephant seal (Charrassin *et al.*, 2008). Oceanographic fronts are important foraging grounds for both species (Bost *et al.*, 2009, Charrassin *et al.*, 2008). Elephant seals, king penguins and many other sub-Antarctic predators direct their foraging trips toward the Polar Front, the sub-Antarctic Front or the

Subtropical Front (Figure 1.2), as these areas provide a predictable source of prey and good conditions for diving predators (Bost *et al.*, 2009).

1.4.1 King penguins

A genetic coalescent study of king penguins from the Crozet Archipelago revealed a rapid population expansion closely following the end of the LGM (Trucchi *et al.*, 2014). The Crozet king penguin effective population size increased from *ca.* 2,000 during the LGM to *ca.* 170,000 today (Trucchi *et al.*, 2014). While many factors may have contributed to this increase in abundance, the key ecological requirements for king penguins are year-round ice-free breeding habitat and access to productive foraging grounds (Hunt, 1991). There is no definitive evidence that Crozet was glaciated during the LGM, and estimates from sediment core microfossil assemblages place the LGM sea ice field south of Crozet (Figure 1.3a), although it should be noted that both the glacial history and the microfossil record in this region are poorly resolved (Gersonde *et al.*, 2005, Hall, 2009, Hodgson *et al.*, 2014). Hence, the overall indication is that ice-free breeding habitat was available on Crozet during the LGM. It may therefore have been a lack of productive foraging that limited the size of the king penguin population. As previously mentioned, biological productivity was likely much reduced during the LGM (Hillenbrand & Cortese, 2006, Kohfeld *et al.*, 2005), furthermore, the location of king penguin foraging grounds may have shifted during this time. King penguins currently forage almost exclusively near frontal zones (Bost *et al.*, 2009). Crozet is situated in the Polar Frontal Zone, equidistant (~400 km) from the Polar Front and the sub-Antarctic Front, making it ideally positioned for king penguin foraging (Figure 1.3a) (Bost *et al.*, 2009). However, it is likely that both of these fronts were displaced *ca.* 5-10° northwards during the LGM (Figure 1.3a) (Gersonde *et al.*, 2005), possibly increasing the travel distance from Crozet. The combination of shifted feeding grounds with lower overall biological productivity may have reduced prey density to a level that was insufficient to support a large population of king penguins. Indeed, given the inherent variability in past population estimates, it is possible that no king penguins were present in the Crozet Archipelago during the LGM, and this colony was subsequently founded during the Holocene once conditions became more favourable. Studies of king penguins from other contemporary breeding colonies could address this hypothesis and also indicate whether the Holocene population expansion on Crozet was an isolated trend, or occurred elsewhere in the species' range.

1.4.2 Southern elephant seals

Southern elephant seals underwent a large latitudinal range expansion during the Holocene, taking advantage of emerging breeding habitat on the Victoria Land Coast in the Ross Sea *ca.* 7,000 years ago, based on radiocarbon dated remains found in the region (de Bruyn *et al.*, 2014, Hall *et al.*, 2006).
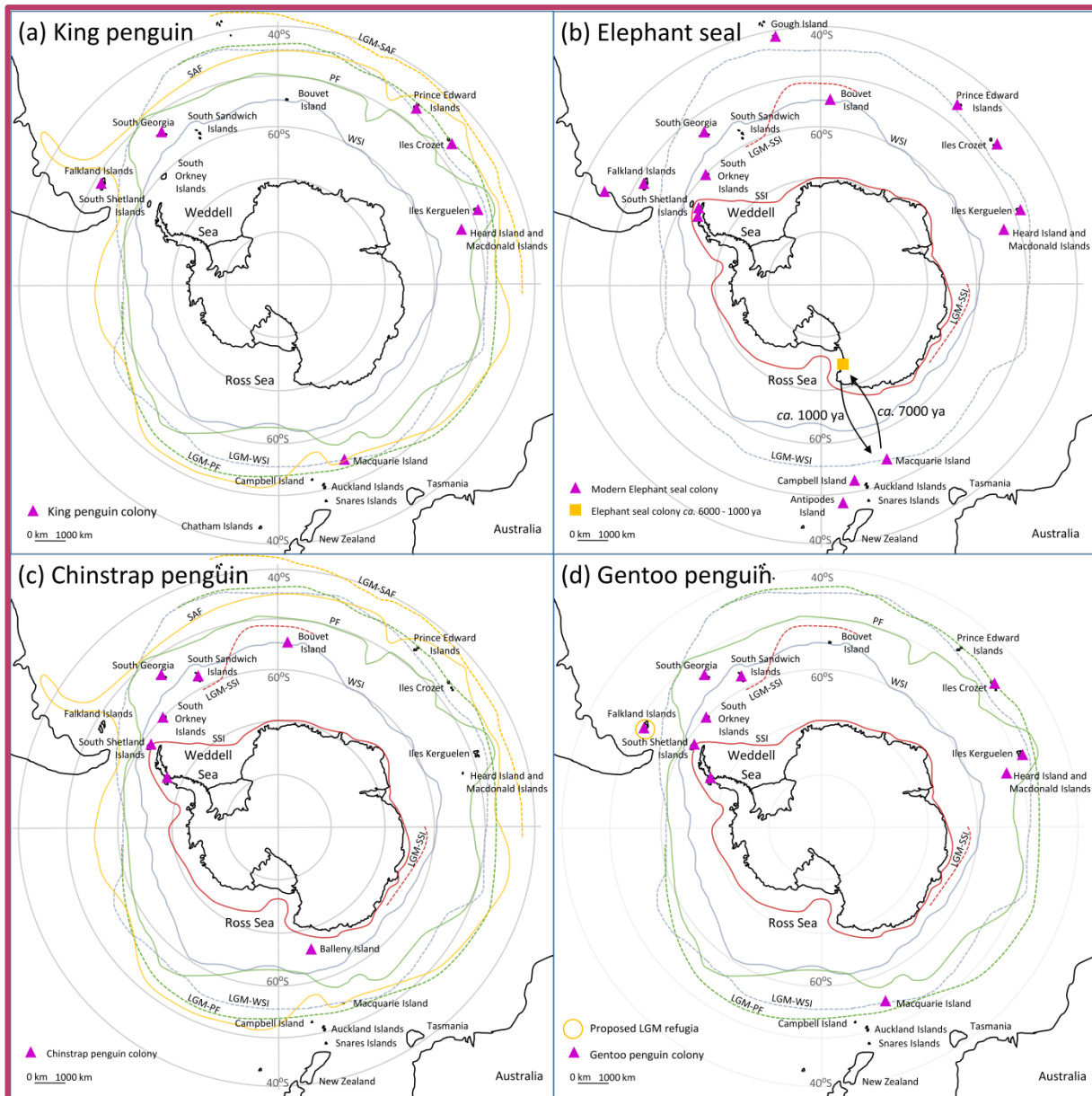
Figure 1.3. Southern Ocean predator breeding distributions showing relevant environmental features.

(e) Adélie penguin

■ Adélie penguin breeding distribution
★ LGM Polynya

(f) Snow petrel

○ Proposed LGM refugia
▲ Snow petrel colony
★ LGM Polynya

(g) Emperor penguin

▲ Emperor penguin colony
★ LGM Polynya

(h) Weddell seal

■ Weddell seal breeding distribution

11
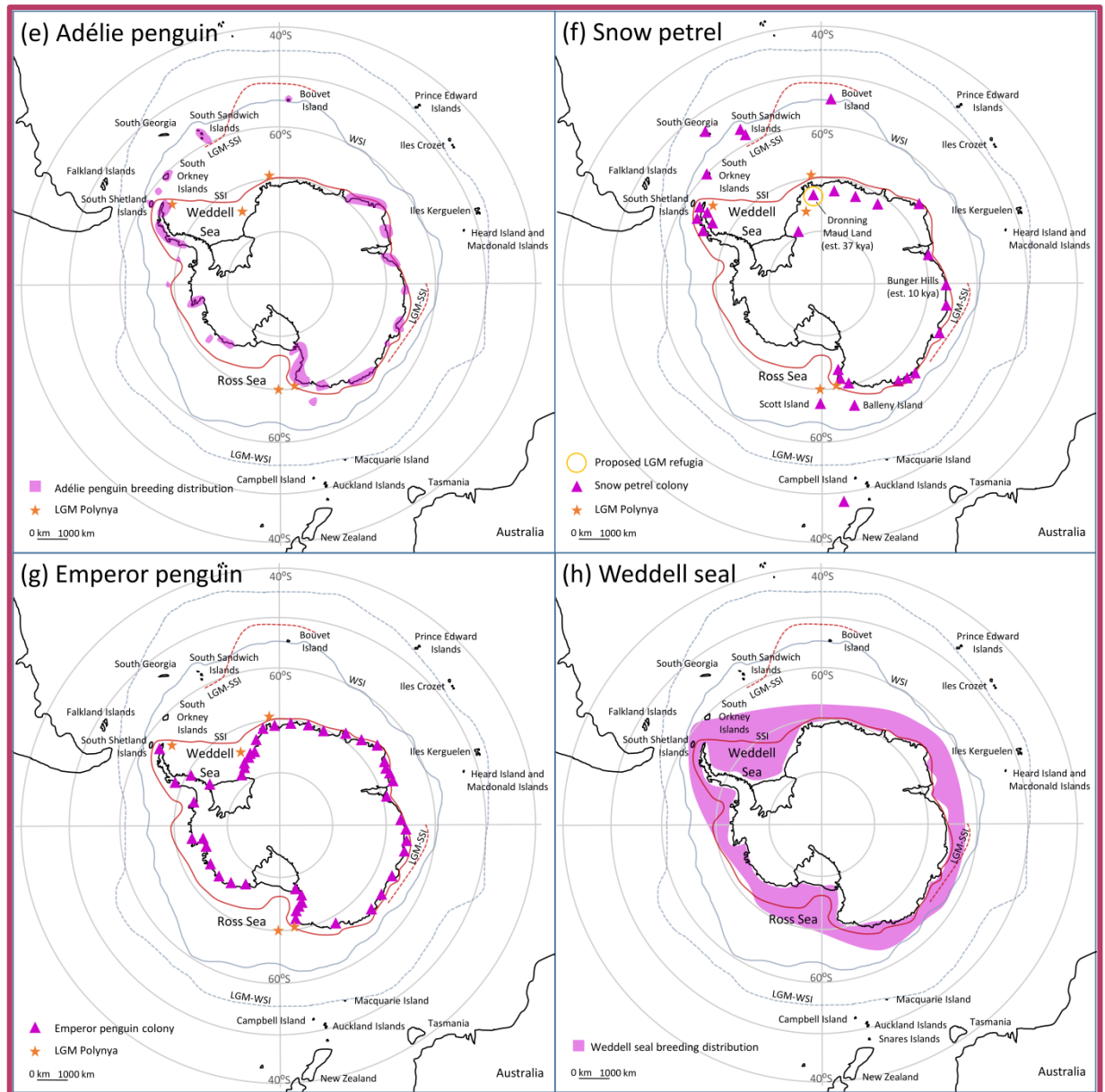
Genetic data indicate that the colonies were most likely founded by individuals originating from Macquarie Island, 2,500 km to the north, representing a long distance colonisation event by a highly mobile species (Figure 1.3b) (de Bruyn *et al.*, 2009, 2014). Elephant seal numbers then fluctuated in the Ross Sea region, until they eventually abandoned the area completely *ca*. 1,000 years ago, retreating to Macquarie Island (de Bruyn *et al.*, 2009, Hall *et al.*, 2006).

It has been postulated that the availability of ice-free breeding habitat, as determined by glaciation, sea ice extent and seasonality, is the major limiting factor of elephant seal distribution (de Bruyn *et al.*, 2009, Hall *et al.*, 2006). Elephant seals currently maintain several small colonies on ice-free areas of the Antarctic continent; however, no breeding or moulting sites exist within the Ross Sea region today, with the Victoria Land Coast presently bounded by fast ice for the majority of the year, thereby precluding the establishment of breeding colonies (Hall *et al.*, 2006). Beaches free of glacial ice were probably released on the Victoria Land Coast between 8,000 and 7,500 years ago, based on the grounding-line retreat of the West Antarctic ice sheet (Conway *et al.*, 1999). Taylor Dome ice core records also indicate warmer conditions coincident with elephant seal occupation of the Ross Sea (Monnin *et al.*, 2004), with these conditions possibly resulting in reduced sea ice concentration in the area and therefore open water adjacent to the shore (Hall *et al.*, 2006). Around 1,000 years ago, coeval with the final abandonment of the region by elephant seals, glacial advance began to overrun the Holocene beaches (Baroni & Hall, 2004, Baroni & Orombelli, 1991, 1994b) and methanesulfonic acid records from nearby Newall Glacier indicate an increase in sea ice extent (Mayewski *et al.*, 1995). It is unlikely that prey distribution was a factor limiting the establishment of the Holocene Ross Sea elephant seal breeding colonies, as they forage in this region today but return to the sub-Antarctic to breed and moult (Hall *et al.*, 2006).

If ice-free breeding habitat suitable for elephant seals was available during the mid to late Holocene in the Ross Sea, then it may have also been suitable for king penguin occupation. However, no king penguin remains have been discovered in the region. The most probable explanation is that king penguins did not venture into the Ross Sea for foraging, and therefore did not have the opportunity to discover emergent breeding habitat in the region. Today, king penguins from Macquarie Island forage almost exclusively in the Polar Frontal Zone, well north of the Ross Sea (Sokolov *et al.*, 2006). While Macquarie Island elephant seals also forage largely in the Polar Frontal Zone, they occasionally forage much further south over the Antarctic continental shelf and in the Ross Sea (Bradshaw *et al.*, 2003, Charrassin *et al.*, 2008, Hindell *et al.*, 1991). It appears that the wide-ranging foraging habits of the elephant seal may convey an advantage in discovering newly available breeding habitat.

Given that many of the sub-Antarctic islands that are currently host to elephant seal breeding colonies were probably glaciated and/or surrounded by sea ice during the LGM (Gersonde *et al.*, 2005, Hodgson *et al.*, 2014), it is likely that elephant seal populations were either reduced in size or displaced northwards, possibly to continental South America, which is currently home to several Southern elephant seal breeding colonies (Lewis *et al.*, 2006, Thatje *et al.*, 2008). The population sizes of other elephant seal colonies during the LGM could be investigated in the future using genetic coalescent methods, as has been done successfully for king and *Pygoscelis* penguins.

1.5 Ice-free breeding species – the *Pygoscelis* penguins

The three *Pygoscelis* penguins are the chinstrap (*Pygoscelis antarctica*), the gentoo (*Pygoscelis papua*) and the Adélie (*Pygoscelis adeliae*). Chinstraps and gentoos currently breed in colonies on sub-Antarctic Islands, the Antarctic Peninsula and on maritime Antarctic islands (Figure 1.3c,d), while the Adélie has a circumpolar distribution with breeding colonies located at ice-free areas of the Antarctic continent and some maritime Antarctic islands (Figure 1.3e) (Borboroglu & Boersma, 2013, Lynch & LaRue, 2014, Schwaller *et al.*, 2013). All three species require ice-free ground for nesting (Borboroglu & Boersma, 2013). They arrive at their breeding colonies when the sea ice is most extensive (Emmerson *et al.*, 2011) and build nests out of small rocks (Hinke *et al.*, 2012). This reliance on ice-free ground suggests that Pygoscelids experienced a restriction of their breeding habitat during the LGM and may have retreated into glacial refugia or range-shifted to lower latitudes, at least for breeding purposes. Given that sea levels were lower during the LGM than now, it is possible that many LGM breeding sites are now submerged (Clucas *et al.*, 2014), making the discovery of physical evidence of these sites unlikely. The palaeoecology of the Pygoscelids has been very well studied relative to other Southern Ocean predators, with a wealth of evidence available concerning their distributions and abundances throughout the Holocene.

A common occurrence for all three Pygoscelid penguins throughout the Holocene is the regular shifting of rookery sites (often local altitudinal shifts) in response to fluctuating sea levels and changing coastlines (Tatur *et al.*, 1997). A clear distinction must be made between the effects of climatic changes, such as changes in sea ice conditions, glaciation, prey availability or predation, as opposed to local geologic changes, such as fluctuating sea levels (Tatur *et al.*, 1997). In the first scenario, climate change may result in changes to the overall numbers of penguins and their ranges, whereas in the second scenario, individual nesting sites could be locally relocated or abandoned even when the population is of constant size and the overall range is unchanged (Tatur *et al.*, 1997). There is evidence for local shifts of Pygoscelid rookeries in response to sea level changes across their

distributions (Baroni & Orombelli, 1994a, Emslie & McDaniel, 2002, Emslie *et al.*, 1998, Emslie & Woehler, 2005, Myrcha & Tatur, 1991, Stonehouse, 1970b, Tatur *et al.*, 1997). However, here I will focus on changes in the overall abundance and distribution of these species in response to large-scale climate changes.

1.5.1 Chinstrap penguins

A genetic coalescent study of chinstrap penguins from the Scotia Arc (West Antarctic Peninsula, South Shetland Islands, South Orkney Islands and South Sandwich Islands; Figure 1.3c), representing almost the entire global population of chinstrap penguins, detected a dramatic increase in chinstrap abundance during the Holocene (Clucas *et al.*, 2014). From an LGM female effective population size of *ca*. 2,000 birds, the population began to increase gradually around 10 kya and then rapidly around 7 kya, eventually reaching an effective female population size of almost 100,000 individuals, equating to a 50-fold increase in abundance (Clucas *et al.*, 2014). The increase occurred coeval with deglaciation of the species current range; deglaciation of the South Shetland Islands was underway by *ca*. 14 kya, with open marine conditions present in Maxwell Bay (adjacent to current colonies) by 10 kya (Cofaigh *et al.*, 2014, Hodgson *et al.*, 2014), facilitating the initial range-expansion and gradual increase in abundance. Chinstrap remains from *ca*. 5 kya have been discovered on King George Island in the South Shetlands, placing a minimum age on the colonisation of this island (Emslie *et al.*, 2011, Tatur *et al.*, 1997). Deglaciation of the South Orkney Islands and West Antarctic Peninsula occurred by *ca*. 7.5 kya and 6.8 kya, respectively (Cofaigh *et al.*, 2014, Hodgson *et al.*, 2014), opening up further chinstrap habitat and facilitating the rapid increase in abundance from *ca*. 7 kya.

The evidence suggests that chinstrap penguins rapidly colonised new breeding habitat as it became available during deglaciation. The locations of chinstrap colonies during the LGM are unknown, but the species was most likely distributed further north than they are now, as their current breeding locations were glaciated at this time.

1.5.2 Gentoo penguins

Modern gentoo penguins are split into two sub-species, the northern (*Pygoscelis papua papua)* and southern (*Pygoscelis papua ellsworthii*) gentoos, which are currently distributed on either side of the Polar Front (Figure 1.3d) (Stonehouse, 1970a). Phylogenetic analyses indicate that the two sub-species diverged either during the LGM or just after it, with their most recent common ancestor dated at between 11 and 59 kya (Clucas *et al.*, 2014). There are two possible scenarios for how the sub-species arose. Firstly, if the divergence occurred during the LGM it is likely that the two sub-species represent two glacial refuge populations that were geographically isolated during the LGM for long enough to diverge genetically (Clucas *et al.*, 2014). In the second scenario, that of divergence

following the end of the LGM, penguins originating from a single LGM gentoo population in the north may have colonised new areas south of the Polar Front as more breeding habitat became available following deglaciation, thus forming the southern gentoo sub-species (Clucas *et al.*, 2014). The second scenario is the more likely, as the current breeding habitat of the northern gentoo penguins on the Falkland Islands was not glaciated during the LGM (Hodgson *et al.*, 2014) and was also north of the maximum sea ice extent (Gersonde *et al.*, 2005), suggesting that it was ice-free and probably suitable for nesting. The Falkland Islands could therefore have supported an LGM population of gentoos (Figure 1.3d), which were able to expand south following the deglaciation of their current range from *ca*. 14 kya (for Maxwell Bay, South Shetland Islands) (Cofaigh *et al.*, 2014). This scenario is supported by genetic coalescent analyses which indicate that while both sub-species increased in number during the Holocene, the size of the northern gentoo population gradually increased by approximately three-fold from *ca*. 9 kya (Clucas *et al.*, 2014), while the southern gentoos increased far more rapidly, by about 70-fold commencing *ca*. 13 kya, consistent with expansion into new habitat (Clucas *et al.*, 2014, Peña *et al.*, 2014). Gentoo penguin remains dated at *ca*. 4.5 kya have been discovered on King George Island in the South Shetlands, placing a minimum date on the colonisation of this island (Del Valle *et al.*, 2002). The slight increase in the northern gentoo population can probably be attributed to increasing biological productivity in the Southern Ocean at this time (Anderson *et al.*, 2009, Denis *et al.*, 2009b, Kohfeld *et al.*, 2005).

1.5.3 Adélie penguins

The palaeoecology of Adélie penguins has been very well studied, more so than any other Southern Ocean predator. The existence of abandoned Adélie penguin rookeries was first noted during the *Terra Nova* expedition (Debenham, 1923) and the first radiocarbon dating of sub-fossil remains was performed in the 1960s (Stonehouse, 1970b). Many studies on the relationship between Adélie penguins and climate changes followed and the topic has been recently reviewed by Millar *et al*. (2012) and Sun *et al*. (2013). In light of this, I will give only a brief overview of Adélie penguin population changes in relation to historical climate change.

A PSMC analysis of an Adélie penguin genome from the Ross Sea revealed a gradual increase in Adélie numbers from *ca*. 1 mya, followed by a rapid increase commencing *ca*. 150 kya (Li *et al.*, 2014). This population expansion is coincident with rapid warming in the Antarctic following the end of the penultimate glacial period (Jouzel *et al.*, 2007), and may have been driven by an increase in ice-free ground for breeding (Li *et al.*, 2014). The abundance of Adélie penguins then declined *ca*. 60 kya during a cold period (Figure 1.1), corresponding with reduced ice-free ground available for nesting (Li *et al.*, 2014). Phylogenetic studies of colonies from the Ross Sea and Scotia Arc found

evidence of two genetic lineages that are suggestive of two refuge populations dating to the LGM (Clucas *et al.*, 2014, Lambert *et al.*, 2002, Ritchie *et al.*, 2004). One of these lineages was comprised solely of individuals from modern Ross Sea colonies, suggesting that a refuge may have been situated somewhere in the vicinity of the Ross Sea during the LGM (Ritchie *et al.*, 2004). The second lineage was comprised of individuals from the Scotia Arc, Ross Sea and East Antarctica (Clucas *et al.*, 2014, Lambert *et al.*, 2002, Ritchie *et al.*, 2004). However, the individuals sampled for the East Antarctic region were from two colonies only and, given the length of the coastline, may not reflect the broader region. As this second lineage shows no strong geographic affinity, the location of its associated LGM refuge is unknown.

A genetic coalescent study of Adélie penguin populations from the Antarctic Peninsula and Scotia Arc (Figure 1.3e) indicated an LGM effective population size roughly one tenth of the current population size (Clucas *et al.*, 2014). The increase in abundance began *ca*. 16 kya (Clucas *et al.*, 2014), following the end of the LGM and coinciding with deglaciation of the region (Cofaigh *et al.*, 2014, Hodgson *et al.*, 2014). It is likely that Adélie penguins in this region were displaced north during the LGM and then underwent a latitudinal range-shift and population expansion as habitat became available to the south, similar to gentoo and chinstrap penguins in the same region (Clucas *et al.*, 2014). It is interesting to note that the expansion of Adélie penguins predates that of the other Pygoscelid penguins by a few thousand years (Clucas *et al.*, 2014). A possible explanation for this is that the Adélie penguin's enhanced proclivity for sea ice compared to the other two Pygoscelid species allowed it to colonise southerly habitat at an earlier stage of sea ice retreat.

More recently, Adélie penguin nesting locations and local abundances have fluctuated regularly throughout the Holocene as glaciation and sea ice cover changed (Baroni & Orombelli, 1994a, Emslie & McDaniel, 2002, Emslie *et al.*, 1998, 2007, Emslie & Woehler, 2005, Huang *et al.*, 2009, Millar *et al.*, 2012). A common finding of many of these studies is of an Adélie penguin "optimum" in the late Holocene (*ca*. 3–5 kya), corresponding to a time of maximum Adélie penguin numbers in many regions of continental Antarctica (Baroni & Orombelli, 1994a, Emslie *et al.*, 2007, Emslie & Woehler, 2005, Huang *et al.*, 2009).

Overall, the distribution of Adélie penguin breeding colonies is thought to be constrained by sea ice conditions (Millar *et al.*, 2012). The Adélie penguin occupies a narrow habitat optimum between too much sea ice (insufficient nesting habitat and access to foraging grounds) and too little sea ice (insufficient foraging grounds), leading to their description as a "bellwether of climate change" (Ainley, 2002, Fraser *et al.*, 1992).

1.6 Ice-free breeding species – flying seabirds of the Antarctic continent

Antarctica is home to breeding populations of several flying seabird species, which nest at ice-free sites along the Antarctic coastline and offshore islands. However, historical population data are only available for the snow petrel; as such, the population trends of Antarctic flying seabirds in relation to past climate change is an area warranting much further study, given that seabirds are particularly vulnerable to contemporary climate change (Jenouvrier, 2013).

1.6.1 Snow petrels

Snow petrels require ice-free rock for their colony sites and typically establish nests in cavities created by large boulders on nunataks, rocky hills or mountains, located anywhere from the coast to up to several hundreds of kilometres inland (Figure 1.3f) (Ainley *et al.*, 2006, Goldsworthy & Thomson, 2000, Verkulich & Hiller, 1994). The locations of snow petrel nesting sites are governed by the availability of suitable cavities with access to productive feeding locations, usually within a day's flight of the sea ice field (Ainley *et al.*, 2006, Fraser & Ainley, 1986, Hiller *et al.*, 1988).

Snow petrel occupation histories are available for several locations based on radiocarbon dating of mumiyo deposits. Snow petrels have been present in Dronning Maud Land from at least *ca*. 37 kya (Figure 1.3f) and were widely distributed in the region throughout the LGM (Hiller *et al.*, 1988, 1995, Steele & Hiller, 1997, Thor & Low, 2011). Interestingly, snow petrels only colonised the ice-free Bunger Hills region *ca*. 10 kya (Figure 1.3f), after which time they were continuously present in the area, with periods of rapid population expansion between 6 and 8 kya and again from 2 kya (Verkulich & Hiller, 1994). The timing of the initial colonisation of the region is noteworthy, as the Bunger Hills were largely deglaciated both prior to and during the LGM (Gore *et al.*, 2001, Mackintosh *et al.*, 2014) indicating that ice-free nesting sites were available, suggesting that alternative factors may have prevented snow petrel colonisation of this area prior to 10 kya.

Access to feeding grounds may have limited the locations of snow petrel colonies during the LGM when sea ice extent was much greater than it is now, placing open water further from nesting sites (Gersonde *et al.*, 2005). It has been proposed that snow petrel persistence in Dronning Maud Land throughout the LGM was made possible by polynyas proximate to the coast that provided feeding grounds (Hiller *et al.*, 1988, Thatje *et al.*, 2008). There is sediment core evidence for polynyas in this region during the LGM, the nearest of which is only 300 km from the LGM nesting sites in Dronning Maud Land (Figure 1.3f) (Thatje *et al.*, 2008). There is no evidence of any LGM polynyas proximate to the Bunger Hills that could have supported snow petrels. Snow petrel settlement of the Bunger Hills

may therefore have been limited by sea ice extent in the region, with settlement occurring in the early Holocene once sea ice had retreated, placing foraging grounds within reach of nesting sites. The Dronning Maud Land population also expanded during the Holocene; this may have been driven by sea ice retreat that provided more foraging habitat than polynyas alone, therefore supporting greater numbers of snow petrels (Hiller *et al.*, 1995). In both regions, increasing biological productivity from 10 kya (Denis *et al.*, 2009b) may have contributed to a rise in snow petrel numbers.

While the rate of snow accumulation at nesting sites is known to be a factor in the breeding success of snow petrels today (Einoder *et al.*, 2014), there is no historical correlation between accumulation rates and snow petrel occupation at either Bunger Hills or Dronning Maud Land, based on ice core records (Severi *et al.*, 2007, van Ommen *et al.*, 2004).

1.7 Antarctic sea ice breeders

Several Southern Ocean predators have life cycles that are closely tied to the seasonal Antarctic sea ice field; these predators use the sea ice as a breeding platform upon which to raise their offspring. These ice-dependent predators are likely to be especially vulnerable to climate change (Jenouvrier *et al.*, 2014), as Antarctic sea ice is expected to undergo substantial declines in the future; in the most extreme climate model scenario (RCP8.5), sea ice would be completely absent from East Antarctica during February by 2081–2100 and only retained in small areas of the Weddell and Ross Seas (Collins *et al.*, 2013a).

Emperor penguins and Weddell seals (*Leptonychotes weddellii*) both form breeding colonies on coastal fast ice (Figure 1.3g,h), whereas the leopard (*Hydrurga leptonyx*), Ross (*Ommatophoca rossii*) and crabeater (*Lobodon carcinophagus*) seals breed on icefloes within the pack ice zone (Budd, 1961, Siniff, 1991). Fast ice is the stable region of sea ice that is attached to the continent, whereas pack ice drifts with the currents offshore. Physical remnants of sea ice predators are scarce, as most physical remains are lost to the ocean when the sea ice undergoes its annual melt. In these cases, genetic studies are particularly useful.

1.7.1 Emperor penguins

A PSMC analysis of an emperor penguin genome from East Antarctica indicated a gradual increase in emperor numbers from *ca*. 1 mya to 100 kya (Li *et al.*, 2014). Throughout the period of expansion the Antarctic climate went through two glacial cycles (Jouzel *et al.*, 2007, Li *et al.*, 2014), which had no noticeable effect on emperor penguin numbers. However, the effective population size during this period was less than the LGM effective population size (Li *et al.*, 2014), suggesting that emperor

penguins may not have reached carrying capacity yet, possibly explaining the continued gradual growth of emperor penguin numbers even during glacial periods.

Thatje *et al*. (2008) hypothesised that the emperor penguin, as the only penguin able to breed on sea ice, may have flourished during the LGM when there was a lack of competition for resources. Based on the PSMC method, emperor penguin effective population size was relatively stable during the LGM and Holocene (Li *et al.*, 2014); however this method is known to have low resolution over shorter timescales (i.e. tens of thousands of years), due to the infrequency of coalescent events in a single genome (Sheehan *et al.*, 2013). Trends in emperor penguin abundance and distribution during the LGM and Holocene could be further investigated using coalescent Bayesian skyline analysis (Drummond *et al.*, 2005, Pybus *et al.*, 2000), which is more sensitive than the pairwise sequentially Markovian method over this time period (Sheehan *et al.*, 2013).

1.8.1 Weddell, crabeater and Ross seals

A genetic study of ice-breeding seals from the Ross Sea determined that both Weddell and crabeater seals in the region underwent historical population expansions based on a mismatch distribution test using 475 bp sequences of the mitochondrial control region, whereas Ross seals showed no evidence of a population expansion based on the same genetic marker (Curtis *et al.*, 2009, 2011). Curtis *et al*. (2009) stated that population expansion occurred 731 kya for Weddell seals and 1.6 mya for crabeater seals. However, these estimates are somewhat implausible, being much older than expansions generally detected using the mitochondrial control region, which is a rapidly evolving region of the genome typically used to detect events that occurred at timescales of tens of thousands of years ago, for example, in elephant seals (de Bruyn *et al.*, 2009). In their follow-up paper (Curtis *et al.*, 2011), the authors corrected the estimate of Weddell seal expansion time to 81 kya, and stated that Curtis *et al*. (2009) had incorrectly reported the expansion time in generations rather than years. However, if the expansion time was 731,000 generations ago, correcting this to years would date the population expansion time at 6.58 mya, based on the generation time of nine years given in (Curtis *et al.*, 2009), which predates the speciation of Weddell seals (which split from the leopard seal between 1.25 and 3.63 mya (Fulton & Strobeck, 2010)), suggesting that there may also be an error in the original estimate of 731,000 generations (Curtis *et al.*, 2009). It is possible that the estimate of generation length used by Curtis *et al*. is inaccurate. The formula for generation length (*tau*) is *tau* = A + [S/(1-S)], A = average age of maturation and S = annual survival probability of adults. Curtis *et al*. estimated generation length as 9 years, however, using the formula, *tau* is estimated at 19 years for Weddell seals based on an average female maturation of 7.62 years and an annual survival rate of 0.92 for breeding females (Hadley *et al.* 2006). However, a generation length of 19 years would date

the expansion even earlier (i.e. 13.9 million years ago), therefore this is probably not the only error in the calculation. An error in the estimate of the mutation rate could also result in an inaccurate estimate of expansion time, however Curtis *et al.* used a good estimate of the mutation rate for the same marker from the closely related elephant seal (Slade *et al.*, 1998). Therefore, the source of the error remains unclear.

Due to the uncertainty in the estimates of Curtis *et al.* (2009) and (2011), the timing of the population expansions of Weddell seals and crabeater seals are unclear, and it is therefore difficult to postulate any underlying environmental driver. The population trends of Antarctic ice-dependent seals in relation to past climate change could be further investigated using Bayesian skyline analysis, which is a widely used method for detecting past population expansions that has been used successfully for other Southern Ocean marine predators (Clucas *et al.*, 2014, Trucchi *et al.*, 2014).

1.9 Key long-term drivers of Southern Ocean predator populations

While the timing and magnitude of population changes varied by species and location (Table 1.1), this synthesis revealed two key factors underlying long-term Southern Ocean predator population changes; 1) the availability of ice-free ground for breeding, and 2) access to productive foraging grounds.

For those species that require ice-free ground for breeding, the processes of glaciation and sea ice fluctuation were key drivers of population change. The distributions and abundances of elephant seals, snow petrels, gentoo, chinstrap and Adélie penguins all responded strongly to the emergence of new breeding habitat as deglaciation and reductions in sea ice opened up new ice-free areas. The rate and extent of range-shifts in response to new breeding habitat appear to be constrained both by the relative environmental tolerance of the species as well as its mobility. For example, Adélie penguins are more tolerant to sea ice than chinstrap or gentoo penguins and were therefore able to expand earlier in the warming period. The wide-ranging elephant seal took swift advantage of emergent habitat 2,500 km from its main breeding grounds, while the less mobile king penguin did not. This finding highlights the need to take a species' environmental tolerance, dispersal ability and current foraging range into account when considering future range-shifts.

Access to productive foraging grounds was another limiting factor for Southern Ocean predator populations. King penguins and snow petrels both had breeding habitat available to them during the LGM, yet their numbers were much smaller than today. Lower biological productivity of the Southern Ocean during the LGM is likely to have reduced overall prey abundance. Additionally, the proximity

of feeding grounds to breeding sites may have been reduced. The preferred foraging grounds of the king penguins are the polar fronts, which were shifted latitudinally northwards during the LGM. Snow petrels breed on the Antarctic continent and fly to the ocean for foraging; during the LGM, the increased sea ice extent placed open water access further away. In the case of the snow petrel, it appears that colonies only persisted in regions that were proximate to polynyas, which could have facilitated foraging. These findings highlight the importance of productive foraging grounds proximate to breeding locations for Southern Ocean predators. With the physical and biological changes that are currently occurring in the Southern Ocean, management bodies need to recognise the importance of foraging grounds in reach of breeding sites and act to minimise potential impacts in these areas from human activities such as fisheries or pollution.

It has been previously hypothesised that polynyas may have played an important role as seabird refugia in the past (Thatje *et al.*, 2008). The range of snow petrels was reduced during the LGM, with a colony persisting in Dronning Maud Land adjacent to a polynya in the region (Thatje *et al.*, 2008). The location of one of the Adélie penguin refugia (Lambert *et al.*, 2002, Ritchie *et al.*, 2004) was also in the vicinity of known LGM polynyas in the Ross Sea (Brambati *et al.*, 2002, Smith *et al.*, 2010). The role of polynyas as past refugia could be further investigated in the future via genetic analyses of other seabird species, in combination with more complete sediment core records to indicate the locations and sizes of polynyas during the LGM.

Future palaeoecological studies could fill several key gaps in our understanding of the climate change responses of Southern Ocean predators. Ice-breeding predators, including emperor penguins and the Weddell, Ross, crabeater and leopard seals, are vulnerable to declines in sea ice extent; however, their responses to past climate regime shifts are largely unknown. Genetic coalescent and phylogenetic studies across these species' ranges could provide crucial information regarding key environmental drivers of their abundances and distributions, their sensitivities to climate change, and identify past refugia that could be used as potential refugia in the future.

Table 1.1. Summary of Southern Ocean predator population changes reviewed, including species, study location, palaeoecological data used, key population trend, the proposed underlying environmental driver for population trend, the corresponding ecological limitation and key citations.

