UNIVERSITY OF CALIFORNIA, SAN DIEGO

Genetic Structuring in a Circum-Antarctic Taxon Investigated using ddRAD Sequencing

A thesis submitted in partial satisfaction of the requirements for the degree of Masters of Science

in

Biology

by

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2015

The thesis of Matthew C. Sasaki is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego

2015

DEDICATION

For my parents. Thank you for the unconditional love and support you have always shown. I am who I am today because of you. Mom, thank you for instilling in me a burning desire to understand the natural world, and for all of the advice (especially when I didn't even know I needed it). Dad, thank you for always being there to talk some sense into me, and for teaching me to stick to my morals and ethics.

For Ruben. Thank you for putting up with all the late nights and weekends in lab. I could not have made it through this without you. On to the next big adventure.

And for the members of Gen 11. Thank you for always being there for me. You all have profoundly impacted my life for the better, and I look forward to a lifetime of friendship. #oneonelove

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ACKNOWLDEGEMENTS

I would first like to thank Dr. Ronald Burton for the many opportunities he provided me in his lab. He has provided constant support throughout each of my projects, and his advice on each undertaking in the lab, coursework, and life in general has been invaluable.

I would also like to acknowledge Dr. Shurin and Dr. Rifkin, as the co-chair and third committee member, respectively, for their part in the completion of my degree. Their time and energy put into this project is much appreciated.

I would also like to thank Dr. Greg Rouse for the opportunity to work on this project, for providing valuable samples for me to use, and for his assistance, advice, and discussion throughout the process. I would also like to thank Jose Carvajal for his patient assistance in obtaining said samples.

And finally, it would be unforgivable to end this section without thanking the other members of the Burton Lab. Without the assistance of Lani Gleason, Tessa Pierce, and Josh Stewart, this project would not have happened. Thank you for putting up with my endless stream of questions. While not directly involved in this project I would also like to thank Ricardo Pereira. His mentorship during my undergraduate research is the reason I am here today. Our conversations have proved critical in my development as an independent scientist. And to Lori. I'm so glad I had someone to go through this process with. Without you to keep things "interesting", this year likely would have been much more difficult, and definitely would have been much less enjoyable.

There are many people, without whom I could not have completed this project, that I have not mentioned above. Nonetheless, your time and energy has been of immense importance to me, and I will be forever grateful.

ABSTRACT OF THE THESIS

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The Southern Ocean hosts an abundant and diverse fauna. The evolutionary history of these taxa has been strongly influenced by the oceanographic and glacial history of this region. Molecular studies have challenged the traditional characterization of many Antarctic taxa as circumpolar, uncovering large amounts of unexpected diversity. In this study,

we investigate the genetic diversity and population structuring of *G. antarctica*. Using double digest restriction-site associated DNA sequencing, we obtain a set of 4301 SNP markers from across the genome for a set of four populations spanning a large portion of this taxa's range distribution. This data set reveals clear evidence of strong population structuring across the Southern Ocean, at both large and small geographic scales. We reject the hypothesis of panmixia within the geographic region investigated. The data presents evidence of the influence of both the Antarctic Circumpolar Current and the last glacial maximum on the genetic structure of G. antarctica

Introduction

The Antarctic biota has traditionally been described as highly endemic, with widespread taxonomic circumpolarity and eurybathy (Dell, 1972; Clarke & Crame, 1989; Brey et al., 1996; Arntz et al., 1997). However, this perspective is changing as studies use molecular techniques to investigate the genetic diversity found within the Southern Ocean. The Antarctic biota has been strongly influenced by isolation from the surrounding regions, imposed by large geographic distances, oceanographic and bathymetric features, and the extreme polar climate, as well as by a long history of glacial activity. Understanding this historical context for Antarctic evolution is essential for interpreting the patterns of diversity observed today. Antarctica provides a unique opportunity to study how contemporary population structuring is influenced by historic climatic and physical forcings, and provides insight into how contemporary climate change may influence gene flow and population connectivity in the Southern Ocean. Understanding the interaction between gene flow and local adaptation is critical for predicting the response of taxa to global change, which has wide-ranging implications {Sanford:2011km}.

Palaeoceanography – a history of isolation

The modern Southern Ocean current system is dominated by the Antarctic Circumpolar Current (ACC), a fast, cold, and saline Eastbound

current encircling the Antarctic continent (Hill et al., 2013). The development of Antarctic isolation began in the Late Cretaceous, but it wasn't until the opening of the Drake Passage between South America and the Antarctic Peninsula, leading to the creation of the ACC, that true Antarctic isolation was initiated (Kennett, 1977; Clarke & Crame, 1989; Pfuhl & McCave, 2005; Lyle et al., 2007). While the timing of formation and subsequent strengthening of this oceanographic feature is still debated (Lawver & Gahagan, 2003; Barker & Thomas, 2004; Stickley et al., 2004; Huber et al., 2004; Lyle et al., 2007), the most recent estimate puts establishment between 23-25 mya (Lyle et al., 2007). The ACC has two main impacts on the Southern Ocean biota. The ACC presents a significant barrier to North-South gene flow and the dispersal of organisms between the Southern Ocean and surrounding waters (Clarke et al., 1999; Crame, 1999). Within the Southern Ocean though, the ACC may act to disperse organisms over vast distances, increasing gene flow between otherwise geographically isolated regions (Fevolden & Schneppenheim, 1989; Rogers et al., 1998; Raupach et al., 2010; Thatje, 2012). This dual role, enhancing both isolation and connection has had a pronounced influence on the evolution of the Southern Ocean biota.

