PHYLOGEOGRAPHY OF *OCULINA* CORALS AND THEIR ALGAL SYMBIONTS: INSIGHTS INTO THE ORIGIN AND EXPANSION OF *OCULINA PATAGONICA* IN THE MEDITERRANEAN

A Dissertation

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This dissertation is dedicated to Brian Leydet, Jr. and
Jean, Chantal, Aline, and Vincent Posbic.

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ABSTRACT

In a world of rapidly changing environmental conditions, species must effectively respond to their changing habitat or risk extinction. The goal of my dissertation is to elucidate the origins and mechanisms underlying the recent successful population expansion of the invasive coral *Oculina patagonica* in the Mediterranean Sea. To do this, I have utilized nuclear markers and next-generation sequencing data for the coral host and its algal symbiont as well as environmental data.

Although only recently first described from the waters of the Mediterranean, genetic, historical demographic, and fossil evidence suggests that *O. patagonica* has not been anthropogenically introduced from the western North Atlantic. Instead, my results support the hypothesis that *Oculina* spp. has had a long history in the eastern Atlantic but remained undetected until it recently began expanding in the Mediterranean to invasive numbers, likely in response to environmental changes. Next, I found that the symbiotic algal communities harbored by *Oculina* corals vary geographically, and that this variation does not match the geographical variation of the host's genetics. Instead, sea surface temperature is better correlated to symbiotic community, particularly in the Mediterranean, which may reflect acclimatization to local thermal conditions. Finally, in a closer inspection of a rapid poleward range expansion of *O. patagonica* along the Spanish Mediterranean coast, I found increased genetic diversity and adaptation to temperature that may have promoted its success.

Together, my dissertation chapters shed light on the mechanisms that have allowed a coral to be successful despite stressful and changing environmental conditions. Unlike many previous studies aimed at assessing the adaptive capabilities and long-term success of tropical corals, my dissertation focusses on the success and adaptive potential of an understudied temperate coral. The findings presented here support the knowledge that *O. patagonica* is able to successfully respond to changing environmental conditions in its native range via both symbiont switching and host adaptation.

CHAPTER 1.
INTRODUCTION

Ecosystems both marine and terrestrial are changing rapidly, largely at the hands of humans (Vitousek 1994). In fact, no marine ecosystem has been unaffected by human influences (Halpern *et al.* 2008). Human disturbances and alterations, such as pollution, habitat fragmentation, coastal habitat modifications, overharvesting, species introductions, and climate change, threaten countless species. The loss of biodiversity over the last few centuries has been exceptionally rapid, indicating a mass human-induced extinction (Ceballos *et al.* 2015). Understanding the response of species to changing ecosystems, including acclimation or adaptation to local environmental conditions, and the particular environmental factors that drive their responses is necessary to inform and implement effective conservation plans.

Although they occupy <0.1% of the world's ocean, coral reefs are home to approximately 25% of marine species (Spalding *et al.* 2001). They provide invaluable ecosystem (Mumby *et al.* 2008) and economic goods and services (Hoegh-Guldberg & Bruno 2010). Unfortunately, corals, the animals responsible for building and maintaining reef ecosystems, are currently declining worldwide as a result of stresses, including increasing sea temperatures, disease, nutrient pollution, and other anthropogenic disturbances (Aronson *et al.* 2003; Hoegh-Guldberg *et al.* 2007; Hughes & Connell 1999; Hughes *et al.* 2003; Pandolfi *et al.* 2003). Understanding how corals respond and adapt to environmental changes and stresses is crucial to predicting their long-term success.

Although corals occurring outside the tropics do not typically form large reefs like tropical corals, we can learn a great deal from studying them because unlike most tropical corals many of these corals are not endangered, but are instead thriving and even invading new habitats (Fenner & Banks 2004; Hoeksema & Vicente 2014; Salomidi *et al.* 2013; Serrano *et al.* 2013). Therefore, understanding the mechanisms that allow these corals to be successful can potentially be applied to corals at risk.

1.1 CORAL HOLOBIONT

Corals constitute a collection of many organisms. The coral animal, its endosymbiotic algae (the zooxanthellae), and its resident microbes compose the coral holobiont (Bourne *et al.* 2009; Rosenberg *et al.* 2007)(Figure 1.1).

Photosynthetic zooxanthellae of the genus *Symbiodinium* provide nutritional resources for the coral host (Falkowski *et al.* 1984). A disruption of the relationship between the coral and its zooxanthellae, known as bleaching, can lead to a nutritionally depleted coral with impaired reproductivity and increased susceptibility to disease (Glynn & De Weerdt 1991; Harvell *et al.* 2002; Szmant & Gassman 1990). Because zooxanthellae are partially responsible for corals' pigmentation, bleaching causes corals to appear pale or white (Figure 1.2). If bleaching is severe or sustained, it can result in coral death.

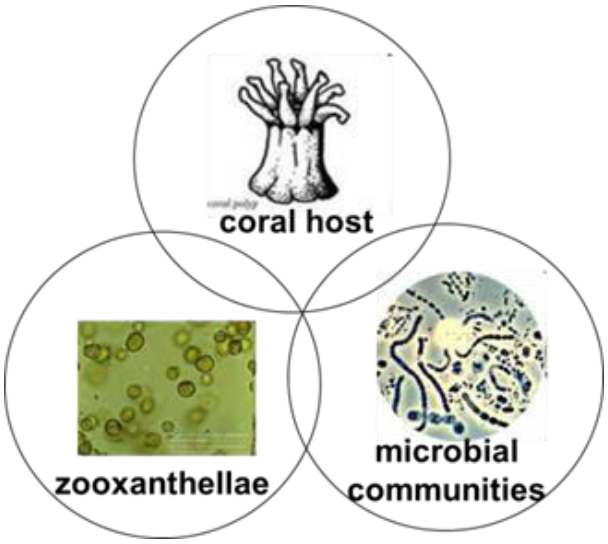

Figure 1.1. Coral holobiont associates: the coral host, photosynthetic algae (zooxanthellae), and the microbial communities including bacteria, archaea, and viruses.

In addition to symbiotic algae, all three coral layers (the surface mucus layer, the coral tissue, and the calcium carbonate skeleton) host large and diverse microbial communities, which include bacteria, archaea, and viruses (Rohwer *et al.* 2010). The benefits, if any, that these microbes confer on the coral host are unknown, although several hypotheses have been proposed. These include preempting available niches within the coral (thus preventing pathogenic microbes from colonizing), producing antibiotics (Chen *et al.* 2012; Kelman 2004; Nissimov *et al.* 2009; Shnit-Orland *et al.* 2012), and providing resistance to thermal stress (Gilbert *et al.* 2012). Furthermore, microbial community shifts have been associated with stress, further implicating their role in coral health (Lee *et al.* 2015; Vega Thurber *et al.* 2009). It is the combination of coral host and its symbionts that is or is not well suited to a particular habitat (Bordenstein & Theis 2015; Parkinson & Baums 2014). To fully comprehend the potential of corals to adapt to a changing world, then, it is necessary to understand its symbiotic associations in addition to the coral host itself.

Figure 1.2. A partially bleached colony of *Oculina patagonica* from Portman, Spain. Adapted from Rodolfo-Metalpa *et al.* (2014), and reproduced with permission from the authors and publisher (Appendix D).

1.2 *OCULINA* AS A STUDY SYSTEM

Over one-third of reef-building coral species face elevated extinction risks (Carpenter *et al.* 2008). Not surprising, most studies have focused on the major tropical reef-building coral species experiencing the worst declines and evaluating how these species are responding to stressors. However, this limits our full understanding of how corals might be responding to anthropogenic change. Understanding the mechanisms that have enabled successful temperate coral species to thrive and even invade new habitats can provide further insight into predicting a coral's adaptive potential and long-term success, and thus serve as a proxy for evaluating other corals that are at risk.

Oculina spp. are scleractinian (hard) corals. Their sexes are separate and they broadcast-spawn, meaning individuals release their gametes into the water column where fertilization takes place (Brooke & Young 2005; Brooke & Young 2003; Fine *et al.* 2001). As such, they acquire their zooxanthellae from the environment after fertilization (i.e., horizontally). They are facultative zooxanthellate corals and can persist in a healthy azooxanthellate state, giving an opportunity to examine this understudied facultative relationship.

Oculina corals occur in the western North Atlantic as well as across the Mediterranean Sea, often inhabiting disturbed habitats, including sites exposed to pollution (Fine *et al.* 2001). While the genus was originally described from the southeastern coast of North America (Miller & Hay 1996), an additional species, *O. patagonica*, is thought to have recently invaded Mediterranean waters (Fine *et al.* 2001; Zibrowius 1974). Like other species within *Oculina*, *O. patagonica* has proven exceptionally hardy, showing comparable reproductive potential between contaminated and uncontained localities (Armoza-Zvuloni *et al.* 2012) and an ability to recover from exposure to acidic conditions (Fine & Tchernov 2007; Movilla *et al.* 2012) as well as from bleaching (Fine *et al.* 2001; Rodolfo-Metalpa *et al.* 2006; Shenkar *et al.* 2006).

O. patagonica was first reported in the Mediterranean in 1966 in the harbor of Savona (Gulf of Genoa), Italy (Zibrowius 1974)(Figures 1.3 and 1.4). In 1973, it was found in the harbor of Alicante, Spain (Zibrowius & Ramos 1983) about 1,000 km away. Today, this coral is well established along the Mediterranean coast of Spain (Izquierdo *et al.* 2007; Serrano *et al.* 2013; Terrón-Sigler *et al.* 2015). Colonies near Marseille, France, were experimentally transplanted from Italian and Spanish sites in the early 1970's (Fine *et al.* 2001; Zibrowius 1974). Records from the eastern Mediterranean are more recent: Egypt (1981), Lebanon (1992), Israel (1993), Turkey (2005), Greece (ca. 2006) (Bitar & Zibrowius 1997; Çinar *et al.* 2006; Fine & Loya 1995; Salomidi *et al.* 2006)(Figure 1.4). *O. patagonica* has also been reported from Algeria (2005, 2007) and Tunisia (2006, 2008) (Sartoretto *et al.* 2008). *O. patagonica*, originally presumed to have a South American origin based on fossils (Zibrowius 1974), was thought to have been accidentally introduced into the Mediterranean coast of Spain via shipping. Such human-facilitated trans-Atlantic crossings have been reported for many other marine organisms including fish (Langeneck *et al.* 2012; Orsi Relini 2002) and corals (Hoeksema *et al.* 2011). From Spain, reports of *O. patagonica* spread east to Italy, south to Egypt, and eventually northward to Israel and Lebanon (Bitar & Zibrowius 1997; Fine *et al.* 2001; Salomidi *et al.* 2006; Sartoretto *et al.* 2008; Zibrowius 1974; Zibrowius & Ramos 1983). A 19-year observational study of *O. patagonica* determined that this coral has quickly spread northward across the

Catalonian coast (Serrano *et al.* 2013). The authors attribute the expansion to increased space availability provided by artificial habitats, higher sea temperatures, and biological features of the coral itself, including high growth rates, early reproduction, and high stress tolerance. Salomidi *et al.* (2013) found that this coral was significantly more abundant in the Aegean Sea on artificial substrates than on natural ones. As such, they proposed that human-mediated coastal habitat modification has facilitated the establishment and spread of this species in this region since its first reported sighting in 2005 (Salomidi *et al.* 2013).

Figure 1.3. Site of the first reported *Oculina patagonica* colony in the Mediterranean in the Gulf of Genoa, Italy. The site was well-described by Zibrowius (1974). (Photo credit: Brian Leydet, Jr.)

While populations of *O. patagonica* share common seasonal reproductive timing across the Mediterranean (Fine *et al.* 2001), they otherwise vary. Western populations have experienced recent recruitment and little or no recent bleaching (Fine *et al.* 2001; Rubio-Portillo *et al.* 2014; Sartoretto *et al.* 2008). In contrast, eastern populations in the Levant (Lebanon and Israel) face annual bleaching and low recruitment (Fine & Loya 1995; Fine *et al.* 2001). This geographic variation may be attributed to regional differences in water temperature. During the hottest month (August), sea surface temperatures in the Mediterranean range from 20-30˚C, with the warmest temperatures along the coasts of Tunisia and the Middle East. During the coldest month of February, sea surface temperatures range from 10-18˚C, with the coldest temperatures generally in the northern most part of the Adriatic Sea and the southern coast of France.

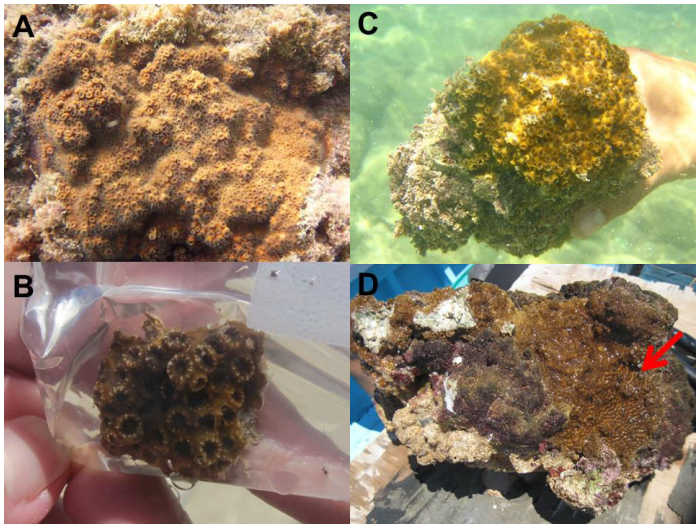

Figure 1.4. Pictures of *Oculina patagonica* colonies from across the Mediterranean: A) Savona Harbor, Italy; B) Athens, Greece; C & D) Tyre, Lebanon. (Photo A credit: Brian Leydet, Jr.)

1.3 DISSERTATION

My dissertation employs molecular techniques to investigate the symbiotic and genetic underpinnings allowing a species to successfully cope with and even thrive in an ever-changing world, as well as the environmental factors that drive adaptation or acclimatization. Specifically, its aim is to understand the origins and mechanisms underlying the rapid and recent population expansion of the invasive coral *Oculina patagonica* in the Mediterranean Sea. It addresses the following questions: 1) Has *O. patagonica* been anthropogenically introduced to the Mediterranean from the New World or has it recently expanded from native populations?, 2) Do *Oculina* spp. harbor different symbiotic algal communities across their geographic range and, if so, does the geographical variation suggest acclimation to local thermal regimes or simply coassociation with similarly geographically differentiated hosts? and 3) What mechanisms have facilitated *O. patagonica's* recent range expansion along the Mediterranean coast of Spain?

1.3.1. Overview of Chapters

My first research chapter (Chapter 2) evaluates the species status of *O. patagonica* by genetically comparing populations from the Mediterranean to populations of other *Oculina* spp. from the western North Atlantic. Using five nuclear genetic markers, I found no genetic or historical demographic evidence to support a recent human-mediated introduction of *O. patagonica* from the western North Atlantic or an expansion across the Mediterranean. Instead, Mediterranean and western Atlantic populations are genetically distinct and appear to have begun diverging about 5 million years ago. I also found evidence of a fossil record of *Oculina* spp. from the eastern North Atlantic millions of years before the present. My results suggest that Mediterranean populations of *O. patagonica* have long been isolated from those in North America and have not been recently introduced from there. Instead, it is more likely that *O. patagonica* has always existed somewhere in the eastern Atlantic, either in undetectable numbers or overlooked and undersampled sites and habitats, and has recently become invasive in the Mediterranean, perhaps in response to human-caused environmental changes.

In my second research chapter (Chapter 3), I examined the role of the algal symbiont *Symbiodinium* in explaining the local success of *Oculina* across the western Atlantic and Mediterranean Sea. Using genetic and environmental data, I found that *Oculina* corals harbor different *Symbiodinium* communities across their western Atlantic and Mediterranean range and that habitat differences in sea surface temperature are better correlated with this geographical variation than the host's genetics, depth, or chlorophyll *a* concentration, particularly in the Mediterranean. These results suggest that although facultative zooxanthellate corals like *Oculina* may be less dependent on their algal partners than obligate zooxanthellate corals, the *Symbiodinium* communities that they harbor may nevertheless reflect acclimatization to environmental variation among habitats. Ultimately, this variation in *Oculina* spp.'s algal symbionts may have allowed these corals to persist over such a wide and environmentally variable habitat range and to endure localized thermal stresses.

My final research chapter explores and identifies mechanisms that have facilitated the range expansion of *O. patagonica* range along the Mediterranean coast of Spain (Chapter 4). Using a modified restriction-site associated DNA sequencing (RAD-Seq) protocol to target coral host

DNA, I collected 595 single nucleotide polymorphisms (SNPs) from 189 individual corals from both long-established core populations and two expansion fronts with different levels of success (less successful to the west, more successful to the north). Although more recent, the northern expansion populations are genetically distinct from the westward expansion and core populations and harbor greater genetic diversity as well. Temperature appears to have driven adaptation along the northern expansion, but not along the westward expansion. One locus (a V-type proton ATPase subunit) under selection associated with temperature along the northern expansion. Finally, I found no evidence of local adaptation to artificial substrate, suggesting that this coral is simply an opportunistic colonizer of free space made available by increasing coastal habitat modifications along the coast of Spain. Together, these results suggest that unique genetic variation and thermal adaptation along the northern expansion front (but not the westward expansion) have facilitated the poleward range expansion of *O. patagonica* in the western Mediterranean.

Finally, in Chapter 5, I synthesize the results from all my research chapters, discuss the implications of my findings for understanding the factors and mechanisms that have allowed a coral genus to be so successful in a stressful and changing environment, and conclude with future directions.

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CHAPTER 2.
THE INVASIVE CORAL *OCULINA PATAGONICA* HAS NOT BEEN RECENTLY INTRODUCED TO THE MEDITERRANEAN FROM THE WESTERN ATLANTIC[1]

2.1. ABSTRACT

2.1.1. Background

Effective policies, management, and scientific research programs depend on the correct identification of invasive species as being either native or introduced. However, many species continue to be misidentified. *Oculina patagonica*, first recorded in the Mediterranean Sea in 1966, is believed to have been introduced in anthropogenic times and expanding in a west to east direction. However, its present identification and status as a recently introduced species remain to be explored. In this study, we used multi-locus genetic data to test whether *O. patagonica* in the Mediterranean has been recently introduced from the western North Atlantic.

2.1.2. Results

We found no genetic or historical demographic evidence to support a recent introduction of *O. patagonica* from the western North Atlantic or an expansion across the Mediterranean. Instead, Mediterranean and Atlantic populations are genetically distinct and appear to have begun diverging about 5 Mya. We also found evidence of a fossil record of *Oculina* spp. existing in the eastern North Atlantic millions of years before the present.

2.1.3. Conclusions

Our results suggest that Mediterranean populations of *O. patagonica* have long been isolated from the western Atlantic, either in undetectable numbers or overlooked and undersampled sites and habitats, and have only recently been expanding to invasive levels as a result of environmental changes. Accurate identification of species' invasive statuses will enable more effective research programs aimed at better understanding the mechanisms promoting the invasive nature of species, which can then lead to the implementation of efficient management plans.

2.2. INTRODUCTION

The number of introduced and invasive species is rising (Baskin 2002), rapidly altering ecosystems around the world, often by out-competing and displacing native species (Morales *et*

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[1] This chapter previously appeared as Leydet, K. P., and M. E. Hellberg (2015) The invasive coral *Oculina patagonica* has not been recently introduced to the Mediterranean from the western Atlantic. *BMC Evolutionary Biology* **15**:79. Copyright rests with the authors. Permission from publisher to reproduce is not required (Appendix E).

al. 2013; Ricciardi *et al.* 1998). Beyond their direct impacts on native species, these invasive species can indirectly lead to cascading effects within a community (Kenis *et al.* 2009), thereby threatening ecosystem functions. Studies concerning successful introduced and invasive species are key to exploring the mechanisms by which these species adapt to and alter their new environment. However, determining whether a species is native or introduced is first necessary, and this is not always an easy task.

Introduced species can be mislabeled as native due to taxonomic misidentification (Genner *et al.* 2004; Turon *et al.* 2003). Native species can also be misidentified as introduced. Zenetos *et al.* (2005) reports that 23% of the 963 reportedly introduced species in the Mediterranean have been misidentified and therefore misclassified. Of the remaining 745 species, 13% remain questionable due to insufficient information and unresolved taxonomic status and many others maintain a "cryptogenic" status, as they cannot be reliably assigned to either "native" or "introduced" (Carlton 1996). In other cases, species are mislabeled as introductions due to a lack of historical records of an obvious presence (Concepcion *et al.* 2010; Hawryshyn *et al.* 2012; Taylor & Bothwell 2014; Zenetos *et al.* 2005). These misidentifications can have profound effects on the assessment of species status (Geller 1999).

Invasive species are often assumed to have been introduced (Concepcion *et al.* 2010; Hawryshyn *et al.* 2012), however species can become invasive within their native range, usually due to human-mediated disturbances (Carey *et al.* 2012; Hierro *et al.* 2006; Simberloff 2011). Effective policies, management, and scientific research programs depend on the correct identification of invasive species as being either native or introduced. Whereas the management of introduced invasives is more concerned with the introduction scenario and conditions enabling subsequent expansion, management of native invasives should ideally be more focused on the changes in environmental conditions that facilitate their invasive characteristics in their native habitat (Simberloff *et al.* 2012; Taylor & Bothwell 2014). Therefore, to implement more effective research and management programs for invasive species, an invasive species must first be identified as either introduced or native.

An introduced invasive population can be distinguished from native invasive populations in several ways. An expanding introduced population is expected to be genetically similar to an external source population where it originated (Perdereau *et al.* 2013). In contrast, a native invasive population will often be genetically distinct from populations outside its range (Concepcion *et al.* 2010; Kim *et al.* 2006). Inferring the divergence time between populations from different ranges can also establish whether or not an invasive population coincides with a recent introduction (estimated divergence time will overlap with the present day), or whether it predates anthropological times (older divergence time) (Brouat *et al.* 2014; Mun *et al.* 2003; Wares *et al.* 2002). Finally, the presence of a fossil record for an invasive species or its progenitors can indicate that the species has a long presence in a particular region (Hawryshyn *et al.* 2012; Taylor & Bothwell 2014).

Corals of the genus *Oculina* were originally described from the southeastern coast of North America (Miller & Hay 1996). Although several nominal species exist in this region, Eytan *et al.* (2009) found no genetic differences among shallow water populations of four named taxa (*O. arbuscula*, *O. diffusa*, *O. varicosa*, and, *O. robusta*), suggesting that these designations do not

represent genetically distinct species. We will refer to these taxa collectively as "western Atlantic (WA) *Oculina* spp."

An additional extant species, *Oculina patagonica*, occurs in the waters of the Mediterranean Sea (Fine *et al.* 2001; Zibrowius 1974). *O. patagonica* was originally described from fossils from the southeastern coast of South America (Zibrowius 1974) (Appendix A, Figure 2.S1), however reports of live specimens from South America are lacking and a recent survey of fouling communities in Argentinian ports failed to find any evidence of this species (Schwindt *et al.* 2014). *O. patagonica* has been thought to have been introduced in anthropogenic times from the western south Atlantic to the western Mediterranean via shipping. *O. patagonica* was first reported from the harbor of Savona (Gulf of Genoa), Italy in 1966 (Zibrowius 1974) and soon after from the harbor of Alicante, Spain (Zibrowius & Ramos 1983), 1000 km away. From the western Mediterranean, it is thought to have spread easterly, and while today reports of this coral in many locations reflect populations limited in number and range, populations in Spain, Greece, and Israel are well-established and expanding (Ballesteros 1998; Bitar & Zibrowius 1997; Çinar *et al.* 2006; Cvitković *et al.* 2013; Fine & Loya 1995; Fine *et al.* 2001; Izquierdo *et al.* 2007; Rubio-Portillo *et al.* 2014; Salomidi *et al.* 2006; Salomidi *et al.* 2013; Sartoretto *et al.* 2008; Serrano *et al.* 2013; Zibrowius 1974; Zibrowius & Ramos 1983).

Alternatively to being recently introduced, *O. patagonica* may be a native species that has only recently been detected due to a recent expansion. The original description and identification of *O. patagonica* is based on fossil remains, not living counterparts (Zibrowius 1974). This is problematic, as morphology is a poor delineation of coral species in general (Fukami *et al.* 2004; Pinzon & LaJeunesse 2011) and *Oculina* species in particular (Eytan *et al.* 2009) (Appendix A, Figure 2.S1). Given that no known populations of *O. patagonica* presently exist outside the Mediterranean, populations of WA *Oculina* spp. are currently the most likely source for a recent introduction.

Here, we ask whether *O. patagonica* has been recently introduced into the Mediterranean from the western North Atlantic, or whether it is an eastern Atlantic native only newly become invasive. First, we use multilocus genetic data to determine whether *O. patagonica* is genetically similar to or distinct from WA *Oculina* spp. If *O. patagonica* has been recently introduced from the western North Atlantic, we expect these populations to be genetically similar. Second, we estimate divergence time between *O. patagonica* populations and WA *Oculina* sp. populations and evaluate whether the estimate is consistent with an anthropogenic introduction. We also search museum collections for evidence of a fossil record of *Oculina* spp. in the eastern Atlantic, which would suggest that *Oculina* has a long history in this region. Finally, we explore whether patterns of genetic diversity in *O. patagonica* are consistent with a west to east expansion across the Mediterranean from a single point of introduction.