Species	Location	Coalescent	Phylogeny	Ancient DNA	Subfossils	Other	Population trend	Environmental driver	Ecological limitation	Citations
King penguin	Crozet Archipelago	X					Population expansion (~85x) following end of LGM	Increase in biological productivity, shift of oceanographic fronts	Prey availability	(Trucchi *et al.*, 2014)
Southern elephant seal	Macquarie Island, Ross Sea	X		X	X		Range expansion to the Ross Sea 7 kya; retreat to Macquarie Island 1 kya	Retreat and advance of glacial and sea ice in the Ross Sea	Ice-free breeding habitat	(de Bruyn *et al.*, 2009, Hall *et al.*, 2006)
Chinstrap penguin	Scotia Arc	X					Population expansion (~70x) commencing 10 kya	Deglaciation of the Scotia Arc and Antarctic Peninsula	Ice-free breeding habitat	(Clucas *et al.*, 2014)
Gentoo penguin	Scotia Arc	X	X				LGM refuge at Falkland Is, southerly range expansion and abundance increase (~70x) commencing 9 kya	Deglaciation of the Scotia Arc and Antarctic Peninsula	Ice-free breeding habitat	(Clucas *et al.*, 2014)
Adélie penguin	Scotia Arc, Ross Sea, East Antarctica, Antarctic Peninsula	X	X	X	X	X	Two glacial refugia, one in the Ross Sea; population expansion (~10x) in the Scotia Arc from 16 kya; optimum 3-5 kya	Glaciation, sea ice and sea level changes	Ice-free breeding habitat, sea ice foraging habitat	(Clucas *et al.*, 2014, Millar *et al.*, 2012, Ritchie *et al.*, 2004, Sun *et al.*, 2013)
Snow petrel	Dronning Maud Land, Bunger Hills					X	Dronning Maud Land refuge during LGM, colonised Bunger Hills 10 kya	Polynyas, sea ice retreat, increasing biological productivity	Productive foraging habitat proximate to breeding locations	(Thor & Low, 2011, Verkulich & Hiller, 1994)

1.10 Statement of thesis aims

The overall aim of this thesis is to investigate and compare the responses of Antarctic, sea ice dependent predators to past climate regime shifts using genetic approaches. As sea ice is expected to decline dramatically by the end of this century, I chose to focus on three species that live largely within the sea ice zone, yet utilise the sea ice in different ways. The emperor penguin and Weddell seal both use fast ice as a breeding substrate, but at different times of year, whereas the Adélie penguin nests on ice-free ground, but forages primarily within the sea ice zone. Because Weddell seals and emperor penguins rely on stable sea ice for breeding I hypothesised that their population sizes would be sensitive to changes in sea ice conditions. As Adélie penguins are less dependent on sea ice, I hypothesised that their populations would respond less dramatically to climate change. All three species were investigated in East Antarctica to allow a comparison of their population trends under the same climate change conditions, which vary dramatically around Antarctica. Emperor penguins were also studied around the entire continent, in order to investigate how regional differences in climate change may have differentially affected populations, and also to more thoroughly investigate where past refugia may have been located. Understanding these species' long-term environmental sensitivities may enable better predictions of how they will respond as habitat availability and quality changes in the future, and aid in assessing the risk of local or widespread extinction. This research is timely given the environmental changes currently underway in the Southern Ocean that may impact sea ice dependent predators. The specific thesis aims are to;

1. Reconstruct the population trajectories of emperor penguins around Antarctica during and since the last glacial maximum using Bayesian coalescent inference with mitochondrial DNA sequence data.

2. Reconstruct and compare the trends in abundance of emperor penguins and Weddell seals in East Antarctica during and since the last glacial maximum using DNA sequence data, to test the hypothesis that, as species with overlapping ecological niches and spatial distributions, they may have responded similarly to past climate changes.

3. Investigate the possibility that there was an Adélie penguin refuge population located in East Antarctica during the last glacial maximum using phylogenetic and Bayesian coalescent analyses of mitochondrial DNA sequences.

4. Investigate the possible locations of emperor penguin refuge populations during the last glacial maximum, which I hypothesised to be located in the vicinity of polynyas, using genome-wide single nucleotide polymorphisms.

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CHAPTER 2

TOO MUCH OF A GOOD THING: SEA ICE EXTENT MAY HAVE FORCED EMPEROR PENGUINS INTO REFUGIA DURING THE LAST GLACIAL MAXIMUM

2.1 Abstract

The relationship between population structure and demographic history is critical to understanding microevolution and for predicting the resilience of species to environmental change. Using mitochondrial DNA from extant colonies and radiocarbon-dated subfossils, we present the first microevolutionary analysis of emperor penguins (*Aptenodytes forsteri*) and show their population trends throughout the last glacial maximum (LGM, 26 – 19.5 kya) and during the subsequent period of warming and sea ice retreat. We found evidence for three mitochondrial clades within emperor penguins, suggesting that they were isolated within three glacial refugia during the LGM. One of these clades has remained mostly isolated within the Ross Sea, while the two other clades have intermixed around the coast of Antarctica from Adélie Land to the Weddell Sea. The differentiation of the Ross Sea population has been preserved despite rapid population growth and opportunities for migration. Low effective population sizes during the LGM, followed by a rapid expansion around the beginning of the Holocene, suggest that an optimum set of sea ice conditions exist for emperor penguins, corresponding to available foraging area.

2.2 Introduction

Genetic data both from modern and subfossil samples, palaeoecological niche modelling, and fossil evidence have become vital tools for reconstructing demographic histories (e.g. woolly mammoths (*Mammuthus primigenius*) (Nogués-Bravo *et al.*, 2008) and lions (*Panthera leo*) (Barnett *et al.*, 2014)). Indeed, such studies have shown that species' patterns of genetic diversity and distribution have varied dramatically under different climatic regimes (Carstens & Richards, 2007). Climatic shifts have been one of the major drivers of species' range shifts, fluctuations in abundance, species' extinctions and also in the formation of genetically distinct populations (Hewitt, 1996). As climate change and habitat degradation potentially take us into the sixth mass extinction (Barnosky *et al.*, 2011), it is critical that we understand how species have coped with change in the past to be able to assess their likely responses and resilience to future climate change (Hoelzel, 2010).

Emperor penguins (*Aptenodytes forsteri*) are an iconic Antarctic species whose population genetic structure has not been studied to date. We know little about dispersal among colonies or how historical climate change may have affected their range and abundance. Thus, we have limited capacity to predict how these birds may fare in the future. Projections for continent-wide declines of emperor penguins have been made based on the demographic responses of the Pointe Géologie colony to changes in sea ice conditions (Barbraud & Weimerskirch 2001, Jenouvrier *et al*., 2009,

Ainley *et al.*, 2010, Jenouvrier *et al.*, 2012, Jenouvrier *et al.*, 2014). However, decadal monitoring data are only available for this single site out of 46 known emperor penguin colonies. As such, the climate change responses of emperor penguins across their entire distribution and over millennial timescales are currently unknown (Ainley *et al.*, 2010).

Emperor penguins are highly reliant on sea ice throughout most of their breeding cycle. Mating and incubation takes place on land-fast sea ice in most of the known colonies (Fretwell *et al.*, 2012, Fretwell *et al.*, 2014). During the breeding season, emperor penguins feed on prey that is also sea ice dependent (Gales *et al.*, 1990). Significant areas of open water exist year-round within the Antarctic sea ice zone in the form of leads and polynyas (Zwally *et al*,. 1985 and references therein). These areas are often important in providing emperor penguins access to their underwater foraging habitat when the fast ice extends far from their colonies (Dewasmes *et al.*, 1980). Polynya formation is driven by either upwelling of Circumpolar Deep Water or by the outflow of katabatic winds that push sea ice away from the coastline (Martin, 2001). Polynyas are associated with enhanced primary production, as the reduction in sea ice volume facilitates an earlier spring melting of sea ice and a resultant earlier start in photosynthetic primary productivity (Martin, 2001). Some polynyas are permanent features of the sea ice zone and create areas of hyperproductivity, such as the Ross Sea polynya (Smith & Gordon, 1997), whilst most are smaller and ephemeral features depending on wind and currents.

Changes in the extent and duration of sea ice around Antarctica show highly regionalized trends with some areas increasing or remaining stable while others are decreasing (Vaughan *et al.*, 2013, Zwally *et al.*, 2002). This has an effect on the population dynamics of emperor penguins because both positive and negative sea ice anomalies can result in negative population growth rates at the local scale (Massom *et al.*, 2009, Ainley *et al.*, 2010, Barbraud *et al.*, 2011, Jenouvrier *et al.*, 2014). Despite uncertainties over the rate and extent of sea ice loss that will occur around Antarctica, all climate models project a reduction in the extent and duration of Antarctic sea ice by the end of the century (Collins *et al.*, 2013). As sea ice declines we might expect emperor penguins to be disadvantaged by a lack of breeding habitat (Jenouvrier *et al.*, 2014), unless they have the capacity to alter their preferred choice of breeding site, or range, by colonising new areas. Recent studies have shown more plasticity than expected in the locations of breeding colonies. Satellite imagery suggests that colonies where the fast ice is inadequate at the onset of the breeding season relocated or partially relocated onto ice shelves or icebergs (Fretwell *et al.*, 2014, LaRue *et al.*, 2014). However, as sea ice declines, emperor penguins may also have to contend with altered prey availability and face new threats from predators as changing conditions differentially affect species at other trophic levels (Trathan *et al.*, 2011).

During the last glacial maximum (LGM, 26 – 19.5 kya), the winter sea ice extent was approximately double the present day values, and seasonal variation in sea ice extent is thought to have been greater (Gersonde *et al.*, 2005). It is unclear how this would have affected emperor penguins. Thatje and colleagues (2008) suggested that they may have migrated with the sea ice to lower latitudes, staying within energetic migration thresholds of the ice edge, and could have maintained breeding populations around Antarctica by foraging in the marginal ice zone at the sea ice edge. Alternatively, they could have remained associated with polynyas. Sediment cores suggest the existence of LGM polynyas in several locations, including the north-western Ross Sea, the south-eastern Weddell Sea off Dronning Maud Land, and the north-western Weddell Sea (Mackensen *et al.*, 1994, Brambati *et al.*, 2002, Thatje *et al.*, 2008, Smith *et al.*, 2010). In either case, reductions in overall primary productivity within what is today's seasonal sea ice zone (Domack *et al.*, 1998, Kohfeld *et al.*, 2005) would likely have been detrimental to emperor penguin populations (Ainley *et al*,. 2010).

Little is known about the level of natal philopatry or migration among emperor penguin colonies. Understanding philopatry is particularly important in light of population models that suggest that emperor penguins may be declining as a result of local climatic shifts (Jenouvrier *et al.*, 2009, Jenouvrier *et al.*, 2014). High emigration rates are conceivable amongst emperor penguin colonies; satellite tracking has shown that they travel thousands of kilometres on their juvenile journeys, often passing other colonies (e.g. Kooyman *et al.*, 1996, Thiebot *et al.*, 2013, Wienecke *et al.*, 2010). Generally, philopatry is high amongst penguins (Dehnhard *et al.*, 2014, Saraux *et al.*, 2011), but population structure is absent in many species (e.g. chinstrap penguins (*Pygoscelis antarctica*) (Clucas *et al.*, 2014)) as even low levels of migration can be sufficient to homogenize populations (Hartl & Clark, 1997).

We analyzed the population structure among eight extant emperor penguin colonies (Figure 2.1) using mitochondrial DNA sequences, and inferred population trajectories during and since the LGM using a combination of ancient and modern DNA sequences in a Bayesian coalescent framework (Drummond *et al.*, 2005). This method reconstructs past changes in abundance by estimating the genealogy from sequence data, and co-estimating the effective population size at different points in time (Pybus *et al.*, 2000). We aimed to: 1) investigate how emperor penguin populations were affected by sea ice conditions during and following the LGM; and 2) to test the hypothesis that emperor penguins comprise one panmictic population as a result of the high dispersal of individuals after fledging, and the lack of obvious ecological barriers to dispersal around the Antarctic coastline.
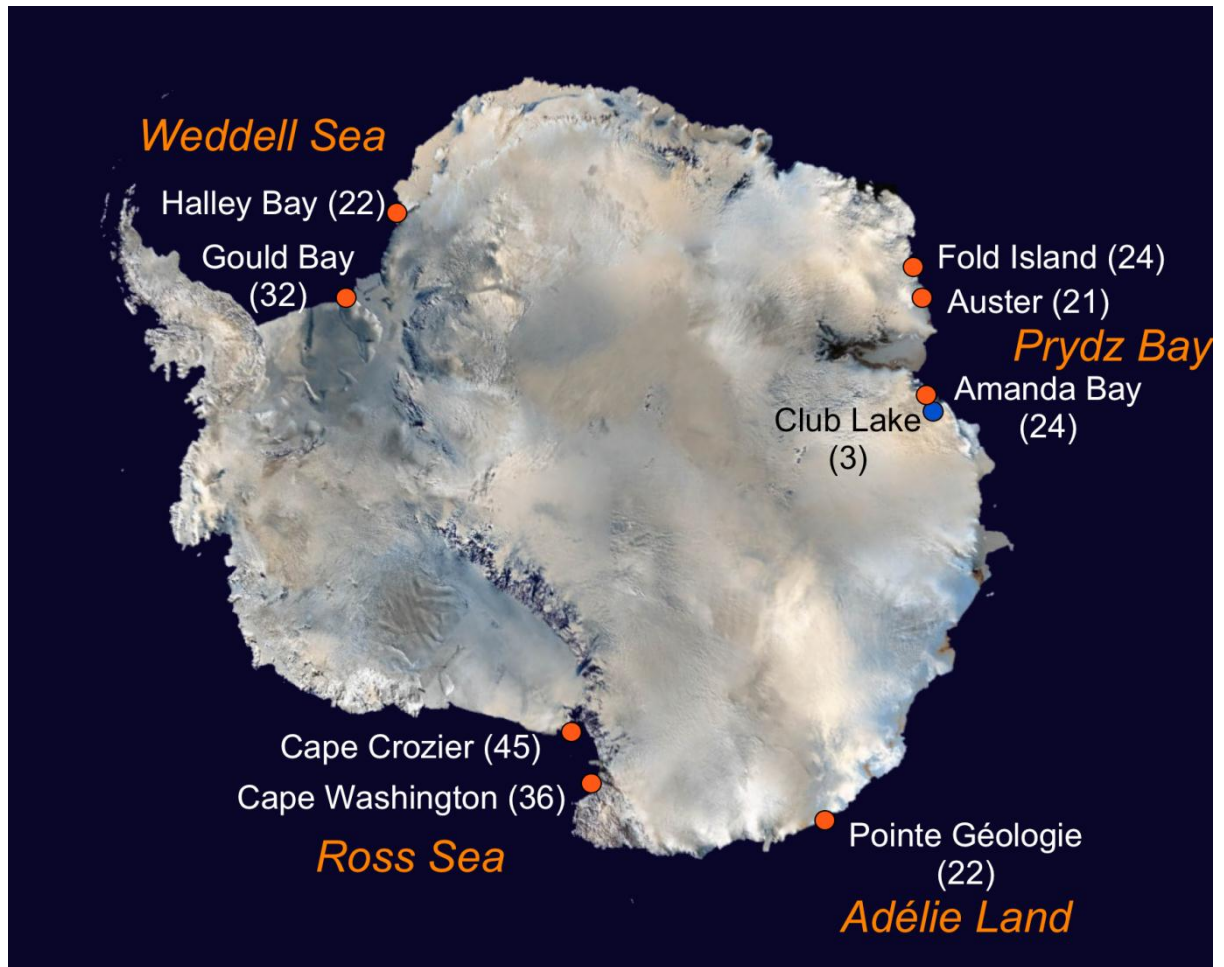
Figure 2.1. Sample locations of emperor penguin colonies. The number of sequences obtained from each location is shown in brackets. Red points indicate the origin of modern samples, the blue point indicates the origin of the subfossil samples.

2.3 Methods

2.3.1 Sample collection

Skin tissue of dead emperor penguins was collected from Halley Bay (see Figure 2.1 for all sample locations) in November 2012 and transported frozen to the UK, where it was transferred to 90% ethanol and stored at -20°C. Blood samples were collected from Gould Bay in December 2013 and transported to the UK at ambient temperature in RNAlater (Life Technologies), and then stored at -20°C. Shed feathers were collected from the Ross Sea between 2010 and 2012, and were transported and stored at -20°C. Shed feathers were collected at least 10 meters apart to minimize sampling the same bird. Pectoral muscle biopsies were collected from dead chicks at Fold Island in September 2010, from Pointe Géologie in December 2010 and from Amanda Bay in December 2012 and 2013. Biopsies were immediately placed in 90% ethanol and stored at -20°C. Whole dead chicks were collected from Auster in September and October in 1993 and 1994 and transported and stored

at -20°C. Bones from the subfossil remains of three penguins were collected at Club Lake in January 2013 and stored at -80°C. Club Lake is an ice-free area in the Vestfold Hills which is currently unoccupied by penguins. The nearest extant colony is Amanda Bay, 95 km away.

Where blood samples were taken, one handler seized the upper body with both hands and restrained the flippers, with the bird's head placed under the arm of the handler to prevent biting and minimize stress (Le Maho *et al.*, 1992). The second handler took blood from the brachial vein using a 25G needle and 1 mL syringe. Total restraint time was generally two to three, but occasionally four, minutes. The bird was then released at the edge of the colony. Sampling was conducted under permits from the UK Foreign and Commonwealth Office, the US National Science Foundation and the Australian Antarctic Division. Each of these permits was issued following independent ethical review of the sampling. All sampling was carried out in accordance with UK Home Office guidelines and also received ethical approval from the University of Oxford, British Antarctic Survey and Australian Antarctic Division. The radiocarbon ages, expressed here as years BP (i.e. before 1950), of the Club Lake remains were determined using accelerated mass spectrometry by GNS Science Rafter Radiocarbon National Isotope Centre, New Zealand. The apparent ages were corrected for the marine-carbon reservoir effect (Gordon & Harkness, 1992) using the calibration program Calib7.0 (ST UI & Reimer, 1993).

2.3.2 DNA extraction, amplification and sequencing

Genomic DNA (gDNA) was extracted from modern samples with the QIAGEN DNeasy Blood and Tissue Kit. The manufacturer's protocols for blood and tissue samples were followed with the following modifications to the digestion step: for blood samples 30 µL of proteinase-K was used and the digestion time was 3 h; for tissue samples 40 µL proteinase-K and an additional 10 µL 1 M dithiothreitol (skin samples only) was used with an incubation time of 32 h. All samples were treated with either 1 µL RNase A (QIAGEN) or 1 µL Riboshredder (Epicentre) according to the manufacturers' instructions. DNA was eluted in 100 µL of elution buffer following an incubation of 5 – 20 min. For subfossil samples ~50 mg of bone was decalcified in 0.5 M EDTA/0.001% Triton X100 at 56°C for 48 h and then extracted using a standard phenol chloroform protocol with ethanol precipitation and a final elution volume of 30 µL. The subfossil samples were extracted in a physically isolated laboratory that had not been used previously for avian samples, with extractions performed inside a laminar flow cabinet to further minimise contamination risk. Negative extraction controls were also used to confirm there was no contamination. The mitochondrial hypervariable region (HVR) and cytochrome *b* (CytB) were sequenced in all modern and ancient DNA samples. HVR is a rapidly evolving region of the mitochondrial genome, and so is suitable for investigations of recent demographic history, whilst

CytB is a conserved gene and can hence give information about longer-term demographic history (Baker & Marshall, 1997). HVR was amplified in all modern samples using primers F-0225 and R-INR (all primer sequences can be found in Appendix 2.2). The reaction mix consisted of 7.5 μL of PCR Master Mix (QIAGEN), 0.2 μM of each primer, and 5 – 10 ng of gDNA, made up to 15 μL with ddH$_2$O. Thermocycling conditions were: 94°C for 3 min; 35 cycles of 94°C for 30 s, 59.5°C for 45 s, 72°C for 1 min; followed by an extension period of 72°C for 10 min. Occasionally, double bands were apparent when the PCR product was visualized by electrophoresis. For these individuals, the shorter 755 bp band was extracted from the gel and purified using QIAGEN or Promega gel extraction kits following the manufacturer's instructions. For Fold Island, Amanda Bay, Auster and Pointe Géologie colonies, CytB was amplified using primers B1 (Baker *et al.*, 2006, Kocher *et al.*, 1989) and B6 (Baker *et al.*, 2006) with a reaction mix consisting of 7.5 μL of GoTaq Green Master Mix (Promega), 0.2 μM of each primer, and 5 – 10 ng of gDNA, made up to 15 μL with ddH$_2$O. Thermocycling conditions were: 95°C for 1 min; 35 cycles of 95°C for 20 s, 52°C for 40 s, 72°C for 50 s; then 72°C for 5 min. For the Cape Washington, Cape Crozier, Gould Bay and Halley Bay samples, primers CytB-F1 and CytB-R1 were used with a reaction mix consisting of 7.5 μL of PCR Master Mix (QIAGEN), 0.2 μM of each primer, and 5 – 10 ng of gDNA, made up to 15 μL with ddH$_2$O. Thermocycling conditions were: 94°C for 3 mins; 35 cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 1 min; then 72°C for 10 min. For the subfossil samples we designed novel, species-specific primers (Appendix 2.2) to amplify short (<150bp) overlapping fragments in order to improve the success rate of amplification from degraded DNA. The sub-fossil PCRs were done separately from the modern PCRs, and set up in a laminar flow hood in a physically isolated laboratory that is never exposed to PCR products. Negative PCR controls were used for both sub-fossil and extant reactions. The reaction mix consisted of 7.5 μL of AmpliTaq Gold 360 Master Mix (Life Technologies), 0.2 μM of each primer, and 25 – 50 ng of gDNA, made up to 15 μL with ddH$_2$O. Thermocycling conditions were: 95°C for 10 min; 42 cycles of 95°C for 20 s, T$_m$ (primer) for 20 s, 72°C for 20 s; 72°C for 5 min. PCR products for Fold Island, Amanda Bay, Auster, Pointe Géologie and the subfossil samples were bi-directionally sequenced by the Australian Genome Research Facility (AGRF) via the Sanger sequencing method using the PCR primer pairs. PCR products for Gould Bay, Halley Bay, Cape Washington and Cape Crozier were sequenced using the Sanger method by Macrogen Europe. The reverse primer for the HVR and the forward primer for CytB were used to sequence each product twice, as these were found to work best in the sequencing reaction. Geneious v5.5.9 was used for alignment. A high number of heteroplasmic sites were found in the HVR and these were re-scored manually according to IUPAC ambiguity codes. No heteroplasmic sites were recorded in the CytB sequences.

2.3.3 Data analysis – summary statistics and population structure

Arlequin v3.5 (Excoffier & Lischer, 2010) was used to calculate summary statistics for HVR, CytB and concatenated HVR and CytB. jModeltest (Posada & Buckley, 2004) was used to estimate the best substitution model for each dataset, and then the following corrections for calculating genetic distances were implemented in Arlequin: HVR - Tamura correction with a gamma distribution for rate heterogeneity with α = 0.016; CytB – Tamura correction; concatenated – Tamura correction with a gamma distribution for rate heterogeneity with α = 0.109 (Tamura, 1992). Arlequin was also used to calculate pairwise genetic distances (θ_{ST}) between colonies and perform analyses of molecular variation (AMOVA) on the concatenated sequences with the Tamura & Nei correction. Network v4.612 (Fluxus Technology Ltd.) was used to draw haplotype networks.

2.3.4 Data analysis - demographic histories

Bayesian phylogenetic analyses and demographic reconstructions were performed using BEAST v1.8 (Drummond *et al.*, 2012). The dataset was partitioned into HVR and CytB, with a nucleotide substitution model of HKY (Hasegawa *et al.*, 1985) with four gamma categories for HVR and TN93 (Tamura & Nei, 1993) for CytB, with ambiguous states permitted. We used the coalescent Extended Bayesian Skyline Plot tree prior (Heled & Drummond, 2008) with a strict molecular clock. For molecular clock calibration, the HVR substitution rate prior was specified as a normal distribution around a mean value of 0.55 substitutions/site/Myr (SD = 0.15), to reflect the substitution rate of the HVR in Adélie penguins (*Pygoscelis adeliae*) (Millar *et al.*, 2008). In the absence of a published substitution rate for CytB in penguins we used a uniform prior of $5x10^{-4}$ to $5x10^{-1}$ substitutions/site/Myr with a starting value of $2x10^{-2}$ (Weir & Schluter, 2008). The corrected radiocarbon ages of the Club Lake samples were input as tip dates, for additional calibration of the molecular clock. Based on these initial priors, substitution rates for our dataset were estimated during the analysis. The posterior distributions of substitution rates, phylogenetic trees and effective population size through time were generated using the Markov chain Monte Carlo (MCMC) sampling procedure, implemented in BEAST, which was run for 120 million generations with samples drawn every 6,000 steps and the first 10% discarded as burn-in. Tracer v.1.5 was used to check effective sample size (ESS) values to confirm convergence with all values >200. Three independent BEAST analyses were performed to ensure reproducibility of the posterior distribution. The population size parameter of the demographic model (N_e*tau) was converted to N_{ef} by dividing the parameter by 14 years, which is the estimated generation length of emperor penguins (Forcada & Trathan, 2009, Jenouvrier *et al.*, 2005). Phylogenetic trees were visualised using FigTree v1.4.

2.4 Results

2.4.1 Present day population structure

We sequenced 226 individuals from eight colonies (Figure 2.1) plus three subfossil birds whose ages ranged from 643 – 881 years BP (after correction for marine reservoir effect). We sequenced 629 bp of the mitochondrial hypervariable region (HVR) and 867 bp of cytochrome *b* (CytB) from each individual (GenBank accession numbers KP644787 - KP645015 and KP640645 - KP640873, respectively). Genetic diversity was extremely high for the HVR, with 220 haplotypes recorded out of the 229 individuals sequenced; the mean number of pairwise differences between haplotypes was 20.62 ± 9.14 (Table 2.1). Genetic diversity was much lower for CytB, with just 59 unique haplotypes recorded.

Our results show a high level of gene flow among all the EAWS colonies (East Antarctica including Adélie Land, and the Weddell Sea) and between the two Ross Sea colonies (Table 2.2), but little exchange between the EAWS and Ross Sea colonies (pairwise θ_{ST} values range from 0.213 to 0.617, Table 2.2). When colonies are grouped into two populations (Ross Sea and EAWS), a high proportion (17.7%) of the genetic variation is explained by the difference between the groups, and there is strong and significant genetic differentiation between them (AMOVA, F_{ST} = 0.196, p < 0.001). This pattern is also evident from haplotype networks (Appendix 2.1), which show that Ross Sea individuals tend to be closely related, whilst sequences from EAWS colonies tend to cluster independently from the Ross Sea haplotypes. However, some Ross Sea sequences are found across the network, and vice versa. This could indicate low-level gene flow between the Ross Sea and EAWS populations.

Table 2.1. Summary statistics by geographic and genetic region. n = number of individuals; N_H = unique haplotypes; N_P = polymorphic loci; H = haplotype diversity; π = mean number of pairwise differences between sequences; significance is indicated for Tajima's D and Fu's F_S test statistic where * denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$.

Geographic Region	Genetic Region	n	N_H	N_P	H	π	Tajima's D	Fu's F_S
All sequences	HVR + CytB	229	222	205	0.999 ± 0.000	23.12 ± 10.21	-1.03	-23.63**
Ross Sea	HVR + CytB	81	80	124	0.999 ± 0.002	18.49 ± 8.28	-0.903	-22.84***
East Antarctic & Weddell Sea	HVR + CytB	148	145	171	0.999 ± 0.001	22.15 ± 9.81	-0.930	-23.80**
All sequences	HVR	229	220	164	0.999 ± 0.001	20.62 ± 9.14	-0.835	-23.67**
Ross Sea	HVR	81	76	109	0.997 ± 0.003	16.81 ± 7.56	-0.836	-17.21*
East Antarctic & Weddell Sea	HVR	148	144	138	0.999 ± 0.001	19.58 ± 8.71	-0.758	-23.85**
All sequences	CytB	229	59	41	0.864 ± 0.016	2.94 ± 1.54	-1.651*	-21.79***
Ross Sea	CytB	81	26	15	0.876 ± 0.028	1.99 ± 1.14	-0.979	-3.43
East Antarctic & Weddell Sea	CytB	148	41	33	0.797 ± 0.031	2.96 ± 1.56	-1.482*	-12.92***

Table 2.2. Pairwise genetic differentiation between colonies. Pairwise θ_{ST} are presented below the diagonal, and associated *p*-values above the diagonal. Significance is indicated by bold text, where * denotes p < 0.05, ** denotes p < 0.01, *** denotes p < 0.001.

	Gould Bay	Halley Bay	Fold Island	Auster	Amanda Bay	Pointe Géologie	Cape Washington	Cape Crozier
Gould Bay		0.596	0.731	0.560	0.186	0.129	**0.000**	**0.006**
Halley Bay	-0.027		0.566	0.798	0.708	0.301	**0.002**	**0.008**
Fold Island	-0.050	-0.032		0.515	0.323	0.462	**0.000**	**0.007**
Auster	-0.026	-0.086	-0.027		0.595	0.797	**0.000**	**0.000**
Amanda Bay	0.055	-0.058	0.014	-0.038		0.576	**0.000**	**0.000**
Pointe Géologie	0.091	0.029	-0.012	-0.085	-0.033		**0.000**	**0.000**
Cape Washington	**0.355*****	**0.440****	**0.468*****	**0.567*****	**0.617*****	**0.596*****		0.509
Cape Crozier	**0.213****	**0.266****	**0.256****	**0.432*****	**0.447*****	**0.428*****	-0.011	

2.4.2 Population history with respect to climate change

There is evidence of past population expansion in emperor penguins across Antarctica as indicated by our extended Bayesian skyline plots (EBSPs) (Figure 2.2). An almost nine-fold increase in abundance of the EAWS population commenced approximately 12 kya. The Ross Sea population expanded three-fold from approximately 9.5 kya. Superimposing expansion signals over the estimated temperature derived from ice cores (Figure 2.2c), it is clear that population expansion followed the end of the LGM. Tajima's *D* and Fu's F_S statistics provide further support for an expansion of both populations (Table 2.1).

Our phylogenetic analyses indicate three highly supported clades (Figure 2.3), which diverged during the late Pleistocene (97 kya, 95% HPD: 50 –154 kya). One of these clades is comprised predominantly of Ross Sea penguins, whereas the other two are dominated by EAWS individuals.
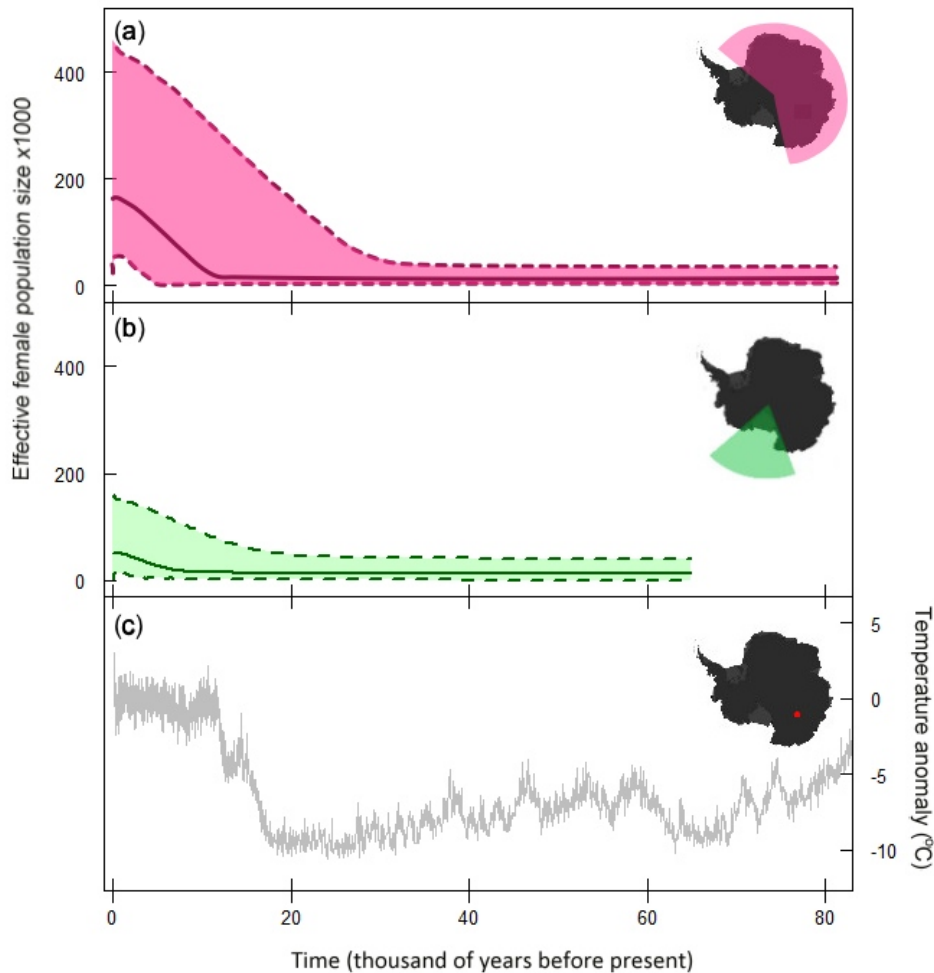
Figure 2.2. Extended Bayesian skyline plots showing the change in effective female population size (N_{ef}). Solid lines show the median estimate; dotted lines show the 95% highest posterior density interval. a) EAWS colonies; b) Ross Sea colonies; c) the Antarctic temperature anomaly (the difference from the average of the last 1000 years) as estimated from the EPICA Dome C ice core (Jouzel *et al.*, 2007), with the ice core location indicated in red.
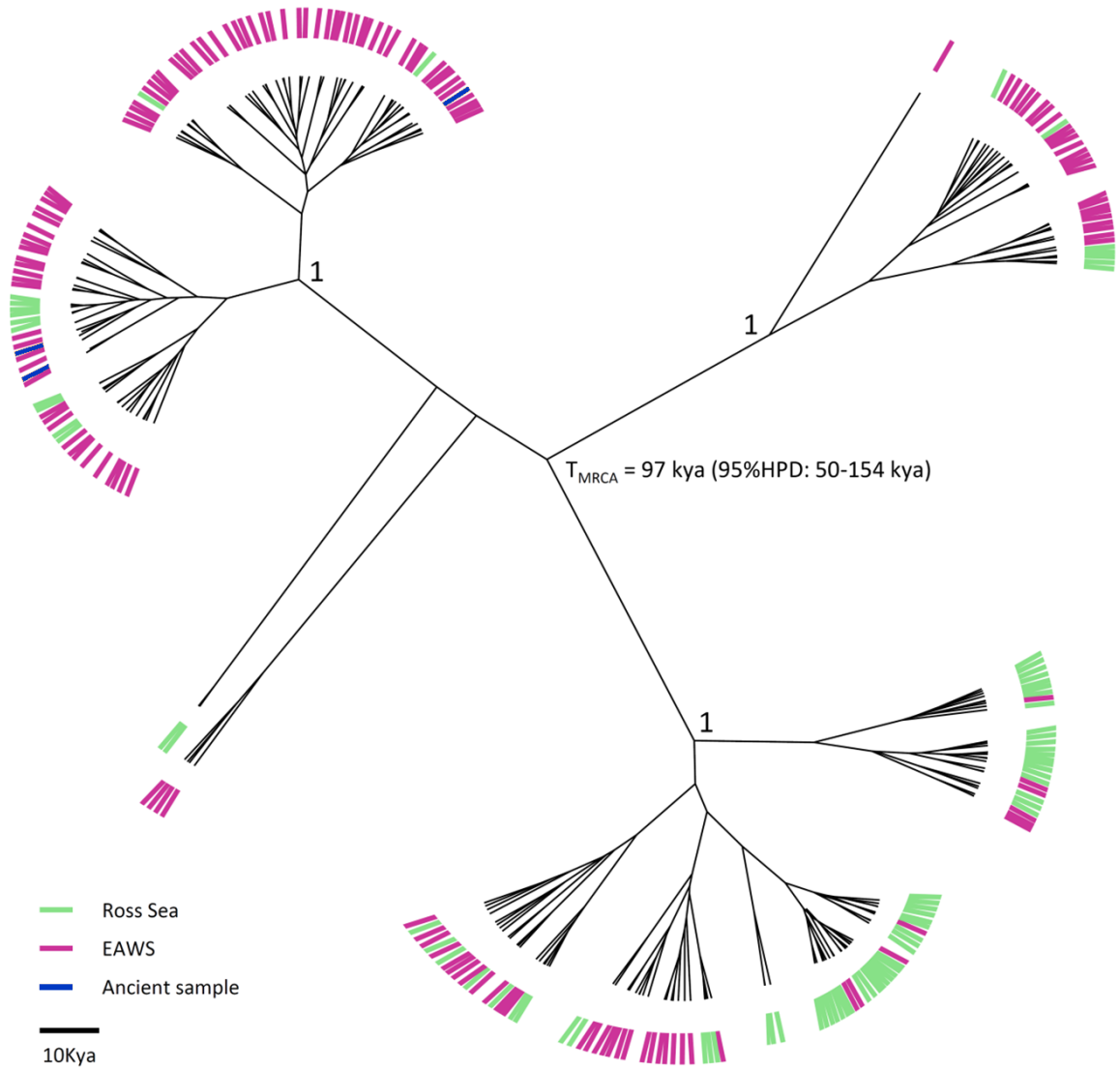
Figure 2.3. Phylogenetic relationships among individuals. Magenta - EAWS individuals; green - Ross Sea individuals; blue – subfossil individuals. The posterior probabilities are shown for the major, strongly supported clades.