Antarctica has been in a polar position since the Late Cretaceous, ~65 mya (Kennett et al., 1975). At that time, Australia and South America were both still attached to the Antarctic Continent, forming the southern landmass Gondwana

(Kennett, 1977). Australia began its northward drift during the Early Eocene (~55 mya), creating a warm, shallow water connection between the southern Indian and Pacific Oceans (Kennett, 1977). The Eocene-Oligocene Boundary (~34 mya) marked a period of rapid climatic change in the Southern Ocean; A rapid drop in water temperatures to near present day levels was accompanied by increased sea ice formation, which in turn led to the initiation of Antarctic Deep Water formation and the beginnings of the contemporary thermohaline circulatory system (Kennett et al., 1975; Kennett, 1977; Mackensen, 2004). At this time, while the Tasman Sea between Antarctica and Australia was totally open to surface water circulation, contributing to this climatic shift (Mackensen, 2004), the Tasman Rise was still a large impediment to deep-water circulation (Kennett, 1977). Significant deep-water circulation was initiated during the mid- to late-Oligocene after the Tasman Rise had moved sufficiently northward (Kennett et al., 1975; Kennett, 1977; Mackensen, 2004; Hill et al., 2013). The opening of the Tasman Gateway allowed for the initiation of localized deep-water circulation, but it wasn't until the opening of the Drake Passage that true circum-Antarctic deep water current activity would initiate (Kennett et al., 1975; Lyle et al., 2007; Heinrich et al., 2011; Lefebvre et al., 2012).

The climatic and geologic history of the Cenozoic before the opening of the Drake Passage had a significant impact on the faunal composition of Southern Ocean communities. Several charismatic groups are absent or

depauperate in the modern Southern Ocean, including decapod crustaceans, bivalve and gastropod molluscs, sharks, and teleost fish, possibly as a result of the climatic events of the early Antarctic history (Clarke & Crame, 1989; Clarke et al., 1999; Aronson & Blake, 2001; Clarke, 2008). However, while important for determining the initial faunal composition, this early history likely had relatively little direct influence on contemporary patterns of genetic diversity, as the initiation of the ACC, as well as other significant climatic changes occurring after the opening of the Drake passage, have since had a strong influence on the Antarctic biota and the distribution of genetic diversity within the Southern Ocean, as will be discussed below.

Lyle et al. 2007 puts the opening of the Drake Passage in the Late Oligocene and strengthening of the current by the early Miocene. A consequence of this strengthening was the creation of the Antarctic Polar Front (APF) (Scher & Martin, 2008). The APF is a zone surrounding the Antarctic continent characterized by intense current flow and a 3-4 ° temperature cline, marking the northern limit of the ACC's cold water mass. The formation of the ACC and APF isolated Antarctica, its outlying islands, and the Southern Ocean in general from other continents and water masses, possibly leading to the pronounced endemism observed today (Arntz et al., 1997; Barnes et al., 2006; Clarke, 2008). This isolation is enforced by several factors; extrinsic physical factors such as the large geographic distance

between Antarctica and the surrounding land masses and the strong oceanographic and bathymetric barriers of the ACC, APF and Southern Ocean, as well as intrinsic physiological barriers imposed by the extreme Southern Ocean and Antarctic climate (Shaw et al., 2004; Rogers et al., 2006).

There is molecular evidence from several diverse taxa supporting the hypothesis that the ACC may act as a strong barrier to gene flow and organismal exchange between Antarctic and South American taxa, leading to population, or even species level divergence between these regions (Patarnello et al., 1996; Bargelloni et al., 2000a; Shaw et al., 2004; González-Wevar et al., 2010; Díaz et al., 2011; Poulin et al., 2014). However, the strength of this isolation is affected by several factors. For example, the strength of the ACC is strongly influenced by the rate of sea ice formation and its control of Antarctic Deep Water formation (Kennett et al., 1975; Barker & Thomas, 2004; Smith et al., 2010; Paillard, 2015). This dependence on sea ice formation creates the possibility for temporal variation in the permeability of the ACC to faunal exchange, with barriers to dispersal weakening during warm inter-glacial periods as sea ice formation decreases, and strengthening during cooler glacial periods when sea ice formation is increased (Clarke et al., 2005; Brandt, 2005; Thornhill et al., 2008). It's also been proposed that rafting may allow for the dispersal of organisms across these major barriers (Helmuth et al., 1994; Nikula et al., 2010; Leese et al., 2010; Thatje, 2012). Additionally,

the APF and ACC are bridged by the Scotia Arc, a chain of islands running eastwards from the southern tip of South America before curving back around to the northern tip of the Antarctic Peninsula. Island hopping via this formation may provide a mechanism for dispersal between South America and the Antarctic (Crame, 1999; Page & Linse, 2002; Griffiths et al., 2009; Strugnell & Allcock, 2012; Poulin et al., 2014).

While the ACC likely plays an important role in maintaining Antarctic isolation, its role as a connective force within the Southern Ocean has also been long discussed (Fevolden & Schneppenheim, 1989). The strong circumpolar current may help to disperse larvae or individuals across vast distances between the regions of the Southern Ocean, increasing gene flow. This dispersive force may help explain the low levels of genetic divergence between geographically distant populations observed in several taxa (Bargelloni et al., 2000b; Sands et al., 2003; Nikula et al., 2010; Raupach et al., 2010; Hoffman et al., 2010a).

Glaciations – A history of disturbance

Physical disturbance via glacial activity has played an especially prominent role in shaping the Antarctic benthos and thus the structuring of genetic diversity contained therein. Antarctic glaciation was initiated approximately 35 mya (DeConto & Pollard, 2003; Davies et al., 2012). This

event was the result of the interaction between a decrease in thermal transport to the Southern Ocean from surrounding water masses, and a global decrease in greenhouse gas levels (DeConto & Pollard, 2003; Goldner et al., 2014). This event marked a critical turning point in the glacial history of the Antarctic. However, modern population structuring has been more strongly influenced by the Quaternary glaciations (Clarke & Crame, 1989; Arntz et al., 1997; Crame, 1997; Clarke, 2008; Allcock & Strugnell, 2012). These glaciations are characterized by cyclical expansions and recessions of the ice caps, with periodicity roughly following that of orbital parameters (Dynesius & Jansson, 2000; Augustin et al., 2004). These orbital parameters determine the amount, the seasonality, and the latitudinal distribution of insolation (Augustin et al., 2004). Records from the Vostok ice core indicate that, beginning around the mid-Pleistocene, the climate has been characterized by a strong 100,000 year cyclicity (Petit et al., 1999; Augustin et al., 2004), and that there have been at least four large amplitude glacial-interglacial cycles over the past 430 kyr (Augustin et al., 2004). These cycles would have had a large impact on the benthic communities of the Southern Ocean. During glacial maximums, grounded ice sheets expanded out from the continent, often scouring the continental shelf all the way out to the continental slope (Dambach et al., 2012; Fraser et al., 2012). Populations of benthic organisms living on the continental shelf would have gone extinct or been forced into ice-free refugia