2.3. METHODS

2.3.1. Sampling and Genotyping

Mediterranean samples (n=66) of *Oculina patagonica* were collected from Spain, Italy, Greece, Lebanon, and Israel during the summers of 2011–13 (Figure 2.1, Appendix A, Table 2.S1).

Individual colonies were sampled by chipping off a small piece of skeleton containing coral tissue and preserving it in 95% ethanol. Samples were generally 10 m from conspecifics and not physically connected to them to avoid collecting clonemates.

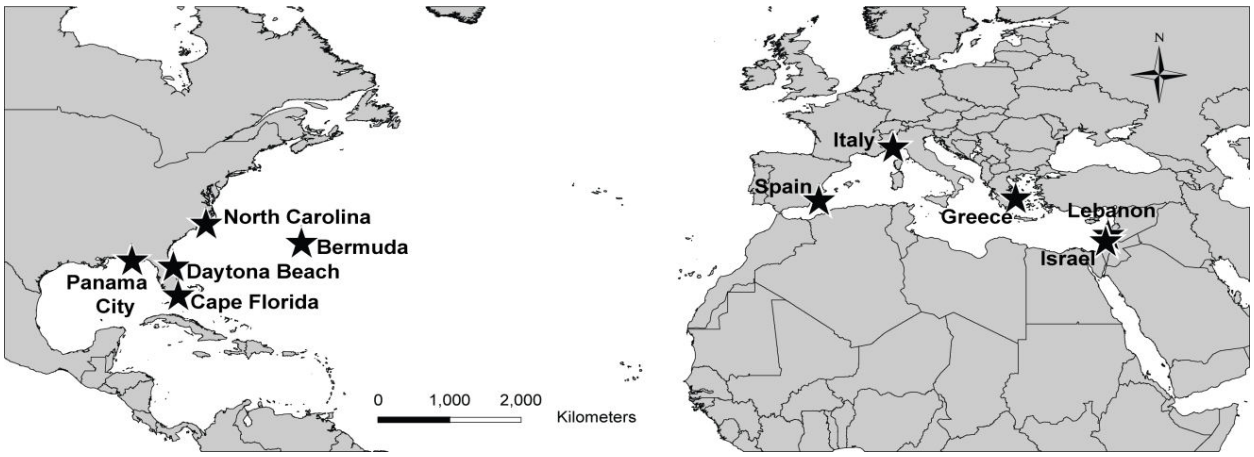

Figure 2.1. Map of collections sites of *Oculina* spp. populations used in this study. Populations along the western North Atlantic included North Carolina, three locations in Florida (Daytona Beach, Cape Florida, and Panama City), and Bermuda. Mediterranean populations included Spain, Italy, Greece, Lebanon, and Israel. This figure was created using maps freely available for use from ESRI.

Western North Atlantic *Oculina* spp. samples (n=56) consisted of a subset of populations along the coast of the eastern United States from Eytan *et al.* (2009). The four populations (North Carolina, Daytona Beach, Cape Florida, Panama City) were chosen to represent the two geographic genetic clusters (North Carolina and Daytona Beach= northern cluster; Cape Florida and Panama City= southern cluster) and include three nominal species: *O. arbuscula*, *O. varicosa*, and *O. diffusa,* although Eytan *et al.* (2009) found no genetic differences among these named taxa. We also obtained 13 new samples of nominal species *O. diffusa* and *O. varicosa* from Bermuda. We will refer to these samples collectively as "Western Atlantic (WA) *Oculina* spp." All sampling was conducted by or with local collaborators in accordance with local and CITES regulations.

We extracted genomic DNA using QIAGEN DNeasy Kit following the manufacturer's protocols with the following modifications. We lysed tissues at 56˚C overnight. We added 200 µl elution buffer and incubated at room temperature for an hour prior to the final centrifuge step. All individuals were genotyped, either previously or in this study, for the mitochondrial cytochrome oxidase I (*COI*) gene and the coding region of five nuclear genes (Appendix A, Table 2.S2). *COI* was genotyped using previously deigned primers (Folmer *et al.* 1994). Three of the nuclear genes (putatively: *fatty acid elongase, elongation factor 1α, and tachylectin-2 motif*) were previously developed to assess subdivision in WA *Oculina* spp. populations (Eytan *et al.* 2009), so only the 13 Bermuda and 66 Mediterranean samples were genotyped for these markers here. Two new nuclear markers (putatively: *crystalline and S-adenosylmethionine synthetase*) were developed using an expressed sequence tag (EST) library (Eytan *et al.* 2009); all samples were genotyped for these.

Polymerase chain reaction (PCR) amplifications were conducted in 25 µl reactions consisting of 2.5 µl of 10x buffer, 10 µM of dNTPs and each primer, and 0.2 µl of One TaqTM DNA polymerase (New England Biolabs Inc.). Amplifications were performed in a Bio-Rad T100 thermocyler under the conditions outlined by Eytan *et al.* (2009). Samples were sequenced in both directions using BigDye v3.1 on an ABI 3130XL at the Louisiana State University Genomics Facility. Sequences were aligned and edited using Geneious 4.5.5 (Drummond *et al.* 2010). Sequences obtained from Eytan *et al.* (2009) were trimmed to align to sequences generated in this study. To resolve alleles in heterozygous individuals, we employed a Bayesian statistical method implemented in PHASE 2.1 (Stephens & Donnelly 2003; Stephens & Scheet 2005; Stephens *et al.* 2001). Individuals with alleles that could not be phased to a probability >90% were cloned using the Invitrogen TOPO TA kit following the manufacture's protocols. At least eight clones per reaction were sequenced to identify the two alleles present in a sample. The phased individuals derived from the cloning reactions were then added to the 'known' PHASE file and the data sets were re-analyzed. This process was repeated until the phase of all individual genotypes was recovered with >90% probability. Individuals heterozygous for an insertion/deletion were resolved using CHAMPURU 1.0 (Flot 2007). In the end, we were able to successfully resolve all 122 individuals' multilocus genotypes.

To prevent clonal reproduction from skewing subdivision and genetic diversity measures, we removed individuals that shared a multilocus genotype with another individual in the same population. The final nuclear data set contained 105 individuals (Appendix A, Table 2.S1). Measures of genetic diversity for each nuclear marker were calculated in DnaSP (Librado & Rozas 2009; Rozas & Rozas 1995) (Appendix A, Table 2.S2). We tested each gene region for intralocus recombination using GARD implemented in Hy-Phy (Delport *et al.* 2010; Pond *et al.* 2005; Pond *et al.* 2006).

2.3.2. Genetic Diversity and Population Subdivision

To visualize the relationships among alleles, we constructed haplotype networks for each locus using statistical parsimony implemented in TCS 1.21 (Clement *et al.* 2000). These networks reveal how alleles for a particular gene are shared among individuals from different populations. We calculated allelic richness for all populations using FSTAT 2.9.3.2 (Goudet 1995) and calculated observed heterozygosity using GENODIVE (Meirmans & Van Tienderen 2004). To test whether Mediterranean populations harbor less genetic diversity than western Atlantic populations, we compared their average allelic richness and observed heterozygosities using two-sample one-tailed t-tests in GraphPad Prism 5. We performed similar comparisons between western Mediterranean (Spain and Italy) and eastern Mediterranean (Greece, Lebanon, and Israel) populations using two-sample two-tailed t-tests. To test for hierarchical genetic subdivision, we performed Analyses of Molecular Variance (AMOVA) implemented in GENODIVE (Meirmans & Van Tienderen 2004) for all populations combined, and for only Mediterranean populations.

The Bayesian clustering analysis implemented in STRUCTURE (Pritchard *et al.* 2000) has been used often to infer species introductions and identify potential source populations (Perdereau *et al.* 2013). Here, we used STRUCTURE 2.3.4 (Pritchard *et al.* 2000) to test whether *O. patagonica* populations in the Mediterranean are genetically similar to or distinct from WA

Oculina spp. populations. We first analyzed all populations together, and then analyzed the pool of Mediterranean populations separately. We ran the program for 1 million MCMC steps and discarded the first 100,000 steps as burn-in. We used the more conservative admixture model with uncorrelated allele frequencies. We performed 10 iterations for each inferred number of genetic clusters, *k*. We used the Evanno method (Evanno *et al.* 2005) implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012) to determine the most likely number of genetic clusters, *k*, in the data. We also used STRUCTURAMA 2.0 (Huelsenbeck & Andolfatto 2007) to explicitly estimate *k* without *a prior* assignment of a range of *k*, as in STRUCTURE. Each trial was run for 20 million generations, sampling every 100, discarding the first million as burn-in. We ran four chains at a temperature of 0.2, and we employed a variety of model options.

2.3.3. Divergence Time

We estimated the time of divergence between the western North Atlantic and Mediterranean populations using a coalescent-based method implement in IMa (Hey & Nielsen 2007). IMa uses Markov Chain Monte Carlo (MCMC) simulations of gene genealogies to estimate the divergence time (t), genetic diversities (θ_1, θ_2, and ancestral θ) and migration rates (m_1 and m_2) for two populations. To convert divergence time (t), which is scaled by mutation in IMa, to years, we used the average nuclear substitution rate for *Porites* corals of 0.138% per Ma (Prada *et al.* 2014), since a rate for *Oculina* corals is unavailable, which was converted to a rate per locus per year for each marker (Appendix A, Table 2.S2). Given that the sequence alignments showed sites with more than two variants and/or haplotype networks contained multiple reticulations, we used the finite-sites model for all genes.

We first performed several IMa runs, subsequently adjusting the upper bounds on the parameter priors, to determine the most efficient search parameters. We then ran four runs that differed only in the starting seed for 3,000,000 total steps sampling every 100 steps for a total of 30,000 saved genealogies following a burn-in of 500,000 steps. The runs yielded similar results. We therefore combined the runs and estimated all parameters
and performed nested model testing on the total saved genealogies in IMa's L mode (load trees). IMa analyses were conducted with high performance computational resources provided by Louisiana State University.

We recorded the maximum-likelihood estimate from the posterior probability distribution for divergence time, adjusted with a two year generation time (Fine *et al.* 2001) and its credibility interval based on the shortest parameter interval containing 90% of the area under the posterior distribution curve. Because the upper end of the posterior probability distribution did not drop to zero (Figure 2.5), we used the lower bound on the distribution as the parameter value at which the probability dropped to zero at the upper bound (McGovern *et al.* 2010). To evaluate all possible scenarios of divergence, which differ in the number of unique divergence parameters and therefore divergence complexity, we used model-based inference and model-based selection to calculate evidence ratios and rank all possible models (Anderson 2008; Carstens *et al.* 2009).

2.3.4. Fossil Record Search

We searched museum collections for evidence of a fossil record of *Oculina* spp. in the eastern Atlantic and/or the Mediterranean. First, we explored the Smithsonian National Museum of Natural History's Department of Invertebrate Zoology records by performing a keyword search of *Oculina* on the IZ collections database website. We also explored the Muséum National d'Histoire Naturelle Paléontologie collections database by performing a general search for *Oculina* on the collections website. From both lists of matches, we searched for fossil specimens of *Oculina* spp. from eastern Atlantic and Mediterranean countries and recorded the catalog number, species name, location, and geologic age. Several of the specimens from the Smithsonian National Museum of Natural History were observed during a visit to the museum in January 2013.

2.3.5. Population Expansion within the Mediterranean

To test for expansion in the Mediterranean, we used LAMARC 2.0 (Kuhner 2006), which estimates parameters including population growth rate using coalescent theory and Metropolis Monte Carlo sampling technique. Three replicates were each run using a Bayesian search strategy and a single final chain. Following a burn-in of 500,000, 5 million trees were sampled every 100 step. Three simultaneous searches were performed at heating temperatures of 1, 1.2, and 1.3, and a swap interval of 10. Trial runs were first conducted and the output files examined in the program TRACER 1.5 (Rambaut & Drummond 2007) to adjust the parameter bounds and assess the run. A "good run" was one in which both the effective sample size (ESS) values were great than 200 and trace plots for each parameter were stationary. LAMARC calculates the overall growth rate across all genes and replicates. Positive values of growth rate indicate that the population has been growing, while negative values indicate that it has been shrinking.

2.4. RESULTS

2.4.1. Genetic Diversity and Population Subdivision

We genotyped 122 samples of *Oculina* spp. from the western North Atlantic (n=56) and Mediterranean (n=66) for the mitochondrial *COI* gene and five nuclear genes. Western North Atlantic populations included North Carolina, Daytona Beach, FL, Cape Florida, FL, Panama City, FL, and Bermuda. Mediterranean populations of *O. patagonica* included Spain, Italy, Greece, Lebanon, and Israel (Figure 2.1). A total of 17 individuals from Spain, Greece, and Israel were removed from the dataset because they shared a multilocus genotype with another individual in the same population, so the final nuclear data set contained 105 individuals (Appendix A, Table 2.S1). All *O. patagonica* samples shared the same *COI* haplotype common to 98% of WA *Oculina* spp. (Appendix A, Figure 2.S2). Because *COI* was nearly invariant, as expected due to the conservation of anthozoan mitochondrial DNA (Hellberg 2006), we used only the five nuclear genes in all analyses. GARD did not detect recombination within any of these five gene regions.

Haplotype networks revealed that, while populations in the western North Atlantic and the Mediterranean share many alleles at all five loci, all markers possess several private alleles

unique to just one region (Figure 2.2). Specifically, for three genes (p14, p62, and p302), over half of the total alleles for each of those genes are unique to the Atlantic. In contrast, the Mediterranean harbors fewer private alleles for all genes (Figure 2.2). T-tests revealed that allelic richness was greater in the western North Atlantic than in the Mediterranean (Figure 2.3a), although this difference is not large (western North Atlantic mean = 2.38±0.09; Mediterranean mean= 2.14±0.09), and therefore likely not biologically significant. Observed heterozygosity did not differ between the western Atlantic and Mediterranean (Figure 2.3a), nor did allelic richness or observed heterozygosity between western Mediterranean (Spain and Italy) and eastern Mediterranean (Greece, Lebanon, and Israel) populations (Figure 2.3b).

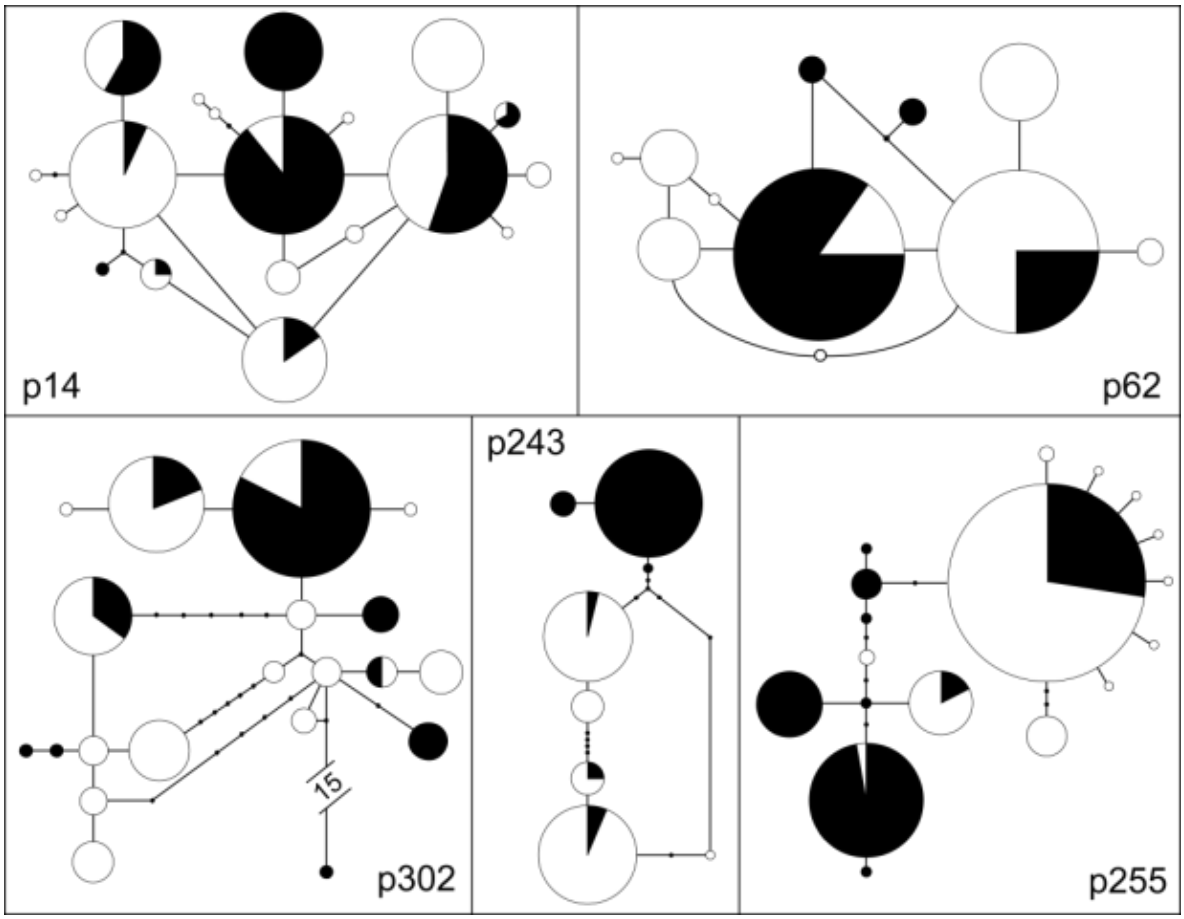

Figure 2.2. Haplotype networks of the five nuclear genes used in this study. Each pie graph represents an allele and the shades represent the proportion of individuals from the different populations that share that particular allele. White represents western North Atlantic *Oculina* spp. populations. Black represents Mediterranean populations of *Oculina patagonica*. Line segments connecting alleles represent a single mutation step separating the alleles, and small black dots represent inferred alleles not present in our dataset.

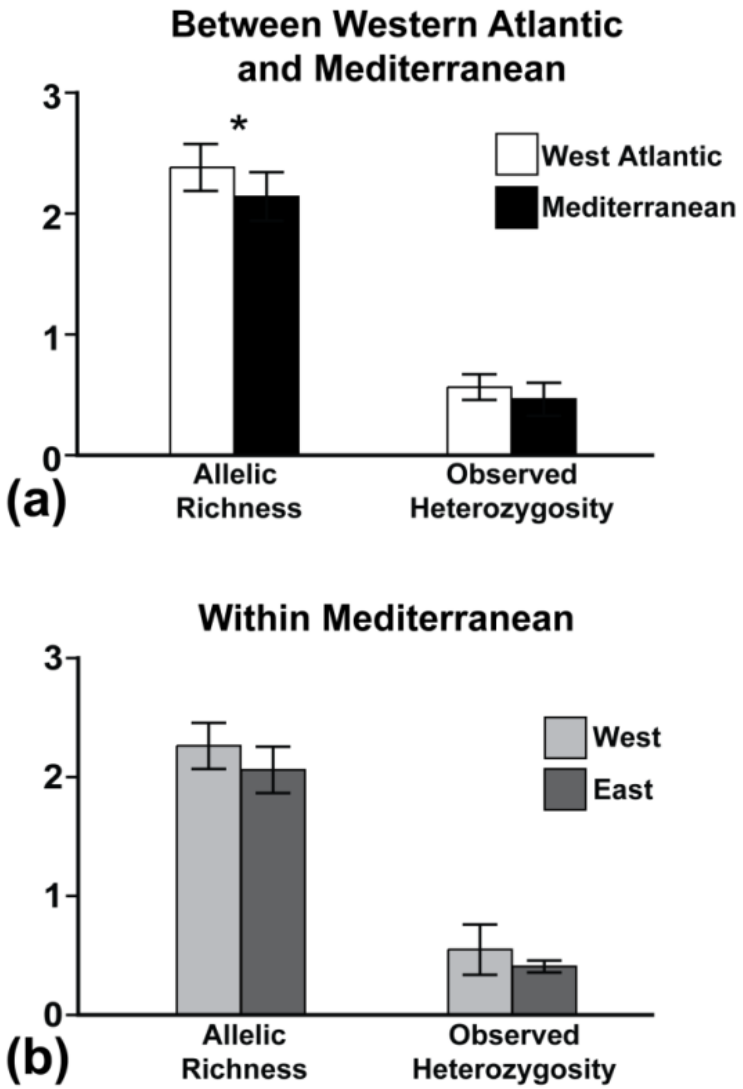

Figure 2.3. Comparison of allelic richness and observed heterozygosity. Means of allelic richness and observed heterozygosity compared between western North Atlantic and Mediterranean populations of *Oculina* spp. (a), and between western Mediterranean (Spain and Italy) and eastern Mediterranean (Greece, Lebanon, and Israel) *Oculina patagonica* populations (b). Bars represent standard deviations. Asterisks represent significant differences as determined by t-tests.

AMOVA revealed significant subdivision among all populations, as well as between western North Atlantic and Mediterranean populations (Table 2.1). However, AMOVA conducted on Mediterranean populations alone revealed no significant subdivision, either among populations or between the west and east (Table 2.2). Instead, variation within populations accounted for 98% of observed variation. These findings suggest that while the western North Atlantic populations are genetically distinct from those in the Mediterranean, populations within the Mediterranean are genetically similar to each other.

Table 2.1. Analysis of molecular variance performed for all populations. Groups= western North Atlantic populations and Mediterranean populations. Significant P-values are in bold.

Source of Variation	%variation	F-value	P-value
Within Populations	58.9%	0.411	NA
Among Populations	8.4%	0.125	**<<0.01**
Among Groups	3.3%	0.328	**<<0.01**

Table 2.2. Analysis of molecular variance performed for Mediterranean populations only. Groups= West (Spain and Italy) and East (Greece, Lebanon, and Israel).

Source of Variation	%variation	F-value	P-value
Within Populations	98.0%	0.02	NA
Among Populations	1.4%	0.014	0.256
Among Groups	0.6%	0.006	0.101

To further test for more subtle genetic differentiation, we used STRUCTURE 2.3.4 (Pritchard *et al.* 2000) and the Evanno method (Evanno *et al.* 2005) implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012) to detect differentiated populations (*k*). When all populations were analyzed according to the Evanno method (Evanno *et al.* 2005), the most likely *k* was two. The visual representation of these two genetic clusters (Figure 2.4a) shows that the western North Atlantic, including Bermuda, and Mediterranean form distinct genetic clusters. At *k* =3, STRUCTURE recovered the two main genetic clusters previously found in the western North Atlantic (Eytan *et al.* 2009) in addition to the Mediterranean cluster (Figure 2.4b). When analyzing the Mediterranean populations alone, the Evanno method (Evanno *et al.* 2005) determined that the mostly likely *k* =4, although the Δk's for the range of *k* tested were very low and similar, suggesting a lack of biologically meaningful clusters. Indeed, the visual representation fails to show any clear individual assignments and geographic association of these clusters, consistent with the Mediterranean populations being genetically similar across the region (Figure 2.4c). STRUCTURAMA runs corroborated these STRUCTURE results.

2.4.2. Divergence Time

We estimated the time of divergence between western North Atlantic and Mediterranean populations using IMa (Hey & Nielsen 2007). We found that the populations diverged 5.4 ± 2.0 million years ago, long before recent times (Figure 2.5). The best supported IM model (Table 2.3) had two parameters for population size and two for migration, suggesting that migration has played a role in the history of WA *Oculina* spp. populations and *O. patagonica* in the Mediterranean. Models of strict isolation were thousands of times less likely than the best model. Migration from the western North Atlantic to the Mediterranean was greater (0.00139 [90% highest posterior density interval= 0.0008– 0.0024]) than the reverse migration (0.000042 [0–0.002]) (Figure 2.6). In fact, the next best model was one in which migration from the Mediterranean to the western North Atlantic was equal to 0.