2.5 Discussion

This first analysis of emperor penguin population structure shows colonies within the Ross Sea are genetically distinct from other Antarctic colonies, whereas those from the rest of the continent and spanning up to 8,000 km of coastline are panmictic (Table 2.2). The admixture of the EAWS emperor penguins supports our hypothesis of limited population structure and indicates a very large dispersal range for the species. Given our genetic evidence of extensive mixing across Antarctica, the unique structure in the Ross Sea emperor penguins is surprising, and interestingly the same pattern was reported for the sympatric Adélie penguin (Ritchie *et al.*, 2004), providing further evidence that the Ross Sea has a unique evolutionary history.

The existence of distinct penguin populations in the Ross Sea is puzzling. There are neither geographic nor oceanographic barriers isolating the Ross Sea from the rest of Antarctica. Furthermore, the relative distance between the Ross Sea and other colonies does not adequately explain its isolation as, for example, the Pointe Géologie colony is approximately 5,600 km closer to the Ross Sea colonies than to those in the Weddell Sea (Figure 2.1). Emperor penguins are known for their extraordinary migrations; satellite tracking showed that juveniles can travel more than 7,000 km in eight months (Thiebot *et al.*, 2013). These observations support our genetic results for the EAWS region and indicate juvenile emperor penguins could comfortably traverse the 1,800 km between Pointe Géologie and the Ross Sea colonies. There are also no clear habitat, environmental or foraging differences between the Ross Sea colonies and those located elsewhere (Budd, 1961, Smith *et al.*, 2012), except that Ross Sea colonies are located closer to the ice edge, and are therefore potentially more resilient to increases in sea ice. We suggest that the divergence of emperor penguins into two populations is historical in origin.

There are three ancestral lineages within modern emperor penguins, providing evidence that populations were isolated in the past (Figure 2.3) and diverged through microevolutionary processes, such as selection or genetic drift, which occur more rapidly in small, isolated populations (Hewitt, 2000). One of these lineages is mostly limited to the Ross Sea, indicating that the isolation of this region has persisted through time. Indeed, emperor penguins occupying the Ross Sea may have become so differentiated that interbreeding with the EAWS penguins occurs at very low rates, perhaps because of genetic, behavioural (Templeton, 1981) or cultural incompatibilities, such as the timing of breeding or the development of regional dialects (de Dinechin *et al.*, 2012, Jouventin & Aubin, 2002, MacDougall-Shackleton & MacDougall-Shackleton, 2001).

Emperor penguins use complex display calls to recognise their mates and offspring (Robisson *et al.*, 1993). Vocalisation is known to be an important part of the courtship process for most penguins (Richdale, 1944, Waas *et al.*, 2000). Interestingly, royal penguins (*Eudyptes schlegeli*) respond more strongly to calls from their own colony members than to calls originating from different colonies, suggesting differences in dialect (Waas *et al.*, 2000). Differences in vocalisations have also been found among gentoo penguin (*Pygoscelis papua*) populations (de Dinechin *et al.*, 2012). If dialects become too different, then courtship may be inhibited, thereby limiting interbreeding. This has been observed in passerine birds, in which genetically distinct groups have unique mating songs (MacDougall-Shackleton & MacDougall-Shackleton, 2001). Emperor penguin vocalisation patterns have only been recorded at Pointe Géologie (Robisson *et al.*, 1993), but our hypothesis could be explored in the future by comparing vocalisations of emperor penguins from the Ross Sea with those of other colonies.

Although the isolation and differentiation of the Ross Sea emperor penguins has persisted, the other two historical lineages show no geographic bias and have now hybridized to form one EAWS population. Incomplete mixing of ancestral lineages is typical of species that have survived the Pleistocene ice-ages in multiple refugia (Hewitt, 1996). Our EBSPs indicate that both the EAWS and Ross Sea populations had reduced effective population sizes during the LGM (Figure 2.2). Thus, contrary to a hypothesis that emperor penguins would benefit from glaciation as a result of reduced competition with other predators (Thatje *et al.*, 2008), it seems that they, like other Antarctic and sub-Antarctic penguin species (Clucas *et al.*, 2014, Ritchie *et al.*, 2004, Trucchi *et al.*, 2014), were adversely affected by the LGM.

We propose that both the reduced abundance and divergence into three lineages were linked to breeding and foraging habitat availability. Today emperor penguins have a circumpolar distribution with suitable habitat spanning the entire continent (Fretwell & Trathan, 2009). However, Antarctica during the LGM looked very different than the continent we know today (Figure 2.4). Most of the continental shelf was covered by ice as a result of both the extension of ice sheets and thick, perennial sea ice, which reduced productivity south of the modern-day Polar Front drastically (Anderson *et al.*, 2002, Anderson *et al.*, 2009, Domack *et al.*, 1998, Gersonde *et al.*, 2005, Kohfeld *et al.*, 2005, Samuel Jaccard pers. comm.). We suggest that the increased sea ice extent would have severely restricted the foraging habitat available for emperor penguins and, coupled with lower primary production, could have resulted in a scarcity of prey resources. Additionally, air temperatures were approximately 13°C colder than the present day (Jouzel *et al.*, 2007), which may have been near the penguins' lower limit of temperature tolerance (Le Maho *et al.*, 1978), potentially impacting both breeding success and adult survival.
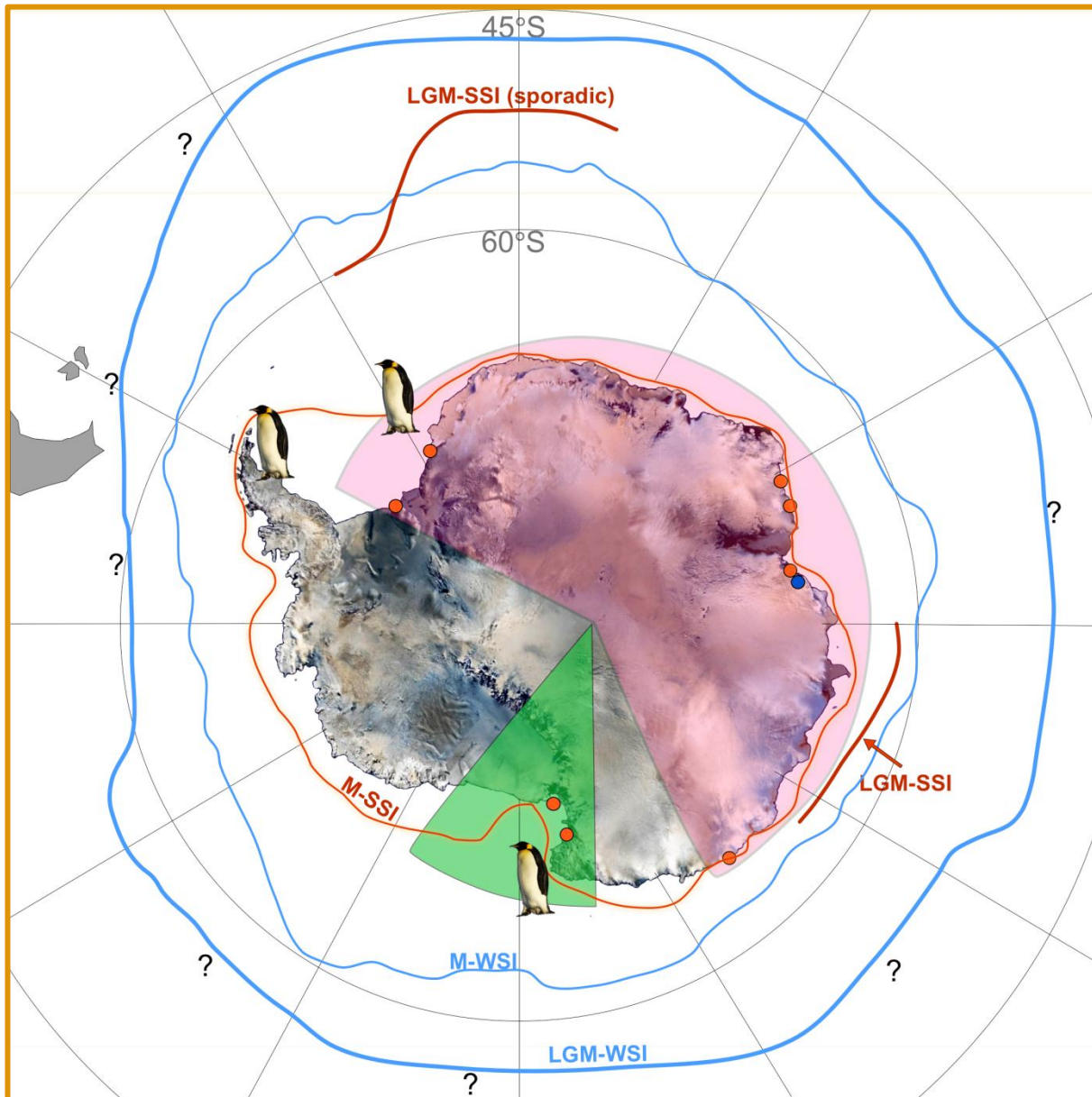
Figure 2.4. Schematic of contemporary population structure and reconstruction of historical conditions. Sampled colonies are indicated by dots, as in Figure 2.1. The magenta and green shading indicate population structure as estimated from this study. Lines represent the sea ice edge, as in Gersonde *et al.*, (2005). M-SSI = modern summer sea ice edge; LGM-SSI = LGM summer sea ice edge; M-WSI = modern winter sea ice edge; LGM-WSI = LGM winter sea ice edge, ? = insufficient data to reconstruct the sea ice edge. Penguins represent hypothesised locations of polynya refugia. Emperor penguin picture: © Samuel Blanc / www.sblanc.com.

The extent and duration of sea ice are important factors in the breeding success of emperor penguins (Massom *et al.*, 2009). Emperor penguins require stable fast ice to breed, but they have to traverse the sea ice to establish colonies in autumn and to forage in winter and spring. The distances between the colonies and potential foraging areas can influence breeding success where the fast ice extent is variable (Massom *et al.*, 2009), but not in locations where the extent is relatively stable (Robertson *et al.*, 2013). We therefore expect that if the winter sea ice extent was substantially greater in the LGM, or if the timing of sea ice retreat was altered, that this would have made some of the extant colony locations energetically untenable during the LGM.

During the LGM, the summer sea ice extent was similar to what we observe today, whereas the winter extent was roughly doubled (Gersonde *et al.*, 2005). Colonies may have been located close to the continent so that the ice remained stable throughout the breeding season, but this would have required adults to walk immense distances to reach foraging areas during winter and spring while provisioning the chick. In that case, the chicks would receive fewer meals and be less likely to survive. The present distribution of colonies close to land (Fretwell & Trathan, 2009) suggests that fast ice proximate to land provides a more stable platform than near the fast ice edge. Also, stable ice close to the coast occurs in predictable locations that might be important for colony establishment and cohesion. Colonies further away from the coast may therefore be difficult to maintain. Our discovery of three distinct lineages provides evidence against a straightforward, latitudinal range shift in line with the sea ice edge, and suggests that emperor penguins may have survived the LGM in three suitably situated, geographically isolated refugia.

Emperor penguin refugia during the LGM may have been linked to the presence of polynyas. The majority of extant emperor penguin colonies are located within walking distance of polynyas (Massom *et al.*, 1998) that are commonly utilised by the birds for foraging during the winter based on satellite tracking studies (Ancel *et al.*, 1992, Kirkwood & Robertson, 1997). Polynyas acted as "hot spots" of primary productivity during the LGM, supporting marine life and flying seabirds (Thatje *et al.*, 2008). Sediment cores in the north-western Ross Sea indicate open water polynya conditions throughout the LGM (Brambati *et al.*, 2002, Thatje *et al.*, 2008) and this polynya could have sustained a refuge population until the Ross Sea began to clear of ice (Figure 2.4). By 9.6 kya most of the northern Ross Sea was open water (Licht & Andrews, 2002). The retreating sea ice and increased upwelling during deglaciation increased productivity in the Ross Sea (Anderson *et al.*, 2009) and likely increased the foraging habitat and prey availability to emperor penguins. Therefore we hypothesise that these factors drove the three-fold expansion of emperor penguins in this region around this time (Figure 2.2). The LGM polynya may have also supported Adélie penguins, accounting for the existence of a distinct Ross Sea clade as previously observed for this species (Ritchie *et al.*, 2004).

Another polynya was located in the south-eastern Weddell Sea off Dronning Maud Land (DML) (Mackensen *et al.*, 1994, Thatje *et al.*, 2008) (Figure 2.4). Colonies of snow petrels (*Pagrodroma nivea*) were present in DML throughout the LGM, associated with this polynya (Wand & Hermichen, 2005), and it may have also provided a refuge for emperor penguins. There is evidence from sediment cores for a third LGM polynya, located in the north-western Weddell Sea (Figure 2.4) (Smith *et al.*, 2010); this would be consistent with our third emperor penguin refuge, given that the refuge is likely to be more proximate to DML than the Ross Sea, since the two refugial lineages hybridized post-glacially while the Ross Sea lineage remained distinct.

We propose that two refuge populations that were isolated in the Weddell Sea expanded their range into Prydz Bay and Adélie Land and merged during the retreat of the East Antarctic ice sheet 14 – 7 kya (Mackintosh *et al.*, 2011). At this time, the onset of more favourable environmental conditions could have resulted in the dramatic, nine-fold increase in abundance shown here (Figure 2.2). A seasonal sea ice cycle was established in Prydz Bay approximately 10.4 kya (Barbara *et al.*, 2010), opening up foraging habitat and coinciding with high levels of primary productivity (e.g. (Anderson *et al.*, 2009, Sedwick *et al.*, 2001). In Adélie Land, primary productivity and the duration of the ice-free season increased from 9 kya (Denis *et al.*, 2009a, Denis *et al.*, 2009b). This new habitat could have facilitated the range expansion of the EAWS lineages.

It should be noted that the timing of the population expansions of emperor penguins do not coincide exactly with the end of the LGM (Figure 2.2). We hypothesise that it is not the temperature change itself, but rather the subsequent change in sea ice conditions and primary productivity that are most likely to affect emperor penguins. Indeed, it has been proposed that there is an optimal level of sea ice at the large temporal / spatial scale for emperor penguins, which roughly corresponds to current conditions (Ainley *et al.*, 2010). Therefore, the greater sea ice extent of the LGM was most likely sub-optimal for emperor penguin populations. The termination of the LGM is measured when temperatures began to increase (19 – 16 kya). However deglaciation, during which ice sheets and sea ice retreated and primary productivity increased, occurred slowly over an extended time period (*ca*. 17 – 11 kya) (Anderson *et al.*, 2009). These events occurred later in the Ross Sea than in East Antarctica, and our results support the hypothesis that ice sheet and sea ice retreat and increasing primary productivity were the main factors controlling emperor penguin abundance, as the Ross Sea emperor penguin population expanded later than the EAWS population (Figure 2.2). Furthermore, emperor penguins produce only one chick per year and take approximately five years to reach sexual maturity (Jenouvrier *et al.*, 2005), so any population expansion would be initially slow. It should also be noted that the timing of the population expansion reported here is approximate rather than definitive.

Our hypothesis of three refugial populations of emperor penguins during the LGM could be tested using a higher density of genetic markers. This would allow for the investigation of clinal variation in genetic diversity arising from founder effects as new areas were colonized following the expansion from refugia after the LGM (Hewitt, 1996). It should be noted that our present study is based on mitochondrial DNA and therefore represents dispersal patterns of females only, but nonetheless supports a plausible explanation for past and present microevolutionary processes in emperor penguins. The next step should be to verify these findings using nuclear markers to account for male-mediated gene flow.

In this continent-wide study of microevolution in an Antarctic penguin we suggest that past climatic changes greatly impacted emperor penguin populations. As conditions became more favourable after the LGM, their global population expanded and the populations from the Weddell Sea and East Antarctic intermixed to form one large, panmictic population. Interestingly, the isolation of the Ross Sea emperor penguins has persisted until today. The reasons for this isolation remain unknown, but we suggest that separate management plans are required for the Ross Sea and EAWS populations. By conserving the full spectrum of genetic variation and, in particular, all phylogeographic lineages, the evolutionary potential of the species can be maximised (D'Amen *et al.*, 2013).

Our study suggests that emperor penguins have shown important historic responses to past climate shifts and their population increase post-LGM was remarkable. However, the projected rate of temperature increase over the next century is an order of magnitude greater than that following the LGM (Collins *et al.*, 2013, Masson-Delmotte *et al.*, 2013, Shakun *et al.*, 2012). At present, emperor penguins become heat stressed around 0°C, so may exist near the upper limits of their physiological tolerance (Wienecke, pers obs). Whether the resilience demonstrated in the past of this highly cold-adapted species will enable it to adapt to projected climate change remains to be seen, as rising temperatures will alter its breeding grounds and foraging space more rapidly than in the past.

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2.7 Appendices

Appendix 2.1 Haplotype networks of phylogenetic relationships
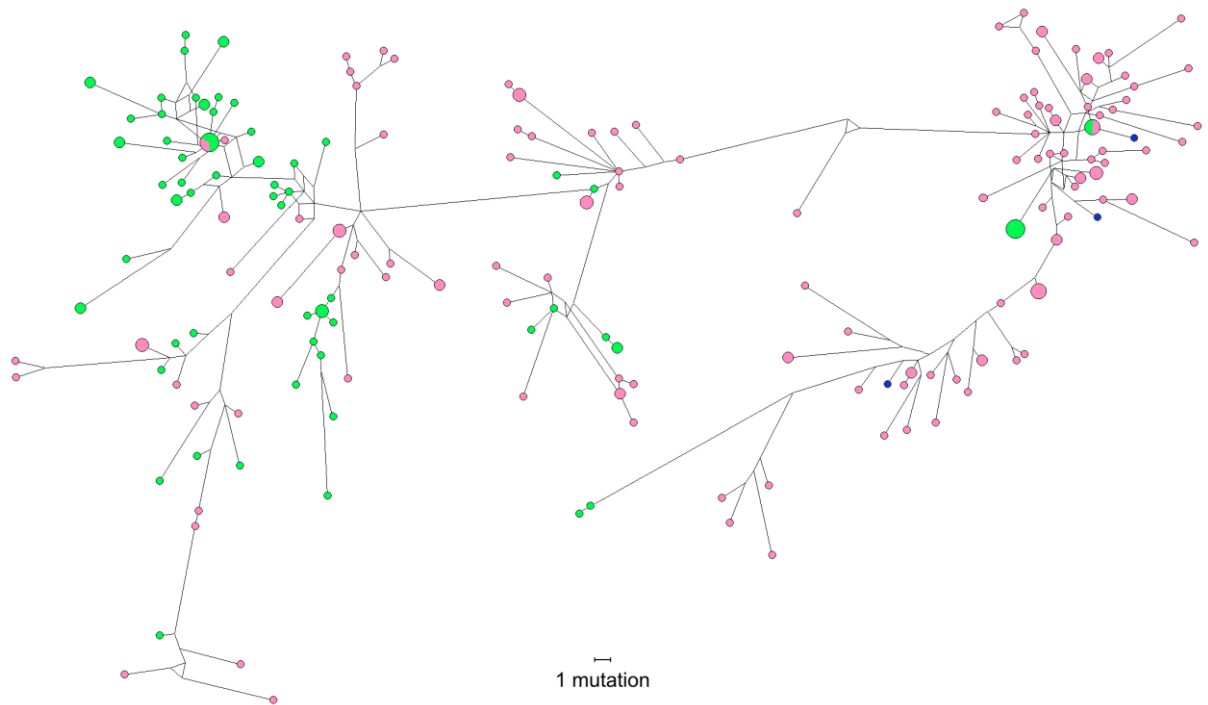
Figure 2A.1 Haplotype network of phylogenetic relationships among all HVR sequences. Magenta – EAWS colonies; green – Ross Sea colonies; blue – subfossil samples; the size of the circle indicates the relative frequency of the haplotype.
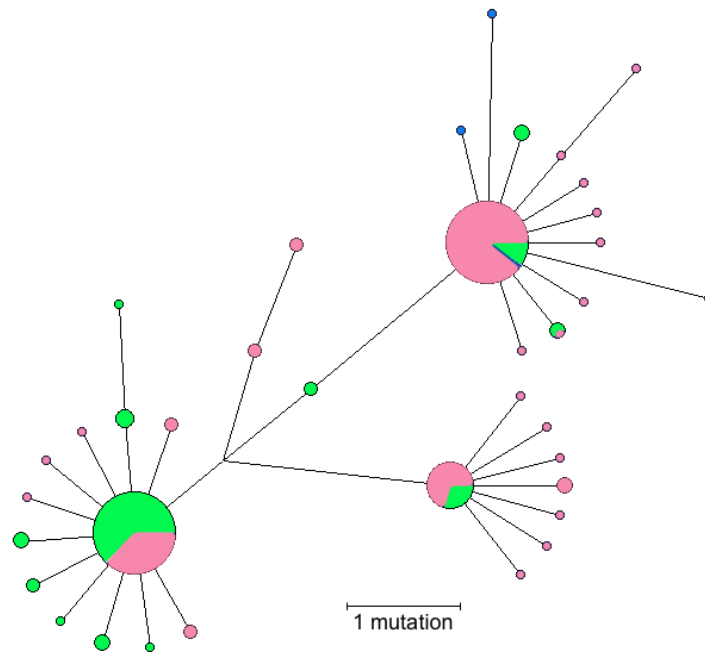
Figure 2A.2 Haplotype network of phylogenetic relationships among all CytB sequences. Magenta – EAWS colonies; green – Ross Sea colonies; blue – subfossil samples; the size of the circle indicates the relative frequency of the haplotype.

Appendix 2.2 Table of primer sequences.

Primer name	Primer sequence 5' – 3'
F-0225	GGAACCTCCCAAAGAGTACCA
RINR	CCAACCAGATGTATCGGTGA
HVR-R1	TGAAAGTATTGCTTTACGTATCCTT
HVR-F2	AAGGATACGTAAAGCAATACTTTC
HVR-R2	AGGAGTAATTGTTGAGTACATGACA
HVR-F3	TCATGTACTCAACAATTACTCCTG
HVR-R3	TCACGTGAGAAGACCGACTAA
HVR-F4	ATCTCCTGAGGCGCTAGCTT
B1	CCATCCAACATCTCAGCATGATGAAA
B6	CCATCCAACATCTCAGCATGATGAAA
CytB-F1	ACTGCAGACACAACCCTAGC
CytB-R1	GGGAAGAGGATCAGGAGGGT
CytB-R1	AATGATGCTCCGTTTGCATGTAGGTT
CytB-F2	ACACATGCCGAAACGTACAG
CytB-R2	GTAGCCTACGAAGGCGGTTG
CytB-F3	GAAACCTGAAACACAGGCATT
CytB-R3	CGGGTTAATGTGGGGTTGT
CytB-F4	CTCAGCCATCCCTTACATTG
CytB-R4	TTGTGGAGTAGTAGGGGTGGA
CytB-F5	CAAATAACCCACTGGGCATC
CytB-R5	TCATTCTGGTTTGATGTGTGG
CytB-F6	CCAGCAAACCCACTAGTCAC
CytB-R6	GGGCTCAGAATAGGAGTTGG
CytB-F7	ATAGCTTTCCGCCCTCTCT

CHAPTER 3

CONTRASTING RESPONSES TO A CLIMATE REGIME CHANGE BY SYMPATRIC MESO-PREDATORS

3.1 Abstract

Models that predict the fate of species under future climate change scenarios often assume that ecological niche and habitat availability are the major determinants of a species' response to climate change. However, species may have very different capacities to adapt to environmental change, as determined by factors such as their dispersal ability, genetic diversity, generation time and rate of evolution. We used mitochondrial DNA sequences to compare the effect of late Pleistocene warming on populations of two key East Antarctic meso-predators. Despite their apparently similar ecological niches and overlapping distributions, our genetic data revealed that the emperor penguin (*Aptenodytes forsteri*) population grew rapidly following the last glacial maximum, but the size of the Weddell seal (*Leptonychotes weddellii*) population did not change. Emperor penguins appear to possess a greater capacity to adapt to environmental change than Weddell seals, and prospered during Holocene warming, most likely due to a higher dispersal ability and hence gene flow among colonies, higher evolutionary rate, and fine-scale differences in their preferred foraging locations. The vastly different climate change responses of two ecologically similar predators suggests that differing adaptive capacities and/or fine-scale niche differences can play a major role in species' climate change responses.

3.2 Introduction

Given the looming threat of a sixth mass extinction (Barnosky *et al.*, 2011), understanding species' resilience to environmental regime shifts is of immediate concern. How a species responds to environmental change depends broadly on three factors: the continued availability of its preferred ecological niche, its capacity to change its distribution in response to niche availability, and its ability to adapt to a different ecological niche if necessary, known as niche evolution (Lavergne *et al.*, 2013, Lynch & Lande, 1993, Walther *et al.*, 2002, Wiens & Graham, 2005). Current models that estimate species' responses to past and future environmental shifts assume that ecological niche and habitat availability are the major determinants of a species' response to climate change (Hazen *et al.*, 2012, Jenouvrier *et al.*, 2014, Laidre *et al.*, 2008, Siniff *et al.*, 2008), leading to predictions that species with comparable ecological niches and habitat should respond in similar ways to environmental change. However, species have different capacities for dispersal and niche evolution (modes and tempos) linked to factors including philopatry, gene flow, genetic diversity and evolutionary rate (Lavergne *et al.*, 2013, Walther *et al.*, 2002). Indeed, species with slower rates of niche evolution may be more prone to demographic decline (Lavergne *et al.*, 2013). The influence of such intrinsic factors in

species' climate change responses, and the degree to which they should be considered alongside the recognised factors of ecological niche and habitat availability, remain unclear.

Understanding responses to environmental change is particularly important in the case of predators, which play critical roles in regulating ecosystems (Baum & Worm, 2009, Heithaus *et al.*, 2008). In Antarctica, there are two key coastal meso-predators that occupy similar ecological niches; the emperor penguin (*Aptenodytes forsteri*) and the Weddell seal (*Leptonychotes weddellii*). Both emperor penguins and Weddell seals have circumpolar distributions (Fretwell *et al.*, 2012, Fretwell & Trathan, 2009, Siniff, 1991) and life-cycles that are closely tied to the seasonality of Antarctic sea ice (Stirling, 1969, Wienecke *et al.*, 2013). Both species use coastal fast ice as a platform upon which to raise their offspring, although the penguin does so during winter, whereas the seal does so during spring (Budd, 1961, Siniff, 1991). The young of both species are fledged/weaned in late December, coinciding with minimum sea ice extent and maximum productivity (Stirling, 1969, Wienecke *et al.*, 2010). Their generation lengths are similar, estimated at 18 years for emperor penguins and 19 years for Weddell seals (Forcada & Trathan, 2009, Hadley *et al.*, 2006). They are both warm-blooded, air breathing species that dive to similar depths and occupy similar trophic levels (Burns & Kooyman, 2001). Direct competition for prey appears to be minimised by temporal and geographic differences in their foraging habitat (Burns & Kooyman, 2001), with latitudinal overlap strongest in near-coastal areas (Raymond *et al.*, 2014). Both emperor penguins and Weddell seals are prey to apex predators. Leopard seals (*Hydrurga leptonyx*) occasionally consume emperor penguins (Kooyman *et al.*, 1990), killer whales (*Orcinus orca*) prey on Weddell seals (Ainley & Ballard, 2012, Pitman & Ensor, 2003) and may also consume emperor penguins (Ainley & Ballard, 2012, Andrews *et al.*, 2008), and southern giant petrels (*Macronectes giganteus*) prey on emperor penguin chicks (Budd, 1961). Because of these many similarities, current forecasting models based largely on ecological niche and habitat availability would likely predict that emperor penguins and Weddell seals would respond in similar ways to changes in the Antarctic coastal environment.

Decadal-scale monitoring studies have been conducted in several locations for both emperor penguins and Weddell seals. Declines in emperor penguin numbers have been observed at Pointe Géologie (Barbraud & Weimerskirch, 2001, Jenouvrier *et al.*, 2005, Jenouvrier *et al.*, 2009) and Haswell Island (Barbraud *et al.*, 2011), and at the Dion Islands the entire breeding colony has been lost (Trathan *et al.*, 2011). These declines were thought to be the result of climatic or oceanographic impacts on breeding success and/or adult survival (Barbraud *et al.*, 2011, Jenouvrier *et al.*, 2005, Trathan *et al.*, 2011). However, new evidence suggests that the colony at the Dion Islands may have relocated 190 km to an area with more favourable sea ice conditions (LaRue *et al.*, 2015) and that the population declines at Haswell Island and Pointe Géologie may be the result of emigration as a

response to locally poor sea ice conditions (Ancel *et al.*, 2014, Barbraud *et al.*, 2011, Fretwell *et al.*, 2014, LaRue *et al.*, 2015). For Weddell seals, numbers declined at Anvers Island, Antarctic Peninsula, over the period of 1973–2002, concurrent with declines in sea ice, and this species has now all but vanished from the region (Siniff *et al.*, 2008). Numbers of Weddell seals have also decreased in eastern McMurdo Sound, Ross Sea since the 1950s–1960s, however, in this region, fast ice conditions are relatively unchanged and are unlikely to be the cause of the decline in seal numbers (Ainley *et al.*, 2015). Rather, Ainley *et al*. (2015) propose that the reduced number of seals in eastern McMurdo Sound could be the result of changes in the distribution and abundance of prey resources, which may have either decreased the survival rates of sub-adult seals, or caused the seals to emigrate to other areas. Interestingly, another decadal-scale monitoring study at the Vestfold Hills, East Antarctica, showed no trend in Weddell seal numbers over the period 1973–2000 (Lake *et al.*, 2008), despite a local shortening of the sea ice season over that period (Massom *et al.*, 2013). Overall, the recent decadal population trends of Weddell seals and emperor penguins are regionally variable and not clearly linked to any one environmental driver. Nevertheless, projected declines in sea ice (Collins *et al.*, 2013) are likely to be problematic for both species, and numbers of emperor penguins and Weddell seals are predicted to decline in the future as climate change alters their breeding and foraging habitats (Ainley *et al.*, 2010, Jenouvrier *et al.*, 2014, Siniff *et al.*, 2008).

In the late Pleistocene, following the Last Glacial Maximum (LGM, 26–19.5 kya), climate and sea ice conditions in Antarctica changed dramatically, providing an ideal scenario to test the climate change induced responses of emperor penguins and Weddell seals over millennial time scales. LGM air temperatures were *ca.* 13°C colder than the present day (Jouzel *et al.*, 2007), the winter sea ice field was approximately double its present size, extending to about 50°S (Gersonde *et al.*, 2005) and, unlike today's seasonality, the LGM sea ice cover was heavy and perennial (Barbara *et al.*, 2010). Levels of primary productivity were also reduced (Kohfeld *et al.*, 2005), possibly having a regulatory effect on the upper trophic levels.

A population's demographic history is encoded in its genome, allowing for a window into its responses to past climate change (de Bruyn *et al.*, 2011, Ho & Shapiro, 2011, Moura *et al.*, 2014). We reconstructed the population trajectories of both emperor penguins and Weddell seals from the late Pleistocene era through the LGM and into the Holocene, using mitochondrial DNA from extant colonies in East Antarctica. We chose to focus our study on East Antarctica due to the spatial heterogeneity of both historical and contemporary climate change trends around Antarctica (Vaughan *et al.*, 2013, Zwally *et al.*, 2002), and the high relevance of regional changes in the context of ecological responses. Using Bayesian coalescent inference (Drummond *et al.*, 2005, Ho & Shapiro, 2011), we analysed changes in effective female population size (N_{ef}) of both species, and assessed

patterns of modern genetic diversity and gene flow. In keeping with the current paradigm, we hypothesized that, as species with overlapping spatial distributions and similar ecological niches, emperor penguins and Weddell seals may have shown similar responses to post-glacial warming following the LGM.

3.3 Methods

3.3.1 Sample collection

Genetic material was collected from extant East Antarctic breeding colonies of emperor penguins and Weddell seals (Figure 3.1). Weddell seal flipper biopsies were collected from 90 individuals across six breeding sites (Figure 3.1, Table 3.1) between 1996 and 2010 and stored at -20°C. Pectoral muscle biopsies were collected from 91 dead emperor penguin chicks at four colonies (Figure 3.1, Table 3.1) between 1993 and 2013 and stored at -20°C (see also Chapter 2, Younger *et al.*, 2015). DNA sequences from radiocarbon dated sub-fossil remains were included in the reconstructions of demographic history as additional calibration points for the molecular clock. Bones from the sub-fossil remains of three penguins and six Weddell seals were collected in the Vestfold Hills and stored at -80°C and -20°C, respectively. The radiocarbon ages, expressed here as years BP (i.e. before 1950), of the sub-fossil remains were determined using accelerated mass spectrometry by GNS Science Rafter Radiocarbon National Isotope Centre, New Zealand. The apparent ages were corrected for the marine-carbon reservoir effect (Gordon & Harkness, 1992) using the calibration program Calib7.0 (ST UI & Reimer, 1993).

Table 3.1. Number of individuals sequenced by species and colony location.

	Emperor penguins	Weddell seals
Auster	21	-
Amanda Bay	24	-
Fold Island	24	-
Pointe Géologie	22	18
Tryne Fjord	-	23
Long Fjord	-	5
Herring Islands	-	21
Swain Group	-	7
Colbeck	-	16
Total extant	**91**	**90**
Vestfold Hills	3	6
Total sub-fossil	**3**	**6**
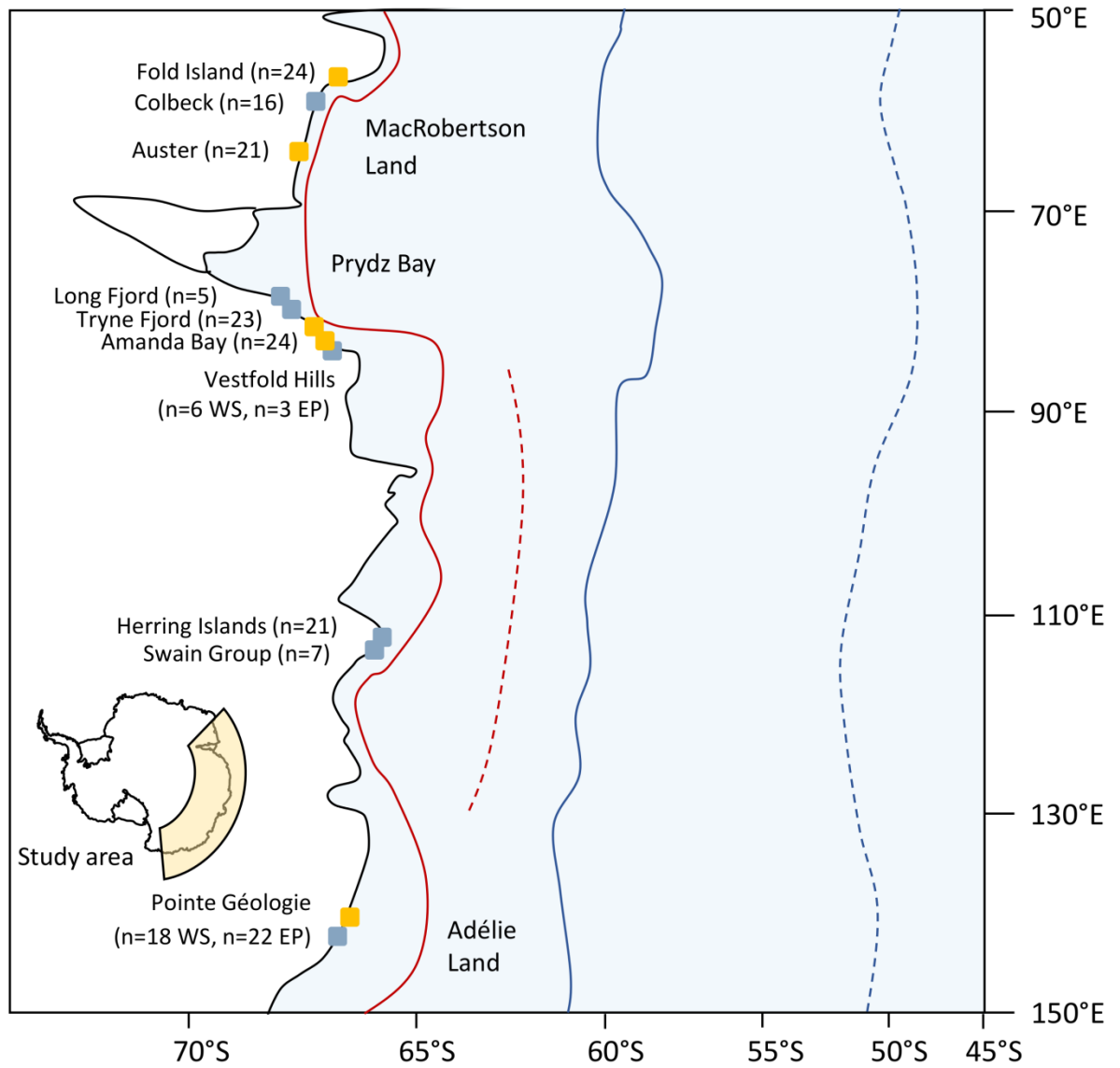
Figure 3.1. Sampled colony locations and sea ice limits. Yellow boxes indicate emperor penguin colony locations, blue boxes indicate Weddell seal colony locations, with the current summer sea ice extent indicated by a solid red line, the current winter sea ice extent indicated by a solid blue line, the LGM summer sea ice extent shown as a dashed red line, and the LGM winter sea ice extent as a dashed blue line. Sea ice extents are as in (Gersonde *et al.*, 2005).