on the shelf, down onto the continental slope or into the deep sea, or out into the sub-Antarctic islands (Clarke & Crame, 1989; 1992; Crame, 1997; Clarke & Crame, 2010; Fraser et al., 2012; Allcock & Strugnell, 2012; Pointing et al., 2014). There is also evidence that the onset of glaciation across the Southern Ocean during glacial maximums was asynchronous, leading to temporal, as well as spatial refugia across the Southern Ocean (Anderson et al., 2002; Davies et al., 2012). Additionally, there may have been many areas that were permanently ice-free due to localized oceanographic or climatic factors (Dayton & Oliver, 1977; Licht et al., 1996; Domack et al., 1998; 1999; Beaman & Harris, 2003; Smith et al., 2010). Isolation in these varied refugia would lead to varying degrees of genetic divergence between populations based on the level of gene flow (Clarke & Crame, 1989; Marko, 2004; Rogers, 2007; Clarke & Crame, 2010). Additionally, persistence in different types of refugium should leave distinct patterns and signatures in the structuring of populations (Allcock & Strugnell, 2012). While these orbitally-forced species range dynamics (Dynesius & Jansson, 2000) would have caused local extinctions across the Southern Ocean in many taxa, the cycle of habitat fragmentation and reconnection may have driven the eventual appearance and establishment of new taxa, increasing the net speciation/diversification rate in the Southern Ocean (Clarke & Crame, 1989; 1992; Wares & Cunningham, 2001).

The integrity of the major ice sheets through time may also have had a

significant influence on contemporary genetic diversity. At several times throughout the Cenozoic and Quaternary periods the total collapse of the West Antarctic Ice Sheet would have created an open seaway between the Ross and Weddell Seas (Pollard & DeConto, 2009). This connection would possibly have allowed for increased historical gene flow between Western Antarctic and Antarctic Peninsula populations, leaving a distinct decrease in levels of genetic differentiation between these two regions (Strugnell et al., 2012).

Biological Perspective – Dispersal, Connectivity, and the Antarctic

The evolutionary history of the Antarctic fauna is tied intimately to the tectonic, climatic, and glacial history of the region. The contemporary patterns of genetic diversity however reflect the interaction between the recent history of glaciation and modern oceanographic and climatic features (Crame, 1997; Clarke et al., 1999; Griffiths et al., 2009; Clarke & Crame, 2010; Allcock & Strugnell, 2012; Poulin et al., 2014).

Population persistence depends on the balance between births + immigration and deaths + emigration. The genetic character of each population however depends on the level of connectivity experienced across various spatial scales through time, with differentiation occurring when gene flow between populations becomes negligible relative to local adaptation or genetic drift (Palumbi, 1994). Equally important as the physical extrinsic

factors influencing connectivity, intrinsic biological factors, such as life history traits, play an important role in determining levels of dispersal capability (Jablonski, 1986). For many benthic invertebrates, adult morphologies are relatively non-dispersive, and most gene flow is due to the exchange of planktonic larvae (Burton, 1983). Pelagic larval stages are therefore critical in determining the level of population connectivity for many benthic invertebrate taxa (Cowen & Sponaugle, 2009).

Dispersal capability is the determined by complex biophysical interactions. The timing and location of larval release, larval behavior, planktonic larval duration (PLD), as well as various physical environmental and oceanographic factors all exert a strong influence on dispersal capability (Cowen & Sponaugle, 2009). The physical forces moving larvae through an environment are extremely complex in themselves, depending on the three-dimensional interaction between coastal topography, bathymetry, and hydrodynamics at various spatial and temporal scales (Largier, 2003; Sponaugle et al., 2005; Mace & Morgan, 2006). Environmental factors affecting marine larval dispersal include water quality and nutrient availability, as well as habitat suitability to induce larval metamorphosis and settlement (Kingsford et al., 2002; Cowen & Sponaugle, 2009). These physical factors play a large role in determining population connectivity by establishing the framework for the movement of larvae between populations. Larval dispersal

capability is also affected by PLD (Cowen et al., 2000; Shanks, 2009), which is itself strongly influenced by food supply, predation rates on the larval form, and physical factors such as water temperature through the influence on larval growth rates (Houde, 1989; Pepin, 1991; McCormick & Molony, 1995; Rombough, 1997). Longer PLD's increase the potential for dispersal simply by allowing more time for the larvae to be dispersed. Despite the clear theoretical ties between PLD and dispersal capability though, many studies have shown that a long planktonic duration does not always a result in high realized dispersal capability (Burton, 1983; Shanks, 2009).