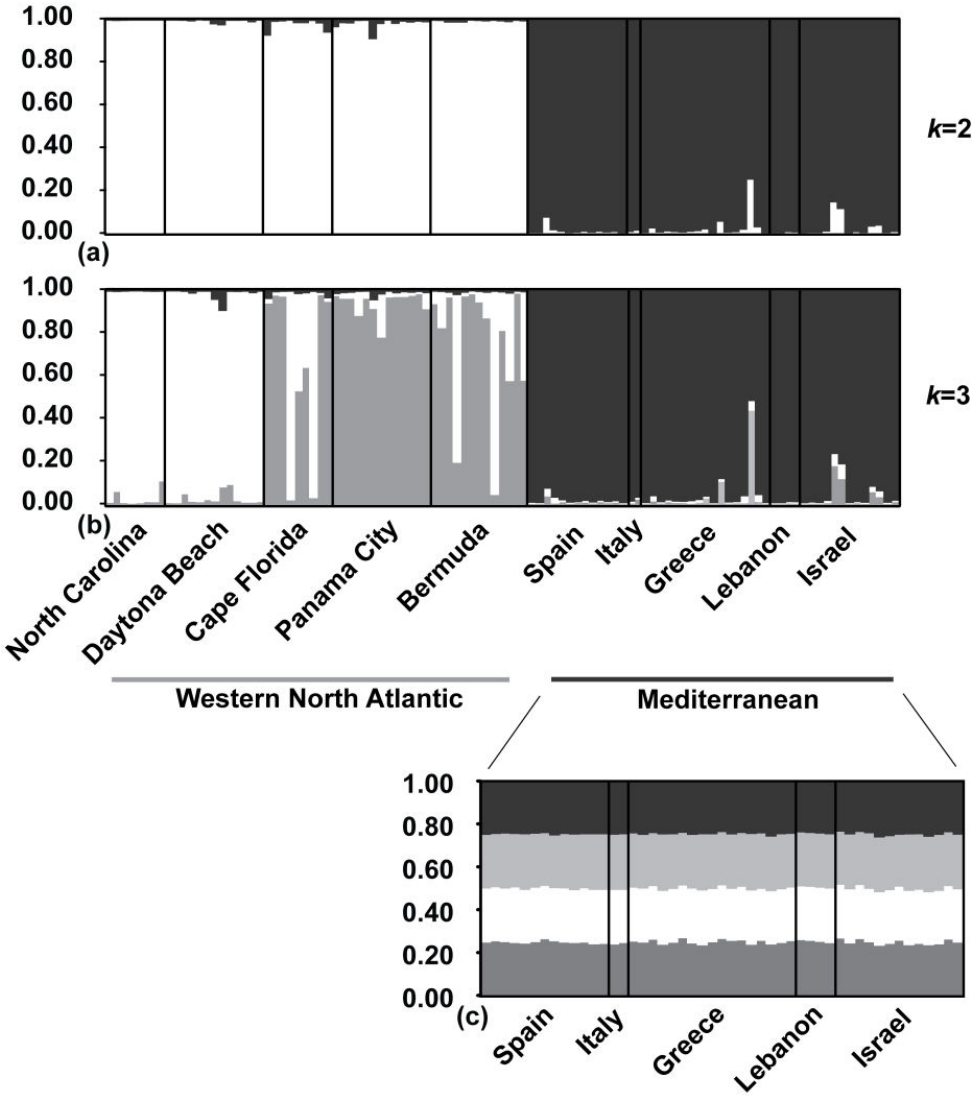

Figure 2.4. STRUCTURE bar plots. Each bar represents an individual. Individuals are grouped by populations along the x-axis. Along the y-axis is the probability of assignment to a particular population represented by different shades. When analyzing all populations, the Evanno method (Evanno *et al.* 2005) determined that the mostly likely number of genetic clusters or populations (*k*) was 2 (a). The visual representation of these two populations (a) shows that individuals cluster geographically (western North Atlantic versus Mediterranean), and that the Mediterranean populations are genetically distinct from the western North Atlantic populations. At *k* =3 (b), the two main genetic clusters previously found in the western Atlantic (Eytan *et al.* 2009) were recovered, while maintaining a genetically differentiated Mediterranean cluster. When analyzing the Mediterranean populations alone (c) there is no clear genetic structure across the Mediterranean, even though the Evanno method (Evanno *et al.* 2005) determined that the most likely number of populations (*k*) was 4.

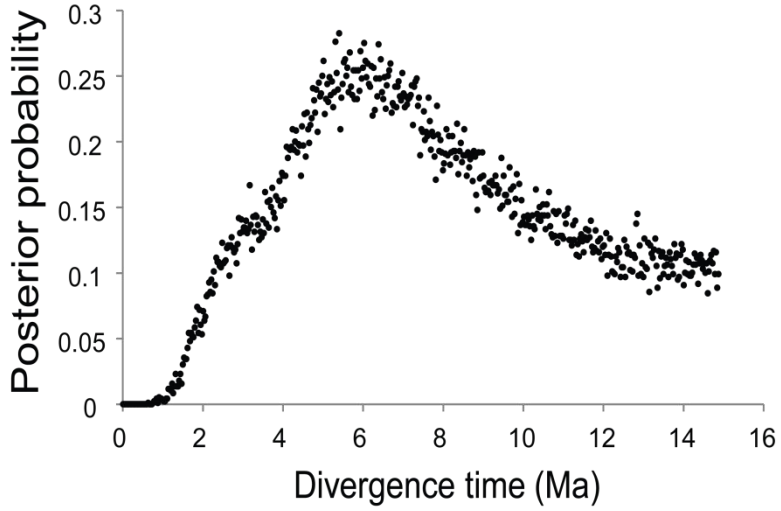

Figure 2.5. Posterior probability distribution for divergence times between western North Atlantic and Mediterranean populations of *Oculina* spp. Divergence time= 5.4 ± 2.0 million years ago.

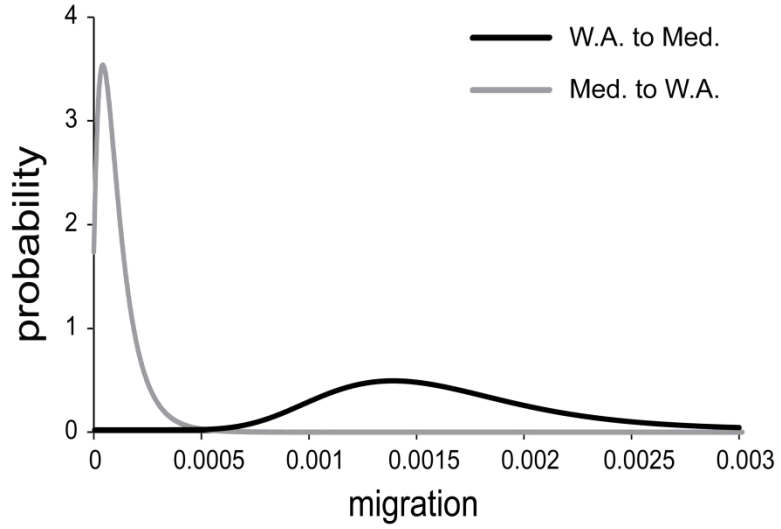

Figure 2.6. Posterior probability distributions for migration between western North Atlantic and Mediterranean populations of *Oculina* spp. Posterior probability distribution for migration (average number of migrants per 1000 generations) scaled by neutral mutation rate between western North Atlantic and Mediterranean populations of *Oculina* spp. Migration from the western North Atlantic to the Mediterranean was 0.00139 (90% highest posterior density interval= 0.0008–0.0.0024). Migration from the Mediterranean to the western North Atlantic was 0.000042 (0–0.002).

Table 2.3. Evidence ratios and ranks of all possible isolation with migration models. Evidence ratios and ranks calculated using model-based selection. For each model, the first three letters represent the three population parameters (θ_1, θ_2, and ancestral θ), and the last two letters represent the two migration parameters (m_1 and m_2), in that order. The best model is the first model listed (ABADE) followed by the next best models in descending order.

Model	k	log(P)	AIC	Δi	Model Likelihoods	wi	Evidence Ratio (best model)
ABADE	4	-1.770	11.540	0.000	1.000	0.364	
ABC0D	4	-1.953	11.906	0.366	0.833	0.303	1.200
FULL	5	-1.446	12.891	1.351	0.509	0.185	1.965
ABBDE	4	-2.723	13.446	1.906	0.386	0.140	2.593
ABADD	3	-7.920	21.840	10.300	0.006	0.002	172.431
ABBDD	3	-8.351	22.702	11.163	0.004	0.001	265.416
ABCDD	4	-7.358	22.715	11.175	0.004	0.001	267.094
AAADE	3	-8.398	22.795	11.255	0.004	0.001	277.994
AACDE	4	-7.866	23.731	12.192	0.002	$<10^{-3}$	443.989
AACDD	3	-10.835	27.671	16.131	$<10^{-3}$	$<10^{-3}$	3182.429
AAADD	2	-12.542	29.084	17.545	$<10^{-3}$	$<10^{-3}$	6452.997
ABCD0	4	-195.238	398.476	386.936	$<10^{-50}$	$<10^{-50}$	$<10^{50}$
ABC00	3	-228.380	462.760	451.220	$<10^{-50}$	$<10^{-50}$	$<10^{50}$
ABA00	2	-234.240	472.480	460.941	$<10^{-100}$	$<10^{-100}$	$<10^{100}$
ABB00	2	-249.428	502.855	491.315	$<10^{-100}$	$<10^{-100}$	$<10^{100}$
AAC00	2	-263.32	530.648	519.108	$<10^{-100}$	$<10^{-100}$	$<10^{100}$
AAA00	1	-272.668	547.337	535.797	$<10^{-100}$	$<10^{-100}$	$<10^{100}$

2.4.3. The Fossil Record

We explored online databases of museum collections for a fossil record of *Oculina* spp. in the eastern Atlantic and/or the Mediterranean. We found 16 fossil specimens of *Oculina* spp. in two independent museum collections: the Smithsonian National Museum of Natural History's Department of Invertebrate Zoology (Appendix A, Figure 2.S1), and the Muséum National d'Histoire Naturelle Paléontologie. All specimens originated from north-northwestern France. The estimated ages of the specimens range broadly, with most from the Eocene (56–34 Ma) to the Miocene (23.03–5.332 Ma)(Table 2.4).

2.4.4. Population Expansion within the Mediterranean

To test for a past population expansion within the Mediterranean Sea, we used LAMARC 2.0 (Kuhner 2006). The overall population growth rate across all genes and replicates was -65, indicating that the population has not been expanding. We treated the Mediterranean as a single population, since we did not detect significantly differentiated populations within the Mediterranean (Figure 2.4c). We also performed analyses on the three Mediterranean populations with the largest sampling sizes (Spain, Greece, and Israel) separately, and found similar results.

2.5. DISCUSSION

2.5.1. *Oculina patagonica* has not been Recently Introduced into the Mediterranean

Our data show that Mediterranean populations of *O. patagonica* are genetically distinct from WA *Oculina* spp. populations (Figure 2.4a). While *Oculina* spp. populations from either side of the Atlantic share many alleles for all markers, there were notable private alleles for both regions (Figure 2.2). Contrary to expectations for a recently introduced and expanding species, Mediterranean *O. patagonica* harbors genetic diversity on par with long-established WA *Oculina* spp. populations (Figure 2.3a). Our IMa results reveal that *O. patagonica* and WA *Oculina* spp. populations diverged millions of years ago (Figure 2.5). Taken together, our results suggest that while *O. patagonica* populations from the Mediterranean are closely related to WA *Oculina* spp. populations, they are genetically differentiated from them and have not been introduced into the Mediterranean from the western North Atlantic in anthropogenic times. Although we did not include *Oculina* spp. samples from the Caribbean, Bermuda likely represents the Caribbean given that in other broadcast spawning corals little genetic variation has been found between the Caribbean and the western Atlantic (Goodbody-Gringley *et al.* 2012).

While there are many similar examples of misidentified native species (Concepcion *et al.* 2010; Taylor & Bothwell 2014; Wares *et al.* 2002), and see below, contrary to our findings for *O. patagonica*, many species have been introduced into the Mediterranean. A well-known example is the green alga, *Caulerpa taxifolia* (Meinesz & Boudouresque 1996). Using nuclear sequence data, Jousson *et al.* (1998) determined that this species was introduced into the Mediterranean from an aquarium in Monaco, which maintained an algal strain of unknown geographical origin cultivated in western European aquaria. Mitochondrial sequence data was used to determine that a Mediterranean clade of sea squirts, *Clavelina lepadiformis*, was recently introduced from

Table 2.4. Museum records of fossil specimens of *Oculina* spp. from the eastern Atlantic.

Museum	Record number	Species	Location	Epoch or Age
Smithsonian National Museum of Natural History	USNM 64539	*Oculina* sp.	Indre-et-Loire, France	Eocene–Serravallian
	USNM I 80806	*Oculina crassoramosa*	France	Miocene
	USNM I 80807	*Oculina crassoramosa*	France	Miocene
	USNM I 80808	*Oculina solanderi*	Oise, France	Lutetian
	USNM I 80809	*Oculina raristella*	France	Eocene
	USNM I 80810	*Oculina* sp.	Oise, France	Lutetian
	USNM I 80811	*Oculina* sp.	Seine-et-Oise, France	Lower Lutetian
	USNM I 80812	*Oculina* sp.	Eure, France	Lutetian
Muséum National d'Histoire Naturelle	MNHN-F-M00169	*Oculina gemmata*	Calvados, France	Bathonian
	MNHN-F-M00170	*Oculina neustriaca*	Calvados, France	Bathonian
	MNHN-F-M00326	*Oculina crassoramosa*	Indre-et-Loire, France	Langhian
	MNHN-F-M00598	*Oculina crassoramosa*	Indre-et-Loire, France	Langhian
	MNHN-F-M00675	*Oculina raristella*	Oise, France	Lutetian
	MNHN-F-M00745	*Oculina crassoramosa*	Indre-et-Loire, France	Langhian
	MNHN-F-M00749	*Oculina crassoramosa*	Indre-et-Loire, France	Langhian
	MNHN-F-M01113	*Oculina explanata*	Sarthe, France	Cenomanian

eastern Atlantic populations (Turon *et al.* 2003). The Mediterranean Sea has experienced an influx of introduced species in recent decades (Galil 2009), which has been attributed to increased sea temperatures, along with coincident range expansions of introduced species and range shifts of native ones (Lejeusne *et al.* 2010).

2.5.2. Where did *O. patagonica* Originate?

The original hypothesis for the origin of Mediterranean *O. patagonica* suggested that, based on its identification, it must have been introduced from South America, where the only evidence (fossils) of this species existing outside the Mediterranean resides (Zibrowius 1974). However, reports of live specimens of *O. patagonica* in South America are lacking, and a recent survey of fouling communities in Argentinian ports failed to find any evidence of this species (Schwindt *et al.* 2014). If living *O. patagonica* are not present in the western South Atlantic, they could not have been recently introduced into the Mediterranean from this region. Although it is possible that *O. patagonica* still resides in the western South Atlantic in low undetected numbers or habitats, there are no grounds to suggest *O. patagonica* recently originated from South America until (if) those specimens are found.

If *O. patagonica* has not recently travelled east across the Atlantic to the Mediterranean, then where did it originate? We found records for 16 fossil specimens of *Oculina* spp. from France (Table 2.4). Along with these multiple records from two museums, *Oculina* spp. fossils have also been reported from the Danish Basin during the Middle Danian (about 64 Mya) (Bernecker & Weidlich 2005) and south Aquitaine, France, during the Late Oligocene (about 25 Mya) (Cahuzac & Chaix 2009). Although all of these fossils originated from outside of the Mediterranean, they suggest that the genus *Oculina* has long been present in the eastern North Atlantic.

A long presence in the eastern Atlantic is consistent with our genetic data, which suggest that the western North Atlantic and Mediterranean populations diverged 5.4 ± 2.0 million years ago (Figure 2.5). This coincides with the Late Miocene Messinian Salinity Crisis (5.33 Ma), when sea levels in the Mediterranean basin dropped, separating it from the Atlantic and killing off many marine species (Hsü *et al.* 1973; Krijgsman *et al.* 1999). The asymmetric migration in the history of *Oculina* spp. (Figure 2.6), with a greater inferred migration from the western North Atlantic to the Mediterranean, may reflect the repopulation of the Mediterranean with Atlantic aquatic fauna following the Messinian Salinity Crisis (Hsü *et al.* 1973; Krijgsman *et al.* 1999; Patarnello *et al.* 2007). However, an ancient introduction would likely leave behind a fossil record, and we found no *Oculina* spp. fossils from within the Mediterranean. This may be due to undiscovered or undocumented fossils, but could also indicate that *O. patagonica* was more recently introduced from elsewhere, likely the eastern North Atlantic (Wangensteen *et al.* 2012). Our finding of no genetic structure within the Mediterranean also suggests that it may not have an ancient presence there. Further survey efforts are needed to determine whether extant *Oculina* spp. populations exist in the eastern North Atlantic, and whether they are the source of *O. patagonica*.

Another hypothesis for the origin of *O. patagonica* lies along the western coast of Africa. *Schizoculina africana* has both a fossil and living presence in Cape Verde (Boekschoten & Best

1988; Monteiro *et al.* 2008). Originally known as *Oculina africana*, this species was split to form a new genus (*Schizoculina)* due largely to a unique way in which polyps bud (Wells 1937; Zibrowius 1974). However, dual modes of budding have been reported within a single coral species (Kai & Sakai 2008) and may therefore not be a good diagnostic trait to differentiate species. Future genetic work is needed to investigate whether *Schizoculina africana* and *Oculina patagonica* are in fact conspecific, and whether *O. patagonica* originated from the northwestern coast of Africa.

Oculinidae is a taxonomically confused family (Fukami *et al.* 2004; Kitahara *et al.* 2010) in need of a more in depth genetic study to better understand the relationships between and within the genera and species in this family. As indicated by mitochondrial and nuclear genes, Oculinidae is paraphyletic, and *Oculina* is more closely related to some members of different families (Faviidae, Caryophylliidae, and Rhizangiidae) than to some members of its own family. Thus, representatives from some extra-familial genera with which *Oculina* has sometimes been allied (*Astrangia* of the Rhizangiidae, *Cladocora* of the Caryophylliidae) (Zibrowius 1974) should also be included in future efforts to trace the origins and taxonomic classification of *Oculina patagonica*.

2.5.3. The Geographical Expansion of *O. patagonica* in the Mediterranean

Direct observations testify to *O. patagonica's* increase in abundance at shallow depths at many localities in the Mediterranean over the past 20 years (Salomidi *et al.* 2013; Serrano *et al.* 2013). Along the Catalan coast, the species spread from just one location in 1992 to 43 by 2010, a rate of northward expansion of 22 km per year (Serrano *et al.* 2013). In 2005, a few colonies of *O. patagonica* were first reported from a single site in the Saronikos Gulf of the Aegean Sea (Salomidi *et al.* 2006). By 2009, *O. patagonica* could be found in 45 of 54 surveyed sites in this region (Salomidi *et al.* 2013).

Fine *et al.* (2001) proposed that *O. patagonica* has been expanding west to east, just as first reports of its presence have. Our tests, however, did not detect a genetic signal of expansion across the Mediterranean. While this may result from low power, the proposed west to east spread is also opposite to most other range expansions in the Mediterranean, which have occurred in a north-westward direction in response to increasing sea temperature (Lejeusne *et al.* 2010). Because in the Mediterranean temperature increases from east to northwest, and rising temperatures have been proposed to be promoting the range expansion of *O. patagonica* (Serrano *et al.* 2013), a west to east expansion would be contrary to expectation, unless it was introduced into the western Mediterranean Sea, which our tests failed to support. Furthermore, some recent first reports have come from the western Mediterranean (Sartoretto *et al.* 2008), and *O. patagonica* was first reported from the Levant prior to Greece (Bitar & Zibrowius 1997; Fine & Loya 1995; Salomidi *et al.* 2006). Finally, if *O. patagonica* was first established in the western Mediterranean and only more recently in the east, then the western populations would likely harbor more genetic diversity; however, we found similar levels of genetic diversity across the Mediterranean. Alternatively, *O. patagonica* could be moving into the Mediterranean from elsewhere in the eastern North Atlantic, but in sufficient numbers to not leave a genetic signature of expansion.

Despite lack of evidence for a demographic expansion from west to east, it appears that *O. patagonica*'s invasive behavior may have "expanded" west to east. Serrano *et al.* (2013) report an expansion along the Spanish coast from 1992-2010. Salomidi *et al.* (2013) reported a later spread along the coast of Greece from 2005-2009. While this eastward trend may owe to chance, it could also be due to human-mediated modifications of shallow coastal habitats occurring earlier in the west, or limiting conditions in the east (Fine *et al.* 2001) that populations have adapted or acclimated to overtime (Armoza-Zvuloni *et al.* 2011).

2.5.4. *O. patagonica* is Native Species Recently Turned Invasive

It seems most likely that *O. patagonica* has always existed somewhere in the eastern Atlantic and has recently become invasive in the Mediterranean, expanding in local regions in response to environmental change (Lejeusne *et al.* 2010), likely mediated by human-modifications of coastal habitats (Salomidi *et al.* 2013; Serrano *et al.* 2013). In a similar way, the snowflake octocoral, identified in Hawai'i as *Carijoa riisei*, was believed to have been recently introduced from its native range in the Caribbean. However, Concepcion *et al.* (2010) used mitochondrial and nuclear sequence data to compare the Hawaiian populations to worldwide populations of *Carijoa* and found that the Hawaiian populations were not genetically similar to the Caribbean and therefore did not originate from there. The originally misidentified native diatom *Didymosphenia geminata* remained undetected in its native range for decades before blooms were documented in the 1990s (Bothwell *et al.* 2014). Today, this native invasive alga is rapidly expanding locally in response to environmental changes (Bothwell *et al.* 2014; Taylor & Bothwell 2014). The gastropod, *Littorina littorea*, has long been thought to have been recently introduced to North American from Europe. However, both mitochondrial and nuclear sequence data indicated that the North American and European populations diverged thousands of years ago. This native gastropod is also believed to have begun expanding along the coast of New England as a result of environmental changes (Wares *et al.* 2002).

Identifying additional mechanisms that facilitate invasiveness in *O. patago*nica will require work aimed at better identifying and characterizing the source populations and population dynamics of well documented locally expanding *O. patagoncia* populations within the Mediterranean, such as along the coasts of Spain (Serrano *et al.* 2013) and Greece (Salomidi *et al.* 2013). The conditions at these invasion localities can then be compared to conditions where *O. patagonica* exits but is not to date invasive to better understand the mechanisms driving its expansion. Such studies may also aid in assessing the future of the newly discovered coral species, *Oulastrea crispate*, in the Mediterranean as it too is expected to rapidly expand its range (Hoeksema & Vicente 2014).

Additional studies are also needed to better understand the ecological consequences of the expansion of *Oculina patagonica*. While marine range shifts may occur at a slower rate than marine introductions, their potential effects on the community are likely to be just as significant (Sorte *et al.* 2010). *O. patagonica* has been shown to successfully compete with bryozoan *Watersipora* sp. (Fine & Loya 2003). Serrano *et al.* (2012) have reported a shift from macroalgal to *O. patagonica* dominance in the Mediterranean. Given that macroalgae are important primary producers, this shift may result in significant changes in ecosystem functioning.

Corals are currently facing worldwide declines as a result of stresses, including increasing sea temperatures, disease, and other anthropogenic disturbances (Aronson *et al.* 2003; Hoegh-Guldberg *et al.* 2007; Pandolfi *et al.* 2003). Understanding the factors and characteristics that promote resilience in *O. patagonica* in the midst of environmental change may shed light into assessing and managing the long-term success of corals that are currently at risk.

2.5.5. Conclusions

Despite years of maintaining that *Oculina patagonica* is a recently introduced coral species in the Mediterranean, we found no genetic or historical demographic evidence to support that claim. Our results suggest that Mediterranean populations of *O. patagonica* have long been isolated from WA *Oculina* spp., and have only recently become invasive in the Mediterranean, most likely due to environmental changes. We advise against hastily identifying a previously unknown species as being introduced without detailed genetic analyses and comparisons to potential source populations. Accurate identification of species' invasive statuses will enable more effective research programs aimed at better understanding the mechanisms promoting the invasive nature of species, which can then lead to the implementation of efficient management plans.

2.6. AVAILABILITY OF DATA

Haplotypes for *COI*, p14, p62, and p302 for western North Atlantic *Oculina* spp. populations obtained by Eytan *et al.* (2009) are available on GenBank [FJ966395–FJ966875]. Haplotypes generated here are available on The European Nucleotide Archive [LN613417–LN614380].

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CHAPTER 3.
DISCORDANT CORAL–SYMBIONT STRUCTURING: FACTORS SHAPING GEOGRAPHICAL VARIATION OF *SYMBIODINIUM* COMMUNITIES IN A FACULTATIVE ZOOXANTHELLATE CORAL GENUS, *OCULINA*[1]

3.1. ABSTRACT

Understanding the factors that help shape the association between corals and their algal symbionts, zooxanthellae (*Symbiodinium*), is necessary to better understand the functional diversity and acclimatization potential of the coral host. However, most studies focus on tropical zooxanthellate corals and their obligate algal symbionts, thus limiting our full comprehension of coral-algal symbiont associations. Here, we examine algal associations in a facultative zooxanthellate coral. We survey the *Symbiodinium* communities associated with *Oculina* corals in the western North Atlantic and the Mediterranean using one clade-level marker (*psbA* coding region) and three fine-scale markers (*cp23S–rDNA*, *b7sym15* flanking region, and *b2sym17*). We ask whether *Oculina* spp. harbor geographically different *Symbiodinium* communities across their geographic range and, if so, whether the host's genetics or habitat differences are correlated with this geographical variation. We found that *Oculina* corals harbor different *Symbiodinium* communities across their geographical range. Of the habitat differences (including chlorophyll *a* concentration and depth), sea surface temperature is better correlated with this geographical variation than the host's genetics, a pattern most evident in the Mediterranean. Our results suggest that although facultative zooxanthellate corals may be less dependent on their algal partners compared to obligate zooxanthellate corals, the *Symbiodinium* communities that they harbor may nevertheless reflect acclimatization to environmental variation among habitats.