3.3.2 Molecular laboratory

DNA was extracted from modern samples with the QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's protocols. For subfossil samples ~50 mg of bone, taken from the interior of the bone specimens following removal of the outer layers with a scalpel, was decalcified in 0.5 M EDTA/0.001% Triton X100 at 56°C for 48 h and then DNA extracted using a standard phenol chloroform protocol with ethanol precipitation. The sub-fossil samples were extracted in a physically isolated laboratory that had never been used before for avian or pinniped samples, with extractions performed inside a laminar flow cabinet to further minimise contamination risk. Negative extraction controls were also used to confirm there was no contamination.

The mitochondrial hypervariable region (HVR) and cytochrome *b* (CytB) were sequenced for all individuals. HVR is a rapidly evolving region of the mitochondrial genome, and so is suitable for investigations of recent demographic history, whilst CytB is a more conserved gene and can hence give information about longer-term demographic history (Baker & Marshall, 1997). HVR and CytB were amplified and sequenced for modern seals using primers TDKD/L15926 (Kocher *et al.*, 1989, Slade *et al.*, 1994) and L-CytB/H-CytB (Arnason *et al.*, 1995) (all primer sequences can be found in Appendix 3.1). HVR and CytB were amplified and sequenced for modern penguins using primers F-0225/R-INR (Chapter 2, Younger *et al.*, 2015) and B1/B6 (Baker *et al.*, 2006). For the sub-fossil samples we designed novel primers (Appendix 3.1) to amplify short (<150bp) overlapping fragments in order to improve the success rate of amplification from degraded DNA. The sub-fossil PCRs were done separately from the modern PCRs, and set up in a laminar flow hood in a physically isolated laboratory that is never exposed to PCR products. Negative PCR controls were used for both sub-fossil and extant reactions.

A number of heteroplasmic sites were observed in the emperor penguin HVR sequences, as has been recorded previously in the HVR of the closely related Adélie penguin (*Pygoscelis adeliae*) (Millar *et al.*, 2008). These were re-scored manually according to IUPAC ambiguity codes if the secondary peak was >40% of the height of the primary peak in both forward and reverse sequences.

3.3.3 Genetic diversity and present day population structure

jModeltest (Posada, 2008) was used to estimate the optimal nucleotide substitution model for each dataset and Arlequin v3.5 (Excoffier & Lischer, 2010) was used to calculate summary statistics by colony and genetic region (Appendix 3.2). Arlequin was also used to quantify genetic differentiation (F_{ST}) between pairs of colonies and to perform analyses of molecular variance (AMOVA), with 10,000 random permutations of the data to determine the statistical significance of the hypothesis of departure from panmixis.

3.3.4 Reconstructions of demographic history

Demographic reconstructions were done using the extended Bayesian skyline plot (EBSP) method in BEAST v1.8 (Drummond *et al.*, 2012, Heled & Drummond, 2008). For molecular clock calibration of the emperor penguin analyses, the HVR substitution rate prior was specified as a lognormal distribution around a mean value of 0.55 substitutions/site/Myr (SD = 0.15), to reflect the substitution rate of the HVR in Adélie penguins (Millar *et al.*, 2008). In the absence of a published substitution rate for CytB in penguins, we used a uniform prior of $5x10^{-4}$ to $5x10^{-1}$ substitutions/site/Myr with a starting value of $2x10^{-2}$ (Weir & Schluter, 2008). The corrected radiocarbon ages of the sub-fossil samples were input as tip dates for additional calibration of the molecular clock. Based on these initial priors, the emperor penguin substitution rates were estimated during the analysis.

In the case of Weddell seals, there are no reliable published estimates of the substitution rates. We therefore conducted initial BEAST analyses to estimate the substitution rates for HVR and CytB using an expanded dataset of 136 Weddell seal sequences. The additional 40 individuals, which we included to incorporate as much genetic variation as possible in our estimate, were sourced from other locations in East Antarctica (*n* = 19) and McMurdo Sound, Ross Sea (*n* = 21) (GenBank accession numbers TBA). The 19 individuals from East Antarctica were excluded from our estimates of population structure and demographic history because the sample sizes from these colonies were less than five, which we used as a minimum cut-off for this study. HVR and CytB sequences for the Weddell seal's closest relative, the leopard seal (Genbank accession numbers HLU03590 and AY377323*),* were incorporated and the divergence time of the two species (2.89 MYA; 95%CI = 1.84-3.97 MYA) (Fulton & Strobeck, 2010) was used as a node calibration. The root height prior was specified as a normal distribution around a mean value of 2.89 million years (SD = 0.65). The corrected radiocarbon ages of the sub-fossil samples were input as tip dates (i.e. ages were assigned to the sequences) for additional calibration of the molecular clock. Based on these initial priors, the Weddell seal HVR and CytB substitution rates were estimated following the BEAST conditions outlined below. For the Weddell seal demographic reconstructions, the substitution rate priors for HVR and CytB were specified as lognormal distributions around a mean value of 0.114 substitutions/site/Myr (SD = 0.15) and 0.0285 substitutions/site/Myr (SD = 0.25) respectively, to reflect our estimates from the initial analyses.

For all BEAST analyses, including the initial estimates of Weddell seal substitution rates, the datasets were partitioned into HVR and CytB, with a nucleotide substitution model of HKY (Hasegawa *et al.*, 1985) with four gamma categories specified for HVR of both species and for Weddell seal CytB, and

the TN93 nucleotide substitution model (Tamura & Nei, 1993) specified for emperor penguin CytB. We used the coalescent extended Bayesian skyline plot (EBSP) tree prior (Heled & Drummond, 2008) with a strict molecular clock for all analyses. The posterior distributions of substitution rates and effective population size through time were generated using the Markov chain Monte Carlo (MCMC) sampling procedure, implemented in BEAST, which was run for between 80 and 120 million generations, depending on the dataset size, with samples drawn every 6000 steps and the first 10% discarded as burn-in. Tracer v1.5 was used to visualise the sampling trace and to check the effective sample size values (ESS) to confirm convergence, with all ESS values >200. Three independent BEAST analyses with different random number seeds were performed for each dataset to ensure reproducibility of the posterior distributions.

The population size parameter of the demographic model (N_e*tau) was converted to female effective population size (N_{ef}) by dividing the parameter by the estimated generation length (*tau*), where *tau* = A + [S/(1-S)], A = average age of maturation and S = annual survival probability of adults. *Tau* was estimated at 19 years for Weddell seals based on an average age of female maturation of 7.62 years and an annual survival rate of 0.92 for breeding females (Hadley *et al.*, 2006). Our estimate of *tau* for emperor penguins was 18 years, based on an age of first breeding of 5.5 years and an annual adult survival rate of 0.925 (Forcada & Trathan, 2009 and references therein). Note that in our previous publication (Chapter 2, Younger *et al.*, 2015) we used the minimum estimate of emperor penguin generation length of 14 years given by Forcada & Trathan (2009), and have now adjusted this to the mean estimate of 18 years, so that it is comparable to our estimate of Weddell seal generation length, which is also a mean estimate. However, it should be noted that any variance in the estimate of generation length, which may be difficult to estimate accurately in the case of emperor penguins at any rate because demographic data are limited to a single site (Jenouvrier *et al.*, 2005), will only affect the absolute values of N_{ef} in our results and has no bearing on either the timing or magnitude of the abundance increases reported, therefore, the timing and magnitude can be directly compared with the estimates in Chapter 2 and Younger *et al.* (2015), despite the different generation lengths used.

3.4 Results

3.4.1 Genetic diversity

We successfully sequenced 629 base pairs (bp) of the HVR and 995 bp of CytB from each of 91 extant and three sub-fossil emperor penguins, and 491 bp of the HVR and 1117 bp of CytB from each of 90 extant and six sub-fossil Weddell seals. The ages of sub-fossil specimens, after correction for marine

reservoir effect, ranged from 643 to 881 years BP for emperor penguins, and from 690 to 1172 years BP for Weddell seals. Genetic diversity was extremely high for the emperor penguin HVR, with 90 haplotypes recorded out of the 91 individuals sequenced; the mean number of pairwise differences between haplotypes was 17.89 ± 8.009. Genetic diversity was much lower based on HVR in Weddell seals (5.102 ± 2.49) and for CytB of both species (seals = 5.658 ± 2.73; penguins = 2.857 ± 1.51) (summary statistics by colony and genetic region are provided in Appendix 3.2). The mutation rates, expressed as substitutions/site/Myr, were higher in emperor penguins (HVR = 0.760 ± 4.55×10^{-3}, CytB = 3.88×10^{-2} ± 3.58×10^{-4}) than in Weddell seals (HVR = 0.114 ± 1.76×10^{-3}, CytB = 2.85×10^{-2} ± 4.48×10^{-4}).

3.4.2 Extant population structure

Present day emperor penguin and Weddell seal populations have very different genetic structures; the seals displayed an order of magnitude more genetic differentiation among colony sites than the penguins (Table 3.2, 3.3). Emperor penguins breeding along the 4,000 km coastline of East Antarctica appear to constitute a single panmictic population based on the mitochondrial DNA (overall $F_{ST} = 0$; *p* = 0.553; pairwise F_{ST} amongst localities *p* > 0.05; Table 3.3). This degree of admixture suggests there are currently few, if any, physical barriers to emperor penguin dispersal along the East Antarctic coastline. Conversely, East Antarctic Weddell seal populations are significantly structured (overall F_{ST} = 0.12602; *p* < 0.001) with 10 of the 15 pairwise comparisons showing significant genetic differentiation (Table 3.2). Small scale spatial structure is strong, with genetically distinct colonies located in embayments separated, in some cases, by as little as 10 km (e.g. Swain Group vs. Herring Islands; pairwise F_{ST} = 0.32629, *p* < 0.001). It should be noted, however, that two of our Weddell seal study colonies, Swain Group and Long Fjord, have low samples sizes (n < 10), therefore the pairwise F_{ST} comparisons for these sites are likely to be less precise than the other locations. Coastal colonies, such as those found at Colbeck and Pointe Géologie, were not genetically differentiated from each other despite a separation of approximately 4,000 km (F_{ST} = 0, *p* = 0.712; Table 3.2), which suggests that occasional migrants travel between these locations. The apparent isolation of the Long Fjord, Tryne Fjord and Herring Islands colonies (Table 3.2) does not appear to have reduced Weddell seal genetic diversity relative to colonies at other locations (Appendix 3.2).

Table 3.2. **Pairwise genetic differentiation (F$_{ST}$) between Weddell seal colonies**. *p*-values are denoted as * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.

	Tryne Fjord	**Long Fjord**	**Herring Is.**	**Swain Gp.**	**Colbeck**
Tryne Fjord (n=23)					
Long Fjord (n=5)	0.17015**				
Herring Islands (n=21)	0.19910***	0.34097**			
Swain Group (n=7)	0.08316*	0.12479*	0.32629***		
Colbeck (n=16)	0	0.14476*	0.18783***	0. 05338	
Pointe Géologie (n=18)	0	0.15265**	0.18522***	0.04685	0

Table 3.3. **Pairwise genetic differentiation (F$_{ST}$) between Emperor penguin colonies.**
All *p*-values are greater than 0.05, indicating no significant differentiation.

	Auster	**Amanda Bay**	**Pointe Géologie**
Auster (n=21)			
Amanda Bay (n=24)	0		
Pointe Géologie (n=22)	0.01175	0.01092	
Fold Island (n=24)	0	0	0

3.4.3 Trends in effective female population size

Our coalescent based 80,000 year reconstruction of effective female population size (N_{ef}) for East Antarctic emperor penguins and Weddell seals showed clear differences in their population trajectories (Figure 3.2). Both population trajectories began with a period of relative stability during climate cooling-warming cycling events during the mid to late Pleistocene and the LGM (Figure 3.2c). Following the LGM, early in the Holocene epoch (*ca*. 10 kya), an expansion in the population of East Antarctic emperor penguins is suggested by the 5.7 fold increase in N_{ef} from *ca.* 10,000 years ago to the present. Over precisely the same 80,000 years of periodic climate and environmental change, Weddell seal N_{ef} remained relatively unchanged (Figure 3.2, 3.3).
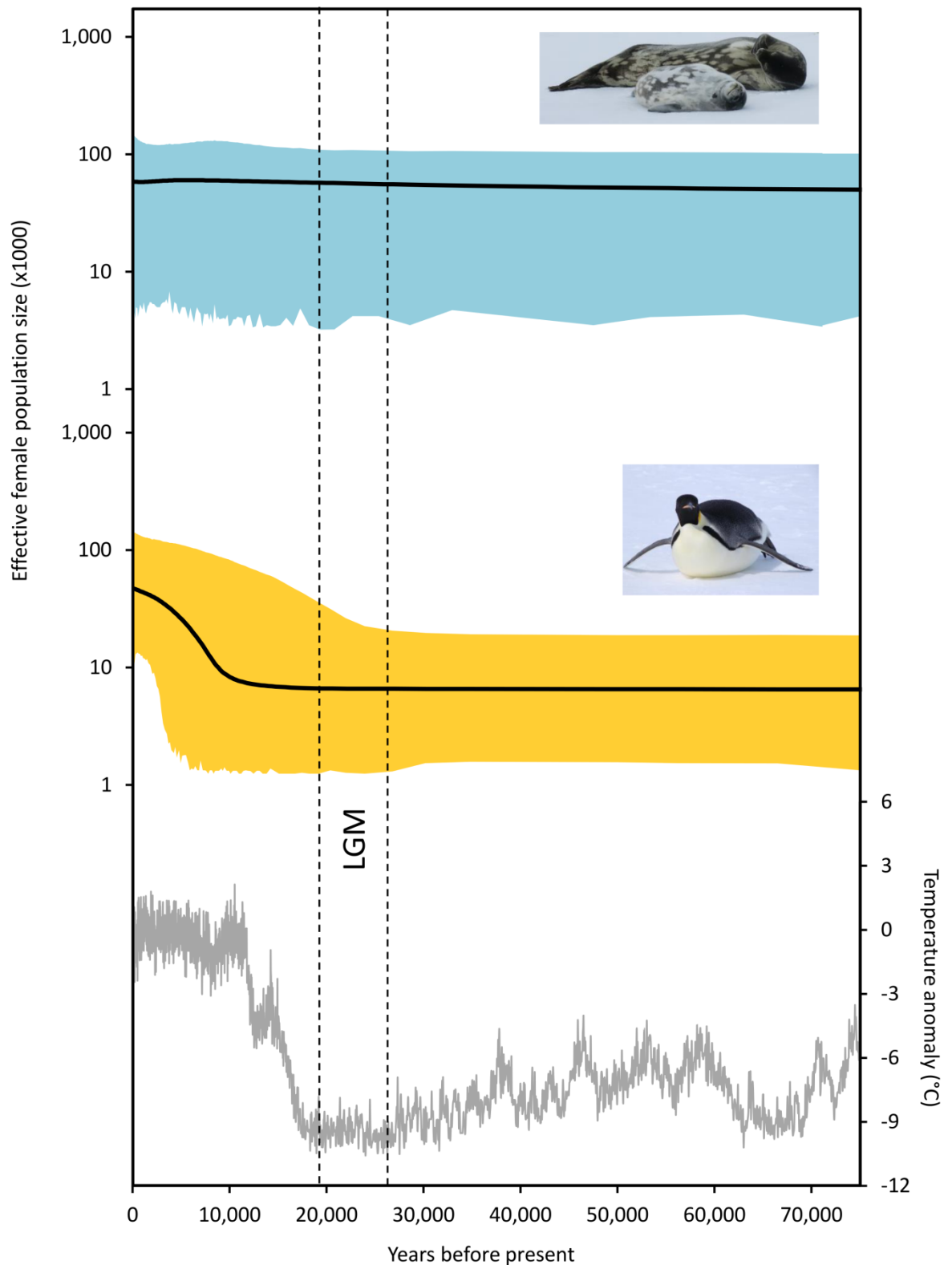
Figure 3.2. Population trajectories of East Antarctic Weddell seals and emperor penguins over the last 80,000 years. Extended Bayesian skyline plots showing the change in effective female population size (N_{ef}), with the black line indicating the median estimate and colour blocks showing the 95% highest posterior density interval. a) Weddell seals; b) emperor penguins; c) the East Antarctic temperature anomaly (the difference from the average of the last 1000 years) as estimated from the EPICA Dome C ice core (Jouzel *et al.*, 2007).

It should be noted that population structure, such as that seen in Weddell seals (Table 3.2), can have confounding effects on Bayesian skyline plots, as an assumption of the method is that the population under consideration is panmictic (Drummond *et al.*, 2005, Heller *et al.*, 2013). The main danger is that Bayesian skyline plots of structured populations can show false signals of population decline when, in fact, there has been no population change (Heller *et al.*, 2013). This does not seem to be the case for the Weddell seal population, which shows no signal of population decline (Figure 3.2). The most appropriate sampling strategy for structured populations is the 'pooled sampling strategy', wherein 2–20 individuals are sampled from 2–20 demes (populations) (Heller *et al.*, 2013). This pooled sampling strategy, which we have applied here for Weddell seals, has been shown to be the most reliable way to capture true signals of population decline and expansion for structured populations (Heller *et al.*, 2013). The use of this sampling strategy combined with the lack of population decline suggests that the population trajectory for Weddell seals is accurate.

3.5 Discussion

Genetic data have revealed that emperor penguins and Weddell seals had very different population responses to past climate change, despite their apparently similar habitat requirements, trophic levels and overlapping distributions. These results suggest that the two predators either do not occupy the same fundamental ecological niche, despite the many similarities in their breeding habitat, distribution and foraging behaviour; or they possess different capacities to adapt to environmental change.

3.5.1 Ecological niche

Warming temperatures at the end of the Pleistocene saw the Earth's environment shift away from characteristic glacial conditions of the LGM into a period of retreating ice sheets, deglaciation, rising sea levels, increased seasonality of a decreasing sea ice cover and increased primary production (Figure 3.3 and references within). These environmental responses to climatic changes happened gradually and asynchronously in the East Antarctic region (Figure 3.3). Given their adaptation to the cold and their apparent obligate requirement to use sea ice as a breeding platform, it has been hypothesised that emperor penguins may have prospered during the LGM (Thatje *et al.*, 2008), although our genetic data indicate that past emperor penguin populations were not favoured during periods of glaciation (Chapter 2, Younger *et al.*, 2015). Indeed, the N_{ef} for emperor penguins only increased to present day values following the end of the LGM. In contrast, while Weddell seals are also dependent on sea ice cover for breeding, their N_{ef} did not change following the LGM. We conclude, therefore, that cold temperatures and an increased sea ice field during the LGM did not
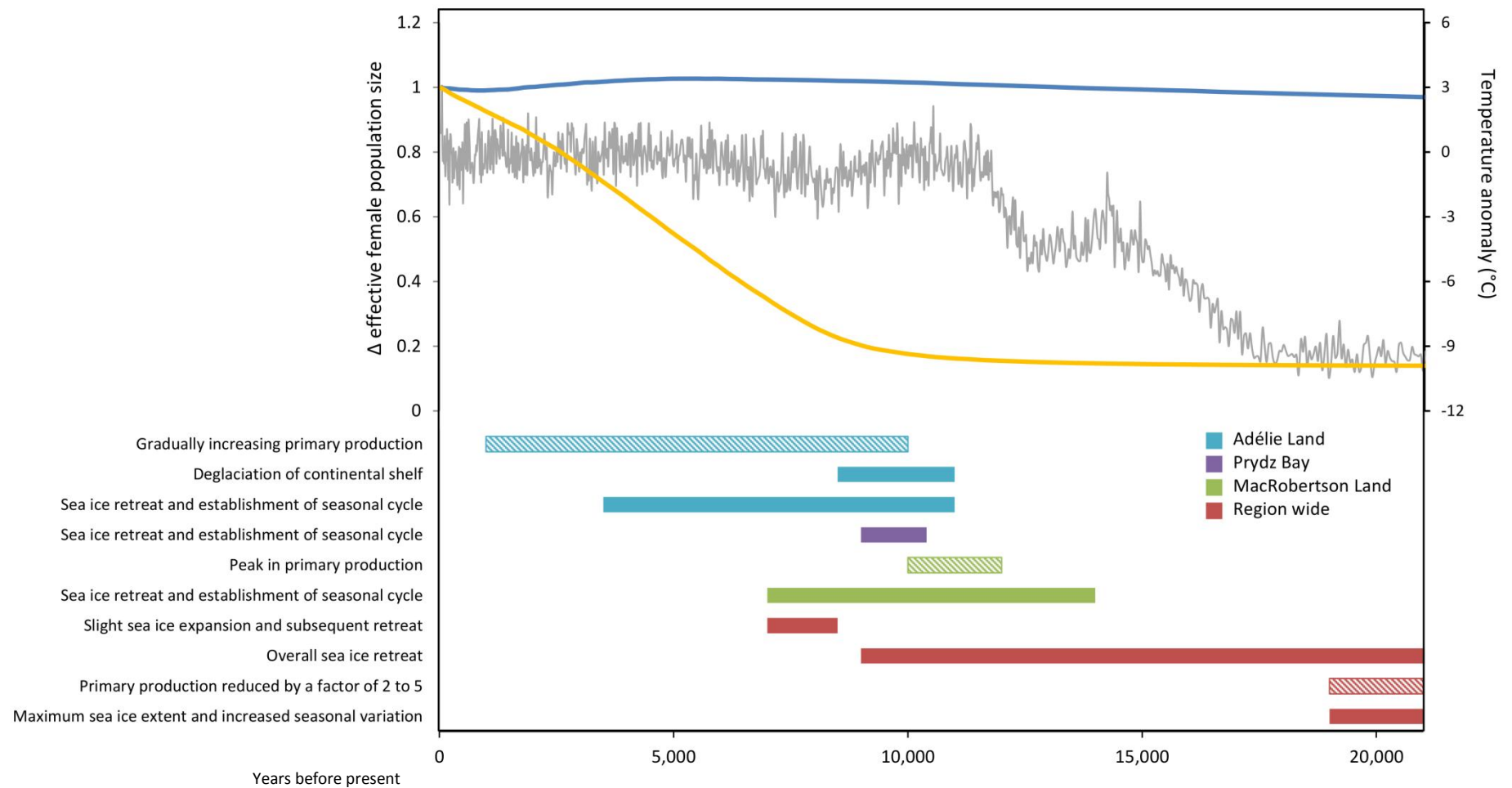
Figure 3.3. Post-glacial and Holocene environmental changes by sub-region compared to abundance trajectories of Weddell seals and emperor penguins. Median estimate of the change in effective female population size (N_{ef}) relative to today; yellow line indicates emperor penguins, blue line indicates Weddell seals. The grey line indicates the East Antarctic temperature anomaly (the difference from the average of the last 1000 years) (Jouzel *et al.*, 2007). Coloured boxes indicate approximate period of environmental changes including; deglaciation (Denis *et al.*, 2009a, Mackintosh *et al.*, 2014), changes in sea ice cover (Barbara *et al.*, 2010, Crosta *et al.*, 2008, Gersonde *et al.*, 2005, Masson *et al.*, 2000) and primary production (Anderson *et al.*, 2009, Denis *et al.*, 2009b, Kohfeld *et al.*, 2005, Mackintosh *et al.*, 2011), where primary production is based on export production indices from sediment records.

positively influence *either* species' population size, but the N_{ef} for emperor penguins has responded positively to other habitat changes during the subsequent post-glacial warming period.

Owing to increases in primary production during the post-glacial warming period (Anderson *et al.*, 2009, Denis *et al.*, 2009b, Kohfeld *et al.*, 2005, Mackintosh *et al.*, 2011), it is possible that prey resources in the East Antarctic region also increased at this time which may have led to the observed increase in emperor penguin numbers. However, based on the static Weddell seal N_{ef} trend, Weddell seals appear to have been unaffected by the same changes in prey availability. This is unexpected as the two predators currently forage at the same trophic level within the meso-pelagic niche (Castellini *et al.*, 1992, Cherel & Kooyman, 1998). While current prey choice does not necessarily reflect historical foraging behaviour, both emperor penguins and Weddell seals are generalist foragers today and should, in theory, be flexible in the prey species that they consume. However, while they do consume similar prey, the seals tend to forage over the continental shelf whereas the penguins usually forage farther north in the pelagic zone (Raymond *et al.*, 2014). These fine scale differences in preferred foraging habitat may explain the differing historical trends, as the pelagic system has more capacity to grow compared to the spatially restricted shelf habitats, and this difference could have favoured emperor penguins. The penguins might also have been more capable of responding to fluctuations in food availability during the early Holocene based on their physiology. Juvenile Weddell seals operate at the edge of their physiological diving ability and cannot increase their effort to exploit deeper foraging areas during periods of reduced prey availability (Burns & Kooyman, 2001). They also cannot reduce their energy requirements by abandoning chicks, as adult emperor penguins can (Burns & Kooyman, 2001). Furthermore, the energy requirements of an individual emperor penguin are substantially less than that of a Weddell seal, owing to their smaller body size. These differences may have allowed emperor penguins to prosper relative to Weddell seals during times of food scarcity.

The depletion of apex predators may result in an increased abundance of meso-predators (Myers *et al.*, 2007), hence, changes in predation pressure during the Holocene could also have affected population trajectories of emperor penguins and Weddell seals. The killer whale is an apex predator that preys both on emperor penguins and Weddell seals (Ainley & Ballard, 2012, Andrews *et al.*, 2008, Pitman & Ensor, 2003). However, killer whale numbers rapidly increased during the Holocene following a population bottleneck during the LGM (Moura *et al.*, 2014). It is therefore unlikely that reduced predation by killer whales during the Holocene was responsible for the increase in emperor penguin population size. Conversely, the increase in emperor penguins, a prey resource of killer whales, may have been a contributing factor to the increase in killer whale numbers during the Holocene. Leopard seals and southern giant petrels also prey on emperor penguins (Budd, 1961,

Kooyman *et al.*, 1990) and a decline in either of these species during the Holocene could have favoured emperor penguin populations, however their population trends throughout the LGM and Holocene are unknown.

While emperor penguins and Weddell seals occupy similar ecological niches, fine scale differences, such as their preferred foraging locations and physiology, may have played important roles in determining their responses to climate change. These subtle differences in ecological niche could have resulted in large differences in their responses to Holocene warming. However, differences in the adaptive capacities of emperor penguins and Weddell seals may also have contributed to their contrasting responses to climate change.

3.5.2 Adaptive capacities

Dispersal facilitates range shifts and promotes gene flow among breeding sites, which can replenish the gene pool of a population with new, potentially adaptive alleles (Walther *et al.*, 2002). We found that emperor penguin colonies across East Antarctica are not genetically differentiated (Table 3.3), suggesting that there is ongoing gene flow among colony sites within the region. This finding also indicates that there are no substantial barriers to dispersal across East Antarctica for emperor penguins, suggesting that the species may be able to emigrate in line with favoured conditions. Our finding of high mobility in emperor penguins is supported by a recent satellite imagery study of colony locations that found six instances of either colony relocation or establishment of a new colony over three years (Ancel *et al.*, 2014, LaRue *et al.*, 2015). A continent-wide genetic study of emperor penguin population structure also found that the penguins were panmictic across *ca.* 8,000 km of coastline, from the Adélie Land Coast to the Weddell Sea (Chapter 2, Younger *et al.*, 2015). Together, these studies strongly refute total philopatry among emperor penguins, and this degree of panmixis must be taken into account in population forecasting studies for emperor penguins, which typically consider breeding colonies as isolated units (Jenouvrier *et al.*, 2014).

Weddell seals, on the other hand, appear to have genetically distinct breeding colonies with a high degree of philopatry, consistent with previous ecological findings that indicate strong site fidelity (Cameron *et al.*, 2007, Davis *et al.*, 2008, Terhune *et al.*, 2008). This finding is based on mitochondrial DNA and therefore represents philopatry of females only, however, Cameron *et al*. (2007) found no difference in the degree of philopatry between male and female Weddell seals, therefore the patterns of genetic population structure should be similar for both sexes. The relative isolation of Weddell seal colonies means that they may be more susceptible to genetic drift and loss of adaptive variation, and are less likely to range shift.

We found that the mitochondrial mutation rates for emperor penguins were greater than for Weddell seals. The faster molecular evolutionary rate of emperor penguins compared to Weddell seals is atypical, as mammals general have higher evolutionary rates than birds (Stanley & Harrison, 1999). This could indicate an increased adaptive capacity of emperor penguins compared to Weddell seals, as higher rates of molecular evolution in mitochondrial DNA are correlated with higher rates of evolution in nuclear genes (Nabholz *et al.*, 2008). Such a notion could be confirmed in future studies by comparing evolutionary rates in potentially adaptive loci, which would allow a more accurate estimation of adaptability.

Overall, emperor penguins appear to possess a greater capacity to adapt to environmental change than Weddell seals, based on their higher rate of gene flow among colonies, greater dispersal ability and higher rate of molecular evolution. This implies that emperor penguins are likely to respond more rapidly to environmental change than Weddell seals, which is supported by our finding that emperor penguin populations expanded during the Holocene while Weddell seal populations did not. As the East Antarctic environment changed during the Holocene, the high dispersal ability of emperor penguins may have facilitated range expansion and the establishment of new breeding colonies, while their higher evolutionary rate may have enabled exploitation of new habitat and resources, leading to increases in population sizes and, most likely, genetic diversity. On the other hand, it must be considered that the stability of Weddell seal N_{ef} throughout several climate oscillations suggests that Weddell seals are robust to environmental changes, and may have a broader range of climatic tolerance compared to emperor penguins. Whether the environmental conditions in East Antarctica over the past 80,000 years have been optimal for Weddell seals is unknown. An investigation of Weddell seal population sizes further back in time, throughout earlier climate regime shifts, could determine whether different climate scenarios are more or less favourable for Weddell seals. This could be achieved using the pairwise sequentially Markovian coalescent method, which allows for the estimation of population sizes over deeper time scales (i.e. 100 kya to 10 mya) (Li & Durbin, 2011). The fact that the Weddell seal population size did not decline during the LGM, the post-glacial period, or the Holocene suggests that the species was able to adapt to environmental change, even if they did not rapidly expand during the Holocene as the emperor penguins did.

3.5.3 Adaptive capacity and contemporary climate change

Emperor penguins are sensitive to changes in sea ice conditions over decadal and yearly timescales (Ainley *et al.*, 2010, Jenouvrier *et al.*, 2005, Jenouvrier *et al.*, 2014). In recent years, when sea ice conditions at their traditional colony sites have been sub-optimal, emperor penguins have demonstrated adaptive behaviour in relation to their breeding strategy by relocating colonies onto

ice shelves (Fretwell *et al.*, 2014, Wienecke, 2012). This behaviour was first observed in 2008, and there are now four recorded ice shelf breeding colonies (Fretwell *et al.*, 2014). This behaviour is thought to be an adaptation to climate change, and is consistent with our findings that emperor penguins may have a high adaptive capacity. Emperor penguins have also shown plasticity in the locations of their breeding colonies, with several cases of relocation of entire colonies by up to 190 km, coincident with sub-optimal sea ice conditions at the former colony sites (Ancel *et al.*, 2014, LaRue *et al.*, 2015, Trathan *et al.*, 2011). In addition to the movement of entire colonies, there is also evidence that emperor penguin individuals are emigrating away from existing colonies to more favourable locales, again as a response to sub-optimal sea ice conditions (Barbraud *et al.*, 2011, LaRue *et al.*, 2015). The findings of colony relocation and emigration show that the dispersal ability of emperor penguins, which our genetic data has shown to be great, is aiding in their response to contemporary unfavourable environmental change, and suggests that this same ability may have facilitated their response to post-glacial warming.

A 30-year study of Weddell seals at Erebus Bay, Ross Sea has shown that Weddell seals use a flexible breeding strategy as a means of demographic buffering when environmental conditions are sub-optimal (Rotella *et al.*, 2012). This strategy was observed in response to the calving of the B-15 iceberg in the Ross Sea in March 2000, which was approximately 10,000 km^2 and persisted in the vicinity of the Erebus Bay seal colony until 2005 (Chambert *et al.*, 2012). Overall reproductive rates were reduced during this period, and there was high inter-annual variability in reproductive rates, possibly as a response to shifts in sea ice conditions and prey availability; however, survival rates were unaffected, and high reproductive rates were observed following the perturbation (Chambert *et al.*, 2012). Whether demographic buffering such as this may have allowed Weddell seals to maintain constant population sizes over the past 80,000 years is currently unknown. Relocation of colonies, or emigration of individuals away from sub-optimal breeding locations, have not been documented for Weddell seals under contemporary climate change to date. This is consistent with our genetic data, which indicates that Weddell seals have low rates of gene flow among colony sites and are not widely dispersive.

3.5.4 Concluding remarks

Our study has revealed that two iconic Antarctic meso-predators with similar niches and distributions responded very differently to late Pleistocene climate warming. Differing adaptive capacities and/or fine-scale niche differences likely played a major role in the responses of these predators to climate change. This is noteworthy, given that broad ecological niche and distribution have been used to forecast declines of several marine predators, without regard to adaptive capacity or fine-scale niche

differences. Individual species are likely to respond quite differently to changing habitats and generalisations based on apparent ecological niche may be misleading. We have shown that dispersal ability, gene flow and evolutionary rate may be indicators of robustness to climate change, and would suggest that genetic indices of population subdivision, diversity and demographic history are included in future risk analyses for predator species.

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3.7 Appendices

Appendix 3.1 Table of primer sequences

Species	Primer name	Primer sequence 5' – 3'
EP	F-0225	GGAACCTCCCAAAGAGTACCA
EP	RINR	CCAACCAGATGTATCGGTGA
EP	HVR-R1	TGAAAGTATTGCTTTACGTATCCTT
EP	HVR-F2	AAGGATACGTAAAGCAATACTTTC
EP	HVR-R2	AGGAGTAATTGTTGAGTACATGACA
EP	HVR-F3	TCATGTACTCAACAATTACTCCTG
EP	HVR-R3	TCACGTGAGAAGACCGACTAA
EP	HVR-F4	ATCTCCTGAGGCGCTAGCTT
EP	B1	CCATCCAACATCTCAGCATGATGAAA
EP	B6	CCATCCAACATCTCAGCATGATGAAA
EP	CytB-F1	ACTGCAGACACAACCCTAGC
EP	CytB-R1	GGGAAGAGGATCAGGAGGGT
EP	CytB-R1	AATGATGCTCCGTTTGCATGTAGGTT
EP	CytB-F2	ACACATGCCGAAACGTACAG
EP	CytB-R2	GTAGCCTACGAAGGCGGTTG
EP	CytB-F3	GAAACCTGAAACACAGGCATT
EP	CytB-R3	CGGGTTAATGTGGGGTTGT
EP	CytB-F4	CTCAGCCATCCCTTACATTG
EP	CytB-R4	TTGTGGAGTAGTAGGGGTGGA
EP	CytB-F5	CAAATAACCCACTGGGCATC
EP	CytB-R5	TCATTCTGGTTTGATGTGTGG
EP	CytB-F6	CCAGCAAACCCACTAGTCAC
EP	CytB-R6	GGGCTCAGAATAGGAGTTGG
EP	CytB-F7	ATAGCTTTCCGCCCTCTCT
WS	TDKD	CCTGAAGTAGGAACCAGATG
WS	L15926	TCAAAGCTTACACCAGTCTTGTAAACC
WS	L-CytB	AGGCGTCGAAGCTTGACATGAAAAGCCATCGTTG
WS	H-CytB	CGAATTCCATTTTTGGTTTACAAGAC
WS	Csec2_R	TCATATATAACATCACTTTCACTGTGC
WS	Csec1_R	CAACCACTTTATGTGCATGCTT
WS	CytBSec1_F	TGACATGAAAAATCATCGTTGT
WS	CytbSec1_R	CGGCAGATGTGTGTAACTGA
WS	CytbSec2_F	CAGGCCTATTCCTAGCCATACA
WS	CytbSec2_R	TGTGAATGTGTAGGAGCCGTA
WS	CytbSec3_F	GGAGCATCCATATTCTTCATCTG
WS	CytbSec3_R	CATTGTACTAAGTCAGTTCCGATGT
WS	CytbSec4_F	AAATATCATTCTGAGGAGCAACC
WS	CytbSec4_R	GGGGTGAAATGGGATTTTGT
WS	CytbSec5_F	CACGAGACAGGATCCAACAA
WS	CytbSec5_R	TTGCGTAGGCAAATAGGAAA
WS	CytbSec6_F	CCACATATCAAACCCGAATG
WS	CytbSec6_R	ATGTAGGGGTGTTCGACTGG
WS	CytbSec7_F	TCAGCCAATGCTTATTCTGACT
WS	CytbSec7_R	TGTTCTCCGTTTTTGGTTTACA

Appendix 3.2 Table of summary statistics of Weddell seal and emperor penguin colonies by genetic region

Overall values for Weddell seals and Emperor penguins refer to extant individuals only, with subfossil individuals shown separately. Number of individuals (n), nucleotide diversity averaged over loci (π), number of unique haplotypes (N_H), mean number of pairwise differences between haplotypes (pairwise) and substitution rate in substitutions/site/Myr (Rate).