The Antarctic fauna appears to have evolved away from pelagic larval stages, containing many examples of brooding larval development, or shortened PLD (Bosch & Pearse, 1990; Pearse et al., 2009). This interesting faunal characteristic has been long discussed, and there are several explanations as to why the Antarctic fauna contains this abundance of brooders, as reviewed in Pearse et al. 2009. On one hand, there may be some level of phyletic constraint of reproductive mode, and the pattern seen in the Southern Ocean may be slightly stochastic (Levin & Bridges, 1995), determined by the initial faunal composition, defined by the break-up of Gondwana, before the onset of an Antarctic climate. The unusually numerous brooding taxa may also be explained by the repeated separation and isolation of populations caused by glaciations, with the cyclic habitat fragmentation

resulting in a high rate of brooder speciation. Various environmental explanations have also been offered, but many of the environmental characteristics proposed to be driving the evolution of brooding and shortened PLD are also observed in the Polar Arctic, which does not possess the same abundance of brooder taxa (Pearse et al., 2009). Therefore, as with most complex traits, the evolution of reproductive strategy in the Antarctic is likely the result of an interaction between many diverse factors. Regardless of its evolutionary origin, brooding and shortened PLD would theoretically increase the likelihood of isolation by distance and genetic differentiation between populations from different regions of the Southern Ocean, despite the homogenizing influence of the ACC. This prediction is supported by Hoffman et al. (2010a), who examined population structuring in two taxa, one with and one without planktonic larval dispersal. Their study found significantly higher levels of gene flow between populations in the taxa with a dispersive larval stage. Several other studies have also found significant population structuring in brooding taxa (Arango et al., 2011; Ledoux et al., 2011). However, brooding does not ensure strong population structuring, just as larval dispersal does not ensure high levels of gene flow. Several studies have found that taxa with brooding larval development can still achieve geographically widespread genetic homogeneity (Mahon et al., 2008; Baird et al., 2011).

Southern Ocean Diversity

The genetic diversity of Southern Ocean organisms has been the subject of numerous studies over the past several decades (Supplementary Table 1). Many of these studies' findings support the idea that the Southern Ocean environment fosters circumpolarity and genetic homogeneity. Fevolden & Schneppenheim (1989) found no evidence of differentiation in *Euphausia superba* from across the Southern Ocean. Other studies have observed a similar lack of population structuring in *Parborlasia corrugatus* throughout the Antarctic and Sub-Antarctic (Thornhill et al., 2008), a species of Notothenioid fish (Matschiner et al., 2009), and two species of shrimp with circumpolar distributions (Raupach et al., 2010). However, many other studies have found that levels genetic diversity greatly exceed expectations. Wilson et al. 2007 found evidence of five species level clades within the "circumpolar" crinoid *Promachocrinus kerguelensis*. Most of these phylogroups existed in sympatry, and had restricted geographic ranges. Another study of *P. kerguelensis* confirmed the presence of these distinct lineages, and added two additional phylogroups not found by the first study (Hemery et al., 2012). Several other studies have also examined supposed circumpolar distributions and found high levels of differentiation (Zane et al., 1998; Held, 2003; Allcock et al., 2011). Allcock et al. (2011) used mitochondrial markers from a large number

of individuals sampled from across the Southern Ocean to assess the circumpolarity of octopods in the genus *Pareledone*. Their study found evidence for circumpolarity in some groups, but evidence of cryptic speciation and limited geographic distributions in others, suggesting a complex evolutionary history not uniformly influenced by the ACC. Held (2003) examined *Ceratoserolis trilobitoides*, a large isopod common in shallow Antarctic and Sub-Antarctic waters. Because of its size and abundance, *C. trilobitoides* is commonly used as a model organism for physiological and ecological studies of the Southern Ocean. However, molecular data uncovered clear evidence of at least one genetically distinct, sympatric cryptic species, mistakenly identified as *C. trilobitoides*. Zane et al. (1998) re-examined the genetic structure of *E. superba*, but, unlike Fevolden & Schneppenheim (1989), found evidence of significant differentiation between populations from different regions of the Southern Ocean.

Other studies have examined population connectivity at smaller spatial scales, and still found surprising levels of differentiation in broadcast spawning taxa (Allcock et al., 1997; Hunter & Halanych, 2010; Hoffman et al., 2010b). Allcock et al. 1997 examined levels of gene flow between three populations of *Pareledone turqueti* from the Scotia Arc. This study found evidence of panmixia around South Georgia, but extremely high levels of differentiation between the South Georgia populations and Shag Rocks, a site only 150 km

away. Hunter & Halanych 2010 found high levels of differentiation between populations of the brittle star *Ophionotus victoriae* from different regions, but also between populations within regions. They observed significant population structure throughout most of the Antarctic Peninsula, as well as evidence of possible cryptic speciation between the Eastern and Western Antarctic Peninsula. Hoffman et al. 2010b found evidence for strong structuring between Antarctic Peninsula and Scotia Arc populations of *Nacella concinna*. These surprising patterns of diversification hold across diverse groups, and are therefore more likely to be explained by broad-scale phenomena than a lineage specific influence (Allcock & Strugnell, 2012). Many of these studies point to the importance of factors other than the ACC in determining the level of population connectivity in the Southern Ocean, and propose that small-scale or localized oceanographic and bathymetric features can exert a strong effect on population connectivity.

Restriction-Site Associated DNA Sequencing

Studies of genetic diversity often rely on sampling as many independent genomic markers as possible in order to maximize the accuracy and power of any results (Brito & Edwards, 2008; Godhino et al., 2008; Carstens et al., 2012). Restriction-site Associated DNA sequencing (RAD seq) (Baird et al., 2008; Hohenlohe et al., 2010; Peterson et al., 2012) is a relatively low cost

and efficient way of obtaining and characterizing single nucleotide polymorphisms (SNPs) from across the genome. RAD loci, or tags, are DNA fragments adjacent to the cut site of a particular restriction enzyme, or combination of two enzymes. Choice of enzyme(s) and size selection of fragments leads to a reduced representation of the genome and can optimize the number of tags for SNP detection, decreasing the computational requirements of downstream analysis (Peterson et al., 2012). RAD seq has been primarily used in model organisms, with the advantage that sequenced loci can be mapped to a reference genome. However, there is a great deal of potential for RAD seq investigations of the population genomics of non-model species (Hohenlohe et al., 2011a; White et al., 2013). While there is no prior information about the location of loci in the genome without a reference, RAD seq can provide a substantially greater number of neutral markers than traditional mitochondrial gene or microsatellite markers. This large panel of SNPs should detect even weak population structuring, giving insight into recent ecological and evolutionary processes (Anderson et al., 2010) and providing reliable inferences of demographic history (Cariou et al., 2013; Rašić et al., 2014). Previous studies using RAD seq for non-model organism population genomic studies have produced results with a high degree of resolution (Emerson et al., 2010; Hohenlohe et al., 2011b; Senn et al., 2013; Reitzel et al., 2013; Pujolar et al., 2013; Macher et al., 2015).