3.2. INTRODUCTION

Coral colonies constitute a partnership between many species. The coral animal itself, its endosymbiotic algae, and its resident microbes compose the coral holobiont (Bourne *et al.* 2009; Rosenberg *et al.* 2007). Photosynthetic algae of the genus *Symbiodinium* contribute nutritionally to the coral (Falkowski *et al.* 1984; Muscatine & Porter 1977) and enhance calcification (Tambutté *et al.* 2011). Bleaching, a disruption of the relationship between the coral and its algae, is associated with a nutritionally depleted coral with impaired reproduction and increased susceptibility to disease and mortality (Glynn 1984; Harvell *et al.* 2002; Szmant & Gassman 1990). Assessing intraspecific coral-algal pairings and the factors shaping this association is crucial to better understanding the functional diversity and adaptive potential of the holobiont (Parkinson & Baums 2014).

The genus *Symbiodinium* is comprised of nine distinct phylogenetic clades (A–I), with numerous types or strains designated within each clade (Pochon & Gates 2010; Pochon *et al.* 2014). Some

[1] This chapter previously appeared as Leydet, K. P., and M. E. Hellberg (2016) Discordant coral-symbiont structuring: factors shaping geographical variation of *Symbiodinium* communities in a facultative zooxanthellate coral genus, *Oculina*. *Coral Reefs*. It is reproduced with permission of the publisher (Appendix F).

subtypes, approximating species-level designations, have also been described (LaJeunesse *et al.* 2012; Parkinson *et al.* 2015). Coral-*Symbiodinium* associations vary in their degree of specificity. At one extreme, the relationship between coral and algal symbionts may be highly specific and stable through space and time (Bongaerts *et al.* 2010; Pinzon & LaJeunesse 2011; Prada *et al.* 2014). Alternatively, the algal symbionts may vary geographically within species (Keshavmurthy *et al.* 2012; LaJeunesse *et al.* 2004), including by depth (Toller *et al.* 2001). Variation in the *Symbiodinium* communities found associated with different coral species may reflect differences in environmental conditions endured by the holobiont, including seasonal fluctuations in temperature (Chen *et al.* 2005; Thornhill *et al.* 2006b), recovery from bleaching events (Jones *et al.* 2008; Thornhill *et al.* 2006b), anthropogenic thermal stressors (Keshavmurthy *et al.* 2012), and irradiance variation (Finney *et al.* 2010; Toller *et al.* 2001). This is because *Symbiodinium* clades and even types vary in their thermal tolerance (McGinty *et al.* 2012; Tchernov *et al.* 2004) and photophysiology (Iglesias-Prieto & Trench 1997; Reynolds *et al.* 2008). These functional differences can affect the holobiont. For example, corals harboring heat-tolerant *Symbiodinium* have a higher bleaching threshold temperature than do counterparts harboring less thermal-tolerant *Symbiodinium* (Berkelmans & Van Oppen 2006; Howells *et al.* 2012).

The particular host-symbiont pairings best suited to local conditions depend not only on the environmental tolerance of the *Symbiodinium*, but also on the life history characteristics of the coral. Brooding corals generally acquire their zooxanthellae from their parent (vertically), while broadcast spawning corals typically acquire their symbionts from the environment (horizontally) (Baird *et al.* 2009; Stat *et al.* 2006). However, the expectations of highly stable and specific associations in the former case and more dynamic associations in the latter (LaJeunesse *et al.* 2004) are not always met (Pettay *et al.* 2011; Stat *et al.* 2009; Stat *et al.* 2013).

Unlike obligate zooxanthellate corals, facultative zooxanthellate corals can persist in a healthy azooxanthellate state. To date, studies have largely focused on obligate tropical zooxanthellate corals and their algal symbionts. For facultative zooxanthellate corals, symbiont types may not be strongly associated with host genetics or environmental conditions because these corals likely do not strongly depend on locally adapted algal symbionts. On the other hand, this lack of dependence between facultative zooxanthellate corals and their symbionts may allow these corals and the symbionts the time and opportunity to be more selective in their associations.

Corals of the genus *Oculina* provide an opportunity to test the determinants of algal associations in a facultative zooxanthellate coral. *Oculina* corals are gonochoristic broadcast spawners (Brooke & Young 2005; Brooke & Young 2003; Fine *et al.* 2001) that acquire their zooxanthellae horizontally. They occur in the western North Atlantic as well as across the Mediterranean Sea. Studies utilizing low-resolution markers and a limited number of populations revealed that *Oculina* corals in the western North Atlantic and the Mediterranean associate with *Symbiodinium* type B2 (LaJeunesse *et al.* 2012; Rodolfo-Metalpa *et al.* 2014; Rubio-Portillo *et al.* 2014a; Thornhill *et al.* 2008), while type B1 has only been reported from a single *Oculina* sp. colony in the western North Atlantic (LaJeunesse 2001). Patterns of *Symbiodinium* communities across *Oculina*'s trans-Atlantic distribution remain to be explored. This is of particular interest given the wide and variable environmental conditions endured by these corals, including water temperatures varying from 13 to 30˚C across their geographic range, and even within a single

location (Armoza-Zvuloni *et al.* 2011; Fine *et al.* 2001; Rosenberg & Ben-Haim 2002; Rubio-Portillo *et al.* 2014c).

Here, we survey the *Symbiodinium* communities associated with *Oculina* corals in the western North Atlantic and the Mediterranean and investigate patterns of *Symbiodinium* intraspecific diversity harbored by these corals using one clade-level marker and three fine-scale markers. We ask whether *Oculina* spp. harbor geographically different *Symbiodinium* communities across their range and, if so, whether the host's genetics or habitat differences in sea surface temperature, chlorophyll *a* concentration, or depth are correlated with this geographical variation.

3.3. METHODS

3.3.1. Sampling and Genotyping

Previous work has shown that shallow (<30 m) temperate western North Atlantic *Oculina* spp. are genetically distinct from *O. patagonica* in the Mediterranean, despite sharing many alleles across five nuclear sequence markers totaling 1002 base pairs (bp) (Leydet & Hellberg 2015). *Oculina* spp. colonies were sampled from five localities in the western North Atlantic (Figure 3.1; Appendix B, Table 3.S1) and include three nominal species (*O. arbuscula*, *O. varicosa*, and *O. diffusa*), although previous genetic work suggests that these morpho-species are not genetically distinct (Eytan *et al.* 2009). *O. patagonica* samples were collected from five localities spanning their Mediterranean distribution (Figure 3.1; Appendix B, Table 3.S1). Colonies were sampled by breaking off a 2-cm^2 piece of live tissue and preserving it in 95% ethanol.

We extracted genomic DNA from the samples using the QIAGEN DNeasy Kit following the manufacture's protocols with the following modifications. We allowed the tissues to lyse at 56˚C overnight, and we added 200 µl AE elution buffer and incubated at room temperature for an hour prior to the final centrifugation step.

To identify the haploid *Symbiodinium* clade(s) present in our samples, we genotyped all individuals for the *psbA* minicircle coding region following previous protocols (Barbrook *et al.* 2006). To investigate fine-scale *Symbiodinium* diversity, population subdivision, and symbiont associations, we genotyped all samples for one chloroplast marker (*cp23S–rDNA*) and two nuclear markers (microsatellite *b7sym15* flanking region and microsatellite *b2sym17*) (Table 3.1). All polymerase chain reaction (PCR) amplifications were conducted in 25µl reactions consisting of 2.5µl of 10× buffer (containing 15mM $MgCl_2$), 2µl of dNTPs (2.5mM), 1µl of each primer (10µM), 0.2µl of One TaqTM DNA polymerase (New England Biolabs Inc.), and 1µl of template DNA. The PCR conditions for all primer pairs consisted of an initial denaturation at 94°C for 3 min, an initial annealing for 2 min, and an initial elongation at 72°C for 2 min, followed by 35 cycles at 94°C for 35 sec, annealing for 1 min, and 72°C for 1 min 15 sec, and a final elongation at 72°C for 10 min. The annealing temperature for a and b regions of *cp23S–rDNA domain V* (Santos *et al.* 2003) was 50˚C. For the few samples for which the above *cp23S* amplification failed, we used alternative primers (Santos *et al.* 2002); annealing temperature 55˚) that amplify a larger portion of the *cp23S–rDNA domain V*. Annealing temperature for the flanking region of the microsatellite marker *b7sym15* (Pettay & Lajeunesse 2007) was 53˚C. For

the microsatellite marker *b2sym17* (Grupstra *et al.* unpublished data), annealing temperatures were 55–59 ˚C. We designed a new forward primer (B2SYM17F2: 5′ GGCAACAATCATATTGACTAGGCC 3′) to amplify *b2sym17* for individuals that failed under the above conditions.

Table 3.1. Markers used to genotype *Symbiodinium* associated with *Oculina* spp. in this study.

Marker	Primers	Reference	Size (bp) [a]
psbA coding region	IA2F IA2R	Barbrook *et al.* 2006	334
cp23S-rDNA domain V (areas a and b)	23SHYPERUP 23SHYPERDNM13	Santos *et al.* 2003	134–182
cp23S-rDNA domain V	23S1M13 23S2M13	Santos *et al.* 2002	134–182[b]
b7sym15 flanking region	B7SYM15F B7SYM15R	Pettay & Lajeunesse 2007	126–145
b2sym17	B2SYM17F, F2 B2SYM17R	Grupstra *et al.* unpublished	27–41

[a] final cropped alignment
[b] cropped to length of *cp23s-rDNA domain V* (areas a and b)

Sequencing was performed using BigDye chemistry v3.1 on an ABI 3130XL at the Louisiana State University Genomics Facility. Sequences were aligned and edited in GENEIOUS 4.5.5 (Drummond *et al.* 2010). Samples were sequenced for *psbA* and *cp23S* in both directions. Preliminary sequencing revealed poor sequence reads through hypervariable repeat regions in *b7sym15* (*n*=34) and *b2sym17* (*n*=26), so most of these samples were sequenced in one direction only (forward for the former, and reverse for the latter).

We cloned a subset of samples for *cp23S* (*n*=15), *b7sym15* (*n*=18), and *b2sym17* (*n*=16) using the Invitrogen TOPO TA kit. This allowed us to resolve haplotypes and validate the haplotype diversity scored from our sequencing efforts. Therefore, we chose a representative subset of samples for each marker that included samples that appeared to contain a mixture of haplotypes as well as some that only contained a single haplotype. We also chose samples that represented the haplotype diversity based on our sequencing efforts. At least six clones per reaction were sequenced in a single direction to identify the haplotypes present in a sample. Two putative haplotypes were scored as distinct if they represented at least 25% of the sequenced clones, because we were only interested in detecting the most common strain(s), not rare diversity. Because *Symbiodinium* are haploid, multiple haplotypes represent coexisting strains within an individual coral. Our cloning efforts corroborated our genotyping via unidirectional sequencing. Five samples (Daytona Beach=2; Panama City=2; and Israel=1) failed to amplify for at least one marker and were therefore not included in the final dataset of 117 individuals (Appendix B, Table 3.S1).

The *psbA* minicircle coding region was nearly invariant among all samples (see below) and was therefore used solely for clade identification by comparison with published sequences (Barbrook *et al.* 2006). Because *cp23S* and microsatellite *b7sym15* flanking region have been utilized in other studies, sequence data for these loci are readily available. This allowed us to perform

nucleotide BLAST (Altschul *et al.* 1990) searches for all haplotypes for these two loci on the NCBI website (http://www.ncbi.nlm.nih.gov) to identify the types of *Symbiodinium* present in our samples. Here, we only report matches with 100% coverage and 100% identity to *Symbiodinium*-type published sequences (see Appendix B, Tables 3.S2 and 3.S3 for all BLAST results).

3.3.2. Geographical Differentiation

We constructed haplotype networks for *cp23S* and *b7sym15* using statistical parsimony implemented in TCS 1.21 (Clement *et al.* 2000). We specified maximum connection steps = 60, and treated gaps as a fifth state. We constructed a haplotype network for *b2sym17* manually by separating each haplotype by repeat number variation. The geographic distributions of these haplotypes were then mapped onto the range. To more quantitatively explore whether *Symbiodinium* communities are geographically differentiated, we performed Analyses of Molecular Variance (AMOVA) implemented in GENODIVE 2.0b27 (Meirmans & Van Tienderen 2004) for all populations combined and for western North Atlantic and Mediterranean populations separately. We also used a Bayesian clustering analysis implemented in STRUCTURE 2.3.4 (Pritchard *et al.* 2000), and the Evanno method (Evanno *et al.* 2005) implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012) to detect significantly differentiated populations (K). We first analyzed all populations together, testing a range of K from 1–10, and then analyzed the western North Atlantic and Mediterranean populations separately (K=1–6). We ran the program for 1 million MCMC steps and discarded the first 500,000 steps as burn-in. We used the more conservative admixture model with uncorrelated allele frequencies. We performed 10 iterations for each K.

3.3.3. Symbiont-host Associations

To test whether the pattern of differentiation we found for *Symbiodinium* was driven by coassociation with similarly differentiated hosts, we compared multi-locus genotypes of the algae to those of their coral host, which consisted of five variable nuclear genes totaling 1002 bp (Leydet & Hellberg 2015). We collapsed the host and symbiont multi-locus genotypes separately into bi-allelic locus genotypes, such that each unique multi-locus genotype had a unique two-digit identifier represented twice to mimic a diploid locus. We did this to meet the format requirements of the program GENEPOP On The Web (Raymond & Rousset 1995; Rousset 2008), which we used to perform genotypic linkage disequilibrium (option 2) to test whether the genotypes at one locus (host's collapsed multi-locus genotypes) are independent from the genotypes at the other locus (symbiont's collapsed multi-locus genotypes).

To examine host-symbiont specificity at a broader scale, we compared the genetic clustering of *Symbiodinium* to the clustering of their coral host to see whether specific algal clusters are associated with specific host clusters. We ran STRUCTURE using the multi-locus genotypes for the hosts, whose algal symbionts we genotyped, using the same parameters as in Leydet & Hellberg (2015). We also used BARRIER version 2.2 (Manni *et al.* 2004), which implements an algorithm using pairwise F_{ST}, to more objectively identify and subsequently compare primary genetic barriers for both the coral host and algal symbiont (see Appendix B, Figure 3.S1 for additional information).

3.3.4. Environmental Correlations of Symbiont Community Composition

To test whether geographical variation in patterns of *Symbiodinium* communities was correlated with habitat differences, we tested for associations between *Symbiodinium* community and three environmental variables: sea surface temperature, chlorophyll *a* concentration, and depth. We chose to investigate these factors because of their relatively easy accessibility, and/or because they have been previously shown to affect *Symbiodinium* community composition (see Introduction).

We used STRUCTURE's output for the most likely number of genetic clusters for *Symbiodinium* (K=3; Figure 3.2) and calculated the average probability of assignment to each genetic cluster for each population. We then plotted these assignment probabilities against four measures of temperature and chlorophyll *a* concentration for each location: at time of sampling, average annual, minimum annual, and maximum annual obtained from the NASA Earth Observations website (http://neo.sci.gsfc.nasa.gov/view.php?datasetId=MYD28M). The annual values were obtained for the year prior to sampling at each location, as these are the ranges of temperature and chlorophyll *a* concentration that the colonies endured most recently prior to being sampled and therefore likely have the greatest effect, if any, on *Symbiodinium* community composition. We also plotted assignment probabilities against the average, minimum, and maximum depths at which the colonies were sampled within each location (Appendix B, Table 3.S1).

We examined whether fine-scale genetic clustering was correlated with habitat differences by analyzing the western North Atlantic and Mediterranean populations separately. For these analyses, we used the STRUCTURE results obtained when the two ranges were analyzed separately. We used the greatest number of genetic clusters that was geographically informative (K=3 for each; Figures 3.3b and 3.3d).

We tested for significant correlations between genetic cluster assignments and environmental variables in GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA). We performed multiple comparisons corrections according to the method described by Benjamini & Hochberg (1995).

3.4. RESULTS

The *psbA* coding region was nearly invariant, with 87% of the samples sharing the same 334 bp haplotype. This most common haplotype matched (100%) previously published *Symbiodinium* clade B sequences sampled from *Bunodeopsis strumosa* from France (accession number AJ884900), *Diploria labyrinthiformis* from Bermuda (AJ884898), and *Madracis decactis* from Bermuda (AJ884908) (Barbrook *et al.* 2006). The remaining haplotype variants included all samples from Daytona Beach (which differed from the most common haplotype by a single bp mutation), two samples from Cape Florida (which differed by an 83 bp deletion), and two Bermuda samples (which differed by a single bp mutation different from the one found in Daytona Beach). These haplotypes all closely matched clade B sequences, thus confirming that all of our coral samples harbored *Symbiodinium* from this clade.

At a finer level of resolution, BLAST searches of *cp23S* and *b7sym15* haplotypes indicated that most haplotypes matched type B2, and in some cases more specifically *Symbiodinium psygmophilum* (within B2). However, some haplotypes from Cape Florida and Bermuda matched type B1, and in some cases more specifically *S. minutum* (within B1) (Figure 3.1; Appendix B, Tables 3.S2 and 3.S3).

3.4.1. Genetic Diversity

Oculina spp. colonies from the western North Atlantic harbored greater *Symbiodinium* diversity both within and between populations than did the Mediterranean populations (Figure 3.1). This is reflected by both the number of haplotypes and the presence of both types B2 and B1 in the western North Atlantic, while Mediterranean populations only harbored type B2. Eastern Mediterranean populations harbored slightly greater *Symbiodinium* diversity than western ones (Figure 3.1). All Spanish and Italian colonies (n=16) harbored a single *Symbiodinium* genotype, whereas Greece, Lebanon, and Israel (total n=49) harbored a total of nine *Symbiodinium* genotypes that varied within and across sampling locations and, in a few cases, within colonies.

3.4.2. Geographical Differentiation

We used haplotype networks (Figure 3.1), AMOVAs (Table 3.2), and STRUCTURE (Figures 3.2 and 3.3) to examine whether *Oculina* corals harbor geographically distinct *Symbiodinium* communities across their range. AMOVA tests revealed significant subdivision of symbionts among all populations considered together (Table 3.2a). In contrast to their coral hosts (Leydet & Hellberg 2015), algal populations from the western North Atlantic were not significantly subdivided from Mediterranean populations. However, when Spain and Italy (the two westernmost Mediterranean populations) were grouped with the western North Atlantic populations, this larger group was differentiated from the eastern Mediterranean populations (Table 3.2a), suggesting that algal communities in the western Mediterranean are more genetically similar to those in the western North Atlantic. AMOVAs performed for the western North Atlantic and Mediterranean populations separately revealed significant subdivision among populations within both of these regions (Table 3.2b).

When all populations were analyzed together using STRUCTURE, the most likely K was three (Figure 3.2). While geographical differentiation of the *Symbiodinium* communities was evident, there was no clear break between the western North Atlantic and Mediterranean populations as seen in the coral host (Figure 3.2). Instead, the western Mediterranean populations were genetically distinct from the eastern Mediterranean, and they were more genetically similar to the western North Atlantic.

When the western North Atlantic populations were analyzed separately, the most likely K was two, with most algal genotypes from Cape Florida and Bermuda falling into a separate cluster from the rest of the western North Atlantic (Figure 3.3a). When K=3, Cape Florida and Bermuda were comprised of two admixed genetic clusters and harbored the most diversity (Figure 3.3b). When the Mediterranean populations were analyzed alone, the most likely K was 2, corresponding to western (Spain and Italy) and eastern (Greece, Lebanon, and Israel) clusters (Figure 3.3c). When K=3, most individuals from Greece fell into a distinct cluster (Figure 3.3d).

Figure 3.1. Haplotype maps showing the distribution and proportion of haplotypes across all populations for the three variable markers used in this study. Populations include North Carolina (NC, *n*=8), Daytona Beach (DAY, *n*=11), Cape Florida (CFL, *n*=9), Panama City (PAN, *n*=11), Bermuda (BER, *n*=13), Spain (SPA, *n*=14), Italy (ITA, *n*=2), Greece (GRE, *n*=18), Lebanon (LEB, *n*=4), and Israel (ISR, *n*=27) (Appendix B, Table 3.S1). For each marker, different colors represent different haplotypes. Pie graphs on the maps show the proportion of each haplotype found at each locality. Haplotype networks for each marker are also shown. We constructed haplotype networks for *cp23S* and *b7sym15* using statistical parsimony implemented in TCS 1.21. We specified maximum connection steps = 60, and treated gaps as a fifth state. The sizes of the circles are directly proportional to the haplotype frequencies, also indicated as percentages. Line segments connecting haplotypes represent a single mutational step separating the haplotypes, and small black dots represent inferred haplotypes not present in our data. We constructed a haplotype network for *b2sym17* manually by separating each haplotype by repeat number variation. The blue haplotype not connected to the network lacks the microsatellite repeat. Haplotypes that match 100% to known published *Symbiodinium*-type sequences are indicated (see Appendix B, Tables 3.S2 and 3.S3 for all BLAST results).

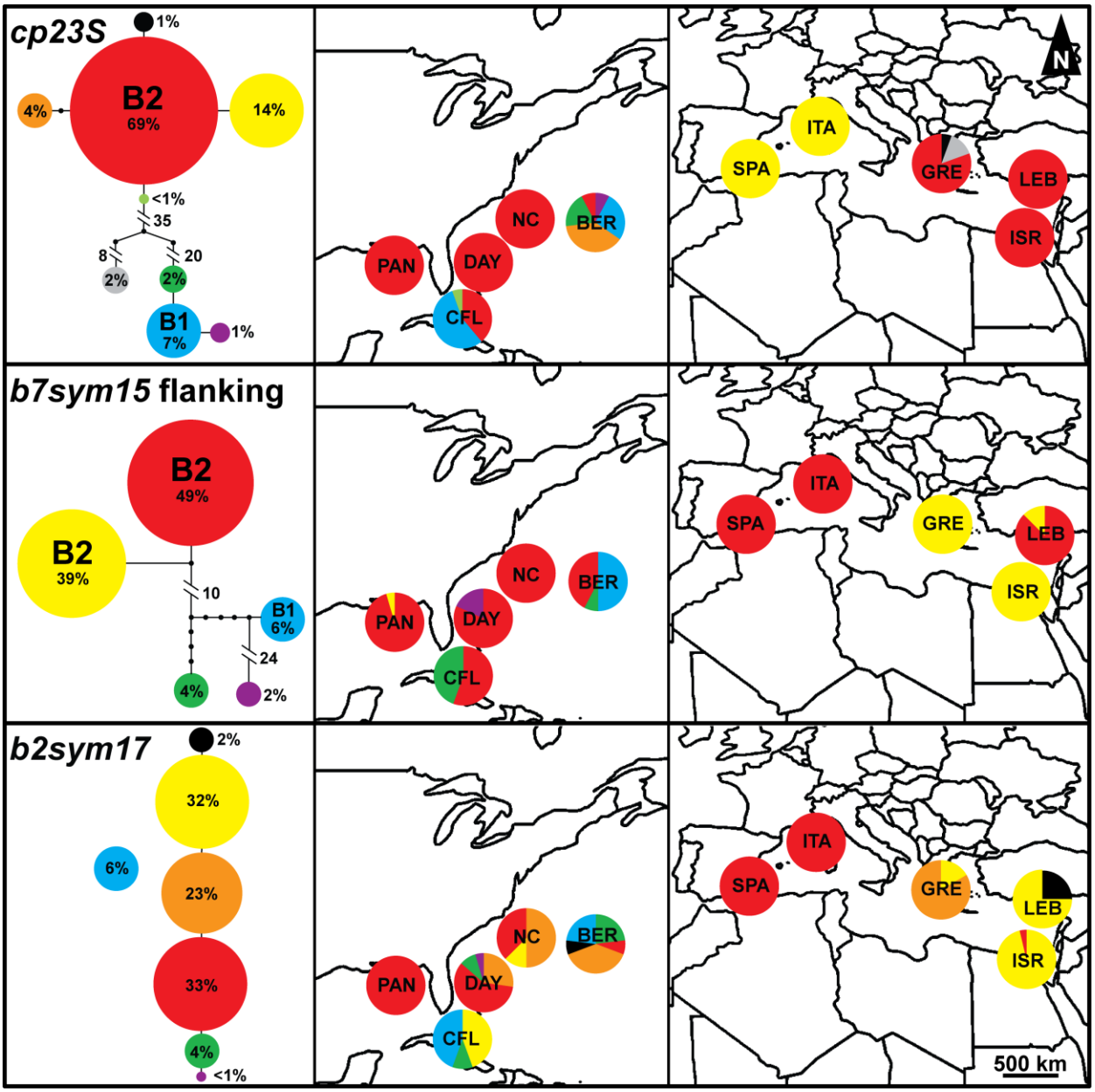
cp23S
1%
B2
69%
4%
14%
<1%
35
8
20
2%
2%
B1
7%
1%
b7sym15 flanking
B2
49%
B2
39%
10
B1
6%
24
4%
2%
b2sym17
2%
32%
6%
23%
33%
4%
<1%
PAN
DAY
NC
BER
CFL
ITA
SPA
GRE
LEB
ISR
N
500 km

Table 3.2. Analysis of molecular variance performed for all populations (a) and the western North Atlantic and Mediterranean populations separately (b).