	HVR					CytB			
	n	π	N_H	Pairwise	Rate	π	N_H	Pairwise	Rate
Tryne Fjord	23	0.010 ± 0.006	11	4.865 ± 2.462		0.005 ± 0.003	10	5.304 ± 2.657	
Long Fjord	5	0.006 ± 0.004	2	2.800 ± 1.768		0.002 ± 0.002	2	2.400 ± 1.556	
Herring Islands	21	0.010 ± 0.006	6	5.104 ± 2.578		0.004 ± 0.002	4	4.180 ± 2.163	
Swain Group	7	0.004 ± 0.003	3	1.714 ± 1.131		0.003 ± 0.002	4	3.904 ± 2.224	
Colbeck	16	0.011 ± 0.006	14	5.208 ± 2.659		0.006 ± 0.003	16	6.500 ± 3.245	
Pointe Géologie	18	0.010 ± 0.006	15	4.830 ± 2.472		0.005 ± 0.003	16	5.169 ± 2.625	
Weddell seals	**90**	**0.010 ± 0.006**	**38**	**5.102 ± 2.498**	**0.114 ± 1.76x10^{-3}**	**0.005 ± 0.003**	**40**	**5.658 ± 2.739**	**2.85x10^{-2} ± 4.48x10^{-4}**
Subfossil WSs	6	0.009 ± 0.006	4	4.466 ± 2.560		0.005 ± 0.003	4	5.133 ± 2.895	
Auster	21	0.024 ± 0.013	20	15.15 ± 7.040		0.003 ± 0.002	7	2.747 ± 1.504	
Amanda Bay	24	0.027 ± 0.014	24	17.00 ± 7.824		0.003 ± 0.002	12	2.960 ± 1.594	
Pointe Géologie	22	0.032 ± 0.016	22	20.28 ± 9.264		0.003 ± 0.002	10	2.960 ± 1.602	
Fold Island	24	0.030 ± 0.015	24	18.70 ± 8.567		0.003 ± 0.002	8	2.889 ± 1.568	
Emperor penguins	**91**	**0.028 ± 0.014**	**90**	**17.89 ± 8.009**	**0.760 ± 4.55x10^{-3}**	**0.003 ± 0.002**	**26**	**2.857 ± 1.516**	**3.88x10^{-2} ± 3.58x10^{-4}**
Subfossil EPs	3	0.0064 ± 0.0054	3	4.000 ± 2.725		0.002 ± 0.001	3	2.000 ± 1.511	

CHAPTER 4

RAPID RESPONSE
OF EAST ANTARCTIC ADÉLIE PENGUINS
TO DEGLACIATION

4.1 Abstract

Major, long-term environmental changes are projected in the Southern Ocean, and these are likely to have impacts for marine predator species such as the Adélie penguin. Decadal monitoring studies have provided insight into the short-term environmental sensitivities of Adélie penguin populations, particularly to sea ice changes. However, given the long-term nature of projected climate change, it is also prudent to consider the responses of populations to environmental change over longer time scales. Using mitochondrial DNA from extant colonies, we report the population trend of Adélie penguins in East Antarctica over the past 22,000 years. We found that the Adélie penguin population expanded rapidly, from approximately 14,000 years ago, during the glacial-interglacial transition. The population expansion is coincident in timing with deglaciation in East Antarctica and therefore an increase in ice-free ground suitable for nesting. While changes in sea ice conditions are a critical driver in Adélie penguin population success over decadal and yearly timescales, ice sheet retreat appears to have been the key driver of population change following the last glacial maximum.

4.2 Introduction

While climate change is a global phenomenon, its environmental effects can vary dramatically in different locations. For example, trends in the extent and duration of sea ice around the Antarctic continent show high spatial heterogeneity (Vaughan *et al.*, 2013, Zwally *et al.*, 2002). Over a 34-year monitoring period, sea ice extent decreased in the Bellingshausen and Amundsen Seas accompanied by a dramatic shortening of the sea ice season by 100 ± 31 days (Stammerjohn *et al.*, 2012, Vaughan *et al.*, 2013). Meanwhile, in the Ross Sea, both extent and duration of the sea ice season increased substantially over the same period (Vaughan *et al.*, 2013). Even within Antarctic regions there have been variations in the extent and duration of sea ice. For example, the East Antarctic region, defined here as between 30°E and 150°E, has demonstrated considerably more complex trends in sea ice seasonality and extent than the rest of the continent (Fraser *et al.*, 2012, Massom *et al.*, 2013). Since 1980, in some East Antarctic areas (between 95°E and 110°E; and isolated pockets between 75°E and 150°E), there has been a significant shortening of the sea ice season by up to 93 days (Massom *et al.*, 2013). Meanwhile, many other localities (west of 105°E; between 40°E and 90°E) have experienced a significant lengthening of the sea ice season (Massom *et al.*, 2013). Antarctic sea ice is expected to undergo further declines in the future; in the most extreme climate model scenario (RCP8.5), East Antarctica would be completely free of sea ice in February by 2081-2100, while only small portions of the Weddell and Ross Seas would retain sea ice (Collins *et al.*, 2013).

Environmental changes that are currently underway in the Southern Ocean, including changes in the timing of sea ice advance and retreat, receding glaciers and shifting oceanographic fronts, could lead to major changes in the terrestrial breeding habitats, marine foraging environment and prey availability for higher order predators (Constable *et al.*, 2014, Smetacek & Nicol, 2005). The Adélie penguin (*Pygoscelis adeliae*) is a prime example of a Southern Ocean predator that is likely to be affected by environmental impacts associated with the Anthropocene (Lewis & Maslin, 2015). The species forms breeding colonies on ice-free land along the Antarctic coastline (Figure 4.1) (Lynch & LaRue, 2014, Trathan & Ballard, 2013) and forages largely in the pack ice zone during the breeding season (Clarke *et al.*, 2006). Adélie penguin populations are known to be sensitive to changes in sea ice extent, the timing of sea ice retreat (Emmerson *et al.*, 2011, Emmerson & Southwell, 2008) and the extent of glaciation (Baroni & Orombelli, 1994). Given the large regional variability in the physical manifestations of climate change, it is likely that geographically distant populations of Adélie penguins will experience very different environmental impacts (Forcada *et al.*, 2006).

East Antarctica is home to approximately 30% of the global population of Adélie penguins, with an estimated abundance of 1.14 million breeding pairs (Lynch & LaRue, 2014). The breeding distribution in this region has expanded over the past several decades, possibly as a result of sustained population growth (Southwell & Emmerson, 2013). Sea ice conditions strongly influence Adélie penguin populations in East Antarctica, although the mechanisms of impact are complex and depend on the nature, extent and timing of the presence of sea ice (Emmerson & Southwell, 2008, Kato *et al.*, 2002). The Béchervaise Island population experienced near total reproductive failure in years with extensive nearshore sea ice during the guard stage (Emmerson & Southwell, 2008). The proposed mechanism for this impact is a reduced efficiency of chick provisioning at a crucial time in the breeding season, as more extensive sea ice increases the duration of the adults' foraging trips and reduces the frequency at which the chicks are fed (Emmerson & Southwell, 2008). The negative impact of unusually extensive sea ice was also felt at the Pétrels Island colony at Pointe Géologie in the 2013/14 breeding season, with emaciated chicks often observed during the summer (Ropert-Coudert *et al.*, 2015). In this case, the negative impact of extensive sea ice was compounded by unusually warm air temperatures that caused snowmelt and unprecedented amounts of rain (Ropert-Coudert *et al.*, 2015). Rain can be fatal to Adélie penguin chicks, as their downy plumage is not waterproof and when wet they may succumb to cold temperatures (Ropert-Coudert *et al.*, 2015). In the case of Pétrels Island, the compounded effects of extensive sea ice and warm temperatures were devastating, resulting in 100% chick mortality in 2013/14 (Ropert-Coudert *et al.*, 2015). In the opposite scenario, at Pointe Géologie the size of breeding populations increased six
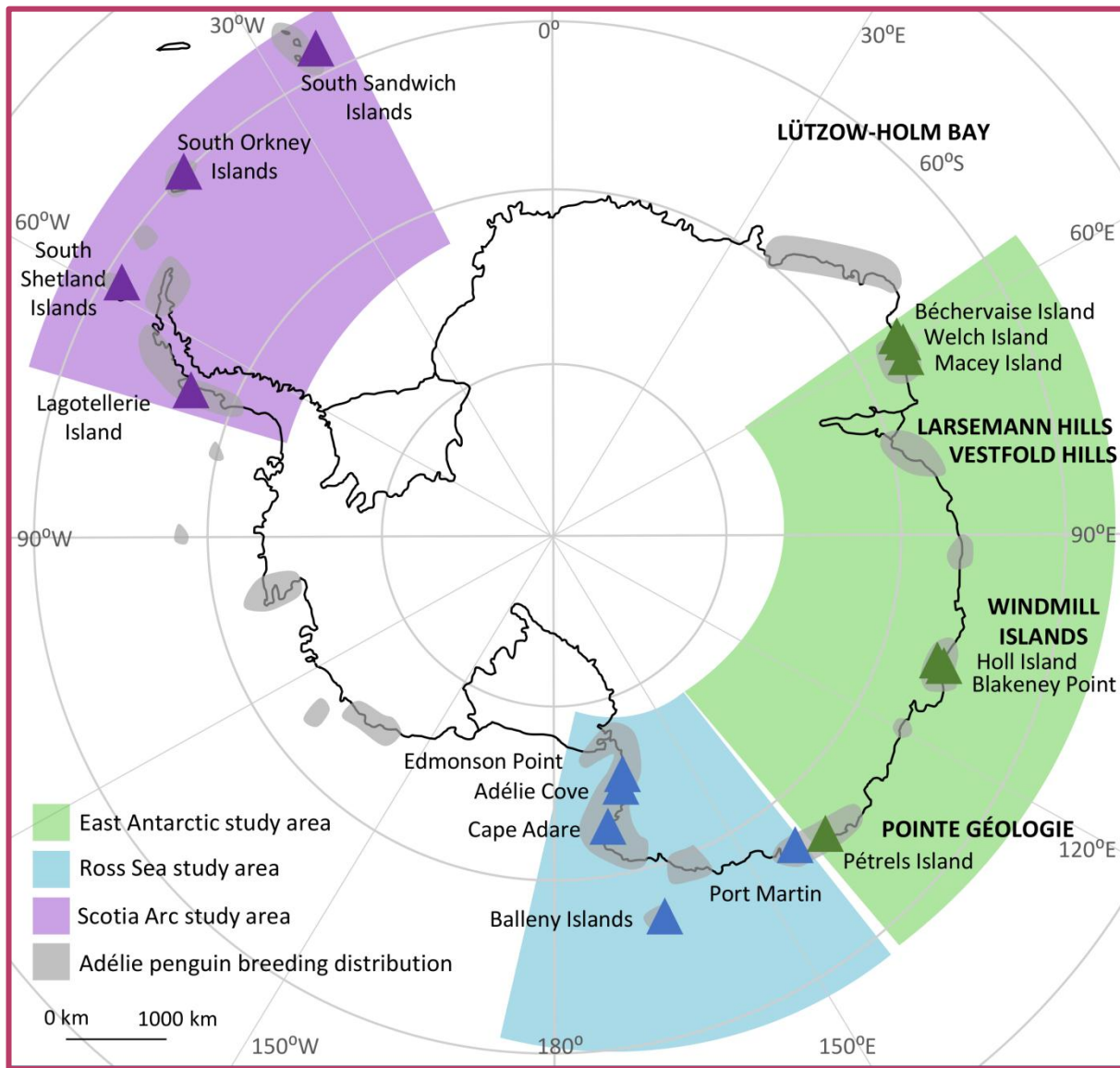
Figure 4.1. Adélie penguin breeding distribution. Grey shading indicates the approximate extant breeding distribution of Adélie penguins, approximated from (Lynch & LaRue, 2014, Southwell *et al.*, in press). Green triangles indicate East Antarctic colonies sampled in this study, blue and purple triangles indicate colonies in the Ross Sea (Ritchie *et al.*, 2004) and Scotia Arc (Clucas *et al.*, 2014) datasets, respectively.

years after a period of low sea ice extent and concentration (Jenouvrier *et al.*, 2006). As Adélie penguins commence breeding between five and six years of age, it is likely that sea ice conditions during the fledgling and yearling stages are important, with lower sea ice extent being favourable for young birds (Jenouvrier *et al.*, 2006). At Béchervaise Island adult penguins were also sensitive to extremes in sea ice concentrations in their winter foraging grounds, with either too much sea ice (> 80% cover) or too little sea ice (< 15% cover) negatively impacting adult survival (Emmerson *et al.*, 2011). Extreme climatic events that alter the sea ice environment may also impact Adélie penguin reproductive success. In 2010 there was a calving of the Mertz Glacier Tongue, which resulted in decreased polynya activity and sea ice production in the area (Dragon *et al.*, 2014, Tamura *et al.*, 2012) and these local changes in the icescape appear to have negatively impacted Adélie penguin reproductive success at the nearby Pointe Géologie populations in the 2011/12 and 2012/13 breeding seasons (Raymond *et al.*, 2014).

While decadal scale monitoring studies have provided invaluable data on the short-term environmental sensitivities of Adélie penguin populations, given the long-term environmental change projected in the Southern Ocean, it is also prudent to consider the responses of populations to environmental change over longer time scales (e.g. thousands to tens of thousands of years) and during climate regime shifts, for example, during the transition from the last glacial maximum (LGM, 26 – 19.5 kya) to the Holocene warming period (11.7 kya – present) (Jouzel *et al.*, 2007). The responses of Adélie penguins to climate change during the LGM and Holocene have been well-studied for populations in the Ross Sea and Scotia Arc regions (Millar *et al.*, 2012). In both locations, Adélie penguin numbers were much lower during the LGM than they are today, which was thought to be the result of reduced ice-free ground suitable for breeding (Clucas *et al.*, 2014, Li *et al.*, 2014). Phylogenetic studies have found evidence of two genetic lineages of Adélie penguins that are suggestive of two refuge populations dating to the LGM (Clucas *et al.*, 2014, Lambert *et al.*, 2002, Ritchie *et al.*, 2004). One of these lineages was comprised solely of individuals from modern Ross Sea colonies, suggesting that a refuge may have been situated somewhere in the vicinity of the Ross Sea during the LGM (Ritchie *et al.*, 2004). The second lineage was comprised of individuals from the Scotia Arc, Ross Sea, and East Antarctica (Clucas *et al.*, 2014, Lambert *et al.*, 2002, Ritchie *et al.*, 2004). However, the samples analysed for the East Antarctic region were limited to two colonies at Gardner and Welch Islands and, given the length of the coastline, may not be representative of the genetic diversity of the broader region. As this second genetic lineage shows no strong geographic affinity, the location of its associated LGM refuge is unknown. Based on a genetic coalescent study, Adélie penguin numbers in the Scotia Arc increased during the Holocene warming period from *ca*. 16

kya (Clucas *et al.*, 2014), roughly coincident with deglaciation of the region (Cofaigh *et al.*, 2014, Hodgson *et al.*, 2014). In the Ross Sea, radiocarbon dated remains suggest that the Adélie penguin distribution expanded from approximately 8 kya, followed by two periods of reduced occupation from 5 – 4 kya and 2 – 1.1 kya (Emslie *et al.*, 2007).

The impacts of past climate regime shifts on Adélie penguins have been less well studied in East Antarctica than in other parts of the Antarctic continent. In the Windmill Islands and Vestfold Hills, radiocarbon dating of remains suggests that Adélie penguins were present as early as 9 kya and 8.5 kya respectively, roughly coincident with local deglaciation and therefore an emergence of available ice-free nesting habitat (Emslie & Woehler, 2005, Huang *et al.*, 2009b). There is also evidence for a peak in Adélie penguin numbers *ca*. 4 kya, coinciding with a mid-Holocene warm period (Emslie & Woehler, 2005, Huang *et al.*, 2009a). During the LGM, the ancestors of individuals currently breeding at Gardner Island in the Prydz Bay region and Welch Island on the Mawson Coast were restricted to a refuge population along with the ancestors of both extant Scotia Arc and Ross Sea colonies (Clucas *et al.*, 2014, Lambert *et al.*, 2002, Ritchie *et al.*, 2004). However, as mentioned previously, the location of this refuge is unknown. Several questions regarding the past dynamics of East Antarctic Adélie penguin populations remain to be answered, including the trends in abundance during and after the LGM, whether an LGM refuge population may have been located in this area, and how populations may have responded to environmental changes across the extensive East Antarctic region. In this study we sought to address these questions using genetic data from multiple extant Adélie penguin colonies across East Antarctica.

4.3 Methods

4.3.1 Field collections

Tissue specimens were collected between 2012 and 2014 from the carcasses of deceased Adélie penguins at Blakeney Point (n = 13), Holl Island (n = 7), Macey Island (n = 2), Welch Island (n = 13), Béchervaise Island (n = 11) and Pétrels Island (n = 10) (Figure 4.1). We refer to these collections as the East Antarctic region samples. Tissue was transported and stored at -20°C.

4.3.2 Molecular laboratory

Genomic DNA was extracted using the QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's protocols. The mitochondrial hypervariable region (HVR) and cytochrome B (CytB) were sequenced, as these markers have been successfully used to reconstruct the demographic

history of the closely related emperor penguin (Chapter 2, Younger *et al.*, 2015), and the use of HVR allowed for comparisons with published Adélie penguin datasets from the Ross Sea (Lambert *et al.*, 2002, Ritchie *et al.*, 2004) and Scotia Arc (Clucas *et al.*, 2014).

HVR was amplified and sequenced for all individuals using primers AP1STR (5'-CCACCCTATACATACAA TTCCCCTCCC-3') (Clucas *et al.*, 2014) and H-A650 (5'-CTGACATAGGAACCAGAGGCGC-3') (Clucas *et al.*, 2014, Ritchie *et al.*, 2004, Roeder *et al.*, 2002). CytB was amplified and sequenced for individuals from Béchervaise Island, Macey Island, Welch Island, Blakeney Point, and Pétrels Island using primers CytB-F1 (5'-ACTGCAGACACAACCCTAGC-3') (Chapter 2, Younger *et al.*, 2015) and CytB-R1 (5'-GGGAAG AGGATCAGGAGGGT-3') (Chapter 2, Younger *et al.*, 2015). For both HVR and CytB, reaction mixes consisted of 7.5 µL of GoTaq Green Master Mix (Promega), 0.2 µM of each primer, and 5–10 ng of gDNA, made up to 15 µL with ddH$_2$O. Annealing temperatures for HVR and CytB PCRs were 52.5°C and 60°C, respectively. Bi-directional Sanger sequencing using the PCR primer pairs was carried out at the Australian Genome Research Facility (AGRF). A number of heteroplasmic sites were present in the HVR dataset and these were re-scored manually according to IUPAC ambiguity codes.

4.3.3 Summary statistics

jModeltest (Posada, 2008) was used to estimate the optimal nucleotide substitution model for each dataset and Arlequin v3.5 (Excoffier & Lischer, 2010) was used to calculate summary statistics for the CytB and HVR datasets and to perform analyses of molecular variance (AMOVA).

4.3.4 East Antarctic demographic reconstructions

The demographic history of East Antarctic Adélie penguins over the past 22,000 years was reconstructed using the coalescent extended Bayesian skyline plot method (Heled & Drummond, 2008) within BEAST v2.1.3 (Bouckaert *et al.*, 2014). The nucleotide substitution model for both HVR and CytB were specified as HKY (Hasegawa *et al.*, 1985) with four gamma categories, which was selected by jModelTest (Posada, 2008) as the optimal model in both cases. A strict molecular clock was used with lognormal substitution rate priors specified for HVR (mean = 0.55 substitutions/site/Myr, SD = 0.15) to reflect the published substitution rate of the HVR in Adélie penguins (Millar *et al.*, 2008); and for CytB (mean = 0.039 substitutions/site/Myr, SD = 1.5) to reflect the substitution rate of CytB in the closely related emperor penguin (Chapter 3).

The posterior distribution of effective population size through time was generated using the Markov chain Monte Carlo (MCMC) sampling procedure, which was run for 120 million generations with samples drawn every 5000 steps. Tracer v1.5 was used to visualise the sampling trace and to check

the effective sample size values (ESS) to confirm convergence, with most ESS values exceeding 1000, and all values exceeding 200. Three independent BEAST analyses with different random number seeds were performed to ensure reproducibility of the posterior distribution.

The effective population size (N_e) (Wright, 1931) is the number of individuals in an ideal population (i.e. a population with an equal sex ratio, random mating and no variation in reproductive success) that undergoes random genetic change at the same rate as the real population (Crow & Kimura, 1970). Because real populations do not usually adhere to these idealised constraints, N_e is almost always considerably smaller than the census size of a population (N); a recent review across a wide range of taxonomic groups found a median N_e/N ratio of 0.14 (Palstra & Ruzzante, 2008). Because mitochondrial DNA was used for this study, our estimate is for female effective population size (N_{ef}). To convert the population size parameter of the demographic model ($N_{ef}*tau$) to N_{ef}, we divided the parameter by the generation length of Adélie penguins, which we have estimated at 13.3 years using the formula $tau = A + [S/(1-S)]$, where A = age of first breeding (estimated at 4.85 for females, (Ainley *et al.*, 1983)) and S = yearly survival rate after reaching breeding age (estimated at 0.894, (Ainley, 2002)). Generation length can be difficult to assess accurately for penguins, as both age at first breeding and annual survival rates may differ by location and be influenced by anomalous environmental conditions. It should therefore be noted that any variance in our estimate of *tau* would affect the absolute value of N_{ef} in our results, but has no bearing on either the timing or magnitude of the abundance increase reported. It should also be noted that the measure of N_{ef} applies to the total breeding population and is not necessarily constrained to our study area, which may be inside the boundaries of a larger panmictic breeding population.

4.3.5 Antarctic wide phylogeny

In order to determine the phylogenetic placement of East Antarctic Adélie penguins within the global population of Adélie penguins, we reconstructed a phylogeny for a dataset of HVR sequences of Adélie penguins from East Antarctica (this study), from the Scotia Arc and Antarctic Peninsula (hereinafter referred to as Scotia Arc) (Clucas *et al.*, 2014), and from the Ross and Somov Seas (hereinafter referred to as Ross Sea) (Ritchie *et al.*, 2004). The Scotia Arc dataset consisted of 36 individuals from the South Shetland Islands, the South Orkney Islands, the South Sandwich Islands, and Lagotellerie Island (Figure 4.1) (GenBank PopSet 634224762; Clucas *et al.*, 2014). The Ross Sea dataset consists of 49 individuals, randomly chosen from within GenBank PopSet 45443792, which contains Adélie penguins sampled from Cape Adare, Port Martin, Adélie Cove, Edmonson Point and the Balleny Islands (Figure 4.1) (Ritchie *et al.*, 2004).

Bayesian phylogenetic analyses were performed on this dataset using BEAST v2.1.3 (Bouckaert *et al.*, 2014). The nucleotide substitution model and substitution rate prior for HVR were specified as described above for the demographic reconstruction, and the coalescent Bayesian skyline tree prior was used (Drummond *et al.*, 2005). The posterior distribution of phylogenetic trees was generated using the MCMC sampling procedure, which was run for 200 million iterations with trees logged every 40,000 steps. Tracer v.1.5 was used to check effective sample size (ESS) values to confirm convergence with all values >200. Four independent BEAST analyses, from different random number seeds, were performed to ensure reproducibility of the posterior distribution. The maximum clade credibility tree (after a burn in of 5%) was selected using TreeAnnotator v2.1.3 and then visualised in FigTree v1.4. The heights specified for each node in the tree are median values.

4.4 Results

4.4.1 Summary statistics

We sequenced 56 individuals from six colonies located in East Antarctica (Béchervaise Island, Macey Island, Welch Island, Blakeney Point, Holl Island and Pétrels Island; Figure 4.1). A 642 bp fragment of the mitochondrial hypervariable region (HVR) was sequenced for each individual [GenBank: KT932437 - KT932492], and a 902 bp fragment of cytochrome b (CytB) was sequenced for 45 individuals [GenBank: KT932493 - KT932537].

Genetic diversity was high for the HVR, with 85 polymorphic sites in the 642 bp fragment, 55 unique haplotypes recorded from the 56 individuals sequenced, and a mean number of pairwise differences between haplotypes of 8.54 ± 4.01. CytB genetic diversity was lower than for the HVR, with only 11 unique haplotypes among the 45 individuals sequenced. We did not detect any significant genetic structure among the colonies (CytB: F_{ST} = 0.023, *p* = 0.170; HVR: F_{ST} = 0.019, *p* = 0.055). The AMOVA analyses for HVR indicated that 98.07% of the observed genetic variation occurred within colonies, with only 1.93% of variation among colonies. The CytB result was similar, with 97.65% of the observed genetic variation within colonies and 2.35% of variation among colonies. These findings indicate that the East Antarctic samples can be analysed as a single population with respect to demographic history. The lack of genetic structure among East Antarctic colonies is consistent with a previous study of Adélie penguin genetic structure, which found genetic homogeneity among Adélie penguins around the continent based on seven microsatellite loci (Roeder *et al.*, 2001).

4.4.2 East Antarctic Adélie penguin abundance over the past 22,000 years

There is evidence of a rapid population expansion in East Antarctic Adélie penguins commencing around 14 kya, towards the end of the glacial-interglacial transition (Figure 4.2). Interestingly, during the period of 22 kya to 15 kya, which encompassed the LGM and the majority of the subsequent glacial-interglacial transition period, the N_{ef} of East Antarctic Adélie penguins was less than 1,000, and based on the 95% highest posterior density interval (HPD) may have been zero (Figure 4.2). It is therefore possible that Adélie penguins were not present in East Antarctica prior to *ca*. 15 kya. The population size then began to rapidly increase *ca*. 14 kya (95% HPD: 11 kya to 19 kya), with a total increase of approximately 135-fold (median estimate, Figure 4.2). This period of population growth is coincident with increasing temperatures and deglaciation in East Antarctica during the Holocene (Figure 4.2).
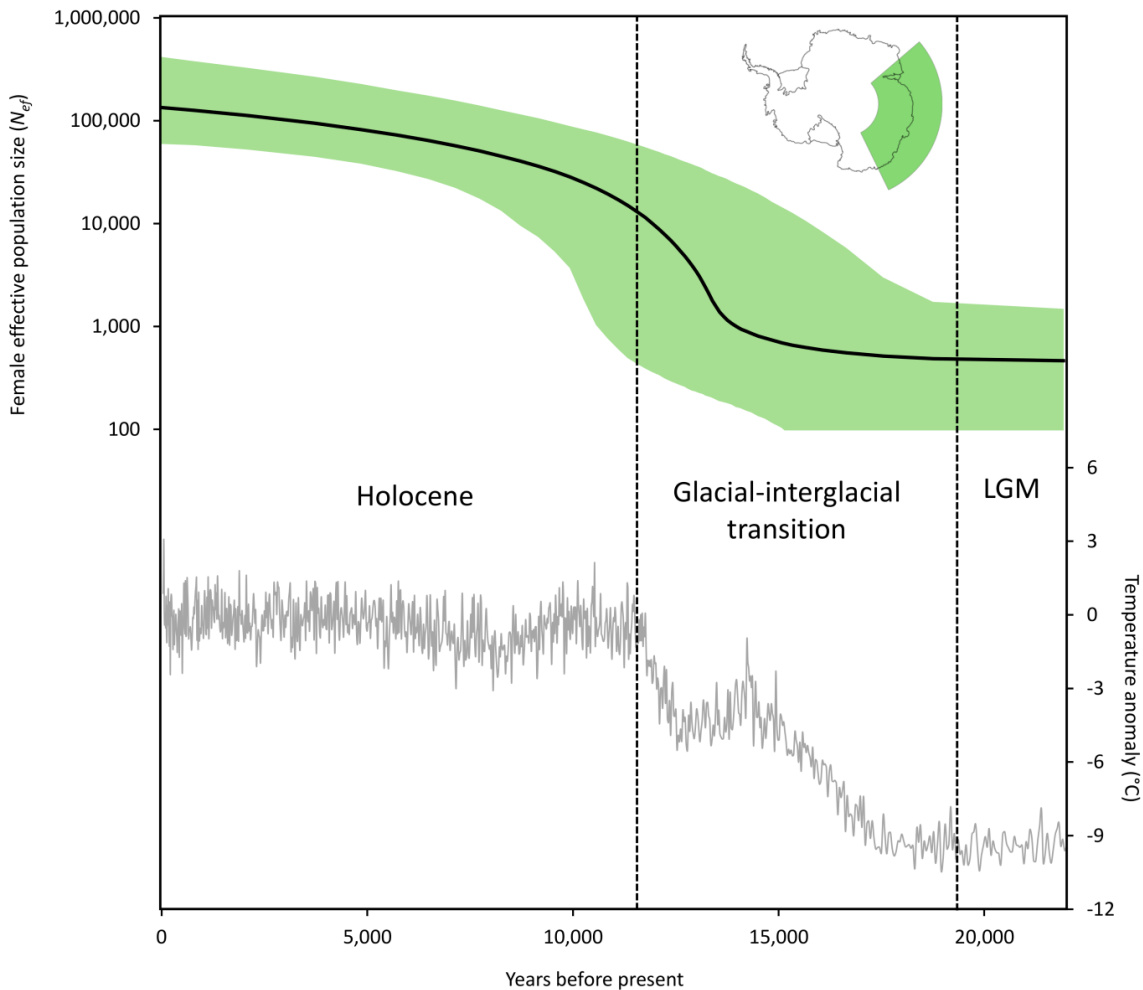
Figure 4.2. Abundance trend of East Antarctic Adélie penguins over the last 22,000 years. Extended Bayesian skyline plot showing the change in effective female population size (N_{ef}), with the black line indicating the median estimate and the colour block showing the 95% highest posterior density interval. The grey line indicates the East Antarctic temperature anomaly (the difference from the average of the last 1000 years) as estimated from the EPICA Dome C ice core (Jouzel *et al.*, 2007).

4.4.3 Antarctic wide mtDNA phylogeny

Our phylogenetic analyses of Adélie penguins from East Antarctica, the Ross Sea (Ritchie *et al.*, 2004) and the Scotia Arc (Clucas *et al.*, 2014), revealed two strongly supported clades (posterior probability = 1; Figure 4.3). One of these clades is comprised solely of penguins sampled at Ross Sea colonies, whereas the other contains individuals from colonies in East Antarctica, the Scotia Arc and the Ross Sea. This confirms the pattern found by Ritchie *et al*. (2004), who noted the presence of one Ross Sea lineage and one "Antarctic" lineage, which comprised individuals from the Ross Sea, Welch and Gardner Islands in East Antarctica, and Torgersen Island on the Antarctic Pensinsula. Clucas *et al*. (2014) compared Scotia Arc penguins to Ross Sea individuals using a haplotype network, and found that individuals from the Scotia Arc also fell into the "Antarctic" lineage. Our study now shows that Adélie penguins from additional locations in East Antarctica, including Pétrels Island, Holl Island, Macey Island, Blakeney Point and Béchervaise Island, also fall into the "Antarctic" lineage.

Our dated phylogeny indicates that both lineages originated during the last glacial period and probably represent two glacial refugia, with the time to the most recent common ancestor (T_{MRCA}) of the Ross Sea lineage estimated at 33 kya (95% HPD: $22-48$ kya), and of the Antarctic lineage estimated at 29 kya (95% HPD: 22–39 kya).

Within the two lineages there is a high degree of topological uncertainty, suggesting very little genetic structure among Adélie penguin populations, aside from the division into two lineages. This is consistent with our F_{ST} and AMOVA results, as well the published findings of genetic homogeneity among Adélie penguins around much of the continent based on microsatellite DNA markers (Roeder *et al.*, 2001).
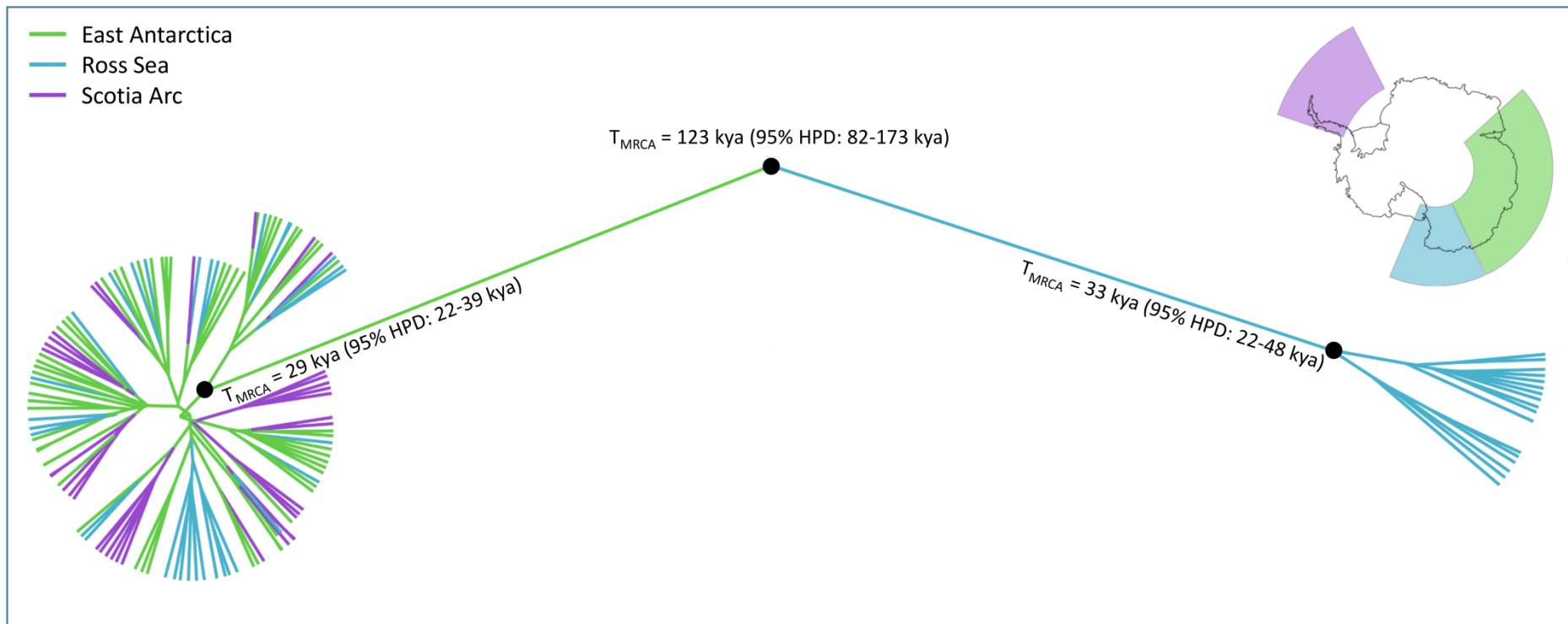
Figure 4.3. Phylogeny of Adélie penguins. East Antarctic penguins are indicated by green, Ross Sea penguins by blue, and Scotia Arc penguins by purple. Black dots indicate strongly supported clades, with a posterior probability of one. The time to most recent common ancestor for the strongly supported clades is indicated as T_{MRCA}, with the 95% highest posterior density interval shown in brackets.

4.5 Discussion

Mitochondrial DNA sequence data has revealed that Adélie penguin numbers in East Antarctica increased 135-fold during the most recent glacial-interglacial transition. This dramatic expansion suggests that the East Antarctic environment is currently much more favourable for Adélie penguins than it was prior to 14 kya. Our genetic analyses shows that the East Antarctic Adélie penguin population began to expand between 19 kya and 11 kya (95% HPD), with a median estimate of 14 kya. This was coincident with a global change in climate regime, from the LGM (26 – 19.5 kya) to the warm Holocene (11.7 kya – present) (Jouzel *et al.*, 2007). During this transitional period, glaciers and ice sheets retreated (Barbara *et al.*, 2010, Bentley *et al.*, 2014, Mackintosh *et al.*, 2014), Southern Ocean primary productivity increased by between two and five fold (Denis *et al.*, 2009, Hillenbrand & Cortese, 2006, Kohfeld *et al.*, 2005), the winter sea ice field halved in areal extent (Gersonde *et al.*, 2005), and sea ice became seasonal, rather than perennial (Crosta *et al.*, 2008). These changes in the East Antarctic coastal environment would have created more favourable conditions for Adélie penguins, by increasing the amount of ice-free ground suitable for nesting, by increasing prey abundance as marine productivity rose, and by creating more accessible marine foraging grounds as sea ice concentrations lessened.

Previous studies based on radiocarbon dated remains, found at the Windmill Islands and Vestfold Hills, placed the colonisation of East Antarctica by Adélie penguins at *ca*. 9 kya (Emslie & Woehler, 2005, Huang *et al.*, 2009b); our data from a more extensive set of sites across the region now show that East Antarctica was colonised at least 2,000 years earlier. Given the confidence interval on our demographic reconstructions (Figure 4.2), it is unclear whether Adélie penguins persisted in East Antarctica throughout the LGM in small numbers, or if they colonised the region during the post-glacial period from refugia located elsewhere.