This Study

Here, we use ddRAD to investigate the genetic diversity and population structuring of a proposed circum-Antarctic taxa across large geographic distances. *Glabraster antarctica* (E.A. Smith, 1876) is a broadcast spawning Poraniid sea star found on the continental shelf and slope throughout the Antarctic and Sub-Antarctic, and as far North as southern South America (Foltz & Mah, 2014). In addition to the wide geographic distribution, *G. antarctica* has a large bathymetric range, recorded at depths from the intertidal to 3,200 m (Brueggeman, 2012). *G. antarctica* was recently synonymized (Foltz & Mah, 2014), leading to a geographically widespread taxa containing high levels of morphological variation. Moore et al. (2015) showed that the various morphotypes synonymized into *G. antarctica* do not represent distinct genetic lineages using mitochondrial COI gene sequences. The larvae of *G. antarctica* are described as facultatively planktotrophic, as they possess both large yolk stores and a complete digestive tract (Bosch, 1989). This larval characteristic indicates a high potential for planktonic dispersal. However, Bosch (1989) also noted a distinct shortening of PLD in *G. antarctica* relative to congeners, suggesting that the reduction in larval duration may mitigate elevated levels of larval mortality.

A previous study using mitochondrial gene sequences found high levels

of population structuring within *G. antarctica* from across the Southern ocean, and proposed that it exists as a single, non-panmictic species with a circum-antarctic distribution (Moore et al., 2015).

In this study, we use nuclear SNPs from across the genome, obtained using ddRAD, to investigate the genetic diversity found within the circumpolar taxa *G. antarctica*. We use this molecular evidence to assess the hypothesis that *G. antarctica* exists as a panmictic taxon across large distances in the Southern Ocean.

Materials and Methods

Sampling and Library Preparation

ddRAD libraries were prepared for a total of 69 individuals from 4 locations. Sampling localities were chosen based on data from Moore et al. (2015). Specimens of *Glabraster antarctica* from the Scotia Arc were collected by benthic Blake trawls during two separate research cruises aboard the RVIB Nathaniel B. Palmer, taking place September – November 2011, and April 2013. Trawl catches were sorted to species and photographed. Tissue samples were preserved in 99% molecular grade ethanol and kept cold until DNA extraction. Additional samples were obtained from ethanol-fixed collections at the National Institute of Water and Atmospheric Research (NIWA, Wellington, New Zealand) and the Australian Antarctic Division (AAD, Hobart, Tasmania). NIWA specimens were collected as part of the New Zealand International Polar Year – Census of Antarctic Marine Life, Ross Sea Biodiversity voyage in 2008.

G. antarctica individuals were collected from four sites (Figure 1): Shag Rocks, South Georgia, Scott A, and Elephant Island. These sites represent locations from the Scotia Arc, the Antarctic Peninsula, and the Ross Sea. The Shag Rocks and South Georgia collection sites are both located in the Scotia Arc. Despite their geographic proximity, individuals from these two sites were shown to be highly divergent by Moore et al. (2015), based on mitochondrial

markers.

Figure 1: Map of sampling localities. Colored circles indicate populations sampled for this study.

Genomic DNA was extracted from each individual using a Qiagen DNeasy Blood & Tissue kit, according to the manufacturer's instructions. Double-digest restriction-site associated DNA sequencing (ddRAD) libraries were prepared as follows: 250 ng genomic DNA were digested using 10 U (1.0 ul) each of two restriction enzymes, the common cutter Mse1 and the rare cutter Xho1, and 4 ul each NEB4 Buffer and BSA (New England Biolabs). The reactions were then purified using a Qiagen Reaction Cleanup kit according to

the manufacturer's protocol. Ampure xP magnetic beads (Beckman Coulter) were used to size select and further clean the fragments, following the manufacturer's protocol. DNA concentration was checked by PicoGreen analysis, according to manufacturer's instructions (Molecular Probes). Barcodes were then ligated onto the fragments. Each individual received a unique Xho1 barcode and an Mse1 barcode common across all samples. Ligation was performed at room temperature with 25 µl 2x Quick Ligase Buffer, 1 µl high-fidelity Quick DNA ligase (X U/mL; New England Biolabs), 1 ul of the Xho1 adaptor, 5 ul of the Mse1 adaptor (IDT), and 19 ul of digested DNA. Reactions were incubated at room temperature for 10 minutes, followed by a heat-kill at 65° for 10 minutes. Samples were then slowly brought back to room temperature before being pooled to the population level. Total volume was reduced using a second Qiagen column clean-up. The pooled samples were then run on a 2% TAE agarose gel for 1.5 hours at 130 V. The gel was stained post-run with GelRed (Biotium, inc.) and then visualized on a UV transilluminator. Gel regions in 300 to 400 base-pair range were excised and purified using the Qiagen Gel Purification Kit. PCR was run with 10 ul of the ligated DNA, 4 ul Phusion HF 5x Buffer (New England Biolabs), 2 ul each of two separate PCR primers (IDT), one common to all reactions and one unique to each population, 1 ul 10 uM dNTP mix (New England Biolabs), and 1 ul of high fidelity Phusion polymerase (New England Biolabs). PCR included an

initial denaturation step at 98° for 1 minute, followed by 12 cycles of denaturation at 98° for 10 seconds, annealing at 65° for 30 seconds, and elongation at 72° for 45 seconds. This was followed by a final elongation step at 72° for 5 minutes. Following PCR, the now barcoded and indexed samples were run out again on a 2% TAE agarose gel for 1.5 hours at 130 V. Again, the gel was stained post-run with GelRed, and then visualized on a UV transilluminator. Indices were expected to add basepairs to the fragments, so gel regions within the 350 to 450 basepair range were now excised and purified using the Qiagen Gel Purification Kit. The libraries were eluted in 25 ul of EB Buffer (Quigen). Final DNA concentrations of the libraries were quantified using PicoGreen. Libraries were sequenced (100 bp paired-end) on an Illumina HiSeq 2500 using version 3 chemistry.