(a)	Western North Atlantic vs. Mediterranean		Western North Atlantic + Western Mediterranean vs. Eastern Mediterranean	
Source of variation	%Variation	*F-value*	%Variation	*F-value*
Within populations	31.3	0.687	27.3	0.727
Among populations	60.6	**0.660**	36.5	**0.572**
Among groups	8.10	0.081	36.2	**0.362**
(b)	Western North Atlantic		Mediterranean	
Source of variation	%Variation	*F-value*	%Variation	*F-value*
Within populations	63.4	—	12.1	—
Among populations	36.6	**0.366**	87.9	**0.879**

Significant *F-values* (α level=0.05) are in bold.

3.4.3. Symbiont-host Associations

We next examined whether the genetic makeup of the host was associated with the geographical structuring in *Symbiodinium* communities. We found that the multi-locus genotypes of *Oculina* were independent from those of its *Symbiodinium* (p=0.655). In most cases, different *Oculina* spp. genotypes harbored similar *Symbiodinium* genotypes. However, in one case in Greece, two *O. patagonica* clones harbored distinct *Symbiodinium* genotypes.

The genetic clustering of *Symbiodinium* was discordant with that of its host (Figure 3.2). In contrast to the host, the algal symbionts were not differentiated between the western North Atlantic and the Mediterranean, nor did they show similar subdivision within the western North Atlantic. They were, however, differentiated between the western and eastern Mediterranean. BARRIER corroborated our STRUCTURE results (see Appendix B, Figure 3.S1 for additional information).

3.4.4. Environmental Correlations of Symbiont Community Composition

We next examined whether habitat differences were associated with the geographical variation in *Symbiodinium* communities. For sea surface temperature, trends were largely similar across all four measures of temperature (Appendix B, Figure 3.S2); so we present only the results for temperature at time of sampling and average temperature. Furthermore, because the results for the western North Atlantic (Appendix B, Figure 3.S3) were similar to the results when all populations were analyzed together, we only present results for all populations here. When all populations were analyzed together, temperature explained at best 22% (p=0.177) of the genetic diversity of the *Symbiodinium* communities (Figure 3.4; Appendix B, Figure 3.S2), although these values were often far lower (0.1%, p=0.939). None of the correlations were significant, although there was a small but consistent trend of one genetic cluster ('black') increasing at the expense of another ('white') as temperature increased. When the western North Atlantic populations were analyzed separately, the trends were similarly weak (0.2%–73%) and not significant (p=0.067–0.942) (Appendix B, Figure 3.S3). Although the 'gray' genetic cluster was

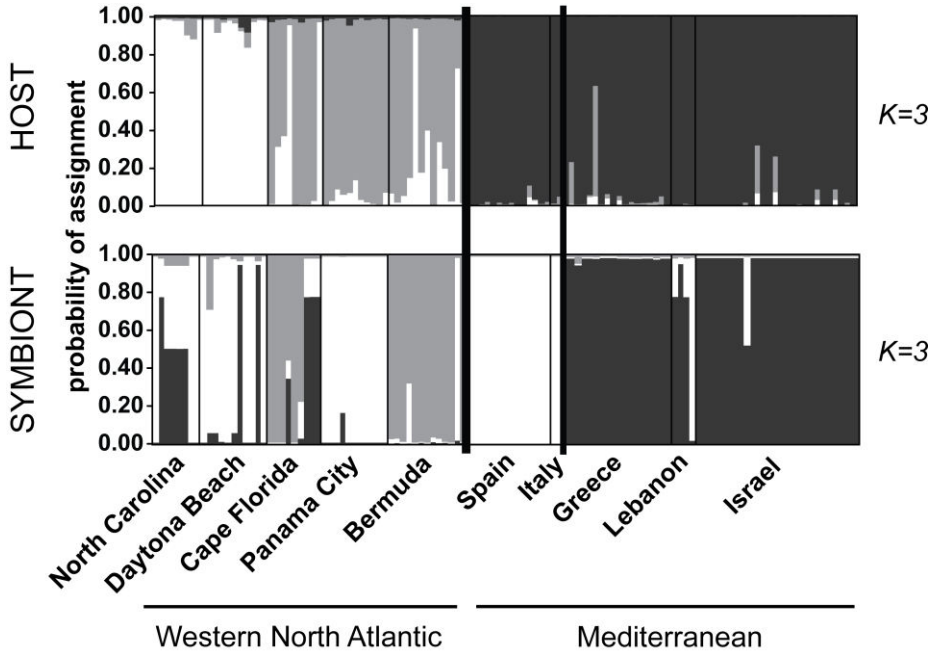

Figure 3.2. STRUCTURE bar plots for the coral host (top) and their respective algal symbiont (bottom) when all populations were analyzed together. Individuals (bars) are grouped by populations along the x-axis, with the probability of assignment to a particular genetic cluster (represented by different shades) along the y-axis. The number of genetic clusters or populations (*K*) is shown for each analysis. Major differences in clustering breaks between host and symbiont are indicated with dark vertical lines.

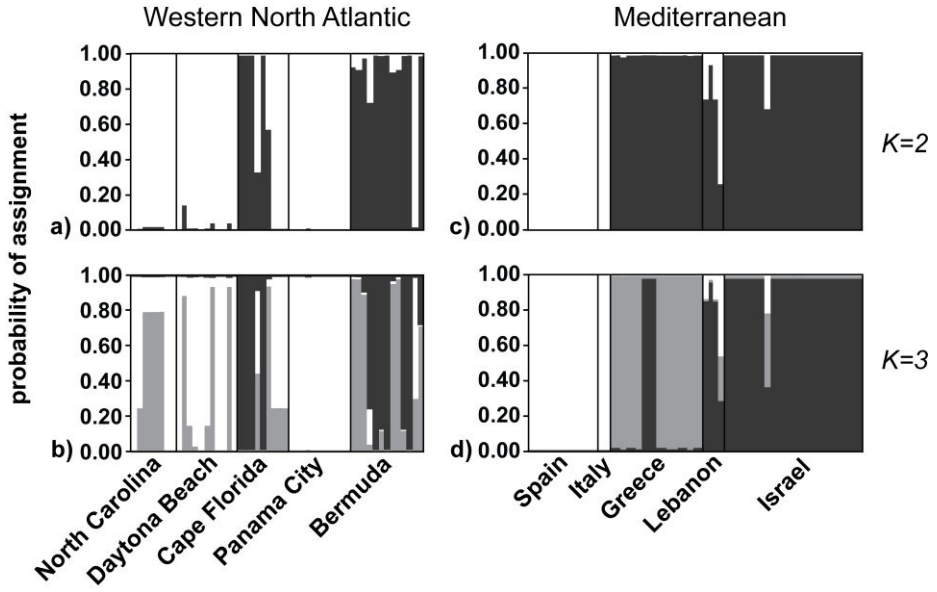

Figure 3.3. STRUCTURE bar plots for the algal symbiont when western North Atlantic (left) and Mediterranean (right) populations were analyzed separately. Individuals (bars) are grouped by populations along the x-axis, with the probability of assignment to a particular genetic cluster (represented by different shades) along the y-axis. The number of genetic clusters or populations (*K*) is shown for each analysis.

significantly negatively correlated with increased maximum temperature (p=0.034) (Appendix B, Figure 3.S3d), this trend was not consistent, nor did it remain significant after correcting for multiple comparisons. When the Mediterranean populations were analyzed separately, the correlation between the 'white' and 'black' genetic clusters and temperature became stronger, with temperature explaining 42%–93% of the variation (p=0.008–0.236) (Figure 3.4; Appendix B, Figure 3.S2). The correlation between the 'black' genetic cluster and temperature was significantly positive for all (p=0.008–0.023) but maximum annual temperature (p=0.061), results that withstood correcting for multiple comparisons.

Overall, chlorophyll *a* concentration did not explain *Symbiodinium* community as well as temperature (Appendix B, Figure 3.S4). Although three correlations were significant, only one remained so following correction for multiple comparisons. The correlation that remained significant was a decrease in the 'black' genetic cluster with increased minimum annual chlorophyll *a* concentration in the Mediterranean (91%, p=0.011) (Appendix B, Figure 3.S4).

Depth was the worst predictor variable. It explained at best 73% (p=0.067) of the *Symbiodinium* communities across all analyses (Appendix B, Figure 3.S5), although these values were often much lower (0.3%, p=0.933). Furthermore, none of the correlations were significant, and there were no consistent trends (Appendix B, Figure 3.S5). We note that nonlinear relationships did not significantly fit the data better (results not shown); therefore, we focus our results on simpler linear relationships.

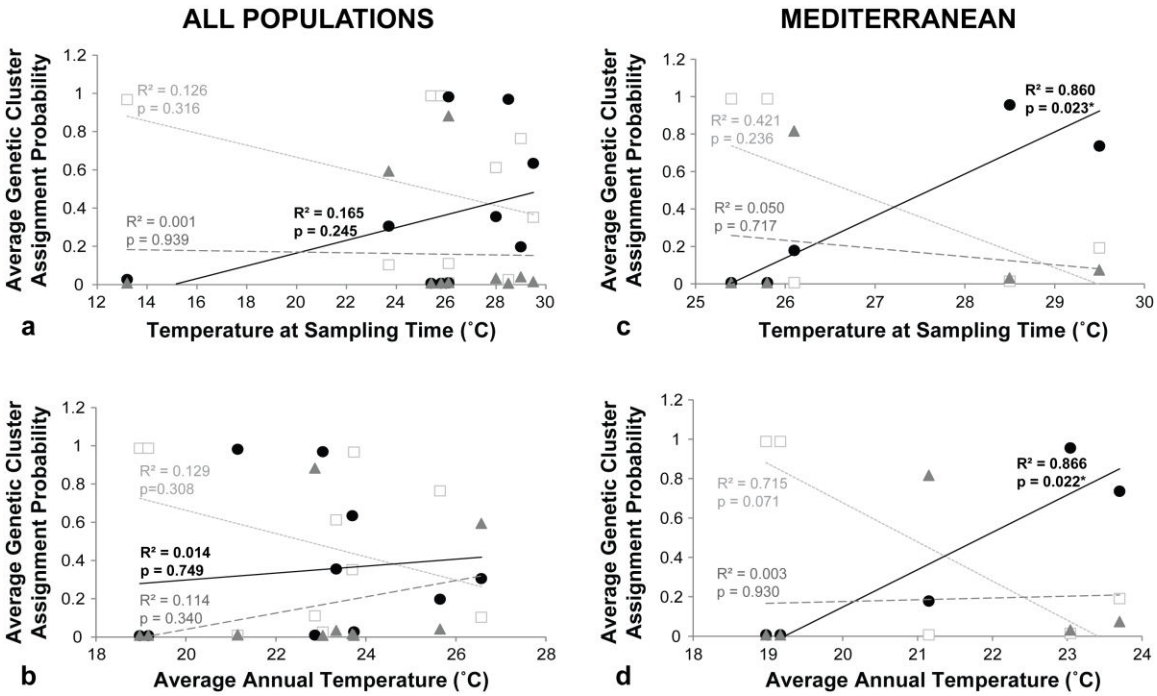

Figure 3.4. Correlation between *Symbiodinium* community composition and temperature (at sampling time and average annual) for all populations (a and b) and Mediterranean populations only (c and d). The white squares, gray triangles, and black circles represent the white, gray, and black genetic clusters, respectively, obtained from STRUCTURE analyses (Figure 3.2 for all populations; Figure 3.3d for Mediterranean). Trend lines are as follow: white= short dashed line, gray= large dashed line, black= solid line. Significant correlations are indicated with an asterisk.

3.5. DISCUSSION

3.5.1. *Oculina* Corals Associate Largely with *Symbiodinium* Type B2 but also Type B1

All of our *Oculina* spp. colonies harbored *Symbiodinium* clade B, in agreement with previous work (LaJeunesse *et al.* 2012; Rodolfo-Metalpa *et al.* 2014; Rubio-Portillo *et al.* 2014a; Thornhill *et al.* 2008). The majority of the colonies harbored type B2, again consistent with earlier studies (Rodolfo-Metalpa *et al.* 2014; Rubio-Portillo *et al.* 2014a; Thornhill *et al.* 2008). We also detected type B1 in several colonies from Cape Florida and Bermuda, which has previously been isolated from *O. diffusa* in Bermuda (LaJeunesse 2001). Identifying *Symbiodinium* species is challenging, given the long history and ongoing taxonomic revisions within the genus, and ultimately depends on a number of diagnostic genetic markers, including *cp23S* and *b7sym15*, and morphological characters (LaJeunesse *et al.* 2012; Parkinson *et al.* 2015; Thornhill *et al.* 2013). Although this study did not employ the full set of diagnostic tools, we did find that some *cp23S* and *b7sym15* haplotypes perfectly matched published sequences for *S. psygmophilum* (within B2) and *S. minutum* (within B1), suggesting at least tentative species identifications.

Symbiodinium type B2 generally dominates in temperate corals (Thornhill *et al.* 2008) and tends to be rare in tropical habitats (LaJeunesse 2002; Thornhill *et al.* 2006a; Thornhill *et al.* 2006b). Type B2 isolated from hosts *Astrangia poculata* and *Oculina* sp. from the western North Atlantic is cold tolerant, with the ability to recover from low temperatures better than tropical types A3, B1, and C2 (Thornhill *et al.* 2008). This type is also resistant to short-term exposure to elevated temperatures (Rodolfo-Metalpa *et al.* 2008; Rodolfo-Metalpa *et al.* 2006; Shenkar *et al.* 2006). These physiological studies suggest that type B2 is able to endure a wide range of temperatures, which may facilitate the ability of *Oculina* corals to survive the variable temperatures they experience across their distribution. Type B1 has been shown to be more thermally sensitive than type B2 (McGinty *et al.* 2012), which may explain why we only detected it in two localities.

3.5.2. *Oculina*'s Symbiont Communities Vary Geographically

Symbiodinium communities associated with *Oculina* corals vary geographically, and algal diversity is greater in western North Atlantic populations compared to Mediterranean populations (Figures 3.1 and 3.2). Within the western North Atlantic, Cape Florida and Bermuda harbor *Symbiodinium* communities genetically distinct from all other populations (Figure 3.3a), likely because these populations possess two *Symbiodinium* types (Figure 3.1).

A stronger pattern of subdivision is evident within the Mediterranean, where we found a clear west versus east division (Figure 3.3c). This pattern may be due to greater thermal stress in the east. Eastern populations have endured annual bleaching events since they were first reported in this region over 20 years ago (Fine & Loya 1995; Fine *et al.* 2001; Rosenberg & Ben-Haim 2002), while bleaching events in western populations have only been reported in the last 5 years (Rubio-Portillo *et al.* 2014c). Furthermore, *O. patagonica* colonies in the east show increased tolerance to bleaching following an initial bleaching event (Armoza-Zvuloni *et al.* 2011). These differences in thermal conditions may have led to differences in *Symbiodinium* communities, whereby eastern populations that recover from annual bleaching events acquire local strains from

the environment that are genetically distinct from western strains. We also found greater *Symbiodinium* diversity in the eastern Mediterranean compared to the west. On the one hand, this increased diversity may help corals cope with environmental stresses. On the other, greater *Symbiodinium* diversity associated with thermal stress is contrary to studies showing that thermal stress reduces symbiont diversity (Fabricius *et al.* 2004; Rowan *et al.* 1997). Future work is needed to elucidate the factors driving these differences in diversity between regions.

3.5.3. Temperature Better Correlates with *Symbiodinium*'s Geographical Variation than Host Genetics, Chlorophyll *a* Concentration, or Depth

Genetic differentiation of the coral host did not correlate with *Symbiodinium* community composition. We found no associations between multi-locus genotypes of *Oculina* spp. and their *Symbiodinium*, nor did we find matching geographical structuring within *Symbiodinium* and its host (Figure 3.2). *Oculina* spp. populations in the western North Atlantic and the Mediterranean show a clear genetic break (Figure 3.2; Appendix B, Figure 3.S1; (Leydet & Hellberg 2015), which was not observed in the symbiont. Instead, western Mediterranean populations of *O. patagonica* harbor symbionts that are more genetically similar to the western North Atlantic than the eastern Mediterranean. Eytan *et al.* (2009) found a genetic division between northern and southern *Oculina* spp. populations in the western North Atlantic, but no such division was evident within *Symbiodinium*. Finally, *O. patagonica* harbors geographically structured *Symbiodinium* type B2 in the Mediterranean, despite being genetically similar across this range (Figure 3.2; Appendix B, Figure 3.S1; (Leydet & Hellberg 2015).

The lack of congruence between host and symbiont geographical variation may reflect the facultative relationship between *Oculina* corals and their algal symbionts. Indeed, obligate symbionts often coevolve with their hosts (e.g., (Bongaerts *et al.* 2010; Prada *et al.* 2014; Symula *et al.* 2011). However, even some obligate coral-algal symbioses show discordant geographical variation between partners (Baums *et al.* 2010; Baums *et al.* 2014; Keshavmurthy *et al.* 2012; Pettay *et al.* 2011), often associated with different environmental conditions (Baums *et al.* 2010; Keshavmurthy *et al.* 2012). For example, while the coral *Platygyra verweyi* shows no genetic differentiation among populations, its *Symbiodinium* composition varies geographically; corals near a hot-water discharge are dominated by the heat-tolerant type D1a, and the abundance of the heat-sensitive type C3 increases with distance from the discharge (Keshavmurthy *et al.* 2012). These differences are attributed to the coral's ability to acclimate to thermal stress by harboring heat-tolerant *Symbiodinium*. Such findings suggest that for corals and other organisms with long generation times that can only slowly generate adaptive genetic diversity within their own genomes, variation in their symbionts may provide a quicker way to respond to local environmental conditions (Baker *et al.* 2004; Rosenberg *et al.* 2007).

Indeed, the patterns of *Symbiodinium* diversity and geographical structuring (particularly within the Mediterranean) suggest that these communities within *Oculina* corals may be shaped by local environmental conditions facing these corals. Overall, temperature explained *Symbiodinium* communities better than chlorophyll *a* concentration and depth, in terms of both significance and consistency of the observed trends (Figure 3.4). We therefore focus our discussion on temperature, while recognizing that environmental variables often covary, and that testing for

casual relationships between environmental variables and community composition will require controlled experiments.

We found strong correlations between temperature and *Oculina*-associated *Symbiodinium* communities within the Mediterranean, where temperature explained as much as 93% of the variation. Despite a strong latitudinal pattern in temperature variation, no such correlation was evident in the western North Atlantic. The difference in associations between the western North Atlantic and Mediterranean regions may be due to host background, since it is the combination of host and algal symbiont (the holobiont) that is or is not well suited to a particular habitat (Parkinson & Baums 2014). The high intraspecific variation and geographical subdivision of western North Atlantic *Oculina* spp. host populations may leave little opportunity for their symbiont communities to adapt to local differences in temperature. However, *O. patagonica* is genetically uniform across the Mediterranean. This simplified host background may have selected for a stronger association between symbiont community and temperature.

As facultative zooxanthellate corals, *Oculina* spp. are readily found in an azooxanthellate state both in the western North Atlantic (Reed 1981) and in the Mediterranean (Fine *et al.* 2001; Koren & Rosenberg 2006). Given the loose dependence of *Oculina* corals on their algal symbionts, we might expect their *Symbiodinium* communities to be random. However, our findings suggest that the *Symbiodinium* communities harbored by *Oculina* corals, particularly *O. patagonica*, may instead reflect acclimatization to varying environmental conditions. This shows that *Symbiodinium* may be integral members of the holobiont even for corals who can survive without them (Dimond & Carrington 2007; Dimond *et al.* 2013).

Given that temperature and geographical distance are correlated in the Mediterranean (temperature increases toward the east), the symbiont-temperature trends we observed could be driven by geographical distance. One way to address this would be to test whether *Symbiodinium* communities fluctuate seasonally within localities. Common garden thermal stress experiments would also be valuable for controlling for host genotype and other microbial communities, and testing whether *Symbiodinium* communities fluctuate with varying temperatures.

While the trends we observed suggest that temperature may play a role in structuring *Symbiodinium* communities associated with *O. patagonica* in the Mediterranean, the question remains whether this genetic diversity and structure reflects any physiological differences for the holobiont. Rodolfo-Metalpa *et al.* (2014) investigated whether *O. patagonica* from localities experiencing different temperature regimes varied in their thermal performance. Despite observing physiological differences *in situ*, laboratory thermal experiments showed little support for substantial geographical variation in host and symbiont physiology in response to temperature variation. However, other environmental factors, such as light intensity, food shortage, and ambient nutrient levels may also be factors driving differences in stress response (Rodolfo-Metalpa *et al.* 2014; Rubio-Portillo *et al.* 2014b). Studies that include multiple manipulated environmental stresses that better reflect natural conditions though challenging are needed to better examine the casual link between *Symbiodinium* diversity and physiological response to stress.

In conclusion, we found that *Oculina* corals harbor different *Symbiodinium* communities across their geographical range and that environmental differences, particularly sea surface temperature, appear to be better correlated with this geographical variation than the coral host's genetics. This study suggests that for facultative zooxanthellate corals the *Symbiodinium* communities that they harbor, although not tightly linked to their host's genetics, may reflect acclimatization to local environmental conditions.

3.6. AVAILABILITY OF DATA

All algal sequences have been deposited in GenBank (*b7sym15* Accession Numbers: KT193928–KT194047) and The European Nucleotide Archive (*psbA* coding, *cp23S*, *b2sym17* Accession Numbers: LN869546–LN869907).

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CHAPTER 4.
HOST-TARGETED RAD-SEQ REVEALS GENETIC CHANGES ASSOCIATED WITH RANGE EXPANSION OF *OCULINA PATAGONICA* ALONG THE SPANISH MEDITERRANEAN COAST

4.1. ABSTRACT

Many organisms are expanding their current ranges in response to changing environmental conditions. Understanding the patterns of genetic diversity and adaptation along an expansion front is crucial to assessing a species' long-term success. While new next-generation sequencing techniques can reveal these changes in fine detail, ascribing these to the evolutionary response of a particular species can be difficult for organisms that live in close association with symbionts. Using a modified restriction-site associated DNA sequencing (RAD-Seq) protocol to target coral DNA, we collected 595 coral-specific single nucleotide polymorphisms (SNPs) from 189 colonies of the invasive coral *Oculina patagonica* from across the entire Mediterranean coast of Spain, which included established core populations and two recent expansion fronts. Surprisingly, populations from the more recent northern expansion populations are genetically distinct from the westward expansion populations and the core populations, and also harbor greater genetic diversity than either of them. Generalized linear models relating genetic structure to environmental variables suggest that temperature appears to have driven adaptation along the northern expansion, but not toward the west. Tests for selection found one candidate gene associated with temperature in the northward expansion, but none in the west. We found no evidence of local adaptation to artificial substrate, which has been proposed for explaining the rapid spread of *O. patagonica*, suggesting that this coral is simply an opportunistic colonizer of free space made available by coastal habitat modifications. Together, these results suggest that unique genetic variation and adaptation to local temperatures along the northern expansion front, but not the westward expansion, may have facilitated the poleward range expansion of *O. patagonica* in the western Mediterranean.

4.2. INTRODUCTION

In the face of current global environmental change, organisms may prove unable to cope and undergo decline (Gibbons *et al.* 2000; Møller *et al.* 2008; Pounds *et al.* 1999). Alternatively, they may evolve *in situ* and adapt to climatic change (Anderson *et al.* 2012; Franks *et al.* 2007; Jensen *et al.* 2008). Finally, they may shift their range to more suitable habitat, which may subsequently lead to local adaptation within the new range (Booth *et al.* 2011; Hickling *et al.* 2006; Parmesan *et al.* 1999; Sorte *et al.* 2010). Understanding how organisms respond to environmental changes is crucial to evaluating their capacity to persist especially with global climate change.

Many species, both native and non-native, are expanding their ranges in response to increasing temperatures (Cavanaugh *et al.* 2014; Serrano *et al.* 2013c; Yamano *et al.* 2011). Species expanding their ranges face challenges. First, invasive species must disperse and establish in a new region. Second, population bottlenecks associated with colonization and establishment may lead to reduced genetic diversity, potentially limiting their ability to adapt and persist (Frankham & Ralls 1998; Lee 2002). This genetic paradox in invasive species (i.e., how bottlenecked

populations can still become successful) has intrigued researchers, who continue to explore possible mechanisms underlining this mystery (Allendorf & Lundquist 2003; Frankham 2005).

Understanding the genetic diversity underlying an expansion can help shed light on the evolutionary response to novel environments during the expansion process, as can identifying putative genes targeted by selection in the newly expanded populations. Linking the function of particular genetic changes to environmental variables can also reveal the ecological forces underpinning evolutionary change (Buckley *et al.* 2012). While examining multiple successful range expansions can reveal general underlying driving forces facilitating successful expansions (Hodgins *et al.* 2015; White *et al.* 2013), comparing a successful expansion with a less successful one (i.e., where populations are not as thriving) can elucidate particular region-specific genetic mechanisms and environmental factors associated with successful evolutionary change during an expansion (Dlugosch & Parker 2008).