Our phylogenetic analyses (Figure 4.3) provide support to the notion that only two Adélie penguin refugia existed in Antarctica during the LGM (Lambert *et al.*, 2002, Ritchie *et al.*, 2004). One refuge was most likely located within the Ross Sea (Ritchie *et al.*, 2004), whereas the location of the other refuge remains unknown, but was clearly the source population for extant colonies in the Scotia Arc (Clucas *et al.*, 2014), parts of the Ross Sea (Lambert *et al.*, 2002, Ritchie *et al.*, 2004), and the full breadth of East Antarctica (Figure 4.3). Given the high genetic differentiation of this lineage from the Ross Sea lineage, it seems unlikely that the second refuge was also located in the Ross Sea, directing attention toward the Scotia Arc or East Antarctica as potential refuge locations.

Our results indicate a median expansion time of the East Antarctic population *ca*. 14 kya, whereas the Scotia Arc population is estimated to have expanded slightly earlier (*ca*. 17 kya, median estimate), coincident with the earlier deglaciation of that region compared to East Antarctica (Cofaigh *et al.*, 2014, Hodgson *et al.*, 2014, Mackintosh *et al.*, 2014). Given that the Scotia Arc population probably expanded first, it may be that the LGM refuge was located in this region and, as the lineage expanded and colonised new areas, it made its way to East Antarctica *ca*. 14 kya. The South Shetland Islands, in the Scotia Arc, had a similar glacial extent during the LGM as they do today (Hodgson *et al.*, 2014), and could therefore have supported a small refuge population of Adélie penguins prior to widespread deglaciation of the Scotia Arc region from 18 kya. Under this scenario, Adélie penguins from a Scotia Arc refuge could have colonised an ice-free area in East Antarctica, such as the Vestfold Hills (Gibson *et al.*, 2009), Lützow-Holm Bay (Hayashi & Yoshida, 1994) or the Larsemann Hills (Burgess *et al.*, 1994) *ca*. 14 kya, subsequently becoming more widespread in East Antarctica as the region deglaciated from 12 kya (Mackintosh *et al.*, 2014). Previous genetic analysis of radiocarbon dated remains indicate that both Adélie penguin lineages were present in the Ross Sea from at least *ca*. 6 kya, suggesting that individuals from the second refuge had spread to the Ross Sea by this time (Lambert *et al.*, 2002, Ritchie *et al.*, 2004).

Given that the 95% HPD intervals surrounding the expansion times of the Scotia Arc and East Antarctic populations are somewhat overlapping, it cannot be ruled out that the East Antarctic population expanded first and the Scotia Arc was colonised by individuals from an East Antarctic refuge. While most of the East Antarctic coastline was covered by glacial ice until at least 12 kya, some areas have been ice-free since before the LGM (Gibson *et al.*, 2009, Mackintosh *et al.*, 2014) and could, theoretically, have acted as a refuge for small Adélie penguin breeding colonies if local sea ice and foraging conditions were favourable. If this were the case, then these sites were likely to be isolated pockets suitable for breeding Adélie penguin populations rather than broad regions of suitable breeding and marine foraging habitat. Previous studies have indicated that ice-free oases during the LGM were located at several sites within East Antarctica, including at the Vestfold Hills (based on lake sediment records; (Gibson *et al.*, 2009)), at Lützow-Holm Bay (based on Holocene raised beach deposits; (Hayashi & Yoshida, 1994)) and at the Larsemann Hills (based on radiocarbon dated moss deposits; (Burgess *et al.*, 1994)). The Bunger Hills were also partially ice-free during and since the LGM (Gore *et al.*, 2001), however, the region is bounded by ice shelves and is therefore inaccessible to penguins. If Adélie penguins were present in East Antarctica at one of these sites before 14 kya, they may have rapidly expanded their range and numbers in the region as ice-free habitat became more plentiful coincident with the widespread retreat of the East Antarctic Ice Sheet

from *ca*. 12 kya (Mackintosh *et al.*, 2014). While there is evidence that these sites were ice-free and could have theoretically supported breeding Adélie penguins, the existence of Adélie penguin glacial refugia in East Antarctica or the Scotia Arc could be determined by radiocarbon dating of penguin remains from these potential refugia sites. The Vestfold Hills, Lützow-Holm Bay and the South Shetland Islands are all home to penguin breeding colonies today and, while no penguins currently breed at the Larsemann Hills, they may have done so in the past.

While ice-free ground suitable for nesting is a key requirement for the existence of Adélie penguin colonies, the species also requires accessible marine foraging grounds and sufficiently abundant prey to survive. Unlike winter during the LGM, when sea ice extent was double the current winter values, the LGM summer sea ice extent is estimated to have been similar to that seen in summer today (Gersonde *et al.*, 2005). Therefore, adult Adélie penguins provisioning for their chicks during the summer breeding season may have encountered similar sea ice extents as they do today. However, the penguins' capacity to forage successfully would largely depend on the amount of fast ice present, which can impede their ability to reach foraging grounds (Ainley, 2002, Emmerson & Southwell, 2008, Ropert-Coudert *et al.*, 2015). The LGM sea ice records refer to total sea ice extent, with no differentiation between fast and pack ice (Gersonde *et al.*, 2005), therefore it is unknown whether the LGM summer fast ice conditions would have been suitable for Adélie penguin foraging. Coastal polynyas could have facilitated Adélie penguin foraging amidst the sea ice field and also acted as hot spots of primary productivity (Thatje *et al.*, 2008). Polynyas are known to have existed in several locations in the Weddell and Ross Seas during the LGM (Brambati *et al.*, 2002, Mackensen *et al.*, 1994, Smith *et al.*, 2010, Sprenk *et al.*, 2014, Thatje *et al.*, 2008). There are currently no records of LGM polynyas in East Antarctica, but this is more likely a result of the sparse sediment core record rather than an actual absence of polynyas, which were believed to have been more widespread during the LGM due to increased katabatic winds compared to today (Smith *et al.*, 2010, Sprenk *et al.*, 2014).

The sea ice extent and seasonality began to shift from LGM conditions *ca*. 10.4 kya in Prydz Bay, with Holocene sea ice conditions similar to today's reached between 10 and 9 kya (Barbara *et al.*, 2010). This decline in sea ice occurred after the initial, rapid increase in abundance of East Antarctic Adélie penguins (*ca*. 14 kya; Figure 4.2), suggesting that sea ice conditions were not the primary driver of population expansion. Recent studies in East Antarctica have shown that sea ice variation is a key driver of Adélie penguin population dynamics and key demographic parameters over yearly and decadal time scales (Emmerson & Southwell, 2008, Emmerson & Southwell, 2011, Jenouvrier *et al.*, 2006, Kato *et al.*, 2002, Southwell *et al.*, in press), and sea ice declines are predicted to result in

decreasing numbers of Adélie penguins in the most northerly latitudes of their breeding range over the coming decades (Ainley *et al.*, 2010). It appears, however, that changes in sea ice extent and seasonality during the glacial-interglacial transition were not the key driver of East Antarctic Adélie penguin population expansion. This suggests that environmental drivers of population trends over thousands of years may differ to drivers over years or decades.

Increases in primary productivity did not commence until between 12 and 10 kya off MacRobertson Land (Anderson *et al.*, 2009, Mackintosh *et al.*, 2011), and from *ca*. 10 kya off Adélie Land (Denis *et al.*, 2009), indicating that changes in primary productivity were not an initial driver of the Adélie penguin abundance increase either. As Adélie penguins began increasing from 14 kya, this suggests that prey abundance was already sufficiently high for penguin survival prior to the increase in East Antarctic primary productivity between 12 and 10 kya. This is supported by evidence from another meso-predator, the Weddell seal, which persisted in East Antarctica throughout the LGM and post-glacial period in similar numbers to today (Chapter 3). For the Weddell seal's population size in the region to be unchanged, the species must have had sufficiently abundant prey over this period; therefore, it is plausible that prey resources for the Adélie penguin, which breeds at the same time of year as the Weddell seal, were also sufficient prior to 14 kya. The overall indication, based on the timing of the East Antarctic Adélie penguin population increase, is that deglaciation, leading to increased ice-free area for nesting, was the initial environmental variable that changed sufficiently to allow the post-glacial abundance increase. Subsequent changes to sea ice and primary productivity after the initial population expansion may have sustained this trend in unison with increasing breeding habitat availability.

The observed post-glacial expansion of Adélie penguins is common to many penguin species, including emperors (Chapter 2, Younger *et al.*, 2015), kings (Trucchi *et al.*, 2014), gentoos and chinstraps (Clucas *et al.*, 2014), which all had populations smaller in size and restricted in range during the LGM, and which expanded post-glacially. Interestingly, the increase in abundance of East Antarctic Adélie penguins began earlier and was far greater than that of the sympatric, closely related emperor penguins (Chapter 3), which were also restricted to refugia during the LGM (Chapter 2, Younger *et al.*, 2015). The East Antarctic emperor penguin population increased in abundance during the Holocene; however, the expansion was only 5.7 fold and did not commence until *ca.* 10 kya (Chapter 3), approximately 4,000 years after the Adélie penguin expansion. This suggests that the two species were influenced by different environmental drivers during the post-glacial period. While both species responded positively to declining ice, for the Adélie penguins it is likely that terrestrial

ice sheet retreat was the key factor, whereas the expansion of emperor penguins was likely to be more closely coupled with reductions in sea ice extent (Chapter 2, Younger *et al.*, 2015).

Our study has shown that East Antarctic Adélie penguins responded similarly to Scotia Arc Adélie penguins during climate warming following the LGM, with both populations undergoing an increase in population size coincident with an expansion of ice-free breeding habitat. Increases in Adélie penguin numbers in these two regions occurred asynchronously, in line with local deglaciation, indicating that Adélie penguins are sensitive to local glacier and ice sheet retreat. As climate change progresses, glaciers and ice sheets in Antarctica are expected to retreat further. This study highlights the possibility that, in regions where sea ice and prey conditions remain favourable, Adélie penguin numbers may expand in line with this deglaciation as additional breeding sites become exposed. In the Ross Sea, such an expansion has already occurred in response to receding glacial ice at the Beaufort Island colony, with an increase of 84% in the Adélie penguin population between 1983 and 2010 concurrent with a 543 m retreat of the glacier field (LaRue *et al.*, 2013). While the future trends in Adélie penguin abundance remain uncertain, our study suggests that ice sheet retreat and the availability of ice-free breeding areas may be overriding factors in determining the millennial scale abundance trends of this species. However, for Adélie penguin populations to expand in line with increasing breeding habitat, prey must be abundant and accessible enough to meet the requirements of the expanding population. Whether this will be the case in the future remains to be seen, as the impacts of climate change on Adélie penguin prey species, such as Antarctic krill (*Euphausia superba*), are currently ambiguous (Atkinson *et al.*, 2012, Constable *et al.*, 2014, Nicol & Brierley, 2010).

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CHAPTER 5

RADSEQ REVEALS A GLACIAL REFUGE FOR EMPEROR PENGUINS AT THE ADARE PENINSULA

5.1 Abstract

The emperor penguin (*Aptenodytes forsteri*) is a remarkable and resilient species that has thrived in the extreme Antarctic environment for millions of years, but may now be threatened by unprecedented climate change. In light of this threat, risk assessments for emperor penguins are necessary and these require accurate data regarding the population structure and geographic boundaries of breeding populations. Furthermore, locations that have been consistently occupied by emperor penguins over tens of thousands of years and have acted as refugia during past climate shifts may be crucial to the future survival of the species. Here a dataset of 20,005 genome-wide single nucleotide polymorphisms (SNPs), generated using restriction site associated DNA sequencing (RADSeq), was used to detect genetic differentiation among eight extant emperor penguin colonies around Antarctica, and to assess the possible locations of emperor penguin refugia during the last glacial maximum (LGM, 26– 19.5 kya), which were hypothesised to be located in the vicinity of polynyas. Six extant breeding populations were delimited among the eight colonies sampled, with ongoing gene flow between colonies located hundreds of kilometres apart providing evidence against strong philopatry in emperor penguins. The genetic evidence strongly indicated that there was an emperor penguin refuge at Cape Roget during the LGM, proximate to the proposed location of an LGM polynya offshore from the Adare Peninsula. This region in the north-western Ross Sea may have been an important penguin breeding habitat for at least 50,000 years and should therefore be considered in any future management for conservation.

5.2 Introduction

The emperor penguin (*Aptenodytes forsteri*) evolved in Antarctica millions of years ago (Baker *et al.*, 2006, Subramanian *et al.*, 2013). They are a remarkable, resilient species that has not only survived in the extreme cold of Antarctica, but thrived, with a current global population of *ca*. 595,000 adult birds in colonies spanning the coastline of the entire continent (Figure 5.1) (Fretwell *et al.*, 2012). The emperor penguin's long persistence in Antarctica suggests a successful endurance of numerous environmental regime shifts, from glacial to interglacial periods and back again (Hewitt, 2000, Jouzel *et al.*, 2007). The Earth is currently in the midst of another transition, from the relatively stable Holocene to a new epoch termed the Anthropocene, that is characterised by changes to land, oceans, the atmosphere and evolutionary pressures (Lewis & Maslin, 2015). The projected rate of temperature increase over the next century is an order of magnitude greater than that following the
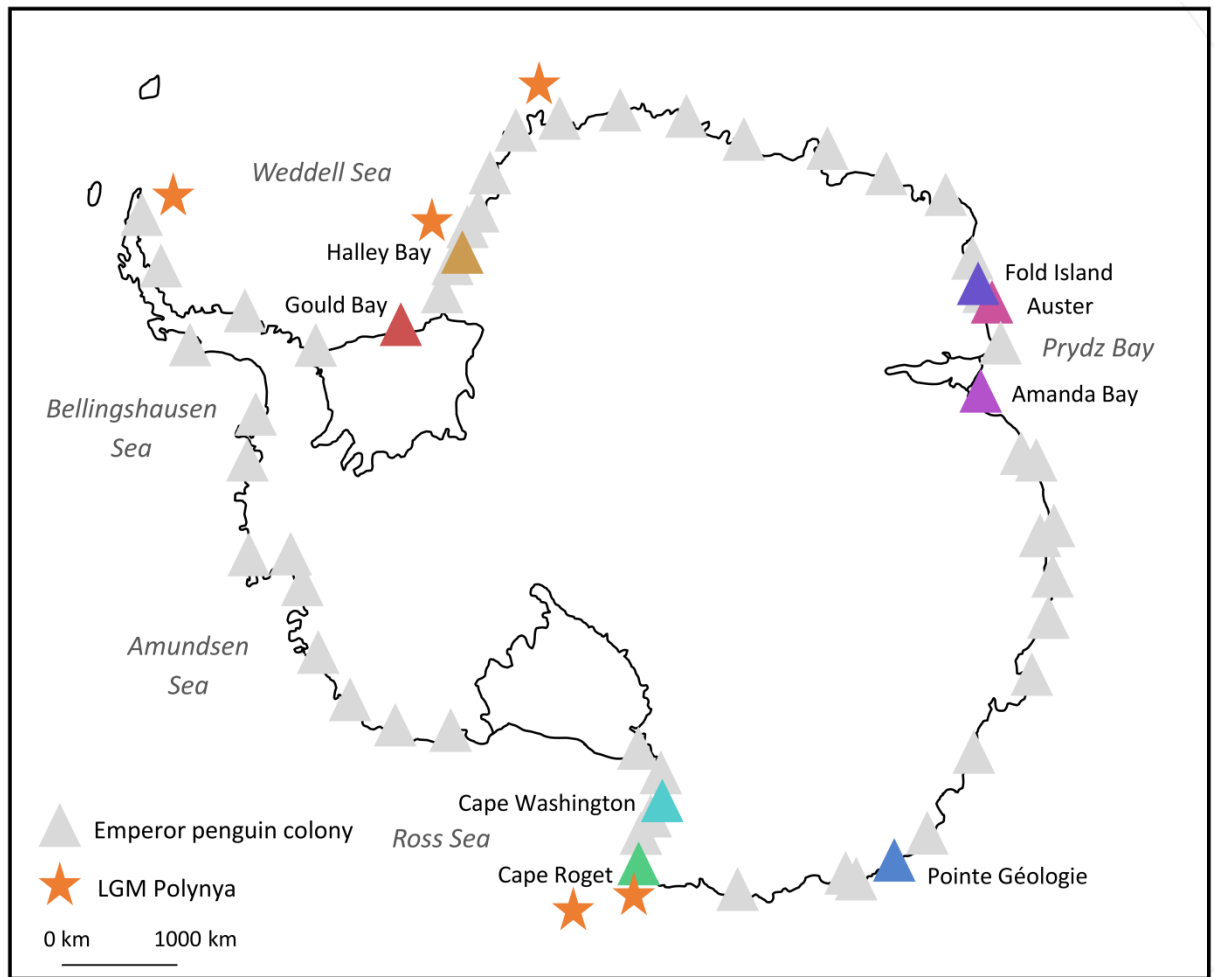
Figure 5.1. Emperor penguin colony locations. Triangles indicate the presence of an emperor penguin colony (Fretwell *et al.*, 2012), coloured triangles indicate colonies sampled in this study. The proposed locations of LGM polynyas, based on sediment core records, are indicated by orange stars (Smith *et al.*, 2010 and references therein, Sprenk *et al.*, 2014).

end of the last glacial maximum (LGM, 26–19.5 kya) (Collins *et al.*, 2013, Masson-Delmotte *et al.*, 2013, Shakun *et al.*, 2012) and the extinction rate during the Anthropocene is estimated at 100 to 1,000 times greater than in previous epochs (Barnosky *et al.*, 2011). It remains to be seen if the past resilience of the emperor penguin will hold in the face of this unprecedented environmental shift.

Arguably the most serious threat to emperor penguins is changing sea ice conditions, including changes in the timing of sea ice advance and retreat, as well as the quality and areal extent of sea ice (Ainley *et al.*, 2010, Jenouvrier *et al.*, 2009). Emperor penguins use sea ice as a substrate throughout their nine-month breeding cycle at the majority of known colony locations (Fretwell *et al.*, 2012, Fretwell *et al.*, 2014). To breed successfully, emperor penguins require breeding sites located in

reasonable proximity to prey resources and with a stable breeding substrate from the start of the courtship period in March through to the fledging of chicks in December. Emperor penguins are known to be sensitive to changes in sea ice conditions and it has been proposed that, until recently, they lived in a "Goldilocks" age of optimal sea ice conditions, in terms of both extent and season duration (Chapter 2, Ainley *et al.*, 2010, Younger *et al.*, 2015).

However, sea ice conditions have begun to change and will continue to change in the future (Collins *et al.*, 2013, Vaughan *et al.*, 2013). Current and historical changes in the extent and duration of sea ice around Antarctica show highly regionalized trends, with some areas increasing or remaining stable while others are decreasing (Vaughan *et al.*, 2013, Zwally *et al.*, 2002). Over a 34-year satellite monitoring period, the sea ice season shortened dramatically by 100 ± 31 days in the Bellingshausen and Amundsen Seas, accompanied by a substantial decrease in extent (Stammerjohn *et al.*, 2012, Vaughan *et al.*, 2013). Meanwhile, in the Ross Sea, both season duration and areal extent increased substantially (Vaughan *et al.*, 2013). The East Antarctic region has demonstrated considerably more complex trends in sea ice seasonality and extent than the rest of the continent, with large local variation in sea ice trends (Fraser *et al.*, 2012, Massom *et al.*, 2013). In some areas (between 95°E and 110°E; and isolated pockets between 75°E and 150°E), there has been a consistent shortening of the sea ice season by 1 to 3 days per year for the past 31 years (Massom *et al.*, 2013). Other localities (west of 105°E; between 40°E and 90°E) have experienced a significant lengthening of the sea ice season (Massom *et al.*, 2013). Overall, Antarctic sea ice is expected to undergo substantial declines in the future; in the most extreme climate model scenario (RCP8.5), sea ice would be completely absent from East Antarctica during February by 2081–2100 and only retained in small areas of the Weddell and Ross Seas (Collins *et al.*, 2013).

In light of this threat, risk assessments for emperor penguins under future climate change scenarios have begun. Jenouvrier *et al*. (2014) predicted the future population trends at 45 emperor penguin colonies, based on a sea ice dependent demographic model paired with the projected changes in local (colony-specific) sea ice conditions. Their findings were dire, with predicted declines of >50% at two thirds of the colonies examined by the year 2100, concordant with a minimum of 19% global decline in emperor penguin numbers (Jenouvrier *et al.*, 2014). They found that the only predicted stable population was in the Ross Sea, notably coincident with the strongest signal of a refugium found in Chapter 2. However, even these colonies are predicted to be in decline by 2100 (Jenouvrier *et al.*, 2014).

One caveat of the Jenrouvier *et al*. (2014) study was that each colony was modelled as an isolated breeding unit, with no exchange of individuals among locations. Several recent lines of evidence suggest this is not a valid assumption for emperor penguins. Satellite tracking studies have shown that juveniles may travel more than 7,000 km in just eight months (Thiebot *et al.*, 2013), and that the travels of juveniles often take them in the vicinity of many different colonies (Kooyman *et al.*, 1996, Wienecke *et al.*, 2010). Furthermore, a recent study of colony locations found six instances of either colony relocation or establishment of a new colony over five years after traditional breeding grounds were lost (Ancel *et al.*, 2014, LaRue *et al.*, 2014). Finally, a continent-wide mitochondrial DNA study of population structure found that emperor penguins were panmictic across *ca.* 8,000 km of coastline, from the Adélie Land Coast to the Weddell Sea (Chapter 2, Younger *et al.*, 2015). Together, these studies refute the notion of strong philopatry among emperor penguins and suggest that individual emperor penguin colonies cannot be considered in complete isolation.

The degree of philopatry in emperor penguins is likely to have a major effect on how they adapt to future climate change, as dispersal among colony sites increases the adaptive capacity of a species in two key ways: 1) by facilitating gene flow among breeding sites, which replenishes the gene pool of a population with new, potentially adaptive alleles; and 2) by facilitating range shifts (Walther *et al.*, 2002). The second point, of facilitating range shifts, is particularly relevant over shorter (i.e. decadal) timescales, as emperor penguins may shift their colony sites and establish new breeding colonies as local sea ice conditions become unfavourable (Ancel *et al.*, 2014, LaRue *et al.*, 2014). Risk assessments for the species would benefit from accurate data regarding the population structure and geographic boundaries of breeding populations. Our preliminary study of emperor penguin population structure found evidence for two breeding populations, one isolated in the Ross Sea and the other encompassing East Antarctica and the Weddell Sea, but that study was based on mitochondrial DNA and therefore represents the genetic structure of a single, uniparentally inherited, locus (Chapter 2, Younger *et al.*, 2015). Multiple independent loci from across the genome of emperor penguins would allow for a more sensitive investigation of genetic differentiation among colony sites.

In addition to identifying the geographic boundaries of populations, it is also important to understand the geography of the species in terms of its most important breeding areas. Locations that have been consistently occupied by emperor penguins over tens of thousands of years and have acted as refugia during past climate shifts may be crucial to the survival of the species, and could act as refugia again in the future. Our past mitochondrial DNA studies indicated the presence of three

emperor penguin refuge populations during the LGM, one of which was probably located in the Ross Sea (Chapter 2, Younger *et al.*, 2015). The locations of the other two refugia are unknown, but were hypothesised to be located in the vicinity of polynyas (Chapter 2, Younger *et al.*, 2015). Polynyas are regions of open water in the sea ice zone, usually formed by katabatic winds that push sea ice away from the coast, that are associated with enhanced primary production because of an earlier spring melting of sea ice and a resultant earlier start in photosynthetic primary production (Martin, 2001). While overall biological productivity of the Southern Ocean was much reduced during the LGM, polynyas may have provided oases of productivity (Thatje *et al.*, 2008) where predators could access food amidst the extensive sea ice field. Sediment cores suggest the existence of LGM polynyas in several locations, including in the Ross and Weddell Seas (Figure 5.1) (Brambati *et al.*, 2002, Mackensen *et al.*, 1994, Smith *et al.*, 2010, Sprenk *et al.*, 2014, Thatje *et al.*, 2008).

In this study, I aimed to: 1) more accurately define the current geographic boundaries of emperor penguin breeding populations, and 2) provide further evidence for the possible locations of emperor penguin refuge populations during the LGM, which were hypothesised to be located in the vicinity of polynyas. To achieve this, I expanded previous mitochondrial DNA analyses (Chapter 2, Younger *et al.*, 2015) with a dataset of 20,005 genome-wide single nucleotide polymorphisms (SNPs) generated using restriction site associated DNA sequencing (RADSeq) (Baird *et al.*, 2008). The SNP analysis allowed the determination of accurate, fine-scale genetic differences among eight emperor penguin colonies around Antarctica (Figure 5.1). Breeding populations among the eight colony sites were delimited, and the locations of extant populations were assessed for indications that they may have been past refugia. The genetic diversity of populations was compared because older, ancestral populations should contain a larger pool of genetic variants, as compared to populations that were founded more recently by fewer individuals (Hewitt, 2000). The demographic history of each breeding population was also assessed and the abundance trends compared to provide further evidence for refugia locations; refuge populations would be expected to expand gradually following the end of the LGM compared to populations that were founded after the LGM, which would be expected to show a rapid expansion.

5.3 Methods

5.3.1 Sampling

A total of 114 emperor penguins from eight colonies (Figure 5.1) were included in this study. Genetic samples included skin and muscle tissue collected from dead emperor penguin chicks at Halley Bay, Fold Island, Pointe Géologie, Amanda Bay and Auster, and blood samples drawn from emperor penguins at Gould Bay, Cape Roget and Cape Washington. For complete details of the blood sampling procedure please refer to Section 2.3.1. All samples were stored at -20°C. Details of DNA extraction and preparation of the mitochondrial HVR sequences are described in Section 2.3.2 (Younger *et al.*, 2015). A total of 114 emperor penguins from eight colonies were genotyped using RADSeq, and the mitochondrial hypervariable region (HVR; 629 bp) was sequenced for 110 of these individuals (Table 5.1; GenBank accession numbers KP644787-KP645015).

Table 5.1. Number of individuals genotyped for each marker and colony.

	HVR	RADSeq
Gould Bay	15	15
Halley Bay	15	15
Fold Island	15	16
Auster	13	16
Amanda Bay	14	16
Pointe Géologie	13	15
Cape Roget	7	10
Cape Washington	8	11
Subtotal	**100**	**114**

5.3.2 Restriction-site-associated DNA sequencing (RADSeq)

RAD libraries were prepared using the SbfI restriction enzyme, which was chosen because it has previously been shown to produce a large number of RAD loci in the closely related king penguin (Trucchi *et al.*, 2014). RADSeq for all individuals was performed at the Edinburgh Genomics Facility, University of Edinburgh (https://genomics.ed.ac.uk/) as described in (Gonen *et al.*, 2014) after (Etter *et al.*, 2011). Briefly, 250 ng of DNA per individual was digested with SbfI-HF (NEB), followed by ligation to barcoded P1 adapters. The uniquely barcoded individuals were pooled into multiplexed libraries of 16 individuals per library, and each library sheared into fragments ~300-400 bp. Fragments were size selected using gel electrophoresis. The libraries were blunted (NEB Quick Blunting Kit) and A-tailed prior to ligation with P2 adapters (IDT). Enrichment PCR was performed to increase yield, followed by product purification with Ampure beads. The pooled, enriched libraries were checked for size and quantity using Qubit and a qPCR assay. Each library was then sequenced in a lane of the Illumina HiSeq 2500 using 125 base paired-end reads in high output mode (v4 chemistry).

5.3.3 Bioinformatics

A dataset of 20,005 SNPs was prepared by the Edinburgh Genomics Facility, University of Edinburgh (https://genomics.ed.ac.uk/). The raw RADSeq reads were demultiplexed using *process_radtags* within the Stacks pipeline (http://creskolab.uoregon.edu/stacks/). The reads were then aligned to the emperor penguin reference genome (http://gigadb.org/dataset/100005) using BWA Fastrack v0.6.1. Duplicate reads were identified with Picard v1.105 before SNPs and indels were called and filtered using GATK v.3.2-2 following the Broad Institute's guidelines. Indels and sequencing errors were removed, and the remaining SNPs were further filtered if their genotype quality was less than 20. SNPs that were genotyped for >75% of individuals in each colony were selected, to give 411,324 SNPs. Rare SNPs with an allele frequency of <1% were also removed, to give a final dataset of 279,433 SNPs. As this dataset exceeded my requirements, the number of SNPs was reduced to 20,005 by selecting every 14th SNP, and this reduced dataset was used in subsequent analyses. The SNPs were then tested for Hardy-Weinberg equilibrium (HWE) at each colony site. None of the 20,005 SNPs were found to be out of HWE at more than four sites, therefore no loci were discarded. The extended Bayesian skyline method cannot be used with SNP data at this time. However, the utility of short sequences for extended Bayesian skyline analysis has been demonstrated in the closely related king penguin (*Aptenodytes patagonicus*) (Trucchi *et al.*, 2014). Therefore, a dataset of nucleotide sequences was prepared for Bayesian skyline analysis by randomly selecting 500 loci from

within the 20,005 SNP dataset and, for these loci, the trimmed 117 bp nucleotide sequences (hereinafter referred to as RAD loci) were used, with individuals heterozygous for a given SNP site encoded with IUPAC ambiguity codes. The PGDSpider data converter was used to convert between various genomic and genetic data formats for analyses (Lischer & Excoffier, 2012).

5.3.4 Delimitation of genetic populations

To identify the number of populations ("clusters") across the eight emperor penguin colonies I used a Bayesian clustering approach with a Markov chain Monte Carlo (MCMC) sampling procedure within STRUCTURE v2.3.4 (Pritchard *et al.*, 2000). The analysis also estimated the membership coefficient of each individual to each of the inferred clusters, effectively assigning individuals to populations. The full 20,005 SNP dataset was used for the analysis and the *correlated allele frequencies* and *admixture* models were selected, as it is highly likely that these colonies have experienced admixture in the past and/or are still exchanging migrants. Models were run both with and without location priors reflecting the colony that each individual was sampled at, in order to detect weak versus strong population structure. The value of *lambda* was inferred in initial runs and then fixed at 0.47 for the main analyses, which were run for a total of 200,000 steps with a burn-in of 100,000. Plots of *alpha* were checked for stability. K values (the number of inferred clusters) from one to nine were tested, with each value of K run a total of eight times. For each K value the results from the eight runs were combined, and then STRUCTURE HARVESTER web v0.6.94 (Earl, 2012) was used to compare K values for each model (i.e. with and without location priors) to determine the most optimal number of clusters in each case. STRUCTURE HARVESTER detects the uppermost level of hierarchical structure in the data by calculating ΔK, the rate of change in the log probability of the data over sequential values of K (Evanno *et al.*, 2005). Replicate runs for the optimal value of K were aligned using CLUMPP (Jakobsson & Rosenberg, 2007) and the membership coefficients of each individual to each cluster were visualised with DISTRUCT v1.1 (Rosenberg, 2004).

The genetic population structure was further analysed using a principal component analysis (PCA) performed on the 20,005 SNP dataset using the R packages *Adagenet* v1.4-2 (Jombart, 2008, Jombart & Ahmed, 2011) and *ade4* v1.6-2 (Dray & Dufour, 2007), with the function *dudi.pca*. The bar plots of the eigenvalues suggested that the first two or three principal components (PCs) were the most important for explaining the variance in the allele frequencies between populations.

Finally, Arlequin v3.5.1 (Excoffier & Lischer, 2010) was used to quantify the genetic differentiation (pairwise F_{ST}) among all pairs of colonies using 20,005 SNPs. The hypothesis of departure from

panmixia was tested with 10,100 random permutations of the data to determine the statistical significance of each pairwise F_{ST} value. A strict Bonferroni correction was applied due to the multiple comparisons (Rice, 1989), so that the cut-off for a pairwise F_{ST} value to be deemed statistically significantly different from zero at the α = 0.05 level was $p < 0.00179$.

5.3.5 Genetic diversity

Arlequin v3.5.2 (Excoffier & Lischer, 2010) was used to calculate the expected heterozygosity (H_e) for the five breeding populations identified by cluster analysis (Weddell Sea, Ross Sea, Mawson Coast, Pointe Géologie and Amanda Bay; see results) using the 20,005 SNP loci dataset. Loci with >5% missing data within a population were excluded from the analysis for that population, and H_e was then calculated for all other loci for each population, with both polymorphic and non-polymorphic loci included. An analysis of variance (ANOVA) was used to test for heterogeneity in H_e among the five populations and *t*-tests were performed to test for statistically significant differences in H_e between all pairs of populations.

5.3.6 Reconstructions of demographic history

In order to investigate the climatic drivers of population trends in emperor penguins, the demographic history of each population over the past *ca*. 50,000 years was reconstructed via the coalescent extended Bayesian skyline plot method (Heled & Drummond, 2008) within BEAST v2.1.3 (Bouckaert *et al.*, 2014), using RAD loci and the mitochondrial HVR. The HVR was used in addition to the RAD loci as it has a well-estimated substitution rate in penguins that can be used to time calibrate the analyses. In cases where HVR sequences were not available for every individual in the population (Table 5.1), an empty sequence was entered in the HVR partition for that individual and the data treated as missing, which is an accepted practice in BEAST analyses and does not affect the accuracy of the result (Drummond & Bouckaert, 2015).

To determine the optimal number of RAD loci to use for the analyses, I first conducted trials using data from a single population, Pointe Géologie, which was chosen for its small number of individuals (15, compared to 30 for the Weddell Sea, 32 for the Mawson Coast and 21 for the Ross Sea) and because an initial skyline analysis using HVR alone revealed a definite population expansion (result not shown). Analyses were conducted using the mitochondrial HVR paired with 10, 20, 50, 100, 250 and 500 RAD loci. The maximum number of loci analysed was 500, for the following reasons: 1) the time to convergence for a 500 loci dataset was approximately one month using high powered

computing, which was deemed excessive; and 2) BEAUti, the application used for generating BEAST XML files, failed with datasets greater than 500 loci (and sometimes even with 500 loci). Analyses were performed for each dataset in triplicate to ensure reproducibility of the results. It was determined that 250 loci was optimal, as the 250 loci dataset resulted in a very similar skyline plot to the 500 loci dataset (albeit with a slightly larger confidence interval; Appendix 5.1), but was considerably less problematic in terms of set up and run time.

The demographic histories of the other four breeding populations delimited by the clustering analyses (i.e. Weddell Sea, Ross Sea, Mawson Coast and Amanda Bay) were reconstructed using 250 RAD loci with the HVR. As the Ross Sea showed indications that it may have been the site of a past refuge population, the demographic histories of the Cape Roget and Cape Washington colonies were also analysed independently, to test for any site-specific differences in history that could suggest one or the other of these colonies as being closer to the site of the refuge.

For all populations, the trimmed RAD loci nucleotide sequences (117 bp for each locus) were used, with the SNPs encoded using IUPAC ambiguity codes. The nucleotide substitution model for both HVR and RAD loci was specified as HKY (Hasegawa *et al.*, 1985) with four gamma categories and with each of the 251 loci assigned unlinked priors. A strict molecular clock was used. The substitution rate for each RAD locus was estimated using a lognormal prior with a mean of 0.022 substitutions/site/Myr (SD = 1.5). This prior was chosen to reflect the average substitution rate found by Trucchi *et al*. (2014) for the king penguin, the closest relative of the emperor penguin, using a RAD loci dataset generated by the same restriction enzyme that we used here (SbfI-HF) (Trucchi *et al.*, 2014). For the HVR substitution rate a lognormal prior was specified (mean = 0.55 substitutions/site/Myr, SD = 0.15) to reflect the published substitution rate of the HVR in the closely related Adélie penguin (*Pygoscelis adeliae*) (Millar *et al.*, 2008). The 250 RAD loci were assigned a shared tree prior; this was necessary as there was not enough information in a single locus to resolve the phylogeny. Both HVR and RAD loci used the extended Bayesian skyline tree prior, scaled according to the ploidy of each locus.

The posterior distribution of effective population size through time was generated using the MCMC sampling procedure with samples drawn every 20,000 steps. The chains were monitored for convergence by periodically inspecting the trace log files in Tracer v1.5. The chains were deemed converged once the effective sample size values (ESSs) for all key parameters exceeded 200 and the sampling traces no longer showed a trend. The chain length required to reach convergence varied according to the dataset, from 118 to 300 million states. Three independent analyses with different

random number seeds were performed for each population to ensure reproducibility of the posterior distribution.

The extended Bayesian skyline plots were generated using EBSPAnalyser with a burn-in of 10%. The population size parameter of the demographic model (N_e*tau*) was converted to effective population size (N_e) by dividing the parameter by the generation length of emperor penguins. Here I have used 14 years, which is the minimum estimated generation length given by Forcada & Trathan (2009). It should be noted that the estimate of generation length determines the absolute values of N_e reported, but has no effect on the timing or magnitude of the observed abundance increases.