RAD Data Sets

Sequenced RAD tags were processed using the Stacks pipeline v. 1.29 (Catchen et al., 2011; 2013). The *process_radtags* script was used for initial quality filtering of tags using the default settings. Because *G. antarctica* does not have a reference genome, we performed a *de novo* assembly of the reads. Detailed information on the modules used within Stacks to accomplish this can be found in Catchen et. al 2011, 2013. Assembly parameters chosen were minimum stack depth (-m), 6; maximum intra-individual distance (-M), 3;

maximum number of mismatches for the alignment of secondary reads (-N), 2; and number of mismatches when building the catalog (-n), 2. Loci with more than two alleles were removed as paralogous genes. The *populations* script allows for the use of different combinations of parameters to filter the initial assembly, resulting in data sets with more or less inclusivity. The final datasets comprised only loci sequenced with more than 20X coverage and $\leq$ 2 SNPs that were present in at least 50% of individuals from each of the four populations. The final data sets were exported in several formats, for ease of further analysis.

Population Inferences

The data set was analyzed in Structure v. 2.3.4 {Hubisz:2009cf, Pritchard:2000fd} to test whether genetic structure could be detected without the need for a priori population identity data. Structure uses a maximum likelihood approach to estimate the most likely number of distinct genetic groups in a sample, as well as which individuals are most likely to belong to each group. We tested likelihood scenarios for k=1, implying no structuring, through to k=8, which would imply that each population sampled contains individuals from multiple distinct groups (such as unsampled populations), with 100,000 MCMC iterations after a burn-in of 10,000, assuming admixture. Structure analyzed the data without prior information about individual

population identities.

Pairwise F_{st} values were calculated in GenoDive (Meirmans & van Tienderen, 2004). A mantel test of isolation by distance was also performed in GenoDive. Significance was estimated using 10,000 replicates. Heterozygosity, with standard deviation, was calculated as part of the Stacks pipeline using the filtered RAD dataset. Nucleotide diversity across loci was calculated in ARLSUMSTAT v. 3.5.2 (Excoffier & Lischer, 2010).

To visualize the phylogenetic relationship between the populations, an unrooted maximum likelihood network was generated in RAxML v. 1.31 {Silvestro:2011gy} under the GTR + G + I model with 10,000 rapid bootstrap replicates. The network was created without an out-group due to the absence of ddRAD data for a related group.

Results

RAD Data Sets

The parameters used for filtering the RAD loci resulted in a final dataset of 4102 loci. Only one SNP was used per loci. Relative nucleotide frequencies in the full alignment were A (0.2346), T (0.2221), G (0.2682), and C (0.2751). Using this data set, we detected significant levels of population structuring between the four sites sampled for *Glabraster antarctica*.

Population Inferences

All pairwise comparisons produced very significant F_{st} values ($p < 0.001$ for all values) (Table 1). The high level of divergence between Shag Rock and the geographically proximate South Georgia observed by Moore et al. (2015) is also observed in our data set ($F_{st} = 0.304$, $p < 0.001$). Shag Rock and Elephant Island were also highly diverged ($F_{st} = 0.380$, $p < 0.001$). Differentiation between Shag Rock the Ross Sea sample was much smaller, but still highly significantly ($F_{st} = 0.123$, $p < 0.001$). South Georgia also shows a less divergence with Scott A than with the other sampling sites ($F_{st} = 0.1.61$, $p < 0.001$). Scott A and Elephant Island showed an intermediate level of divergence ($F_{st} = 0.266$, $p < 0.001$). Observed heterozygosity is very low in all populations, with values comparable between all four populations (Table 2).

While Nucleotide diversity was also low in all four populations (Table 3), there is variation between populations, with the diversity observed being almost twice as large in Scott A as seen in Shag Rock (0.462537 +/- 0.223329 versus 0.271433 +/- 0.133807 respectively).

Table 1 : Pairwise F_{st} values among populations of *Glabraster antarctica*. $P < 0.001$ for all comparisons.

	Shag Rock	South Georgia	Scott A	Elephant Island
Shag Rock	---			
South Georgia	0.304	---		
Scott A	0.123	0.161	---	
Elephant Island	0.38	0.269	0.266	---

Table 2 : Observed Heterozygosity, with associated variance and standard error, as calculated in the Stacks pipeline for the filtered RAD data set.

	Obs. Het.	Var.	SE
Shag Rocks	0.0662	0.0178	0.0021
South Georgia	0.0558	0.0121	0.0017
Scott A	0.0559	0.0102	0.0016
Elephant Island	0.0515	0.0138	0.0018

Table 3 : Genetic diversity across loci calculated in ARLSUMSTAT for the four popualtions of *Glabraster antarctica*.

	Gene Diversity
Shag Rock	0.271433 +/- 0.133807
South Georgia	0.331461 +/- 0.160502
Scott A	0.462537 +/- 0.223329
Elephant Island	0.372779 +/- 0.180008

A Mantel test, comparing pairwise F_{st} values to linear geographic distances (Figure 2) between the four populations yielded significant results, but a negative r-value ($r = -0.833$, $p = 0.031$). This result was most likely driven by the affinity between the geographically distant Shag Rock and Scott A populations. When Shag Rock is excluded, a Mantel test indicated no significant correlation between genetic and geographic distances ($r = 0.135$; $p = 0.494$). Thus, an isolation-by-distance model does not explain the variation we observe in the pairwise Fst values.