High-throughput sequencing approaches have greatly improved our understanding of range expansions by identifying genetic signatures of adaptation associated with expansion (White *et al.* 2013; Zenni & Hoban 2015) and environmental factors driving local adaptation (Buckley *et al.* 2012). The development of high-throughput sequencing of reduced-representation libraries, such as genotyping-by-sequencing and restriction-site associated DNA sequencing (RAD-Seq), has allowed researchers to collect hundreds to thousands of single nucleotide polymorphisms (SNPs) for multiple samples from multiple populations without a reference genome. Basic genotyping-by-sequencing methods sequence all of the DNA extracted from a sample. However, many animals and plants are now recognized as holobionts, consisting of a host and communities of microbial symbionts (Rosenberg & Zilber-Rosenberg 2013). Consequently, for organisms that harbor endosymbionts, such as corals, reduced-representation libraries can be contaminated with unwanted symbiont DNA, thus allocating time, money, and sequence data to the wrong organism and resulting in low coverage across host contigs (Leese *et al.* 2012; Toonen *et al.* 2013). Furthermore, symbiont variation or switching may provide a more rapid and versatile way to respond to environmental conditions than genetic mutation and selection of the host itself (Rosenberg *et al.* 2007; Rosenberg & Zilber-Rosenberg 2011). If not accounted for during next-generation sequencing methods, measures of genetic diversity and selection may be confounded due to aberrant sequencing and analyses of symbiont loci. Therefore, new next-generation sequencing techniques are necessary to target host DNA and separate its evolutionary response from that of its symbionts.

The coral *Oculina patagonica* was thought to have been introduced into the Mediterranean in the mid-20th century (Fine *et al.* 2001; Zibrowius 1974), however recent genetic work suggests it has had a far longer history in the eastern Atlantic (Leydet & Hellberg 2015). At odds with this long history, it was only first reported from the Mediterranean in 1973 (Zibrowius & Ramos 1983) off the coast of Spain. Since then it has quickly expanded along the entire Spanish coast, possibly due to increased sea surface temperatures and coastal habitat modifications (Rubio-Portillo *et al.* 2014; Serrano *et al.* 2013c). Based on abundance and size structure (Serrano *et al.* 2013a, b; Serrano *et al.* 2013c)(R. Comas & M. Ribes unpublished data), populations of *O. patagonica* along the southeastern coast of Spain (SOE) are believed to make up the core zone of Spanish establishment (Figure 4.1). From there, *O. patagonica* appears to have expanded westward into the Alboran Sea (ALB), although population densities there suggest only modest

success. A more recent northern expansion along the Catalan coast has been more successful, with colonies rapidly increasing in abundance and density (Serrano *et al.* 2013c; Terrón-Sigler *et al.* 2015) (R. Comas & M. Ribes unpublished data). This dual colonization allows us the opportunity to genetically compare two expansions with different levels of success.

Here we investigate the range expansion of *O. patagonica* along the Spanish coast using a modified RAD-Seq protocol to enrich for coral DNA. We ask: 1) Are the two expansions genetically similar or different, and do they exhibit reduced genetic diversity relative to the core populations? 2) Is geographic distance or environmental variation (temperature and/or substrate use) driving patterns of genetic diversity along the expansions? and 3) Are any genes under selection and potentially facilitating the expansions, and are they the same for both fronts?

4.3. METHODS

4.3.1. Sampling and Sequencing

Oculina patagonica samples were collected between August 20-31, 2014 from 14 localities spanning their distribution along the Mediterranean coast of Spain (Figure 4.1, Table 4.1). We obtained 13 samples from each site except for the northernmost (site 14 in Fig. 4.1), from which 20 were collected. Although *O patagonica* is found on both natural and artificial substrate throughout the Spanish coast (Rubio-Portillo *et al.* 2014; Serrano *et al.* 2013c; Terrón-Sigler *et al.* 2015), in this study, samples from all but two sites (10 and 13 in Fig. 4.1) came from natural substrate. Depth of each sample was also noted at time of collection and averaged for all samples analyzed from each site (Table 4.1). Sea surface temperatures at each location were measured at the time of sampling.

For this study, populations were grouped into four zones based on survey data from Serrano *et al.* (2013a,b,c)(R. Comas & M. Ribes unpublished data). They found that of the surveyed locations (n=90), colonies of *O. patagonica* were present in 95% of locales on the southeastern coast, with occurrence decreasing to 50% in the Alboran Sea, and as low as 45% along the northeastern Spanish coast. Furthermore, colonies along the Catalan coast (most north) have been well monitored for 19 years (Serrano *et al.* 2013c). In this region, abundance of *O. patagonica* increased from being present in only one location in 1992 to colonizing 19% of the surveyed locations in 2010. These data suggest that populations along the southeastern coast (SOE) are core populations, populations in the Alboran Sea (ALB) represent a westward expansion from the core, and northeastern (NOE) and Catalan (CAT) populations represent a northern expansion from the core.

Colonies were sampled via SCUBA, breaking off a 2 cm^2 piece of live tissue and preserving it in 95% ethanol. We also obtained a "symbiont-free" *Oculina* sp. sample lacking algal endosymbionts (*Symbiodinium*) from a deep-water (80 m) population off the southeastern coast of North America (Eytan *et al.* 2009). This sample was necessary to target coral-specific sequence reads (see below). Based on five nuclear genes, the level of sequence divergence between *Oculina* spp. from this deep population (*Oculina* Banks) and a Spanish population (Cabo de Palos) is about 0.02 nucleotide substitutions per site (Leydet & Hellberg 2015), demonstrating its genetic similarity to our target species.

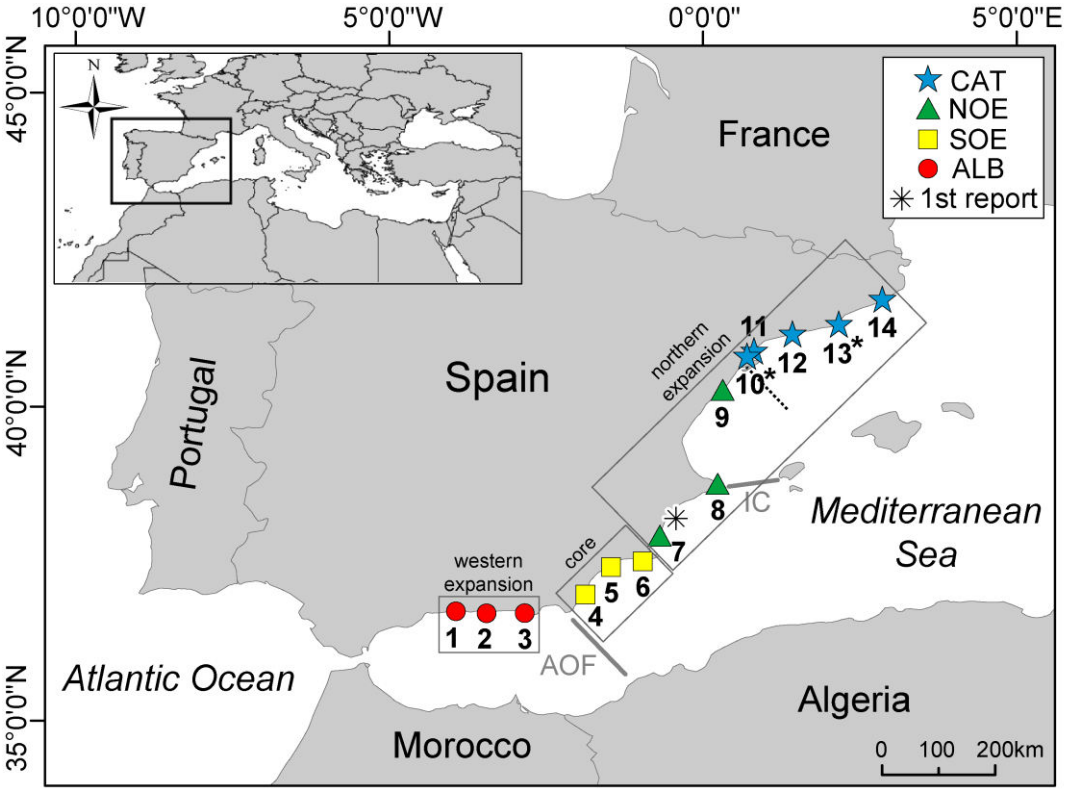

Figure 4.1. Map of the collection sites along the Mediterranean coast of Spain, by zone and site number (Table 4.1). Populations 4-6 in SOE (Southeast) are considered the core populations. Populations 1-3 in ALB (Alboran Sea) represent the westward expansion, while populations 7-9 in NOE (Northeast) and 10-14 in CAT (Catalan coast) represent the northern expansion, CAT being more recent. The 1st reported site is the Alicante Harbor where *O. patagonica* was first reported in Spain in 1973 (samples not collected). The dashed line at Ebro Delta represents the northern limit of well-established populations. The asterisks associated with sites 10 and 13 indicate that samples from these sites were sampled from artificial substrate. The Almeria-Oran Front (AOF) and Ibiza Channel (IC) are indicated.

We extracted genomic DNA from the samples using the QIAGEN DNeasy Kit following the manufacture's protocols with the following modifications: we allowed tissues to lyse at 56˚C overnight; immediately following lysis samples were treated with RNase A (4 µl of 100 mg/ml) (QIAGEN) and then incubated for 2 minutes at room temperature; and DNA was eluted in 200 µl of AE buffer after incubation at room temperature for an hour to maximize DNA yield. Species identification was confirmed by sequencing the mitochondrial cytochrome oxidase I (*COI*) gene using previously deigned primers (Folmer *et al.* 1994) and protocols (Leydet & Hellberg 2015).

To prescreen for potential clonemates, we sequenced a variable nuclear marker (p14: fatty acid elongase; 206 bp; Leydet & Hellberg 2015). During the p14 screening, a sample collected from Barcelona (site 13) was identified as the Mediterranean native coral *Cladocora caespitosa.* We used this sample as well as four *C. caespitosa* colonies from Pantelleria Island to test for possible hybridization between this species and *O. patagonica* (see below and Appendix C, Figure 4.S5

Table 4.1. Collection sites. Site numbers correspond to those in Figure 4.1. SOE (Southeast) is considered the core zone. ALB (Alboran Sea) represents the westward expansion, while NOE (Northeast) and CAT (Catalan coast) represent the northern expansion.

Zone	Site	n^a	n^b	Substrate	Latitude	Longitude	depth[c]	T^d
ALB	1. Torrox	13	13	natural	36°43'34"N	3°57'16"W	1.0	26
	2. Sacratif	13	12	natural	36°41'40"N	3°27'55"W	2.4	27
	3. Punta peña del Moro	13	11	natural	36°41'53"N	2°51'30"W	2.8	27
SOE	4. Carboneras	13	11	natural	36°59'40"N	1°53'21"W	1.4	28
	5. Cabo Cope	13	9	natural	37°25'39"N	1°30'03"W	1.2	27
	6. Muelle del Curra	13	11	natural	37°35'16"N	0°58'33"W	1.9	27
NOE	7. La Zenia	13	6	natural	37°55'03"N	0°43'12"W	0.9	27
	8. Xàbia	13	10	natural	38°45'51"N	0°13'26"E	2.2	27
	9. Alcossebre	13	7	natural	40°15'33"N	0°18'11"E	1.8	26
CAT	10*. L'Ampolla	13	13	artificial	40°48'29"N	0°42'39"E	3.6	26
	11. Roca de l'Illot	13	13	natural	40°50'49"N	0°45'24"E	2.6	26
	12. Torredembarra	13	13	natural	41°08'34"N	1°24'53"E	2.6	26
	13*. Barcelona	13	13	artificial	41°17'43"N	2°09'09"E	6.1	26
	14[†]. Roca Muladera, Es Bullents, Fenals, Punta Santa Ana, Sa Palomera	20	19	natural	41°41'39"N	2°50'42"E	8.0	22

[a] number of samples collected and sequenced (n=189)
[b] number of samples analyzed (n=161)
[c] average sampling depth (meters) of samples analyzed
[d] temperature at time of sampling (˚C)
* sampled from artificial substrate
[†] also referred to as 'Northernmost population'

for additional details). Sequencing was performed using BigDye chemistry v3.1 on an ABI 3130XL at the Louisiana State University Genomics Facility.

The "symbiont-free" status of the deep *Oculina* sp. sample was verified by attempted PCR amplification of several alga-specific markers: ITS2 (LaJeunesse 2002), *psbA* minicircle coding region (Barbrook *et al.* 2006), cp23S–rDNA domain V (Santos *et al.* 2002), and the flanking region of the microsatellite marker B7SYM15 (Pettay & Lajeunesse 2007). The lack of amplicons confirmed that no *Symbiodinium* was present in the "symbiont-free" coral sample.

DNA quantification and concentration of all samples was determined using NanoDrop and Qubit 2.0 Fluorometer at the LSU Genomics Facility. Samples were submitted to SNPsaurus (Eugene, OR) and sequenced using a modified RAD-Seq protocol. Because *Oculina* often harbor endosymbiotic algae with large genomes, traditional genotype-by-sequencing methods can result in an overrepresentation of unwanted symbiont sequence fragments (Leese *et al.* 2012; Toonen *et al.* 2013). To maximize coverage of the host coral genome, we employed the following approach. A RAD library was created from 100 ng genomic DNA from the "symbiont-free" *Oculina* sp. sample. This DNA was double-digested with PstI-HF and MfeI-HF (New England Biolabs) and ligated to complementary adapters that allowed the resulting amplified fragments to be converted to biotinylated RNA baits. Fragments with insert sizes 100-350 bp in size were isolated by gel extraction from a portion of the ligated product prior to amplification and the *in vitro* transcription reaction to create the RNA baits. This bait library template was also converted into one that could be sequenced along with the captured libraries described below. Nextera sequences and indices were added to the bait fragments using long primers matching the adapters in a short PCR reaction to create the baits library. Shotgun sequencing libraries were prepared from 189 submitted samples, plus one "no-capture" control replicate, using 5 ng DNA of each sample in a 1/10th Nextera (Illumina, Inc.) reaction with unique dual-indices to distinguish the individuals. The samples were pooled and size-selected for insert sizes 170-370 bp. The pooled libraries were then used in two successive overnight hybridizations to the biotinylated bait library, followed by capture using Dynabeads® MyOne™ Streptavidin C1 magnetic beads (Thermo Fisher) and amplification. The final captured libraries were sequenced in two paired-end Illumina HiSeq 3000 runs (one 2×100 bp and one 2×150 bp) at the Center for Genome Research and Biocomputing, Oregon State University.

4.3.2. Genotyping

SNPsaurus processed raw sequence reads using a custom pipeline and scripts.
Reads that passed the default Illumina pipeline quality control were merged into long pseudo-reads with bbmerge (BBMap, Bushnell B, sourceforge.net/projects/bbmap/). The pseudo-reads and individual paired-end reads were then assembled into longer contigs with tadpole (BBMap). The bait reads were collapsed into unique reads and aligned to the contigs using bbmap. The longest contigs that matched bait reads were selected to represent each bait locus. These representative loci were further collapsed to remove redundancy, ultimately creating a contig reference. Next, the paired-end reads were trimmed to remove Nextera adapter sequences using bbduk (BBMap). Then, 21 very abundant (present > 0.2% of the total reads) sequences found in the samples were removed so that misalignment of these reads to the reference set of loci would not cause artifacts. The trimmed reads were aligned to the contig reference with bbmap using an

88% identity threshold given the sequence diversity seen in the reads. After alignment, the sample contigs were converted to a variant call format (VCF) genotype table with SAMtools (Li *et al.* 2009), and then filtered for depth (>9 reads), minor allele frequency (≥ 0.05) and presence (≤ 25% missing data in a population) with VCFtools (Danecek *et al.* 2011). To minimize linkage of SNPs, only a single SNP from each sample contig was retained. The VCF was then filtered using a custom script to remove probable duplicated loci (loci that were heterozygous in nearly all samples, suggesting two fixed paralogous loci were aligned to the same reference). Individuals missing >50% data and loci missing >20% data were excluded from further genetic analyses. The final VCF file was converted into file formats necessary for subsequent analyses using PGDSPIDER 2.0.9.0 (Lischer & Excoffier 2012). Searches for contigs representing the SNPs were performed in BLASTn and BLASTx (Altschul *et al.* 1990).

4.3.3. Genetic Diversity

To test for genetic clones (in addition to our initial p14 marker screen), we calculated the allele dissimilarity between all pairs of individuals within all populations using the R package *poppr* (Kamvar *et al.* 2015; Kamvar *et al.* 2014). Dissimilarities equal to or close to zero would indicate that those pairs of individuals are likely clones. We also used *poppr* to calculate the number of privates alleles found in each population and in each zone.

To compare genetic diversity between core and expansion populations, we calculated gene diversity (*Hs*) using FSTAT 2.9.4 (Goudet 1995), which corrects for variation in population sample size. We also calculated average *Hs* for each zone and compared these using two-sided tests in FSTAT. We ran 1000 permutations for all pairwise comparisons.

4.3.4. Population Subdivision

To test for genetic subdivision, we first performed an Analyses of Molecular Variance (AMOVA) implemented in GENODIVE 2.0b27 (Meirmans & Van Tienderen 2004). We analyzed the two expansions separately. The westward expansion consisted of the core zone SOE and the westward expansion zone ALB (Fig. 4.1). The northward expansion consisted of core zone SOE and the northern expansion zones NOE and CAT. We ran 10,000 permutations. We performed principle component analysis (PCA) using the EIGENSOFT package (Patterson *et al.* 2006). We plotted the two components explaining the most variance of each PCA to examine genetic differentiation associated with populations, zones, temperature at time of sampling (Table 4.1), and sampling substrate.

We used a Bayesian clustering analysis implemented in STRUCTURE 2.3.4 (Pritchard *et al.* 2000), which detects significantly genetically differentiated clusters or populations (*K*), to examine population subdivision. Using the admixture model with correlated allele frequencies, we ran the program for 500,000 MCMC steps following a burn-in of 100,000 steps. We set sampling locations as *a prior* (LOCPRIOR model) because we expected structure signal to be weak (Hubisz *et al.* 2009) based on our previous survey of subdivision within *O. patagonica* in the Mediterranean (Leydet & Hellberg 2015). We performed 10 iterations for each inferred number of genetic clusters, *K*=1–6, and used the Evanno method (Evanno *et al.* 2005)

implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012) to determine the most likely number of genetic clusters.

4.3.5. Factors Associated with Genetic Diversity

We used a hierarchical Bayesian method implemented in GESTE 2.0 (Foll & Gaggiotti 2006) to evaluate how much of the variation in genetic subdivision can be explained by variation in geographic or environmental factors. GESTE estimates population-specific F_{ST} values and then relates them to environmental factors using a generalized linear model. We examined the effects of geographic location, sampling substrate (natural versus artificial), and temperature at time of sample (Table 4.1). For the westward expansion, we examined only the effects of longitude and temperature since all samples from these populations where collected from natural substrate. A total of 5 models were evaluated for the westward expansion, which included a combination of a constant term representing only random effects, environmental factors, and their interactions. For the northward expansion, we evaluated latitude, temperature, and substrate in a total of 8 models. Because we considered more than two factors, interaction terms were not allowed. We ran 40 pilot runs of 5,000 iterations each to adjust acceptance rates for each parameter of the MCMC chain. An additional burn-in of 100,000 iterations was run followed by a sample size of 100,000 iterations with a thinning interval of 20. Posterior probabilities of the models were used to assess which model (containing a set of factors) has the greatest effect on genetic structure.

4.3.6. Loci under Selection

To identify putative loci under selection, we used the F_{ST}-based genome-scan method implemented in BayeScEnv 1.1 (de Villemereuil & Gaggiotti 2015). BayeScEnv incorporates environmental differentiation and locus-specific effects to discriminate between signals of local adaptation relating to a particular environmental factor and spurious signals left by other processes, such as allele surfing, differences in mutation rate among loci, and background selection, thus improving its ability to control for false positives.

To investigate loci putatively driving the expansions, we first examined geographic location as the environmental factor for each expansion front separately. For the westward expansion, we used longitude to represent the spread to the west. For the northern expansion, we used latitude to represent a poleward spread. We standardized latitude and longitude by dividing each by their respective standard deviation. We also investigated local adaption to temperature for each expansion. We standardized temperature at time of sampling by first computing the mathematical distance from the mean, and then dividing by the standard deviation. Finally, we tested for local adaptation to substrate (natural=1, artificial=2) in the northern expansion populations. To minimize any confounding effects due to geographic distance and/or varying temperature, we analyzed populations 10–13, which are in relatively close proximity and share the same sampling temperature. Sites 11 and 12 represent natural substrate, and sites 10 and 13 represent artificial substrate (Table 4.2).

We ran 20 pilot runs of 5,000 iterations each to adjust acceptance rates of the MCMC chain to the recommended range of 0.2–0.4. An additional burn-in of 10^6 iterations was run followed by a sample size of 10^6 iterations with a thinning interval of 20. The prior parameters were π=0.1 and

p=0.25. We used the R package *coda* (Plummer *et al.* 2006) to ensure chain convergence and acceptable auto-correlation (Appendix C, Table 4.S1). BayeScEnv calculates two kinds of false discovery rate (FDR) related statistics: Posterior Error Probability (PEP) and q-value. We used an FDR cut-off of 5% to determine which loci were putatively under selection (Funk *et al.* 2016).

Table 4.2. Summary of tests of local adaptation performed in BayeScEnv.

Factor	Categories	Populations/zones tested
Expansion	longitude	SOE, ALB
	latitude	SOE, NOE, CAT
Temperature	26, 27, 28˚C	SOE, ALB
	22, 26, 27, 28˚C	SOE, NOE, CAT
Substrate	natural (1) *vs* artificial (2)	11+12 *vs* 10+13

4.4. RESULTS

4.4.1. Sampling, Sequencing and Genotyping

The average sampling depths for zones ALB, SOE, NOE, and CAT were 2.1m, 1.6m, 1.7m, and 4.6m, respectively. An ANOVA revealed that these zone averages were significantly different (F=29.37, p<0.0001), and post-hoc Tukey's multiple comparisons tests indicated that the average sampling depth for zone CAT was significantly greater than all other zones (p<0.0001).

RAD-sequencing of the baits resulted in 611,000 reads. After these reads were sorted and collapsed, reads with counts between 2 and 39 were retained, yielding 14,000 loci. 18,866,444 raw reads were used to construct the reference. After trimming, removing high-repeat reads and merging, there were 821,531 sequences. These merged reads were assembled with the unmerged reads, producing 637,646 contigs, which were aligned to the bait reads. The alignment to the longest contig was used to select a representative contig locus for that bait locus, ultimately resulting in 595 SNPs (one per contig). Contig length ranged from 180-1272 bp (mean = 317 bp). BLASTn (Altschul *et al.* 1990) searches resulted in only 8 contigs matching to nucleotide sequences. However, BLASTx (Altschul *et al.* 1990) searches returned 341 contigs that matched to proteins inferred from sequences from other corals (189 contigs had an E-value $\leq 1\times10^{-5}$). No contigs aligned to *Symbiodinium,* whose full genome has been sequenced (Lin *et al.* 2015; Shoguchi *et al.* 2013), indicating that the protocol was successful in removing symbiont sequences.

Twenty-eight of the 189 samples did not pass quality cutoffs (low number of sequence reads and >50% missing data, Appendix C, Figure 4.S1) and were therefore excluded from further analysis. Of the remaining 161 individuals, missing data ranged from 0–48.1%, with most (88%) of individuals containing <20% missing data (Appendix C, Figure 4.S2). The percent missing data per locus for these 161 individuals was no more than 14.3% (Appendix C, Figure 4.S3). All loci were therefore retained for analysis.

Missing data were skewed among populations in that southern populations (those besides zone CAT) had a higher proportion of individuals with >10% missing data (Appendix C, Figure 4.S2). To determine whether inclusion of these individuals would alter our results, we ran preliminary

genetic analyses for a subset of 127 individuals that did not exceed 10% missing data in FSTAT 2.9.4 (Goudet 1995). These alternative datasets produced similar results (not shown), suggesting that missing data did not significantly alter our results. The final dataset thus consisted of 161 individuals (Table 4.1) and 595 SNPs.

4.4.2. Genetic Diversity

Sequencing of the variable nuclear marker p14 indicated ≥4 genotypes in each of the sampled populations, suggesting that the populations were not overwhelming comprised of clonemates. The average pairwise allele dissimilarity between individuals from RAD-seq SNP data across all populations was 18.7% (Appendix C, Figure 4.S4). The lowest average dissimilarity within a population was 15.6% (site 5), while the highest was 21.1% (site 10). The lowest dissimilarity between any two individuals was 7.3%, meaning that those two were 92.7% similar in their multi-locus genotype. Only eight pairs of individuals out of all possible pairwise comparisons had dissimilarities <10%. Although establishing a cut-off is somewhat arbitrary, given that we observed no dissimilarities <5%, we are confident that clonality was not a significant factor in our dataset and subsequent genetic analyses.