5.4 Results

5.4.1 RADSeq genotyping success

The 114 samples yielded 1.94–62.4 million reads per individual, with an average of 13.2 million reads (Figure 5.2a). On average, 93.42% of reads successfully mapped to the emperor penguin reference genome (Figure 5.2b). The mean coverage depth per individual ranged from 10X to 330X, with an average of 71X (Figure 5.2c). SNP calling and filtering protocols resulted in an average of 175,680 mapped SNPs per individual, with a range of 76,547 – 231,273 (Figure 5.2d).

5.4.2 Emperor penguin breeding populations

The optimal number of clusters (K) when all 114 individuals were included were K=3 and K=2 for the STRUCTURE analyses with and without location priors, respectively. Inspection of the individual assignment plots (Figure 5.3a) showed that four individuals sampled at Halley Bay and Gould Bay were heavily influencing the structure by being assigned with very high likelihood to one or two separate populations. Those four individuals were therefore excluded and the analyses repeated using the same settings in order to assess population structure among the other 110 individuals. With the four divergent individuals excluded, K=5 was inferred for both *locprior* and *nolocprior* analyses (Figure 5.3b).

The five populations inferred by the STRUCTURE analyses are: 1) the Weddell Sea population, 2) the Mawson Coast population, 3) the Pointe Géologie population, 4) the Ross Sea population, and 5) an unnamed population with no significant geographic bias amongst the sampled colonies (Figure 5.3b). Gould Bay and Halley Bay individuals exhibit high membership coefficients for the Weddell Sea population; Fold Island and Auster individuals have high membership coefficients for the Mawson

Coast population; Amanda Bay individuals exhibit signals of admixture, mainly between the Weddell Sea population and Pointe Géologie, with lesser signals from the Mawson Coast population and the fifth unnamed population; and, finally, Cape Washington and Cape Roget have high membership coefficients for the Ross Sea population. A principal components analysis resolved the same population structure as the STRUCTURE analysis, but with an indication of subtle differentiation between the Auster and Fold colonies (i.e. Ross Sea cluster, Weddell Sea cluster, Pointe Géologie cluster, Fold Island cluster, Auster cluster, and a signal of admixture for Amanda Bay; Appendix 5.2).

Our measures of pairwise F_{ST} (Table 5.2) indicate that Gould Bay and Halley Bay are not significantly differentiated from each other at the α = 0.05 level (F_{ST} = 0.0042), suggesting that there is enough gene flow between the two sites that they should be considered as a single breeding population. Similarly, Cape Roget and Cape Washington are not differentiated (F_{ST} = 0.0000). Both findings are supported by the clustering analyses, which resolved clusters for the Weddell Sea and Ross Sea.

Based on the pairwise F_{ST} analysis, the Fold Island and Auster colonies are significantly differentiated at the α = 0.05 level (Table 5.2), which is supported by the principal components analysis, but not the STRUCTURE analysis, where these two colonies grouped together as the Mawson Coast population. This is most likely because STRUCTURE tends to pick up big genetic differences and is less adept at detecting fine-scale genetic differentiation. It is therefore possible that Fold Island and Auster were a single panmictic population in the past that have since diverged to form two breeding colonies with minimal contemporary gene flow.

Overall, the analyses suggest that there are either five or six breeding populations of emperor penguins among the eight colonies analysed. In the five population scenario, the groupings are Amanda Bay, Pointe Géologie, the Weddell Sea (comprising Gould Bay and Halley Bay), the Ross Sea (comprising Cape Roget and Cape Washington) and the Mawson Coast (comprising Fold Island and Auster). In the six population scenario, the Mawson Coast population is split into Fold Island and Auster. For the analysis of demographic history and genetic diversity I used the five population scenario, as this is more likely to represent the genetic structure over longer time scales (i.e. during the LGM and post-glacial period).
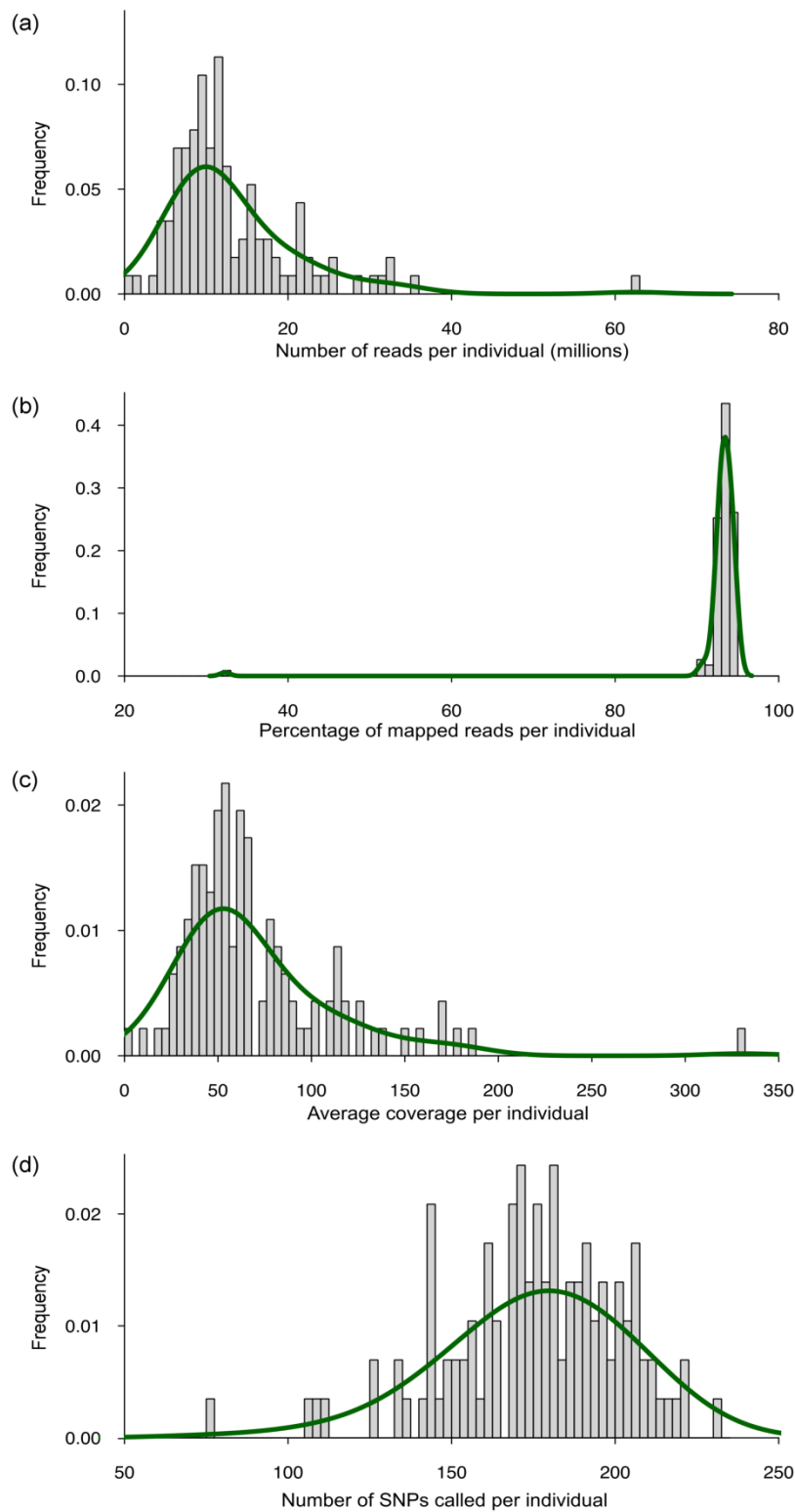
Figure 5.2. Sequencing success. Probability density distributions showing: a) the number of reads per individual; b) the percentage of those reads that mapped to the emperor penguin genome per individual; c) the average coverage of the mapped reads per individual; and d) the number of SNPs called per individual. The green line represents the smoothed kernel density estimate.
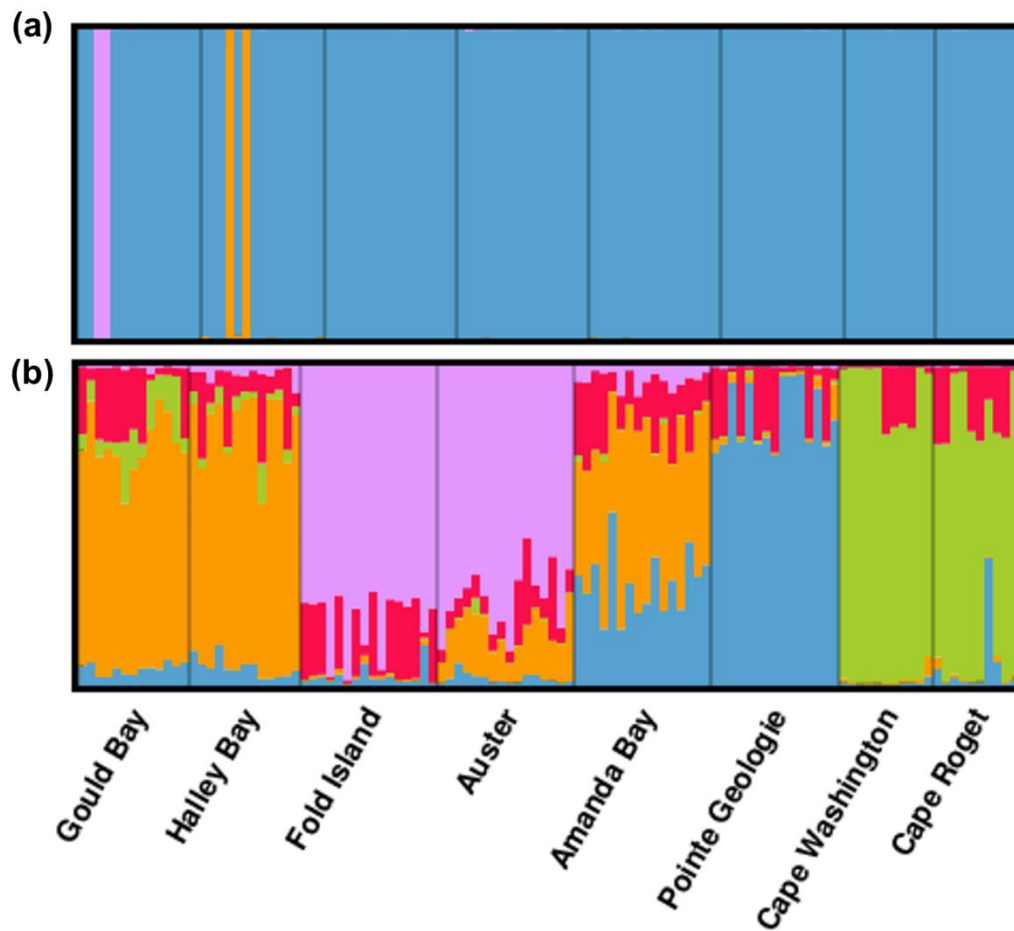
Figure 5.3. Population assignment of individuals by Bayesian clustering. Membership coefficients for each individual are shown by vertical bars with the clusters represented by colours. Only the result for the analyses with the location prior are shown, as the results without the location prior were effectively the same. a) three clusters were resolved when all 114 individuals were included in the analysis: 1) the two Gould Bay outliers, 2) the two Halley Bay outliers, and 3) the other 110 individuals; b) five clusters were resolved when the four divergent individuals were removed from the analysis; 1) the Weddell Sea population (orange), 2) the Mawson Coast population (purple), 3) the Pointe Géologie population (blue), 4) the Ross Sea population (green), and 5) an unnamed population with no significant geographic bias amongst the sampled colonies (red).

Table 5.2. Pairwise genetic differentiation between colonies. Pairwise F_{ST} values are presented below the diagonal. Results that are significantly different from zero at the α = 0.05 level, following Bonferroni correction, are indicated with asterisks.

	Gould Bay	Halley Bay	Fold Island	Auster	Amanda Bay	Pointe Géologie	Cape Roget
Gould Bay							
Halley Bay	0.0042						
Fold Island	0.0051*	0.0060*					
Auster	0.0050*	0.0041*	0.0018*				
Amanda Bay	0.0039*	0.0040*	0.0041*	0.0023*			
Pointe Géologie	0.0034*	0.0049*	0.0054*	0.0035*	0.0028*		
Cape Roget	0.0020	0.0023	0.0012	0.0043*	0.0023*	0.0000	
Cape Washington	0.0054*	0.0045*	0.0059*	0.0048*	0.0039*	0.0044*	0.0000

5.4.3 Divergent individuals in the Weddell Sea

Both clustering analyses revealed four individuals that were highly divergent from the other 110 emperor penguins in the dataset (Figure 5.3a, Appendix 5.2). These four individuals were sampled in the Weddell Sea, with two of them sampled at the Gould Bay colony and the other two at the Halley Bay colony. Interestingly, the outliers form two very divergent groupings in the clustering analyses (Figure 5.3a, Appendix 5.2) and these are geographically confined. It should be noted that the genetic samples for these individuals were blood draws and tissue samples from dead chicks, therefore their identity as emperor penguins is not in dispute. Furthermore, the four individuals had HVR haplotypes that grouped with the other emperor penguins in the dataset. These four individuals had average levels of RADSeq genotyping coverage, ranging from 48.46X to 77.15X within a dataset range of 10–330X with a dataset average of 71X. The mapping rates of reads from these individuals to the emperor penguin reference genome were 93.25%, 93.81%, 94.36% and 94.55%, which are normal when compared to an average mapping rate across the 114 individuals of 93.42%. Importantly, this is a very high rate of mapping to the emperor penguin reference genome, indicating that the DNA is most likely from emperor penguins and is unlikely to be contaminated. The SNP filtering protocols that I selected were strict (section 5.3.3) and therefore the genetic differentiation of these individuals is unlikely to be the result of genotyping errors. Furthermore, a locus-by-locus pairwise F_{ST} comparison between the two outlier groups with the other 110 individuals did not show any fixed SNP differences. The pairwise F_{ST} between the Gould Bay outlier individuals with the other 110 individuals was 0.1222, with a *p*-value of 0.000 based on 10,000 permutations for significance. For the Halley Bay outliers, compared with the other 110 individuals, pairwise F_{ST} = 0.1123, *p* = 0.00079. The overall indication is that these four individuals are genuinely divergent emperor penguins, with several orders of magnitude greater genetic differentiation than among the other 110 emperor penguins.

5.4.4 Locations of glacial refugia

Based on the pairwise F_{ST} analysis, the Cape Roget colony is not significantly differentiated at the α = 0.05 level from Gould Bay, Halley Bay, Fold Island or Pointe Géologie (F_{ST} = 0.0020, F_{ST} = 0.0023, F_{ST} = 0.0012, F_{ST} = 0.0000, respectively; Table 5.2). Given the genetic structuring among the rest of the colonies, and the large geographic distance between Cape Roget and the Weddell Sea and Mawson Coast (Figure 5.1), it seems unlikely that there are large amounts of contemporary emigration between Cape Roget and these sites. I therefore propose that the genetic similarity of Cape Roget to distant colonies is historical in origin, i.e. individuals from a refuge population located at Cape Roget

in the past may have colonised the Weddell Sea, Fold Island and Pointe Géologie, accounting for the genetic similarities. It is notable that the other Ross Sea colony, Cape Washington, did not show the same pattern, and was significantly differentiated from all colonies other than Cape Roget at the α = 0.05 level. This suggests that out of the two Ross Sea colonies, Cape Roget is more likely to have been a past refuge site.

The Ross Sea population had the highest genetic diversity, measured as H_e, whereas Amanda Bay had the lowest (Table 5.3). An ANOVA confirmed that there was statistically significant heterogeneity in H_e among the five populations (p = 9.21×10^{-22}) and *t*-tests between all pairs of populations confirmed that H_e of the Ross Sea population was significantly higher than for any other population (Table 5.4; *p*-values range from 0.0000 to 0.0013), while H_e of the Amanda Bay population was significantly lower than that of all other populations (Table 5.4; p = 0.0000). There was no significant difference in H_e among the Weddell Sea, Mawson Coast and Pointe Géologie (Table 5.3, 5.4). This suggests that the Ross Sea population may have been associated with a past refuge, whereas the Amanda Bay population may have been founded more recently. Given the indications that the Ross Sea may have been the location of a refuge, the H_e of the Cape Roget and Cape Washington colonies were compared in case one of these sites showed stronger indications of being associated with a past refuge. Cape Roget had the higher genetic diversity (H_e = 0.1940 compared to H_e = 0.1713 for Cape Washington) and a *t*-test indicated that the difference was significant (p = 6.45×10^{-18}).

Table 5.3. Expected heterozygosity by population. n = number of individuals genotyped; % poly. = the percentage of genotyped loci that were polymorphic; H_e = expected heterozygosity; SD = standard deviation of expected heterozygosity.

	n	% poly.	H_e	SD
Weddell Sea	30	87.7	0.1715	0.1587
Mawson Coast	32	88.2	0.1723	0.1584
Amanda Bay	16	74.9	0.1598	0.1594
Pointe Géologie	15	74.7	0.1720	0.1669
Ross Sea	21	81.4	0.1784	0.1651

Table 5.4. Pairwise comparisons of genetic diveristy. p-values indicate the statistical significance between the expected heterozygosities (H_e) of pairs of populations.

	Weddell Sea	Mawson Coast	Amanda Bay	Pointe Géologie
Mawson Coast	0.6675			
Amanda Bay	**0.0000**	**0.0000**		
Pointe Géologie	0.7702	0.8898	**0.0000**	
Ross Sea	**0.0003**	**0.0013**	**0.0000**	**0.0009**

My reconstructions of effective population size (N_e) over the past *ca*. 50,000 years indicated that all populations have expanded in size during this period (Figure 5.4). However, the timings and magnitudes of the increases differ by locality (Figure 5.4). All six populations were smaller during the LGM than today; however, the median magnitude of increase since the end of the glacial period (19 kya) varies from 3-fold for the Ross Sea to 15-fold for the Mawson Coast (Figure 5.4b), although it should be noted that the 95% highest posterior density (HPD) intervals around these estimates are large (as expected for BSPs) and somewhat overlapping. Interestingly, the Ross Sea population appears to have been increasing in size since *ca*. 45 kya, and did not experience a bottleneck during the LGM (Figure 5.4). Based on the 95% HPD, it is possible that there were no penguins at any of the populations except for the Ross and Weddell Seas during the LGM (Figure 5.4c). The expansion in the Weddell Sea population appears to have begun slightly earlier than in other localities, followed by expansions at the Mawson Coast, Cape Washington and Pointe Géologie, which were roughly coincident with the termination of the glacial period and, finally, the Amanda Bay population, which expanded several thousand years later. The late expansion of the Amanda Bay population is

consistent with the lower genetic diversity of this population, with the overall indication being that this site was colonised last out of the study sites. Within the Ross Sea, the expansion of the Cape Roget population size since the end of the LGM was 2-fold, whereas the Cape Washington expansion was 7-fold. This disparity, while subtle, again indicates that Cape Roget is the more likely candidate for a past refuge site.

5.5 Discussion

5.5.1 Emperor penguin population structure and philopatry

I found evidence for six extant breeding populations among the eight emperor penguin colonies analysed. The two colonies in the Weddell Sea – Gould Bay and Halley Bay, located 550 km apart – comprise a single, panmictic breeding population. Likewise, the two colonies in the Ross Sea, located 300 km apart, are similarly panmictic. At the Mawson Coast, the Fold Island and Auster colonies are separated by only 190 km and grouped together in the clustering analyses, but were subtly differentiated based on pairwise F_{ST} (Table 5.2). This pattern may indicate that the two colonies were founded from the same ancestral population, which may have been a refugium, and have now diverged into distinct breeding populations. It is unclear why these two colonies are now genetically distinct from one another given their close proximity, as there are no geographic or oceanographic boundaries separating them that might act as barriers to emperor penguin dispersal; indeed, fledgling emperor penguins that were satellite tracked from Auster travelled past the Fold Island colony (Wienecke *et al.*, 2010), therefore emigration between these sites is not limited by dispersal ability. Whether there is low level gene flow between these sites is currently unclear, but rates of emigration between these colonies, and indeed among all breeding populations, could be quantified in the future as the number of migrants per generation using a method such as MIGRATE (Beerli & Felsenstein, 2001). The Amanda Bay colony has genetic similarities to the Pointe Géologie, Weddell Sea and Mawson Coast populations (Figure 5.3), yet is significantly genetically differentiated from all colonies (including Pointe Géologie, Weddell Sea, Fold Island and Auster) at the α = 0.05 level based on pairwise F_{ST} (Table 5.2). As the Amanda Bay colony is 3,230 km away from Pointe Géologie and 4,640 km away from the Weddell Sea, there is unlikely to be
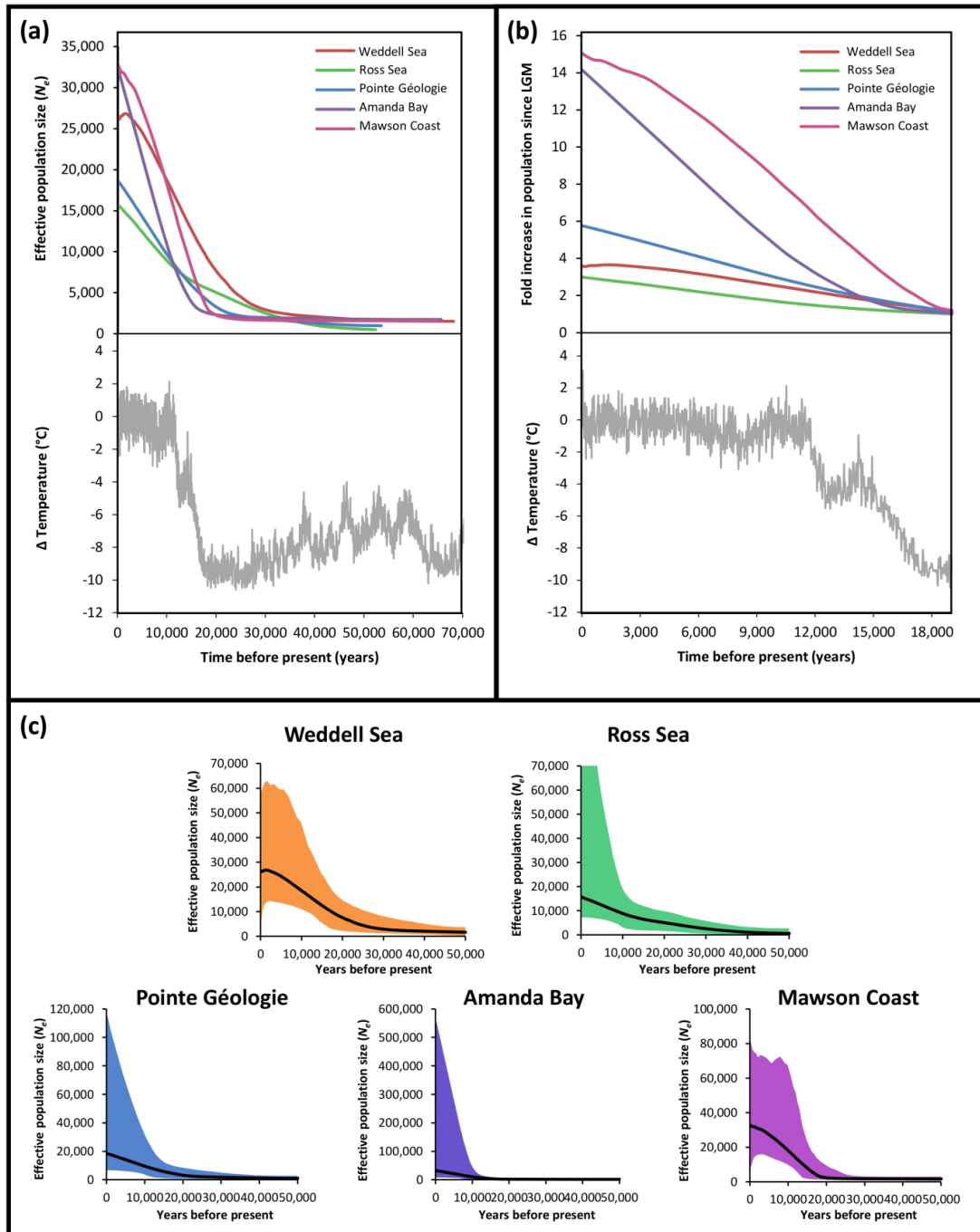
Figure 5.4. Trends in emperor penguin population sizes through time. a) Extended Bayesian skyline plots showing the change in effective population size (N_e) over the past 70,000 years for each population (median estimate only); b) the ratio increase in each population size since the termination of the Last Glacial Maximum, 19 kya; c) the extended Bayesian skyline plots for each population, where the black line indicates the median estimate of N_e and the colour blocks show the 95% highest posterior density interval – note that the y-axes have different scales to accommodate the data. In panels a) and b) the Antarctic temperature anomaly (the difference from the average of the last 1000 years), as estimated from the EPICA Dome C ice core (Jouzel et al., 2007), is indicated with a grey line.
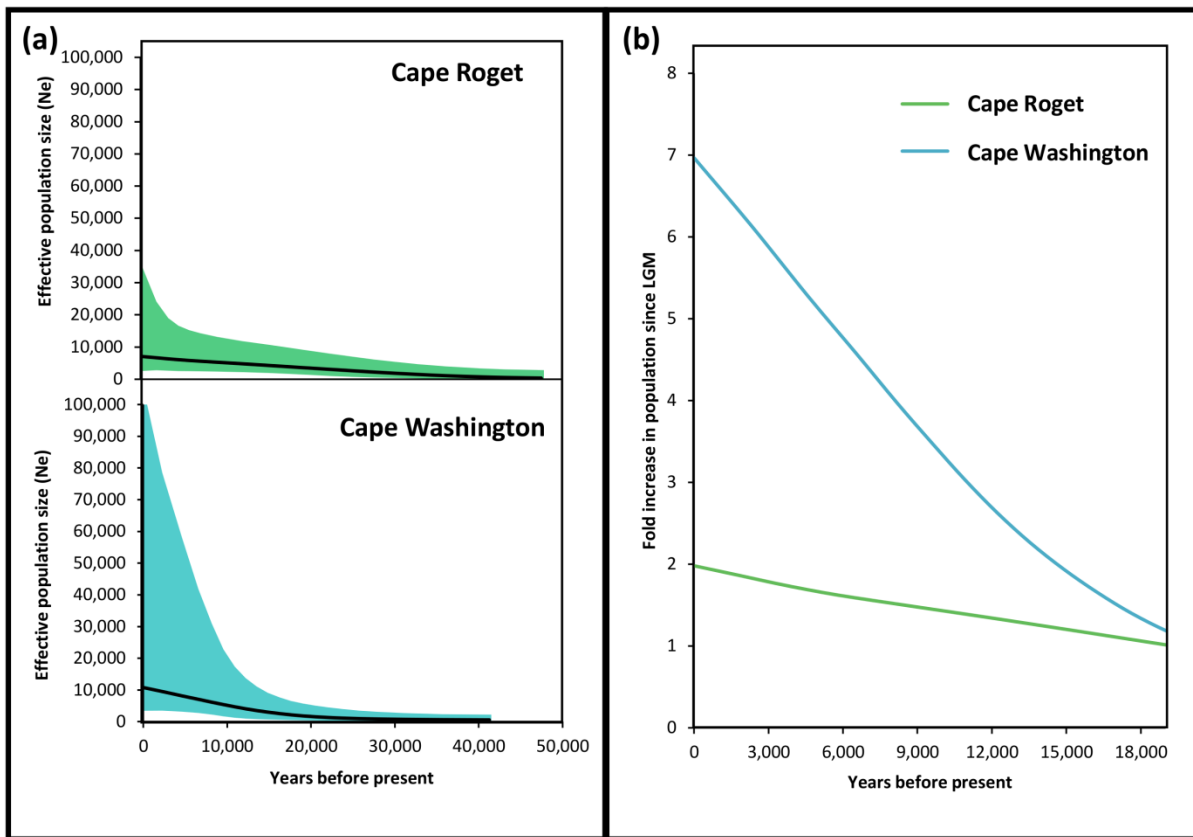
Figure 5.5. Comparison of the demographic histories of colonies in the Ross Sea. a) Extended Bayesian skyline plots for each colony, where the black line indicates the median estimate of N_e and the colour blocks show the 95% highest posterior density interval; b) the ratio increase in each colony's effective population size since the termination of the last glacial maximum, 19 kya.

contemporary gene flow between these sites. The more likely explanation for the observed signature of admixture is that the Amanda Bay population was founded by individuals from multiple glacial refugia during the post-glacial colonisation period, and has retained signatures of these ancestral populations within its genetic variation. This is consistent with my Bayesian skyline analyses, which indicated that Amanda Bay was colonised last out of the populations in this study. Overall, the patterns of genetic differentiation found among the eight colonies highlight the unpredictability of the boundaries of emperor penguin breeding populations, and demonstrate that geography alone cannot explain their population structure.

There is a growing body of evidence that emperor penguins lack strong philopatry. My genetic evidence suggests that individual penguins are moving hundreds of kilometres between colony sites within the Weddell Sea and Ross Sea regions, a notion supported by a recent satellite imagery study that observed movements of penguins among other colonies, including six instances in the past five years of shifts in colony sites of up to 190 km, occurring when breeding habitat at the usual site

became unfavourable (Ancel *et al.*, 2014, LaRue *et al.*, 2014). The characteristics of emperor penguin philopatry have implications for the future responses of the species to climate change. If penguins are not strictly philopatric, they should be able to respond to local, unfavourable environmental changes by shifting to other breeding sites if suitable conditions are available elsewhere. Movement of individuals between colony sites can also increase the adaptive capacity of populations by facilitating an influx of new, potentially adaptive alleles. The number of penguins moving among colonies could be quantified in the future with emigration analysis using the genetic dataset of genome-wide SNPs. This was not possible within the timeframe of this thesis, but is planned as immediate future work.

Here I have ascertained the population structure among eight breeding colonies, out of a total of 54 emperor penguin breeding colonies. Since the boundaries of emperor penguin breeding populations do not appear to be constrained by distance alone, it would be desirable to genetically sample additional colonies across the species' distribution in order to assign colonies to breeding populations. This would greatly improve the accuracy of future population forecasting studies by allowing forecasts to be made for true populations rather than colony sites. Understanding the boundaries of breeding populations around the continent would also assist Southern Ocean management, so that conservation planning can be implemented at a relevant spatial scale.

5.5.2 Mystery migrants to the Weddell Sea

My clustering analyses revealed four individual penguins that are highly divergent from all other individuals in the dataset (Figure 5.3a, Appendix 5.2). The overall indication is that this finding is not the result of genotyping errors or accidental sampling of another penguin species, but that these individuals are genetically distinct emperor penguins of unknown origin.

One possibility is that these individuals are hybrids of emperor penguins with another penguin species. Mitochondrial HVR and CytB sequences of the divergent individuals grouped with other haplotypes in the emperor penguin dataset, but if the female parent was an emperor, hybridisation would not be detected based on mitochondrial DNA alone. The only other species that shares the *Aptenodytes* genus is the king penguin and, while emperors and kings could be related closely enough to interbreed, they are not sympatric and rarely encounter each other, as the king penguin breeds on sub-Antarctic islands and generally forages only as far south as the Polar Front (Bost *et al.*, 2013). The two species also have very distinct breeding cycles. The emperor penguin initiates courtship and mating during late March, whereas the king penguin does so in October. The Adélie

penguin is sympatric with the emperor penguin, but is not in the same genus and initiates breeding in September. Overall, the emperor penguins' breeding biology is so distinct, with egg incubation occurring on sea ice throughout the winter months, that it seems extremely unlikely that they could successfully hybridise with any other penguin species. However, this could be definitively ruled out in the future by genetic comparisons of the outlier individuals to king and Adélie penguins using nuclear DNA.

Another possibility is that the individuals were migrants from two unsampled origin populations and, given that these individuals were several orders of magnitude more genetically differentiated than any of the other 110 penguins were from each other, the origin populations could comprise sub-species of emperor penguins. There are sub-species divisions within other penguin species, for example the gentoos (*Pygoscelis papua*), which split into northern (*Pygoscelis papua papua*) and southern (*Pygoscelis papua ellsworthii*) sub-species either during or just after the LGM (Clucas *et al.*, 2014). The emperor penguin species may be similarly sub-divided; as this thesis is the first study of emperor penguin population genetic structure, it is very possible that there are sub-species of emperor penguins that remain to be discovered. Either way, the presence of these individuals in the Weddell Sea is a mystery, as the origin populations must have been isolated from all other colonies in this study for a long time to show this level of genetic differentiation.

In order to determine the geographic origin of these individuals, it would be necessary to sample more colonies until genetically similar individuals could be located. Large stretches of coastline were excluded from this study and could be home to the divergent population; these include between the Weddell Sea and Mawson Coast, between Amanda Bay and Pointe Géologie, and Western Antarctica. Given that these four individuals are highly divergent from emperor penguins in the Weddell Sea, East Antarctica and the Ross Sea, I speculate that their colony of origin could be located somewhere in West Antarctica, such as the Bellingshausen or Amundsen Sea, which are somewhat isolated from the Weddell Sea by the Antarctic Peninsula. West Antarctica has experienced large declines in sea ice extent (–4.3% per decade) and sea ice season duration (–100 ± 31 days) over a 34-year satellite monitoring period (1978 – 2012) (Stammerjohn *et al.*, 2012, Vaughan *et al.*, 2013). Furthermore, the Amundsen Sea is undergoing an increase in the outflow of glacial ice, accompanied by calving events, which are destabilising the marine ice shelf and local sea ice conditions (Mouginot *et al.*, 2014). The local destabilisation and decline of sea ice, the emperor penguins' breeding habitat, may have resulted in penguins leaving West Antarctica for more favourable locales. There is some evidence for local emigration of emperor penguins on the western side of the Antarctic Peninsula, with satellite

imagery revealing the presence of a new breeding colony at Alexander Island during the 2012-2013 breeding season that was not present in 2009 (LaRue *et al.*, 2014). This new colony is located 190 km from Dion Island, which was home to a small emperor penguin colony that was abandoned sometime prior to 2009 coincident with local declines in the duration of the sea ice season (LaRue *et al.*, 2014, Trathan *et al.*, 2011). West Antarctica was excluded from the current study because of its remoteness and the logistical difficulties associated with sample collection. I suggest that a genetic sampling of West Antarctic colonies would be highly prudent, given the rapid decline of favourable emperor penguin habitat in the region, and would allow an assessment of population structure. In turn, this would enable accurate forecasting studies for West Antarctic populations.

5.5.3 Molecular markers for delimiting population structure

My previous study of emperor penguin population genetic structure, based on the mitochondrial HVR and CytB, found that penguins in the Ross Sea were distinct from those in East Antarctica and the Weddell Sea (Chapter 2, Younger *et al.*, 2015). This finding was supported by my current analyses using genome-wide SNPs; however the SNPs revealed further population subdivisions within the East Antarctic and Weddell Sea region that were not detected using mitochondrial DNA alone. This suggests that, in future studies of emperor penguin population structure, SNPs should be the molecular marker of choice.

5.5.4 Glacial refugia

My previous findings, based on mitochondrial DNA, indicated that emperor penguins were restricted to a few small refuge populations during the LGM, one of which was probably located in the Ross Sea, and then increased in abundance during the post-glacial period (Chapter 2, Younger *et al.*, 2015). The habitat changes that emperor penguins experienced during the last glacial period and subsequent transition to the interglacial Holocene were discussed in detail in Chapter 2 and will not be re-visited here.

My new findings based on RADSeq suggest that there was an emperor penguin LGM refuge located at or near Cape Roget in the north-western Ross Sea (Figure 5.6), based on extended Bayesian skyline analyses (Figure 5.4, 5.5), comparisons of genetic diversity (Table 5.3, 5.4) and pairwise F_{ST} (Table 5.2). The genetic evidence for a refuge at Cape Roget correlates with the proposed location of an LGM polynya offshore from the Adare Peninsula (Figure 5.6) (Smith *et al.*, 2010 and references therein). There is also evidence for an LGM refuge population of Adélie penguins in the Ross Sea (Lambert *et al.*, 2002, Ritchie *et al.*, 2004) that could have been supported by the same polynya.

Interestingly, this region is currently home to the world's largest populations of both Adélie and emperor penguins (Fretwell *et al.*, 2012, Lynch & LaRue, 2014, Schwaller *et al.*, 2013). The Adélie penguin colony at Cape Adare had an estimated abundance of 338,231 breeding pairs during the 2010/11 breeding season (Lynch & LaRue, 2014), and the Coulman Island emperor penguin colony, located just 150 km south of Cape Roget, contained an estimated 25,298 breeding pairs during the 2009/10 breeding season, while the Cape Roget colony itself was estimated at 9,505 breeding pairs (Figure 5.6) (Fretwell *et al.*, 2012). Furthermore, southern elephant seals (*Mirounga leonina*) rapidly colonised the north-western Ross Sea from Macquarie Island, 2,500 km to the north, during a warm period associated with reduced sea ice conditions in the mid-Holocene (de Bruyn *et al.*, 2009, 2014, Hall *et al.*, 2006). The species maintained colonies on the Victoria Land Coast for 6,000 years before abandoning the area *ca*. 1,000 years ago when sea ice concentrations increased (de Bruyn *et al.*, 2009, Hall *et al.*, 2006). The overall indication is that this region in the Ross Sea has been an important breeding habitat for Southern Ocean predators for at least 50,000 years. It is therefore likely that the north-western Ross Sea could act as a breeding refuge in the future and should be considered in any future management for conservation.

