Potential mechanisms driving the geographic distribution pattern of defensive secondary metabolites in the Antarctic red seaweed *Plocamium cartilagineum*

Introduction

Macroalgae are important ecosystem engineers in near-shore marine ecosystems worldwide because they provide living space as well as nursery grounds for many organisms. They are also important primary producers. In Antarctica, the biomass produced by macroalgal forests is comparable to temperate *Macrocystis* kelp forests (Wiencke & Amsler, 2012). On the Western Antarctic Peninsula macroalgal forests support very dense assemblages of amphipods and other mesograzers (Huang *et al.,* 2007) but most macroalgae are chemically defended against herbivory by their inhabitants.

Plocamium cartilagineum is a finely-branched red macroalga, also supporting a high density of amphipods (Huang *et al.*, 2007). We investigated the chemical defenses produced by *P. cartilagineum* at 19 sites around Palmer Station in 2016 and found a total of 12 different chemogroups (i.e., unique mixtures of secondary metabolites) at different sites. Some chemogroups were only found at a single site or a single depth, whereas others were found at multiple sites. Sequencing a portion of the *cox1* gene revealed two genetically distinct groups that corresponded to different chemogroups. Therefore, differences in chemogroup synthesis could be driven by (i) the environment (i.e., light/ depth, nutrient availability), (ii) barriers to gene flow, or (iii) a mixture of the two.

During the 2017 and 2018 field seasons, transplant experiments were conducted to address driver (i). The experiments were deployed for a year and preliminary data indicates that there is no change in chemogroup production (*unpubl data*). To address whether (ii) is the underlying reason for some of the site specificity of the chemogroups observed thus far, microsatellite loci for population genetic analysis are currently being developed.

Methods

Samples were collected at Palmer Station on the Western Antarctic Peninsula during the 2017 and 2018 field seasons. Transects were run perpendicular to the shore using SCUBA. Where possible, three transects were surveyed, each 30m apart from each other. Five individuals were collected from every depth at 3m depth intervals between 5m and 29m. At some sites, *P. cartilagineum* was not present at high enough abundance to be collected from every depth. A total of five sites had all three transects surveyed, another three sites had one full transect surveyed and five sites had partial transects surveyed. Samples were sorted and preserved upon return to Palmer Station. Their reproductive state was recorded, a photograph was taken, a sample was frozen at -20°C for later chemogroup analysis at the University of South Florida and another sample was dried on silica gel for DNA extraction at the University of Alabama at Birmingham (UAB). If enough material was left over, it was pressed and returned to the herbarium collection of Dr. Amsler at UAB.

Ecogenics generated SSR-enriched sequence data for the microsatellite development. Upon quality control of the library that was produced, putative microsatellite loci were selected to test for amplification. I am still in the process of the microsatellite development as unforeseen circumstances have delayed the progress. However, I have been able to develop six out of the 12 planned microsatellite loci following Krueger-Hadfield *et al.* (2011) which are looking promising thus far (they amplify across individuals and are polymorphic).

The six microsatellite loci were amplified across 40 individuals which had already been described for their chemogroup production. A principal component analysis of the genotypes was conducted using the distance-based function in GenAlEx 6.51b2 (Peakall & Smouse, 2012). And a PCoA plot was generated using ggplot2 in R version 3.5.1 (R Core Team, 2018, Wickham, 2016).

Preliminary Results

The chemogroups show site specificity as well as depth distribution at some of the sites (*unpubl data*). **Figure 1** represents the chemogroup distribution at two selected sites. Some chemogroups are absent as not all of the chemogroups found during field seasons were present at the transect sites. Chemogroup assignment is still preliminary. All but one chemogroup belong to one haplotype and the green chemogroup belongs to the second haplotype. Both of these correspond to the haplotypes identified before. A total of 93 individuals from Norsel Point and Stepping Stones (5.73km apart) were used for testing the microsatellite loci developed so far. Out of the 93 individuals, 40 had been described for their chemogroup production and included in the Principal Coordinates Analysis (**Figure 2**). Individuals producing the green chemogroup clustered separately from the other individuals. Individuals from Norsel Point which produced a different chemogroup to the green one clustered together with individuals from Stepping Stones. Those patterns are expected to further evolve as more microsatellite loci are developed, and more individuals as well as more sites are analyzed.

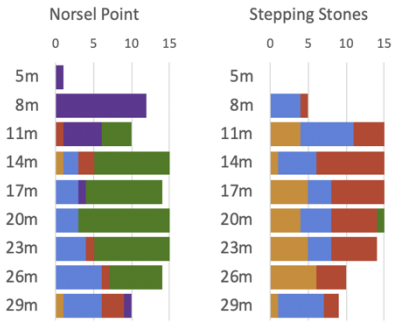

Figure 1. Chemogroup distribution across varying depths at two different sites. Chemogroup assignment is preliminary. Each color represents a different chemogroup.

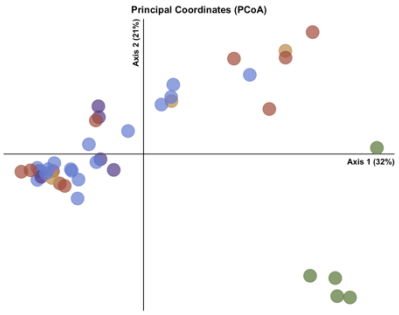

Figure 2. PCoA of genetic distances of *Plocamium cartilagineum* individuals from two sites based on six microsatellite loci. Color assignment for chemogroups are the same as in Figure 1.

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