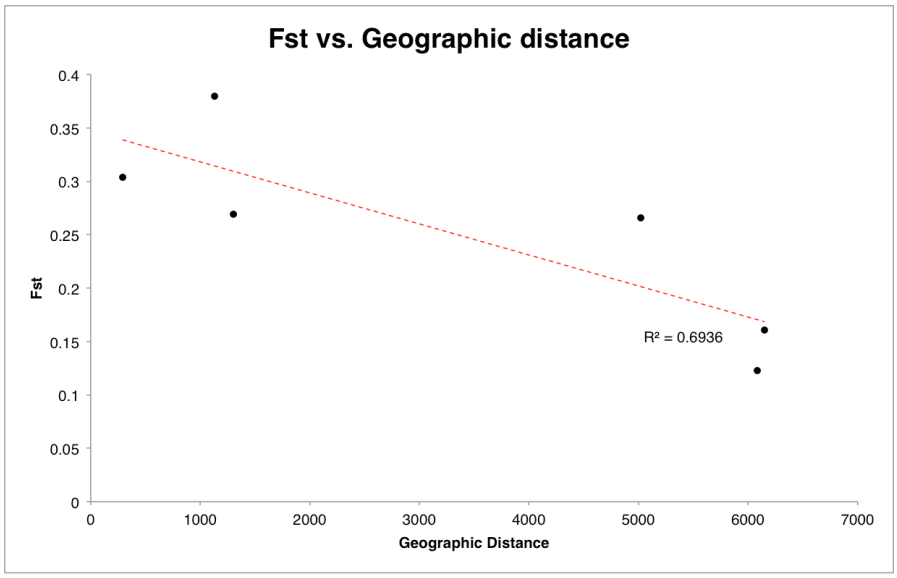

Figure 2 : Plot of genetic distance (F_{st}) by geographic distance.

The STRUCTURE plot shows clear population structuring in the data set. Five replicate runs were conducted for each possible number of groups (k). The true number of groups is often interpreted from the maximal value of Ln P(D), which estimates the posterior probability of the data. This statistic peaked at k=4, indicating strong support for four distinct groups. Membership coefficients for the four inferred groups are visualized in Figure 2, where each bar represents an individual. The proportions of each color indicate the relative probability of membership to each inferred group.

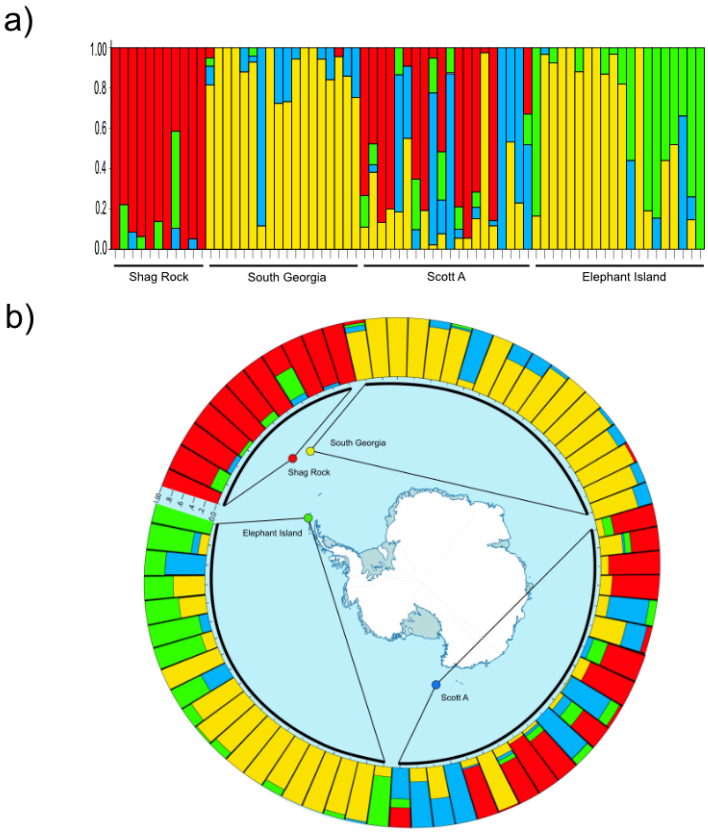

Figure 3 : a) Group membership coefficients derived from STRUCTURE for 69 *Glabraster antarctica* individuals. Each individual is represented by one bar. Shading represents the probability of membership to each of the four inferred populations. Individuals are ordered by collection site. b) Membership coefficients visualized spatially. Individuals are grouped by collection site, indicated on the map.

The unrooted ML network (Figure 3) shows individuals clustering by collection locality. Several individuals from Scott A and one individual from South Georgia fall in with other populations. These individuals may represent migrants between populations, as individuals collected from a distinct geographic population with a genetic makeup more similar to individuals from

a different geographic population. As seen in the pairwise F_{st} values, Shag Rock groups more closely with Scott A, the Ross Sea sample, than any other, including the geographically proximate South Georgia.