My previous phylogenetic analysis of emperor penguins suggested that there were at least two other LGM refugia, probably located in East Antarctica or the Weddell Sea (Figure 2.3; Chapter 2, Younger *et al.*, 2015). I have now determined that refugia were most likely not located at either Amanda Bay or the Mawson Coast, as these populations underwent dramatic expansions consistent with colonisation in the post-glacial period (Figure 5.4b). Amanda Bay also had significantly lower genetic diversity than all other populations (Table 5.4; *p* = 0.0000), consistent with a more recently founded population, and signatures of admixture (Figure 5.3), consistent with post-glacial colonisation from multiple refugia (Hewitt, 2000). The post-glacial expansion of the Pointe Géologie and Weddell Sea populations were less extreme and with an earlier onset than the expansions of the Mawson Coast or Amanda Bay populations (Figure 5.4). However, at this time there is insufficient evidence to propose that these were refugia.
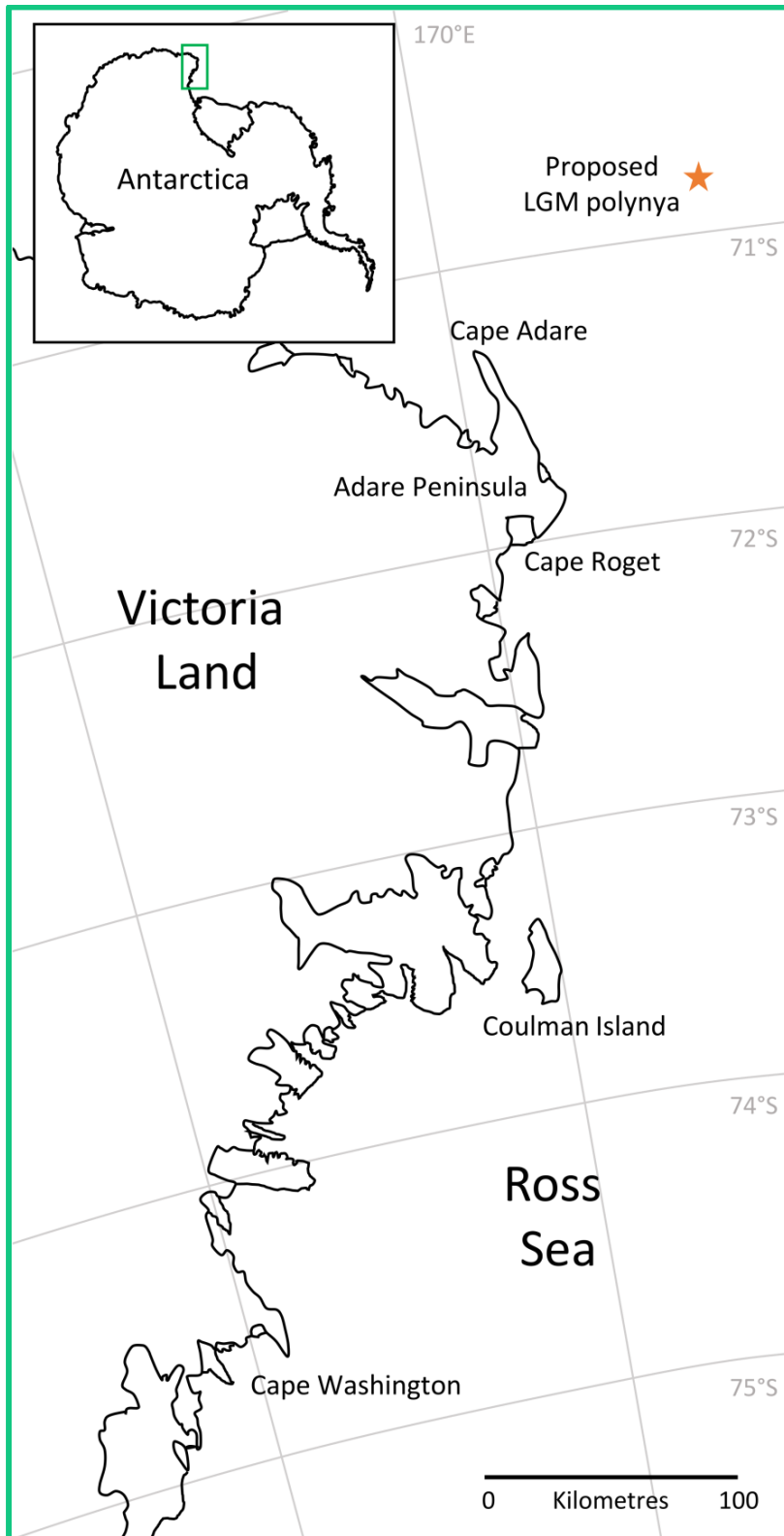
Figure 5.6. Victoria Land Coast of the north-western Ross Sea. Genetic evidence suggests there may have been a glacial refuge of emperor penguins located near Cape Roget. Extant emperor penguin colonies are located at Cape Roget, Coulman Island and Cape Washington. The largest extant Adélie penguin breeding colony is located at Cape Adare. The orange star indicates the possible location of an LGM polynya, as proposed by Smith *et al*. (2010).

The hypothesis of polynyas as Antarctic seabird refugia during glacial periods was first proposed by Thatje *et al*. (2008), and is now supported by evidence from at least two species – emperor penguins and snow petrels. My genetic data strongly indicate the presence of an emperor penguin refuge near the Adare Peninsula that may have been supported by an LGM polynya offshore, and this same polynya may have also supported the foraging of an LGM population of Adélie penguins (Ritchie *et al.*, 2004). Snow petrels were widely distributed in Dronning Maud Land throughout the LGM (Hiller *et al.*, 1995, Hiller *et al.*, 1988, Steele & Hiller, 1997, Thor & Low, 2011), and must have had foraging habitat nearby to support these colonies. Given the expansive sea ice field during the LGM (Gersonde *et al.*, 2005), foraging most likely took place in the LGM polynya located approximately 300 km away (Hiller *et al.*, 1988, Smith *et al.*, 2010, Thatje *et al.*, 2008). The hypothesis that polynyas acted as LGM refugia for emperor penguins could be further investigated with analysis of the demographic history of extant colonies in the vicinity of known LGM polynya sites. In addition to the Ross Sea polynya, which I have investigated here, there are three known LGM polynya sites in the Weddell Sea. One is near the Atka Bay emperor penguin colony, and another is near the Snow Hill colony (Smith *et al.*, 2010, Sprenk *et al.*, 2014, Thatje *et al.*, 2008). There may have been polynyas in other locations around the continent during the LGM (Smith *et al.*, 2010), however, there are no empirical data to confirm this at present, as the sediment core record around Antarctica is sparse. The identification of areas that have sustained breeding populations of emperor penguins continuously for tens of thousands of years, throughout adverse environmental conditions, may prove crucial for the future conservation of this species.

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5.7 Appendices

Appendix 5.1 Extended Bayesian skyline plot trials to determine the optimal number of RAD loci for analysis
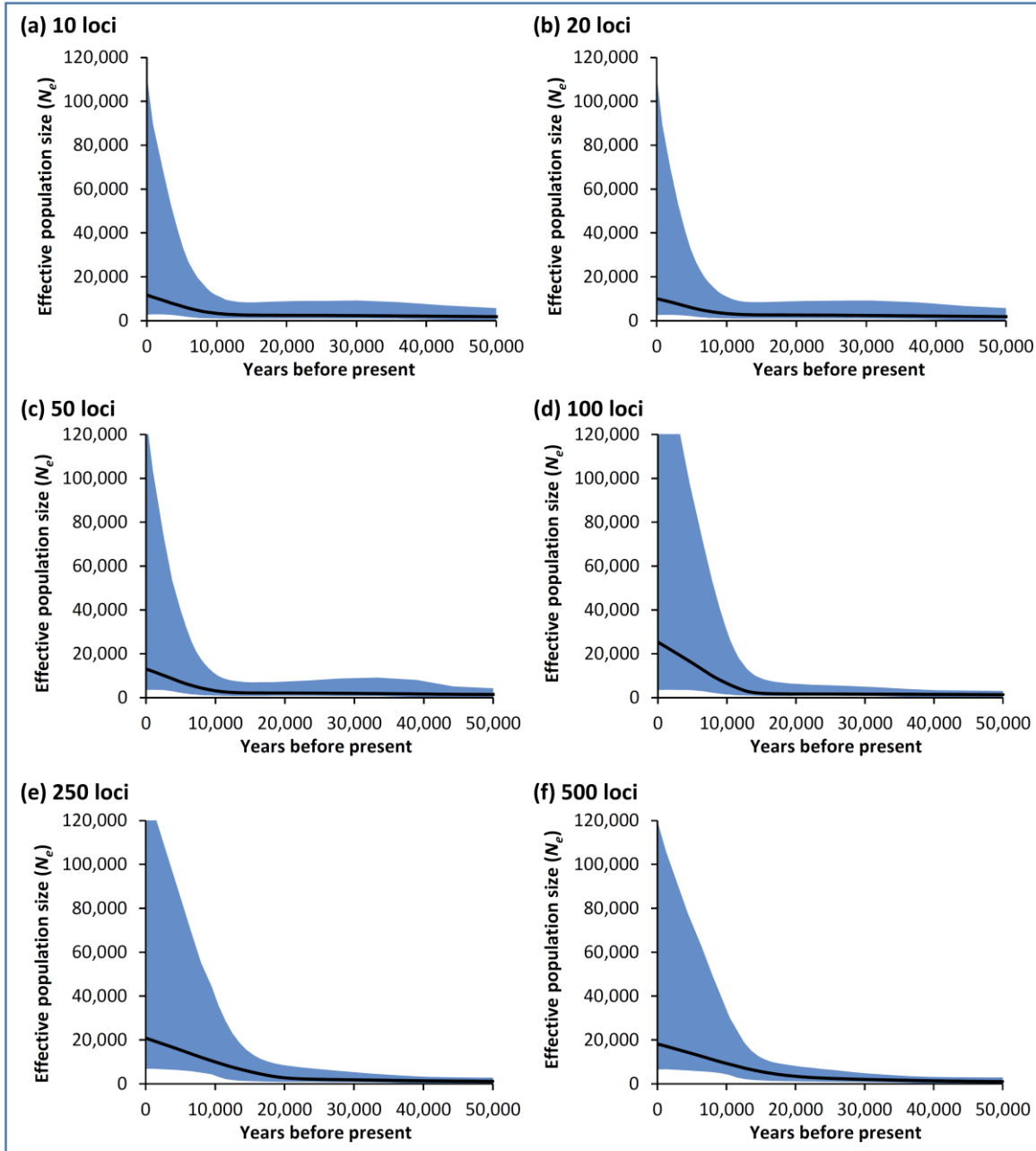
Figure 5A.1. Extended Bayesian skyline plot trials to determine the optimal number of RAD loci for analysis. Extended Bayesian skyline plots showing the change in effective population size (N_e) of the Pointe Géologie population, as inferred from a) 10, b) 20, c) 50, d) 100, e) 250 and f) 500 RAD loci. The 250 loci dataset was used for all further analyses, as 500 loci gave a similar result but had a considerably longer run time.

Appendix 5.2 Principal components analysis

The same four individuals from Halley Bay and Gould Bay were identified as highly divergent from all of the other individuals when PCs 1 and 2 were plotted (Figure 5A.2a). To investigate more fine-scale structure, these four individuals were removed and the PCA repeated to better visualise the structure among the remaining 110 individuals. The proportion of variance explained by each of the axes was very low, at 1.221% for PC1, 1.179% for PC2 and 1.109% for PC3. While it is generally assumed that only PCs with large eigenvalues represent structure in the data, this may not always be the case for data such as bi-allelic SNPs, as each SNP contains relatively little information compared to highly variable microsatellites; therefore, a small contribution to the total variance in SNP genotypes should not be cause to exclude PCs from the analysis (Dadousis *et al.*, 2014). In this case, the population structure resolved was very similar to that found via STRUCTURE analysis. In the plots of PCs 1 versus 2 (Figure 5A.2b) and PCs 1 versus 3 (Figure 5A.2c) the inertia ellipses, which contain 67% of the individuals from each colony, are separated into three clusters: the Ross Sea colonies (Cape Washington and Cape Roget) form one cluster with very little overlap with other clusters; the Mawson Coast colonies (Fold Island and Auster) form a cluster separate from the other colonies, but with some differentiation between the two; whilst the Weddell Sea colonies, Pointe Géologie and Amanda Bay overlap with one another. In the plot of PCs 1 versus 3, Gould Bay and Halley Bay sit almost atop one another, with little overlap with Point Géologie, whilst Amanda Bay sits intermediately between these two groups, reinforcing the admixture signal found by the STRUCTURE analysis.

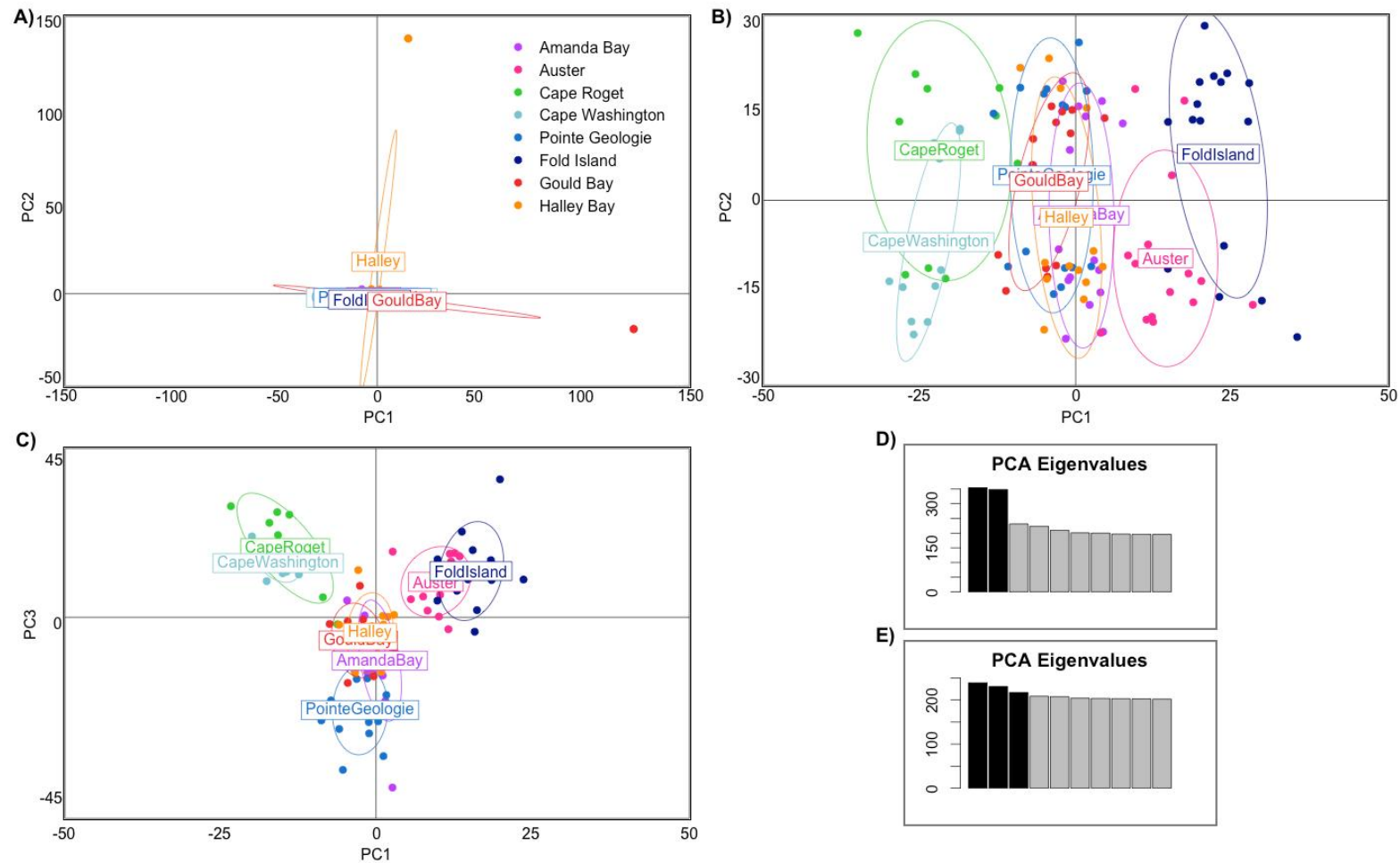**Figure 5A.2. Principal components analysis.** The plots show: a) PCs 1 versus 2 when all individuals are included in the analysis; b) PCs 1 versus 2 when the divergent individuals are removed; and c) PCs 1 versus 3 when the divergent individuals are removed. The eigenvalues for the first 10 PCs when all individuals are included are shown in d), and when the divergent individuals are removed in e).

CHAPTER 6

DISCUSSION

In this thesis I compared the responses of three Antarctic sea ice dependent predators, the Weddell seal, the emperor penguin and the Adélie penguin, to historical climate regime shifts. The findings were surprising and many of my hypotheses were disproven, leading to new and unexpected insights into the relative vulnerabilities of Southern Ocean predators to long-term climate change. I found that numbers of both Adélie and emperor penguins were smaller during the LGM than they are today and were restricted to two and three glacial refugia, respectively (Chapter 2, 4, Younger *et al.*, 2015). Populations of both penguin species expanded during the post-glacial period; Adélie penguins expanded 135-fold from *ca*. 14 kya, apparently driven by an increase in ice-free ground suitable for nesting (Chapter 4), and emperor penguins expanded 4,000 years later by between 2- and 15-fold, depending on location, coincident with reductions in sea ice that possibly corresponded with more accessible prey (Chapter 2, 5, Younger *et al.*, 2015). On the other hand, Weddell seals have had a stable population size in East Antarctica over approximately 80,000 years, and were apparently unaffected by the environmental changes that drove abundance increases in their compatriots (Chapter 3).

6.1 Long-term vs. short-term drivers of population change

Contemporary ecological studies suggest that the availability of suitable breeding habitat and access to productive foraging grounds are crucial to the success of Southern Ocean predator populations in the short-term (i.e. over decadal timescales) and my palaeoecological data have highlighted the importance of these same variables over longer, millennial, timescales.

Following the end of the LGM, the emergence of new ice-free breeding habitat drove population expansions of Adélie penguins in East Antarctica (Chapter 4), the Scotia Arc (Clucas *et al.*, 2014) and the Ross Sea (Lambert *et al.*, 2002, Ritchie *et al.*, 2004), of elephant seals in the Ross Sea (de Bruyn *et al.*, 2009, Hall *et al.*, 2006), and of gentoo and chinstrap penguins in the Scotia Arc (Clucas *et al.*, 2014). On the other hand, increased foraging habitat during the glacial-interglacial transition, in the form of decreased sea ice, increased primary productivity and shifting oceanographic fronts, allowed the proliferation of emperor penguins (Chapter 2, 5, Younger *et al.*, 2015), snow petrels (Thatje *et al.*, 2008, Verkulich & Hiller, 1994) and king penguins (Trucchi *et al.*, 2014). Notably, the latter three species all had much smaller and spatially restricted populations during the LGM, despite the availability of suitable breeding habitat.

My palaeoecological findings suggest that the long-term environmental drivers of population change are consistent within species at broad spatial scales; for example, the timing of Adélie penguin

population expansions in East Antarctica and the Scotia Arc differed by a few thousand years (Chapter 4, Clucas *et al.*, 2014), but in both cases the timing of expansion was coeval with local deglaciation. Similarly, emperor penguin populations in the Weddell Sea, Ross Sea and East Antarctica expanded at different times, but in all cases were broadly coincident with local changes in sea ice conditions (Chapter 2, 5, Younger *et al.*, 2015).

While the two key requirements of suitable breeding habitat and access to productive foraging ground are absolute, the environmental drivers of population change for a given species are not necessarily the same over all temporal scales. While sea ice conditions are known to be a critical driver in Adélie penguin population success over decades and years (Ainley *et al.*, 2010, Emmerson *et al.*, 2011, Emmerson & Southwell, 2008, Jenouvrier *et al.*, 2006), the post-glacial expansion of Adélie penguin populations both in East Antarctica (Chapter 4) and the Scotia Arc (Clucas *et al.*, 2014) pre-dated changes in sea ice conditions by several thousand years, instead occurring shortly after the local retreat of ice sheets (Cofaigh *et al.*, 2014, Hodgson *et al.*, 2014, Mackintosh *et al.*, 2014). Meanwhile, the abundance and breeding distribution of emperor penguins has been influenced by changes in sea ice conditions over millennia (Chapter 2, 5, Younger *et al.*, 2015), decades (Jenouvrier *et al*. 2005, Trathan *et al.*, 2011), and even years (Ancel *et al.*, 2014, LaRue *et al.*, 2015). This indicates that while the short-term environmental drivers of population change may also drive change over millennia, in some cases they do not. This is an important finding given that the climate change currently underway represents a marked shift in the Earth's environmental state, considered the dawn of the new Anthropocene epoch, rather than a short-term fluctuation in climate (Lewis & Maslin, 2015). My findings suggest that a combination of palaeoecological and contemporary data provide the most complete understanding of the environmental sensitivities of species, and a complementary approach seems prudent given the long-term nature of projected climate change.

6.2 Sensitivity to climate change

This study has demonstrated that the timing and magnitude of population responses to climate change can vary by both species and region, suggesting that regional, species-specific conservation plans will be required under future climate change scenarios. The differences among species' responses to climate change may be attributed to differences both in their key environmental sensitivities and their adaptive capacities, which includes factors such as dispersal ability, genetic diversity and generation time.

The effects of differing environmental sensitivities can be observed in studies of different penguin species. After the end of the glacial period, Adélie penguin populations expanded coincident with local ice sheet retreat and an increase in available breeding habitat (Chapter 4, Clucas *et al.*, 2014), whereas emperor penguin populations expanded several thousand years later, coincident with local changes in sea ice conditions (Chapter 2, Younger *et al.*, 2015). In the Scotia Arc, Adélie, chinstrap and gentoo penguins all took advantage of the emergence of new breeding habitat following deglaciation, but as Adélie penguins are more tolerant to sea ice than chinstrap or gentoo penguins, they were able to expand their territory slightly earlier in the warming period (Clucas *et al.*, 2014).

The effects of differing adaptive capacities can be observed by comparing sympatric seal and penguin species. I originally hypothesised that emperor penguins and Weddell seals, as sympatric meso-predators with similar ecological niches, may have undergone similar population trends in response to past climate shifts. Furthermore, I anticipated that both species were likely to have prospered during the LGM due to an increase in their sea ice breeding habitat and reduced competition from other, less cold-tolerant, predators such as the Adélie penguins. However, I have now shown that the two species responded very differently to post-glacial warming. While emperor penguins responded to warming with an increase in numbers and range expansion from refugia (Chapter 2, 5, Younger *et al.*, 2015), the Weddell seal population size in East Antarctica has been consistent over the past 80,000 years (Chapter 3). This may be the result of differing adaptive capacities between the two. Emperor penguins disperse among colony sites over distances up to 550 km (Chapter 5), whereas Weddell seals have genetically distinct colonies separated by as little as 10 km (Chapter 3). This suggests a higher dispersal ability for emperor penguins, which could facilitate both range shifts and the movement of potentially adaptive genetic variants among sites. Emperor penguins were also found to have higher evolutionary rates of the mitochondrial genome, despite the similar generation times of the two species (Chapter 3). The overall indication is that the greater adaptive capacity of emperor penguins allowed them to exploit new habitat and resources during the Holocene by expanding in number and range, whereas Weddell seals did not.

In another example, during the mid-Holocene warm period, elephant seals from Macquarie Island took swift advantage of emergent habitat in the Ross Sea 2,500 km away (de Bruyn *et al.*, 2009), while Macquarie Island king penguins, which have very similar breeding habitat requirements as the elephant seals, did not. The key difference in this case was most likely the relative foraging ranges of the two species. King penguins do not venture into the Ross Sea for foraging, therefore did not have the opportunity to discover emergent breeding habitat in the region. Elephant seals, on the other hand, do occasionally forage over the Antarctic continental shelf and in the Ross Sea (Bradshaw *et*

al., 2003, Charrassin *et al.*, 2008, Hindell *et al.*, 1991), and it appears that this wide foraging range conveyed an advantage in discovering newly available breeding habitat.

Similar observations have been made in other ecosystems. In Europe, the red fox (*Vulpes vulpes*) experienced very little change in its effective population size throughout the LGM and post-glacial warming period, which the authors attributed to the high dispersal ability and adaptability of the species (Teacher *et al.*, 2011). On the other hand, the Arctic fox (*Alopex lagopus*) became extinct in large areas of Europe during the post-glacial warming period and did not range shift north to Scandinavia in line with its preferred Arctic habitat (Dalén *et al.*, 2007). The Arctic fox population in Scandinavia was founded by emigrants from the Siberian population some time later in the Holocene (Dalén *et al.*, 2007). This difference in ability to range shift in line with preferred habitat during climate change shows a clear difference in adaptability of these two fox species. In another example, two small mammal prey species with similar ecological niches in the Arctic tundra, the collared lemming (*Dicrostonyx torquatus*) and narrow skulled vole (*Microtus gregalis*), demonstrated markedly different responses to post-glacial climate warming, which the authors attributed to small differences in their thermal tolerance (Prost *et al.*, 2013).

These findings highlight the need to take a species' sensitivity to climate change into account when considering its future responses to climate change, where a species' sensitivity encompasses its environmental tolerance, dispersal ability, evolutionary rate, genetic diversity and current foraging range. While many risk-assessments for species under future climate change scenarios focus primarily on exposure (i.e. the magnitude of climate change the species will face), sensitivity is an equally important determinant of species' responses.

6.3 Implications for the future of Adélie penguins, emperor penguins and Weddell seals

Both the breeding and foraging habitats of Southern Ocean predators are currently under threat, with climate models projecting substantial, long-term changes (Collins *et al.*, 2013, Constable *et al.*, 2014). Based on the available palaeoecological data, these projected environmental changes are likely to have impacts on the distributions and abundances of many Southern Ocean predators, including the three species investigated in this study.

Adélie penguin numbers have begun to expand in recent years in the Ross Sea, in line with local deglaciation that has resulted in additional breeding sites becoming exposed (LaRue *et al.*, 2013). In

other areas, such as the Antarctic Peninsula, Adélie penguin numbers are in decline, possibly as a result of declining prey abundance in the region (Lynch *et al.*, 2012). As climate change progresses, glaciers and ice sheets in Antarctica are expected to retreat further, potentially leading to further penguin expansions in regions where prey is sufficiently abundant and accessible enough to meet the requirements of the expanding population. It is possible that in the Anthropocene epoch, Adélie penguin abundance will be limited by foraging availability rather than the availability of ice-free breeding habitat. Under this scenario, it is difficult to predict the future abundance of this species, as climate induced changes in the abundance and distribution of Adélie penguin prey, such as Antarctic krill (*Euphausia superba*), remain ambiguous at this time (Atkinson *et al.*, 2012, Constable *et al.*, 2014, Nicol & Brierley, 2010).

Emperor penguins have begun to show plasticity in their breeding sites in response to unfavourable sea ice conditions, consistent with my findings that they may have a high adaptive capacity. There have been six recorded instances of colony relocation, or establishment of a new colony, over the past five years, coincident with deterioration of sea ice quality at some traditional breeding locations (Ancel *et al.*, 2014, LaRue *et al.*, 2015). There have also been declines in the number of emperor penguins breeding at colony sites with unfavourable sea ice conditions, which were originally interpreted as decreased survival rates and/or breeding success (Barbraud *et al.*, 2011, Barbraud & Weimerskirch, 2001, Jenouvrier *et al.*, 2005). However, there is now a growing body of evidence to suggest that large numbers of emperor penguins are emigrating from these colony sites to more favourable locales (Barbraud *et al.*, 2011, LaRue *et al.*, 2015). This is supported by my findings that emperor penguins disperse among colony sites up to 550 km apart (Chapter 5). Furthermore, the species appears to have adopted a new breeding strategy in some locations in response to locally poor sea ice conditions, by relocating colonies onto ice shelves (Fretwell *et al.*, 2014, Wienecke, 2012). This supports my conclusion based on palaeoecological evidence that emperor penguins may have a high capacity to adapt to environmental change (Chapter 3).

My palaeoecological data indicate no change in East Antarctic Weddell seal abundance over the past 80,000 years, suggesting that breeding habitat and prey availability were both sufficient to maintain a constant population size throughout this entire period. This is plausible, as the Weddell seal's breeding habitat (sea ice) has been consistently available throughout this period (Gersonde *et al.*, 2005, Jouzel *et al.*, 2007). Whether this was also the case in other parts of Antarctica, such as the Ross and Weddell Seas, could be investigated in the future with Bayesian coalescent analyses for seals from these locations. Whether environmental conditions in East Antarctica have been optimal for Weddell seals, or have just been consistently sub-optimal, for 80,000 years is unknown and could

be investigated in the future using the pairwise sequentially Markovian coalescent method to estimate past population sizes further back in time (Li & Durbin, 2011). The responses of Weddell seals to contemporary, decadal-scale climate change have been regionally variable. The species has almost disappeared from Anvers Island at the Antarctic Peninsula, coincident with declining fast ice over the period of 1973–2002 (Siniff *et al.*, 2008). In eastern McMurdo Sound, Ross Sea, Weddell seal numbers have also declined; however sea ice conditions are unchanged and the decline appears to be related to changing prey availability (Ainley *et al.*, 2015). Meanwhile, at the Vestfold Hills, East Antarctica, there was no change in Weddell seal numbers between 1973 and 2000, in spite of a shortening of the local sea ice season (Lake *et al.*, 2008, Massom *et al.*, 2013). Overall, the past, present and future responses of Weddell seals to climate change are still somewhat unclear and, in light of projections of decline in their breeding habitat (Collins *et al.*, 2013), deserve further study.

6.4 Glacial refugia

Areas that have sustained breeding populations of Antarctic meso-predators continuously throughout climate regime shifts may have an important role to play in the future survival of a diverse array of Antarctic species. Therefore, the identification of past glacial refugia may directly inform future management plans for conservation. The potential role of polynyas as oases of productivity and Southern Ocean predator refugia during glacial periods was proposed by Thatje *et al*. (2008) with supporting evidence from snow petrels in Dronning Maud Land (Hiller *et al.*, 1995, Hiller *et al.*, 1988, Steele & Hiller, 1997, Thor & Low, 2011). My genetic data for emperor penguins strongly suggest that there was an emperor refuge in the vicinity of the extant Cape Roget colony on the Adare Peninsula, in close proximity to the proposed location of an LGM polynya (Smith *et al*. 2010) that could have supported the foraging requirements of the refuge population (Chapter 5). My phylogenetic analysis suggests there were at least two other emperor penguin refugia during the LGM, most likely in the Weddell Sea or East Antarctica (Figure 2.3) but probably not proximate to any of the colonies included in my study (Chapter 5). The location of these refugia could be further explored by analysing the population history and genetic diversity of additional breeding populations in East Antarctica and the Weddell Sea, particularly focusing on colonies in the vicinity of known LGM polynyas (Smith *et al.*, 2010, Sprenk *et al.*, 2014, Thatje *et al.*, 2008), such as the Atka Bay and Snow Hill colonies.

The evidence for the locations of Adélie penguin refugia is currently limited. It seems likely that the species had only two refugia during the LGM (Chapter 4) and one of these may have been in the Ross

Sea (Ritchie *et al.*, 2004). An Adélie penguin LGM refuge would require ice-free ground for nesting and a suitable foraging area, which could have been provided by a polynya. It seems possible that, as for emperor penguins, a refuge may have been located in the vicinity of the Adare Peninsula. Given that Adélie penguins nest on ice-free ground, their sub-fossil remains are often left in situ at abandoned breeding sites and can be radiocarbon dated to determine the time of occupation (Stonehouse, 1970). However, despite extensive radiocarbon dating of Adélie penguin remains from the Ross Sea, there have been no records of any remains dated to the LGM, although remains that pre-date the LGM have been recovered (Emslie *et al.*, 2007). As the locations of Adélie penguin refugia remain a mystery, they could be further investigated using similar methods as suggested for emperor penguins, via a combination of Bayesian skyline and genetic diversity analyses, with studies targeted toward extant colonies in the vicinity of LGM polynyas (Smith *et al.*, 2010, Sprenk *et al.*, 2014, Thatje *et al.*, 2008). Additionally, emphasis could be placed on sites that were known to have ice-free ground during the LGM, such as the Vestfold Hills, Lützow-Holm Bay, the South Shetland Islands and the Larsemann Hills (Berkman *et al.*, 1998, Burgess *et al.*, 1994, Gore *et al.*, 2001, Hayashi & Yoshida, 1994, Hodgson *et al.*, 2014). In the case of Adélie penguins, any genetic indications of refugial areas could be further investigated by radiocarbon dating of penguin remains from proposed refuge sites. This approach of genetic studies combined with radiocarbon dating of remains could be extended to other Southern Ocean predators that may have been restricted to refugia during the LGM, such as snow petrels, king penguins and elephant seals.

6.5 Comparison of molecular markers for ecological studies

In this work I used a combination of mitochondrial DNA sequencing (specifically, the hypervariable region and cytochrome *b*), RADSeq generated SNPs and RADSeq short reads of 117 bp length. Mitochondrial DNA sequencing has been used in thousands of published studies of genetic population structure, gene flow, phylogeography and demographic history (Zink & Barrowclough, 2008) but has also been widely criticised as an inappropriate marker for such studies, largely on the basis that it represents an incomplete view of species' histories e.g. (Ballard & Whitlock, 2004). However, other reviews have refuted these claims and suggest that, in most cases, mitochondrial DNA is a robust indicator of population history, geographic population structure and phylogeographic patterns, and has superior resolution compared to nuclear DNA sequences for detecting more recently derived population structure and demographic events (Zink & Barrowclough, 2008). RADSeq is a method for the rapid and (relatively) inexpensive genotyping of hundreds of thousands of genome-wide SNPs (Baird *et al.*, 2008) that has gained popularity in recent years. The method has

been applied to a broad spectrum of taxa and ecological questions, from population structure of marine invertebrates (Reitzel *et al.*, 2013) to adaptation in three-spine sticklebacks (Hohenlohe *et al.*, 2010) and species delimitation in cichlids (Wagner *et al.*, 2013).

I analysed population genetic structure of emperor penguins using mitochondrial DNA sequences (Chapter 2) and using 20,005 SNPs (Chapter 5), and found that SNPs were superior for detecting fine-scale population structure. In the mitochondrial analysis, I found that penguins in the Ross Sea were distinct from those in East Antarctica and the Weddell Sea, a finding that was supported by genome-wide SNPs. However, SNPs revealed further population subdivision within the East Antarctic and Weddell Sea region, with four distinct genetic populations resolved among the six colonies studied, which were grouped as a single genetic population using mitochondrial DNA alone. Analyses of population structure of other penguin species, including Adélies (Clucas *et al.*, 2014, Ritchie *et al.*, 2004, Roeder *et al.*, 2001) and chinstraps (Clucas *et al.*, 2014, Freer *et al.*) using either mitochondrial DNA or microsatellites, have resolved similar levels of population differentiation as found in my mitochondrial DNA analysis of emperor penguins. It therefore seems possible that other penguin species may have higher levels of genetic differentiation than reported based on mitochondrial DNA or microsatellites, which could be detected using genome-wide SNPs.

My analyses of demographic history for emperor penguin populations were conducted using mitochondrial DNA (Chapter 2) and 250-loci datasets of RADSeq short reads (Chapter 5). The only population analysed using both methods was the Ross Sea (because the East Antarctic/Weddell Sea population resolved by the mitochondrial DNA was subdivided in the RADSeq analysis), and both methods found a population expansion commencing at approximately the same time and with a same magnitude of abundance increase (*ca*. 3-fold), although the confidence intervals were slightly reduced with the addition of the RADSeq markers. I would therefore suggest that mitochondrial DNA alone is sufficient for resolving population histories, as the extra cost and effort involved in the RADSeq genotyping and analysis had minimal pay-off in this case.

6.6 Future research directions

There are many questions that remain to be answered regarding the long-term responses of Southern Ocean predators to climate change. In addition to the future work already proposed, other suggested lines of inquiry include palaeoecological studies of Southern Ocean flying seabirds and pack ice seals, both of which are particularly vulnerable to projected climate change (Jenouvrier, 2013, Siniff *et al.*, 2008). Pack ice seals, including the Ross, crabeater and leopard seals, breed on

pack ice and are therefore vulnerable to declines in sea ice extent; however their past and present climate change responses are unknown. It is possible that they may be robust to climate changes, as their fast ice breeding cousins the Weddell seals were. However, their pack ice breeding habitat is different to fast ice. Genetic coalescent and phylogenetic studies across these species' ranges could provide crucial information regarding key environmental drivers of their abundances and distributions, their sensitivities to climate change, and identify past refugia that could be used as potential refugia in the future. As the Earth shifts into the Anthropocene and faces unprecedented extinction rates and environmental change, comparative palaeoecological studies can provide valuable insight into the key environmental drivers underlying changes in species' abundance and distribution and, in combination with contemporary ecological studies, will be invaluable to future conservation strategies.

6.7 References

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