We compared gene diversity (*Hs*) between all zones and found that the more recent and more successful northward expansion front (zone CAT) had higher heterozygosity (pairwise p=0.026, 0.007, and 0.011), albeit slightly (0.288 vs. 0.272, 0.270, and 0.271), than the others (Figure 4.2). Zones ALB, SOE, and NOE did not differ from one another (pairwise p=0.748, 0.921, and 0.854). Although no populations or zones harbored any private alleles, zones ALB and CAT shared 3 SNPs not present in SOE and 10 other SNPs not observed in NOE (Appendix C, Table 4.S2).

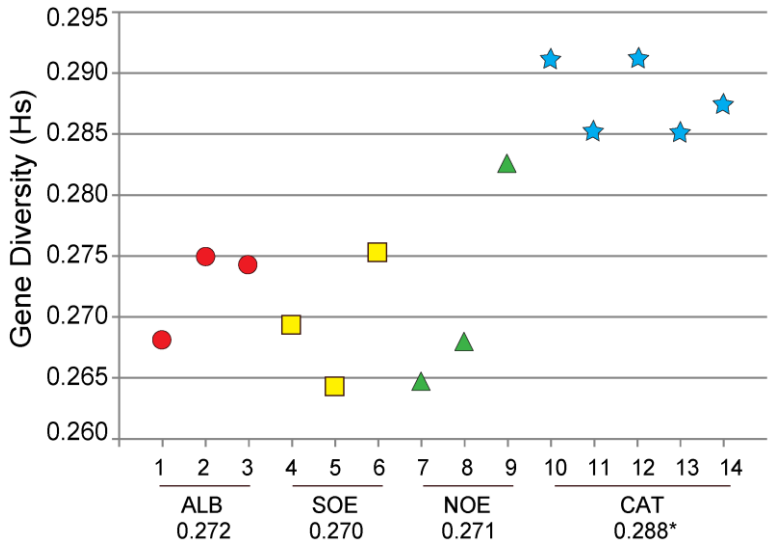

Figure 4.2. Gene diversity (*Hs*) calculated for each population. Zone averages are shown below the graphs. The asterisk indicates that zone CAT had significantly greater *Hs* average compared to all other zones. *P*-values of pairwise comparisons are as follows: ALB vs SOE (p=0.748), ALB vs NOE (p=0.921), ALB vs. CAT (p=0.026), SOE vs NOE (p=0.854), SOE vs CAT (p=0.007), NOE vs CAT (p=0.011).

4.4.3. Population Subdivision

AMOVA revealed significant subdivision among populations and zones along the northern expansion, but not the less successful westward expansion front (Table 4.3). PCA showed genetic differentiation associated with zones and with temperature, but not with population or habitat (Figures 4.3 and 4.4). Although significant, these effects were not particularly strong, with the first and second eigenvectors explaining only 3.24% (p<0.01) and 2.42% (p=0.01) of the variation, respectively.

Table 4.3. Analysis of molecular variance performed for a. westward expansion populations, and b. northward expansion populations. Significant F-statistic values are **in bold**.

a. Source of variation	% variation	*F-stat*	b. Source of variation	% variation	*F-stat*
within populations	0.997	0.003	within populations	0.985	0.015
among populations	0.001	0.001	among populations	0.007	**0.007**
among zones	0.002	0.002	among zones	0.009	**0.009**

The Evanno method ΔK (Evanno *et al.* 2005) and LnP(K) agreed that the most likely number of genetic clusters inferred by STRUCTURE was two. The two clusters represent a southern and northern cluster (Figure 4.5). ALB and SOE are entirely assigned to the southern cluster and CAT entirely to the northern cluster. NOE is comprised of a mix of individuals, with individuals from southern sites 7 and 8 assigned with ALB and SOE, while the northern site 9 clusters with CAT. We also found that two individuals within the northernmost population have a high probability of assignment to the southern genetic cluster.

Together, these results suggest that the westward expansion is genetically similar to the core populations, whereas the more successful northern expansion is genetically distinct from the westward expansion and the core populations, indicated by a genetic break within the NOE zone.

4.4.4. Hybridization in Northern Populations

We investigated whether the genetic distinctness and relatively high genetic diversity of northern populations (9-14) could be the result of hybridization with a co-occurring and morphologically similar coral, *Cladocora caespitosa*. The haplotype network for the variable nuclear marker p14 shows that all sequences from *Oculina* spp. (including *O. patagonica* from this study) share similar alleles across a large geographic scale, and are distinct from *Cladocora caespitosa* (at least 20 mutation steps between *Oculina* and *Cladocora* alleles), suggesting that these species are not hybridizing (see Appendix C, Figure 4.S5 for additional information).

4.4.5. Factors Associated with Genetic Diversity

We examined the effects of geographic location, temperature at time of sampling, and substrate (natural versus artificial) on patterns of genetic structure on the two expansion fronts separately (Table 4.4). Along the westward expansion, model 5 that included all factors and their interaction had the highest probability (Table 4.4a). This implies a complex interaction of geographical and environmental factors shaping patterns of genetic diversity, but does not point

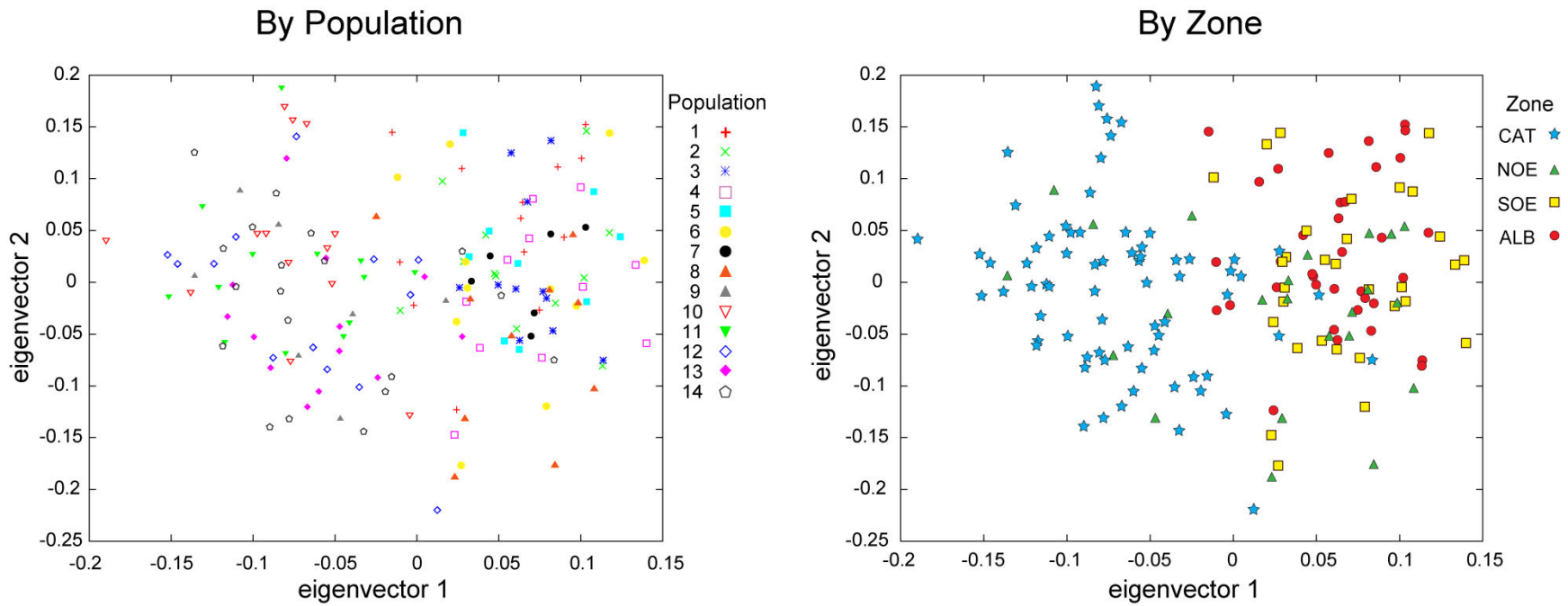

Figure 4.3. Principal component analysis plots of individuals grouped by location: population and zone. The first eigenvector explains 3.24% of the variation (p<0.01). The second eigenvector explains 2.42% of the variation (p=0.01).

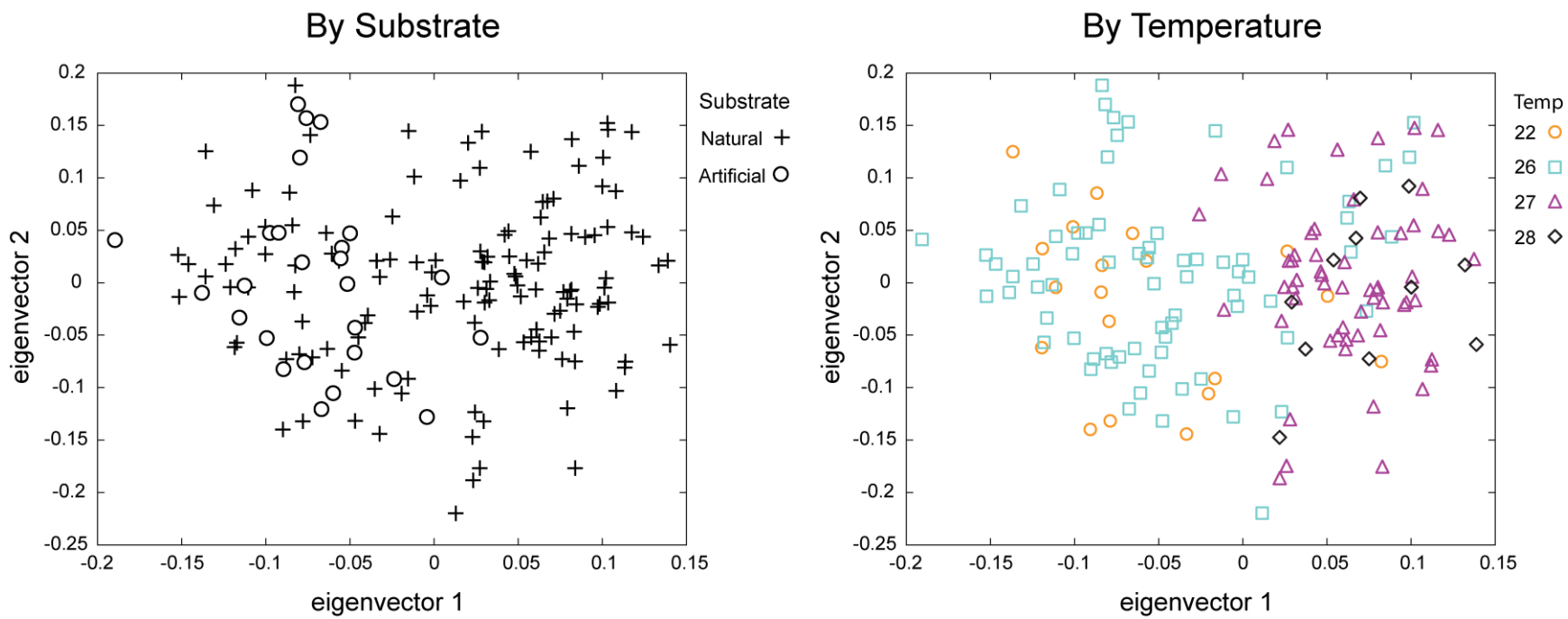

Figure 4.4. Principal component analysis plots of individuals grouped by environmental variables: substrate, and temperature at each locality. The first eigenvector explains 3.24% of the variation (p<0.01). The second eigenvector explains 2.42% of the variation (p=0.01).

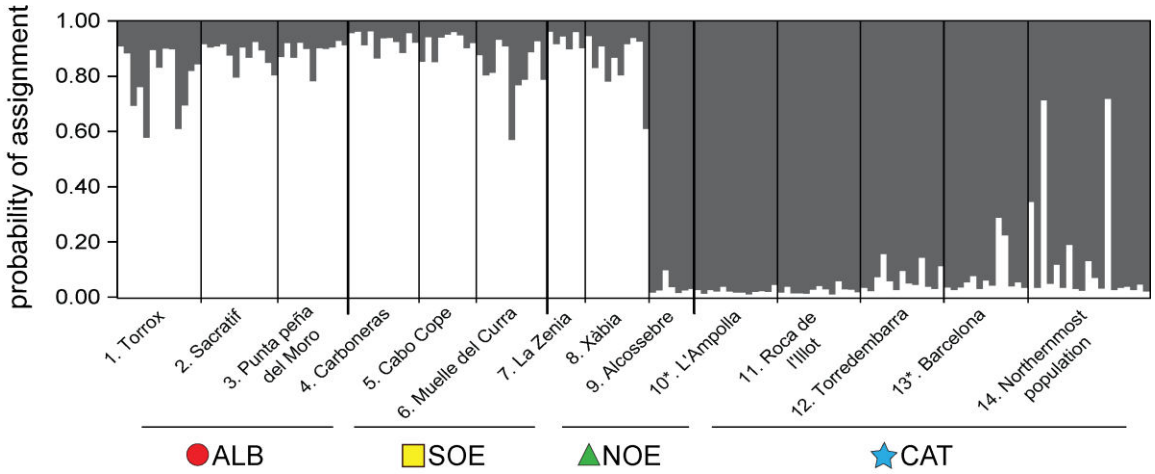

Figure 4.5. STRUCTURE bar plot for *K*=2. Individuals (bars) are grouped by population along the x-axis, with the probability of assignment to a particular genetic cluster (represented by different shades) along the y-axis.

to a single environmental factor explaining the majority of the variation. For the northern expansion, however, temperature alone stood out as being the single factor most driving genetic structure. The model (3) with the highest probability included the constant and temperature (Table 4.4b). Furthermore, all models that included temperature had the highest probabilities, and the sum of all models containing temperature was 99.8%, compared to just 0.2% for those without temperature.

Table 4.4. Model probabilities (P(*M*)) for all models examined, a. along the westward expansion, and b. along the northward expansion.

a. Westward Expansion

Model	Factors included	P(*M*)
1	Constant	0.00
2	Constant + Longitude	0.00
3	Constant + Temperature	0.00
4	Constant + Longitude + Temperature	0.00
5	Constant + Longitude + Temperature + Longitude*Temperature	1.00

b. Northern Expansion

Model	Factors included	P(*M*)
1	Constant	0.00077
2	Constant + Latitude	0.00086
3	Constant + Temperature	0.302
4	Constant + Latitude + Temperature	0.263
5	Constant + Substrate	0.00057
6	Constant + Latitude + Substrate	0.00061
7	Constant + Temperature + Substrate	0.247
8	Constant + Latitude + Temperature + Substrate	0.186

4.4.6. Loci under Selection

Along the westward expansion front, we found no signal for selection associated with either geographic location or temperature. Along the northern expansion, we also found no evidence for loci under selection associated with location. However, when we tested for local adaptation to temperature along the northern expansion, we found one candidate locus under selection, as indicated by an FDR value < 0.05. A BLASTx search of the contig matched (with high probability; E-value = 4×10^{-8}) a V-type ATPase 116 kDa subunit (accession number XP_015752902), part of a protein family previously shown to be down-regulated under thermal stress in the coral *Acropora aspera* (Rosic *et al.* 2014). Three other loci had FDR values between 0.06 and 0.30, while the remaining 591 loci had values above 0.85, with the majority (99%) of these >0.90. We found no loci putatively under selection associated with substrate.

4.5. DISCUSSION

In this study, we investigated the range expansion of *O. patagonica* along the Spanish coast using a RAD-Seq protocol modified to target coral DNA. Our goal was to compare the genetic and environmental differences between a successful northward expansion and a less successful westward expansion.

4.5.1. RAD-Seq Targeted to Host DNA

Although the advent of next-generation sequencing of reduced-representation libraries has vastly improved our ability to study non-model organism at a genomic scale, this can still be a daunting task for organisms that harbor endosymbionts. Unwanted symbiont DNA can contaminate these libraries, especially in corals, which harbor vast numbers (as much as 6×10^{6} *Symbiodinium* cells per cm^2 of coral tissue)(Stimson *et al.* 2002) of intracellular algal symbionts with large genomes (ca. 1200-1500 Mbp)(Lin *et al.* 2015; Shoguchi *et al.* 2013). By comparison, the genome of the coral *Acropora digitifera* is 420 Mbp (Shinzato *et al.* 2011), about one-third the size. Combined with the high densities of *Symbiodinium* within coral tissue, the signal from coral genes could be swamped by its symbiont's DNA. Dissociating changes to host and symbiont genomes is crucial, given that symbiont variation ('switching') may provide a quick way to respond to environmental change (Domaschke *et al.* 2013; Jones *et al.* 2008; Leydet & Hellberg 2016). Thus, the response of the symbiont community may confound any coral-specific responses if their genomes are not carefully disentangled.

To focus sequencing efforts on the coral host, we implemented a new RAD-Seq method to capture and sequence coral sequence fragments. Although, we were unable to take advantage of the reference genomes available for coral and symbiont (due to phylogenetic divergence and partial genome assembly, respectively), most contigs matched best to coral proteins in BLASTx searches. Furthermore, we only retained contigs that aligned to our 'symbiont-free' sample as an extra precaution. We are therefore confident that the majority of our loci are indeed coral-specific.

4.5.2 The Two Spanish Expansions are Genetically Distinct

We found that the westward and northern expansions of *Oculina patagonica* are genetically distinct (Figure 4.5). While the westward expansion is similar to more long-established core populations, the northern expansion, although more recent (Serrano *et al.* 2013c), is genetically distinct from the core, although two individuals within the northernmost population have a high probability of assignment to the southern populations. The break between the two genetic clusters occurs within the northern expansion, within zone NOE between populations 8 and 9 (Fig. 4.1). This region coincides geographically with a decrease in temperature at time of collection (27˚C at population 8 and 26˚C at population 9). Indeed, according to our PCA plots (Figure 4.4) and GESTE results (Table 4.4), temperature appears to be a factor associated with population structure along the northern expansion. A similar shift in temperature along the westward expansion (between populations 1 and 2), however, is not associated with a genetic break.

Aside from a temperature shift, the observed genetic break within the northern expansion could also stem from a barrier at the Ibiza Channel (IC in Fig. 4.1). The Ibiza Channel coincides with genetic breaks in other marine organisms (García-Merchán *et al.* 2012; Mokhar-Jamai *et al.* 2011), including the coral *Cladocora caespitosa* (Casado-Amezúa *et al.* 2014). Circulation across the Ibiza Channel is often blocked by the Northern Current, which carries waters south to the Ibiza Channel and then diverts northeastward (Pinot *et al.* 2002; Ruiz *et al.* 2009). In the summer, the northeastern deflection of the Northern Current, caused by the formation of a gyre, is more intense (Pinot *et al.* 2002). Given that *Oculina patagonica* spawns at the end of the summer (Fine *et al.* 2001), these currents may restrict gene flow between populations on either side of the Ibiza Channel, thus explaining the observed genetic break between populations 8 and 9.

Elsewhere within our sampled range, the Almeria-Oran front (AOF between ALB and SOE in Fig. 4.1) coincides with a genetic break in some marine species (Patarnello *et al.* 2007), but not others (García-Merchán *et al.* 2012). In *Oculina patagonica*, this front does not appear to be a genetic break, as populations on either side of the Almeria-Oran front are genetically similar (Figure 4.5).

4.5.3. Increased Genetic Diversity in the Northern Expansion

Unlike many invasions (Cahill & Levinton 2016; Herborg *et al.* 2007; Tsutsui *et al.* 2000), genetic diversity within the northern expansion was higher than that in the other, longer-established populations (Figure 4.2). In contrast, heterozygosity within the westward expansion was the same as in the core populations. Although genetic diversity does not necessarily predict invasion success (Roman & Darling 2007), genetic variation is the raw material for evolution and adaptation. Therefore, having more genetic variation may provide the northern populations with more raw material on which adaptation can act.

Although uncommon, increased genetic diversity in invasive populations has been documented in other organisms. This has been most often attributed to admixture of distinct populations within the invaded range, due either to multiple introductions from different source populations

(Kolbe *et al.* 2004; Novak & Mack 1993; Roman 2006) or to hybridization within the newly invaded range (Ayres & Strong 2001; Hohenlohe *et al.* 2013).

Could *Oculina patagonica* be hybridizing within the northward expanded range? The closest relative to *O. patagonica* in the Mediterranean is *Cladocora caespitosa* (Fukami *et al.* 2004; Kitahara *et al.* 2010), even though classical taxonomy has long placed them in separate families. The two are easily mistaken for each other in the field (C. Grupstra, personal observation) and their *COI* haplotypes differ by only a single SNP over 606 bp (*C. caespitosa COI* accession number KR297263). *C. caespitosa* occurs along the entire Spanish Mediterranean coast (Casado-Amezúa *et al.* 2014) and spawns at the end of summer (August– October) in the western Mediterranean, when seawater temperatures are declining (Kersting *et al.* 2013). This coincides with the spawning time of *O. patagonica* (Fine *et al.* 2001). However, *C. caespitosa* is more abundant at deeper depths (> 10 m) than *O. patagonica* (Casado-Amezúa *et al.* 2014; Kersting & Linares 2012), suggesting the two species segregate by depth. In addition, according to a variable nuclear marker (p14), *C. caespitosa* is highly genetically distinct from *Oculina* spp. (including *O. patagonica* along the northern Spanish expansion) (Appendix C, Figure 4.S5). Whereas the average number of nucleotide differences for p14 within *Oculina* spp. is 1.4, the average number of nucleotide difference between *Oculina* spp. and *Cladocora* is 20.5. Our data thus suggest that interspecific hybridization does not explain the relatively high genetic diversity in the northern expansion.

Could the increased genetic variation in the northern expanded range be the result of multiple introductions? The European green crab, for example, was first reported in North American waters (southern Massachusetts) in 1817, but has only recently (last 50 years) expanded north into Nova Scotia. Surprisingly, greater allelic diversity was found in these more recent northern populations compared to the older southern populations, a pattern attributed to admixture with secondary introductions from northern Europe (Roman 2006). Candidate populations for a source of genetically differentiated secondary invaders of *O. patagonica* occur nearby.

Close by, *O. patagonica* occurs along the Mediterranean coasts of France and Italy, although they are not well established in either country. Our recent work (Leydet & Hellberg 2015), however, indicates that the Spanish and Italian populations are not genetically differentiated from each other, nor indeed are any of the populations we sampled across the Mediterranean, although this result could stem from the relatively low power of our 5-locus dataset. The significant differentiation we found here at a much smaller geographic scale using 595 SNP loci suggests that we cannot rule out possible gene flow from genetically differentiated Italian populations, whose distinctness was too subtle to detect with our previous dataset.

In addition to admixture, an influx of genes from different habitats could boost northern genetic diversity. Depth is increasingly being implicated in driving genetic structuring within coral populations (Pérez-Portela *et al.* 2016; Prada & Hellberg 2013; Serrano *et al.* 2014). Along the coast of Spain, *O. patagonica* is most commonly reported from depths less than 10 m (Coma *et al.* 2011; Serrano *et al.* 2013c; Terrón-Sigler *et al.* 2015), however colonies have been found along the Catalan coast as deep as 28 m (Serrano *et al.* 2013c). Indeed, samples collected from the two most northern sites (13 and 14), were from greater depths compared to the other sites

(Table 4.1), and may harbor allelic variation from genetically differentiated deep populations. Sampling along a depth cline is needed to test this hypothesis (Prada & Hellberg 2013).

4.5.4. Environmental Factors Facilitating Northern Range Expansion

Similar to other invasions (Cavanaugh *et al.* 2014; Pateman *et al.* 2012; Yamano *et al.* 2011), temperature has been linked to the geographic and demographic spread of *O. patagonica*, mainly by extending its growth period (Serrano *et al.* 2013c). We found that populations along the Spanish Mediterranean coast appear to the genetically differentiated along a temperature gradient and that temperature variation is driving patterns of genetic structure. Furthermore, we found one locus (a V-type proton ATPase subunit) under selection associated with temperature, but not latitude (which is correlated with temperature). This suggests that temperature itself is the environmental factor driving selection at the locus. V-ATPases are involved in cell membrane transport. Interestingly, another V-type proton ATPase proteolipid subunit is down-regulated in the coral *Acropora aspera* in response to thermal and nutrient stress (Rosic *et al.* 2014). Gene expression studies comparing core and expanded populations are needed to more effectively test for adaptation to cooler temperatures within the northern expansion front (Lancaster *et al.* 2016).

Modification of coastal habitats has also been linked to the Mediterranean expansion of *O. patagonica* (Coma *et al.* 2011; Salomidi *et al.* 2013; Serrano *et al.* 2013c; Terrón-Sigler *et al.* 2015). We did not find genetic differentiation associated with substrate, nor did we find any loci under selection when we compared populations sampled from different substrates. Although additional paired-sampling from both substrate types along the entire Spanish coast are needed to provide a more powerful test for local adaption to artificial substrate, our results suggest that *O. patagonica* may not be locally adapted to artificial substrate. Instead, it seems more likely that it is an opportunistic colonizer, taking advantage of the increased space availabilities provided by artificial habitats (Serrano *et al.* 2013c).