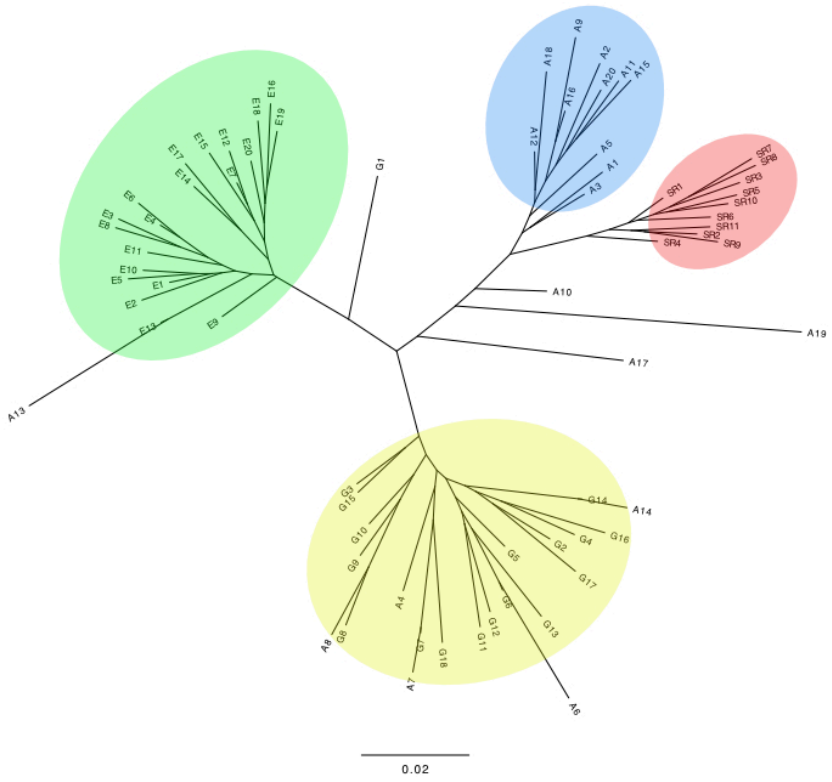

Figure 4 : Maximum likelihood network calculated in RAxML for the four populations of *Glabraster antarctica.*

Discussion

Population Structuring

Our data shows clear evidence of population structuring in G. antarctica, with limited levels of connectivity. We therefore reject the hypothesis of panmixia. While G. antarctica produces very large, buoyant eggs and facultatively planktotrophic larvae (Bosch, 1989), increasing dispersal potential, realized dispersal appears to be limited by some factor, or the interaction between multiple factors. Through direct observation of larval development, Bosch (1989) noted that the PLD of *G. antarctica* is shortened in culture relative to other congeners, as well as to other Antarctic asteroids. This decrease in larval duration may be interacting with localized oceanographic features to promote the local retention of larvae over larval dispersal to other populations. However, the Mantel test yielded no significant results, suggesting that a simple isolation by distance model does not explain the differentiation observed in the data set. The combination of high levels of population differentiation, with no evidence for isolation by distance, suggests that dispersal is not limited by distance, but instead, some physical, environmental, or biological factor. It is interesting to note that the surprising divergence between Shag Rock and South Georgia populations has been observed in several other taxa (Allcock et al., 1997; Linse et al., 2007; Wilson

et al., 2009). These locations, while geographically proximate, are separated by a stretch of very deep water (>1000 m depth). This is proposed to act as a significant barrier to larval dispersal between the two localities, even given the large, buoyant character of these taxa's eggs. A more thorough understanding of why this barrier exists will require a better understanding of how the bathymetric features of this area interact with the local current systems. The similarity in findings between diverse taxa suggests that Antarctic population connectivity is strongly affected by the physical environment, that diverse taxa responded in a similar way to the climatic and glacial fluctuations over the past 500,000 years, persisting in discrete refugia.

Relict Diversity

The differential levels of genetic diversity and surprising genetic relationships between populations we observe suggest that *G. antarctica* populations did not respond uniformly to glaciation events of the last glacial maxima. We observed the highest levels of genetic diversity in Scott A. This suggests that population size during the last glacial maximum remained relatively large. Strugnell et al. 2012 suggests that higher levels of genetic diversity may be found in populations that persisted in deep sea refugia during the last glacial maximum, where gene flow between populations was higher compared to levels between populations in isolated shelf refugia. Additionally,

the similarity we observe between the Scotia Arc populations and Scott A suggests that individuals from the Ross Sea may have played an important role in the recolonization of the Scotia Arc after glacial recession. The lower genetic diversity, and higher level of genetic differentiation between Scott A and Elephant Island however, suggests that the Antarctic Peninsula was recolonized as populations expanded outwards from isolated shelf refugia, rather than by immigration from other regions.

The ACC Influences Larval Dispersal

Despite being the largest and strongest current in the Southern Ocean, the ACC is not the only oceanographic feature to influence the distribution of genetic diversity in the Antarctic. Many taxa with dispersive larval stages, which should show large-scale genetic homogeneity due to the influence of the ACC, exhibit surprising levels of population differentiation (Wilson et al., 2007; Hunter & Halanych, 2010; Brandão et al., 2010; Hoffman et al., 2010b; Moore et al., 2015). It's becoming clear that local environmental conditions and the fine scale structuring of eddies and currents in the Southern Ocean can play a large role in determining the level of connectivity between populations (Ragu Gil et al., 2004).

Our data highlights the importance of these fine scale features as factors influencing population structuring. We observe high levels of

divergence between the geographically proximate populations Shag Rock and South Georgia. This divergence suggests a large, stable barrier to gene flow between these locations, strong enough to override the influence of the ACC. However, we also observe relatively low genetic differentiation between the Scotia Arc populations and the geographically distant population Scott A, suggesting that long distance gene flow is occurring, or has occurred in the past, most likely assisted by the ACC. This study shows that both large scale, and fine scale features of the Southern Ocean can strongly influence population structuring.

Conclusion

Our study highlights the importance of molecular ecological studies of the Southern Ocean. Our ddRAD libraries provided a large number of SNP markers, which detected distinct population structuring. Several pairwise estimates of differentiation were surprising, showing high differentiation between two proximate populations, separated by only about 150 km, and low differentiation between samples from the Scotia Arc and the Ross Sea, regions separated by more than 6000 km. This study adds to the body of work showing that the ACC may not universally act as the homogenizing agent, as proposed during the early Antarctic investigations of genetic diversity. Future studies will need to increase the geographic coverage to investigate

relationships at both larger, and smaller spatial scales than we examined. A future study should include populations from the Eastern Antarctic, as well as the Magellanic Region of far Southern South America. Moore et al. 2015 observed several interesting patterns in the mitochondrial diversity between these regions and the regions we surveyed. A proper understanding of the distribution of genetic diversity, and population structuring of *G. antarctica* and other circumpolar taxa relies on representative sampling across the Southern Ocean. Genetic studies play an important role in determining effective conservation and management efforts. Together with studies of local adaptation, understanding the balance between population differentiation and gene flow between populations can assist in predicting the response of taxa to climate change. In the Antarctic, these biological studies will need to be paired with studies of how the complex environmental factors affecting populations will also change, if biodiversity in this region is to be conserved.

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