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CHAPTER 5.
CONCLUSIONS

In the face of global environmental changes, species must adapt, acclimate, or move, or they will perish. The goal of my dissertation was to understand the origins and mechanisms underlying the rapid and recent population expansion of the invasive coral *Oculina patagonica* in the Mediterranean Sea. Using nuclear markers and next-generation sequencing data, I explored whether *O. patagonica* has been recently introduced into the Mediterranean or whether it is a native invasive, as well as the mechanisms that have allowed this coral to be so successful within the Mediterranean Sea, where it is locally expanding its range.

In my first research chapter (Chapter 2), I found no genetic or historical demographic evidence to support a recent human-mediated introduction of *O. patagonica* from the western North Atlantic into the Mediterranean. Instead, I found that Mediterranean and Atlantic populations are genetically distinct and appear to have begun diverging about 5 million years ago. Fossil evidence of *Oculina* spp. in the eastern North Atlantic millions of years ago further supports the hypothesis that *Oculina* spp. has had a long history in this region. My results suggest that Mediterranean populations of *O. patagonica* have not been recently introduced from North America. Instead, it is more likely that *O. patagonica* is a native invasive species, and that it has always existed somewhere in the eastern Atlantic, either in undetectable numbers or overlooked and undersampled sites and habitats, and has recently begun expanding to detectable numbers in the Mediterranean, likely in response to anthropogenic environmental changes.

Next (Chapter 3), using genetic and environmental data I found that *Oculina* corals harbor different symbiotic algal communities across their western Atlantic and Mediterranean range, and that habitat differences in sea surface temperature are better correlated with this geographical variation than the host's genetics, depth or chlorophyll a concentration. This was particularly evident for populations within the Mediterranean. These results suggest that the *Symbiodinium* communities that *Oculina* corals harbor may reflect acclimatization to local temperature conditions, thus allowing these corals to endure localized thermal stress. The work also demonstrates the importance of these algal symbionts even for facultative zooxanthellate corals that can survive without them.

In my final research chapter (Chapter 4), I used a modified restriction-site associated DNA sequencing (RAD-Seq) protocol to target coral host DNA and separate its adaptive response from that of its symbionts. I collected hundreds of single nucleotide polymorphisms (SNPs) from *O. patagonica* from across the entire Spanish Mediterranean coast, where this coral has been expanding westward and northward from the southwestern core populations. I found that the more successful northern expansion populations harbor greater genetic diversity than the less successful westward expansion populations or the core populations. I also found that the northern most populations are genetically distinct from all other populations. I did not find evidence of local adaptation to artificial substrate, suggesting that this coral is simply an opportunistic colonizer, a trait likely to have facilitated its rapid expansion. In contrast to substrate, I found that temperature appears to have driven local adaptive only along the northern expansion; a V-type proton ATPase subunit gene was found to be putatively under selection associated with temperature. Together, these results suggest that unique genetic variation and adaptation along

the northern expansion front may have promoted the rapid and very successful poleward range expansion of *O. patagonica* in the western Mediterranean.

Altogether, my dissertation highlights the factors and mechanisms that have allowed a coral to be so successful despite its stressful and changing environment. Most research is focused on studying threatened tropical corals and assessing their adaptive capabilities and long-term success. In contrast, my dissertation focusses on the success and adaptive potential of an understudied temperate coral in the face of environmental changes, including increasing temperatures and coastal habitat modifications. My findings suggest that *O. patagonica* is able to successfully respond to changing and stressful environmental conditions via symbiont switching and host adaptation, thus facilitating its expansion in its native range.

5.1 FUTURE DIRECTIONS

While I examined the association between *Oculina* corals and their algal symbionts in driving acclimation to different habitats, the coral holobiont constitutes a congregation of many other microorganisms including endolithic algae (Fine & Loya 2002; Rubio-Portillo *et al.* 2014a) bacteria, archaea, and viruses (Nissimov *et al.* 2009; Rubio-Portillo *et al.* 2014b). These communities have also been implicated in coral health and survival (Gilbert *et al.* 2012; Nissimov *et al.* 2009). Furthermore, microbial community shifts have been associated with stress, lending further support that these communities play a role in coral health (Ainsworth & Hoegh-Guldberg 2009; Lee *et al.* 2015; Vega Thurber *et al.* 2009). Given that the bacterial communities associated with *O. patagonica* vary seasonally in the eastern Mediterranean (Koren & Rosenberg 2006), future work should examine bacterial and other microorganismal variation associated with thermal and other stressors to better understand their role in the successful expansion of the coral host.

Another potential explanation for *O. patagonica's* widespread success across the Mediterranean Sea is a variable stress response allowing it to cope with the wide range of environmental conditions across the sea (Rodolfo-Metalpa *et al.* 2014). Gene expression in corals has been shown to vary in response to stress (DeSalvo *et al.* 2010; DeSalvo *et al.* 2008; Meyer *et al.* 2011; Polato *et al.* 2010). Location-specific variation in gene expression in response to stress occurs in corals despite significant gene flow, suggesting that even genetically similar populations can adapt to different habitats by varying their gene expression (Polato *et al.* 2010). Furthermore, gene expression variation has been associated with invasion where 'native-core' and expanding populations differ in gene expression profiles, potentially revealing candidate genes contributing to invasiveness (Hodgins *et al.* 2013). Exploring the role of gene expression plasticity in *O. patagonica* adaptation will require an RNA-Seq approach, a method based on the deep sequencing and quantitative analysis of short cDNA reads (Meyer *et al.* 2011), which can then be used to determine significant gene expression patterns that may be unique to particular locations across the Mediterranean. Transcriptomic data will be particularly useful to further investigate the mechanisms driving *O. patagonica*'s northward expansion in the western Mediterranean. Using genomic data, we were able to identify a candidate gene under selection showing promise to identify additional evolutionary responses using transcriptomic data, which is more suitable for exploring functional adaptation.

Finally, the dissertation work suggests that *Oculina* corals, in particular *O. patagonica*, are able to overcome the challenges of changing environmental conditions. However, little work has been conducted on evaluating the long-term adaptive potential of corals if temperatures continue to increase, coasts continue to be affected by human modifications, and the environmental changes that we are witnessing worsen in the coming decades. Such studies are needed to better assess the future of corals, and will require a multidisciplinary approach to identify all of the key environmental factors affecting corals' survivorship (and their threshold values), and predictive computer modeling.

5.2. REFERENCES

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APPENDIX A.
SUPPLEMENTARY MATERIAL FOR CHAPTER 2

Table 2.S1. Collection sites of all *Oculina* spp. samples used in this study.

	Country	Locality	n[a]	n[b]	Latitude	Longitude	Depth (m)
Western Atlantic	United States	North Carolina	8	8	34˚42′ N	76˚40′ W	2–4
		Daytona Beach, Florida	13	13	29˚15′ N	80˚45′ W	21–23
		Cape Florida, Florida	9	9	25˚40′ N	80˚9′ W	2
		Panama City, Florida	13	13	30˚3′ N	85˚51′ W	28–29
	Bermuda	Tynes Bay	13	13	32˚18′ N	64˚46′ W	3–10
Mediterranean	Spain	Cabo de Palos	14	13	37˚38′ N	0˚41′ W	3–8
	Italy	Savona	2	2	44˚20′ N	8˚30′ E	1–2
	Greece	Athens	18	17	37˚53′ N	23˚43′ E	0.5–1
	Lebanon	Tyre	4	4	33˚16′ N	35˚11′ E	4
	Israel	Caesarea	10	4	32˚30′ N	34˚54′ E	2–5
		Hadera	8	4	32˚27′ N	34˚55′ E	2–5
		Sdot-Yam	10	5	32˚29′ N	34˚53′ E	2–5
Total			122	105			

[a] sample size prior to removing clonal genotypes
[b] sample size after removing clonal genotypes

Table 2.S2. Nuclear markers used to genotype all *Oculina* spp. samples in this study.

Marker	(putative gene) Primer sequences	Length (bp)	# alleles	S^a	k^b	π^c	Θ^d	rate[e]
p14[*]	(Fatty acid elongase) 5′ TGTACCACTTGGGATGAACG 3′ 5′ TCAAGCTTCCAGTCTTGTGAAA 3′	206	19	18	1.54	0.0075	0.0148	2.8×10^{-7}
p62[*]	(Elongation factor 1α) 5′ TGATTGTCCTCAACCATCCA 3′ 5′ CTCCTGACAGACTTTCGATGG 3′	249	11	6	1.03	0.0042	0.0048	3.4×10^{-7}
p302[*]	(Tachylectin-2 motif) 5′ TTATACGGCGTCACAAACGA 3′ 5′ TCGTCATCACCCTTTTATTCC 3′	226–229	20	28	2.17	0.0098	0.0228	3.2×10^{-7}
p243	(Crystalline) 5′ TCCCCAGAATGTCAACAACA 3′ 5′ ATTCYTTMCGAATGCTCTGC 3′	124	8	11	4.42	0.0357	0.0150	1.7×10^{-7}
p255	(S-adenosylmethionine synthetase) 5′ GCCAGGTGGATTGCTAAGTC 3′ 5′ CRTCTKTGTTTAAATAAAGCAAACATT 3′	197	18	18	3.91	0.0200	0.0155	2.7×10^{-7}

[*] Eytan *et al.* 2009[1]
[a] segregating sites
[b] average number of nucleotide differences
[c] nucleotide diversity per site
[d] theta-W per site based on number of segregating sites
[e] substitution rate per locus per year used in IMa analyses

[1] Eytan RI, Hayes M, Arbour-Reily P, Miller M, Hellberg ME (2009) Nuclear sequences reveal mid-range isolation of an imperilled deep-water coral population. *Molecular Ecology* **18**, 2375-2389.

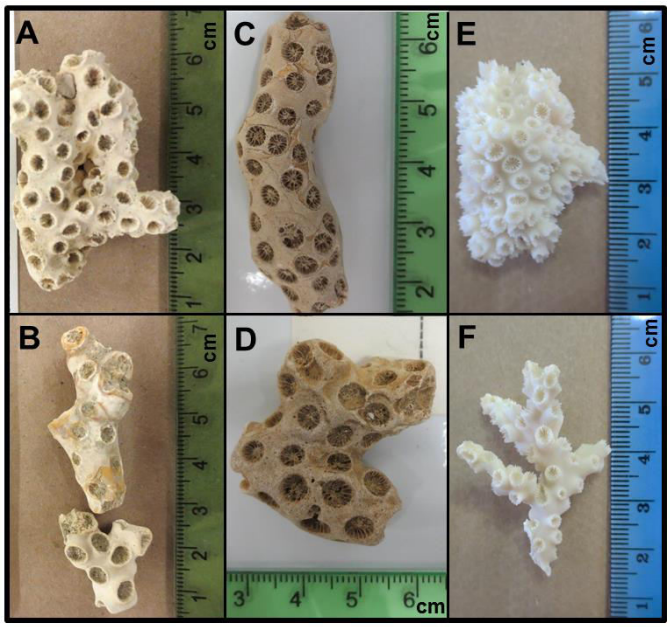


Figure 2.S1. Photographs of *Oculina* spp. specimens. A–D are *Oculina* spp. fossil specimens from the Smithsonian National Museum of Natural History. A and B are *O. patagonica* from South America (USNM 75199 and USNM 75205, respectively). C and D are *O. crassoramosa* from France (USNM I 80807). E is a skeletal specimen of extant *O. patagonica* from the eastern Mediterranean. F is a skeletal specimen of extant *O. diffusa* from Panama City, Florida (USA).

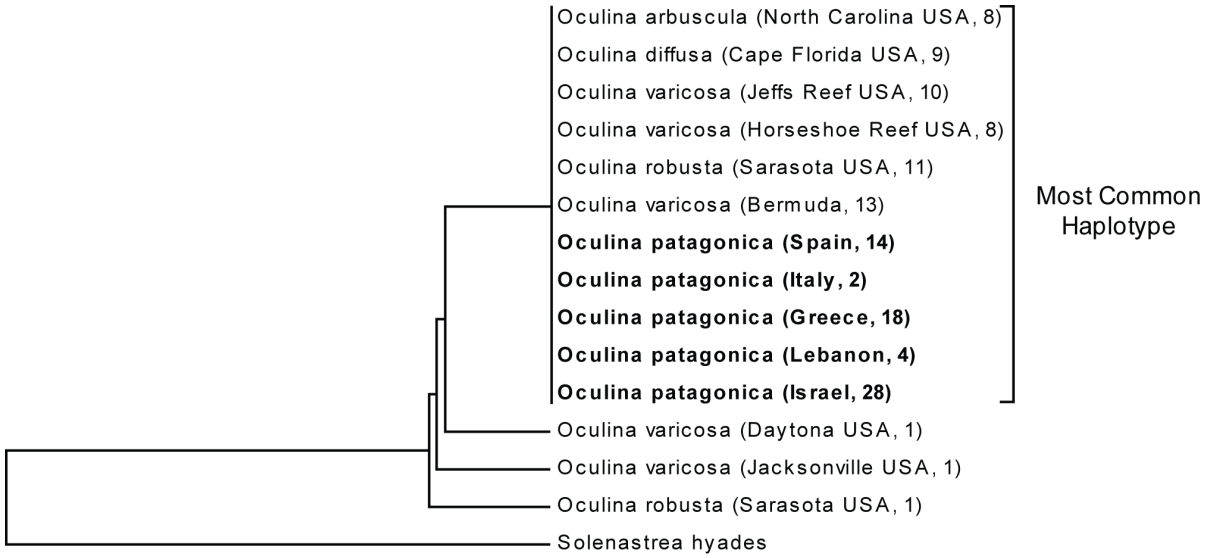


Figure 2.S2. *COI* Neighbor-Joining Tree. Neighbor-joining tree constructed using *COI* haplotypes from western North Atlantic *Oculina* spp. populations and *O. patagonica* populations from the Mediterranean, with *Solenastrea hyades* as the outgroup. Numbers represent the number of individuals from each locality that share that haplotype. The tree shows that *O. patagonica* (bolded) shares the same haplotype common to most western North Atlantic *Oculina* spp.

APPENDIX B.
SUPPLEMENTARY MATERIAL FOR CHAPTER 3

Table 3.S1. Collection sites of all samples used in this study.

	Country	Locality	Latitude	Longitude	n^a	Depth (m)	Date collected
Western Atlantic	United States	North Carolina	34˚42′ N	76˚40′ W	8	2–4	August 2003
		Daytona Beach, Florida	29˚15′ N	80˚45′ W	11	21–23	July 2005
		Cape Florida, Florida	25˚40′ N	80˚09′ W	9	2	March 2004
		Panama City, Florida	30˚03′ N	85˚51′ W	11	28–29	January 2004
	Bermuda	Tynes Bay	32˚18′ N	64˚46′ W	13	3–10	October 2005
Mediterranean	Spain	Cabo de Palos	37˚38′ N	00˚41′ W	14	3–8	August 2011
	Italy	Savona	44˚20′ N	08˚30′ E	2	1–2	July 2013
	Greece	Athens	37˚53′ N	23˚43′ E	18	0.5–1	July 2011
	Lebanon	Tyre	33˚16′ N	35˚11′ E	4	4	August 2011
	Israel	Caesarea, Hadera, Sdot-Yam	32˚28′ N	34˚54′ E	27	2–5	July 2012

[a] number of samples (this final dataset included all individuals with no missing data)

Table 3.S2. Top BLAST matches of all *cp23S* haplotypes obtained in this study. Haplotype color identifications correspond to the color designations in the haplotype network in Figure 3.1. Coverage and identity matches are indicated as both percentages and base pairs (bp). Asterisks indicate haplotypes with 100% coverage and 100% identity to *Symbiodinium* type published sequences.

Haplotype	Accession #	Reference	Type	Species	Coverage	Identity
Red*	JX213589, JX213590	LaJeunesse *et al.* 2012	B2	*S. psygmophilum*	100% (181/181 bp)	100% (181/181 bp)
	JN557993–JN557995	Pochon *et al.* 2012	B2	–	100% (181/181 bp)	100% (181/181 bp)
Yellow	JX213589, JX213590	LaJeunesse *et al.* 2012	B2	*S. psygmophilum*	100% (181/181 bp)	99% (180/181 bp)
Orange	JX213589, JX213590	LaJeunesse *et al.* 2012	B2	*S. psygmophilum*	100% (182/182 bp)	99% (180/182 bp)
Light Green	JX213589–JX213591	LaJeunesse *et al.* 2012	B2	*S. psygmophilum*	100% (180/180 bp)	99% (179/180 bp)
Black	JX213589, JX213590	LaJeunesse *et al.* 2012	B2	*S. psygmophilum*	100% (181/181 bp)	99% (180/181 bp)
Grey	JX213589–JX213591, JX213593	LaJeunesse *et al.* 2012	B2	*S. psygmophilum*	74% (134/181 bp)	100% (134/134 bp)
Blue*	JX213588	LaJeunesse *et al.* 2012	B1	*S. minutum*	100% (141/141 bp)	100% (141/141 bp)
	JN557992	Pochon *et al.* 2012	B1	–	100% (141/141 bp)	100% (141/141 bp)
Dark Green	JX213587, JX213588	LaJeunesse *et al.* 2012	B1	*S. minutum*	100% (141/141 bp)	99% (140/141 bp)
Purple	JX213588	LaJeunesse *et al.* 2012	B1	*S. minutum*	100% (141/141 bp)	99% (140/141 bp)

Table 3.S3. Top BLAST matches of all *b7sym15* haplotypes obtained in this study. Haplotype color identifications correspond to the color designations in the haplotype network in Figure 3.1. The blue haplotype also matched sequences downloaded from DRYAD entry doi:10.5061/dryad.r84n5 (Thornhill *et al.* 2013). Coverage and identity matches are indicated as both percentages and base pairs (bp). Asterisks indicate haplotypes with 100% coverage and 100% identity to *Symbiodinium* type published sequences.

Haplotype	Accession #	Reference	Type	Species	Coverage	Identity
Red*	JX263428	LaJeunesse *et al.* 2012	B2	*S. psygmophilum*	100% (139/139 bp)	100% (139/139 bp)
Yellow*	JX263429	LaJeunesse *et al.* 2012	B2	*S. psygmophilum*	100% (139/139 bp)	100% (139/139 bp)
Green	JN602468	Reichman & Vize 2014	B?	–	100% (139/139 bp)	100% (139/139 bp)
Blue*	JX263427	LaJeunesse *et al.* 2012	B1	*S. minutum*	100% (145/145 bp)	100% (145/145 bp)
	DRYAD download	Thornhill *et al.* 2013	B1	*S. minutum*	100% (145/145 bp)	100% (145/145 bp)
Purple	JN602460	Reichman & Vize 2014	B?	–	100% (126/126 bp)	100% (126/126 bp)
	KT149354	Parkinson *et al.* 2015	B1	*S. endomadracis*	99% (125/126 bp)	100% (125/125 bp)
	EF212868	Pettay & LaJeunesse 2007	B7	–	100% (126/126 bp)	99% (125/126 bp)

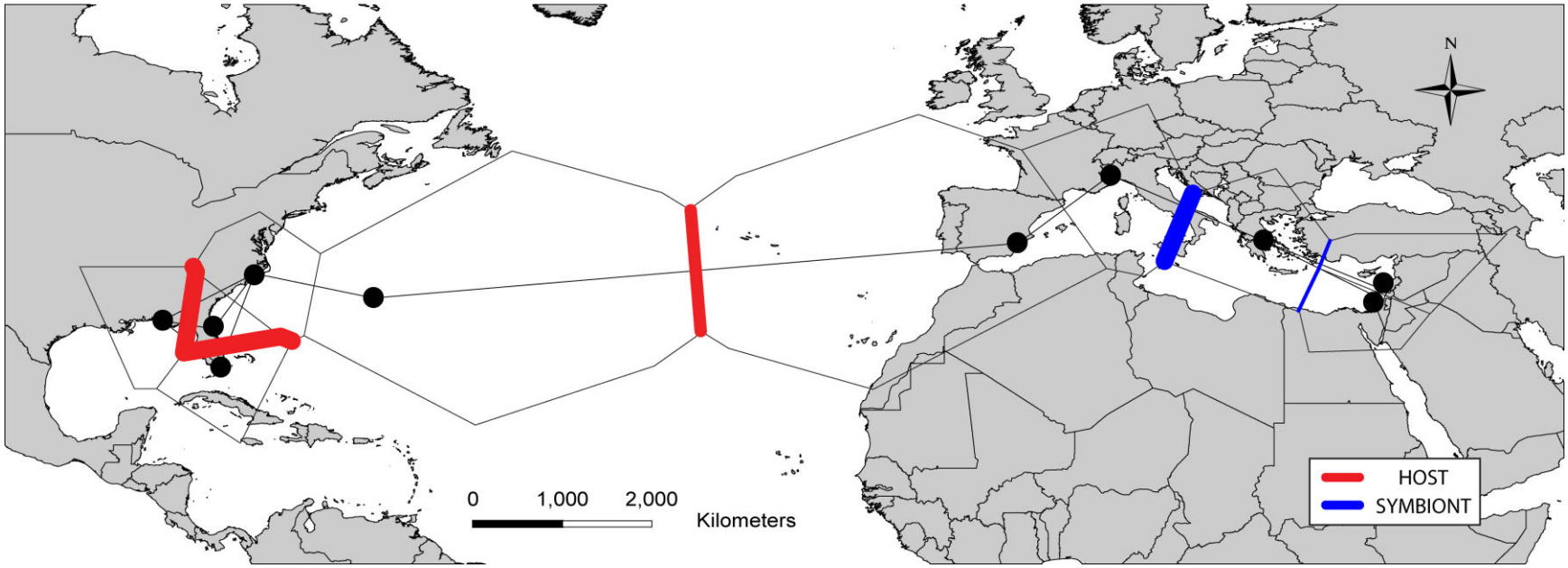


Figure 3.S1. Genetic barriers of the coral host (red) and algal symbiont (blue) computed in BARRIER version 2.2 (Manni *et al.* 2004). BARRIER uses Voronoï tessellation (the polygons on the map), genetic distance matrices, and Monmonier's maximum difference algorithm to identify the zones where differences between pairs of populations are largest (i.e., genetic barriers on a map). We computed F_{ST} matrices for host and symbiont separately in GENODIVE 2.0b27 (Meirmans & Van Tienderen 2004). We performed the analyses on all loci for each partner (host=5 loci; symbiont=3 loci) and composite the strongest barrier for each locus and each partner on a single map. The thickness of the barriers indicates their robustness (i.e., agreement across loci), where thicker barriers are more robust. BARRIER recovered the two main breaks in *Oculina* spp. previously described by Eytan *et al.* (2009) and Leydet & Hellberg (2015). BARRIER also recovered the two main genetic breaks in the *Symbiodinium* communities that we describe in this study. These results corroborate our STRUCTURE results, showing that the main barriers identified distinguish the main genetic clusters obtained by STRUCTURE (Figures 3.2 and 3.3), and further show that the host and symbiont are structured differently.

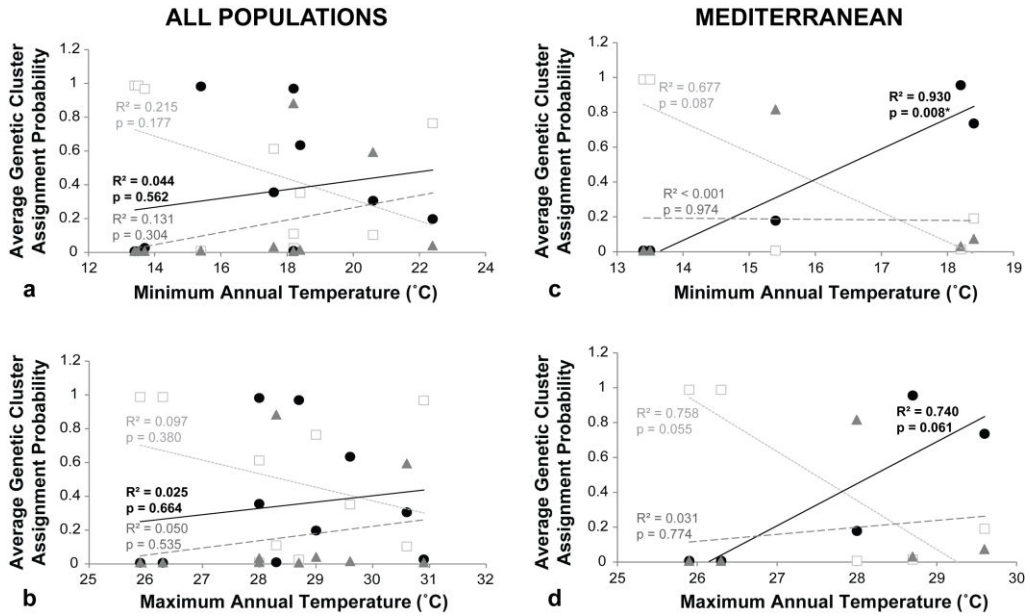


Figure 3.S2. Correlation between *Symbiodinium* community composition and temperature (minimum and maximum annual) for all populations (a and b) and Mediterranean populations only (c and d). The white squares, gray triangles, and black circles represent the white, gray, and black genetic clusters, respectively, obtained from STRUCTURE analyses (Figure 3.2 for all populations; Figure 3.3d for Mediterranean). Trend lines are as follow: white= short dashed line, gray= large dashed line, black= solid line. Significant correlations are indicated with an asterisk.

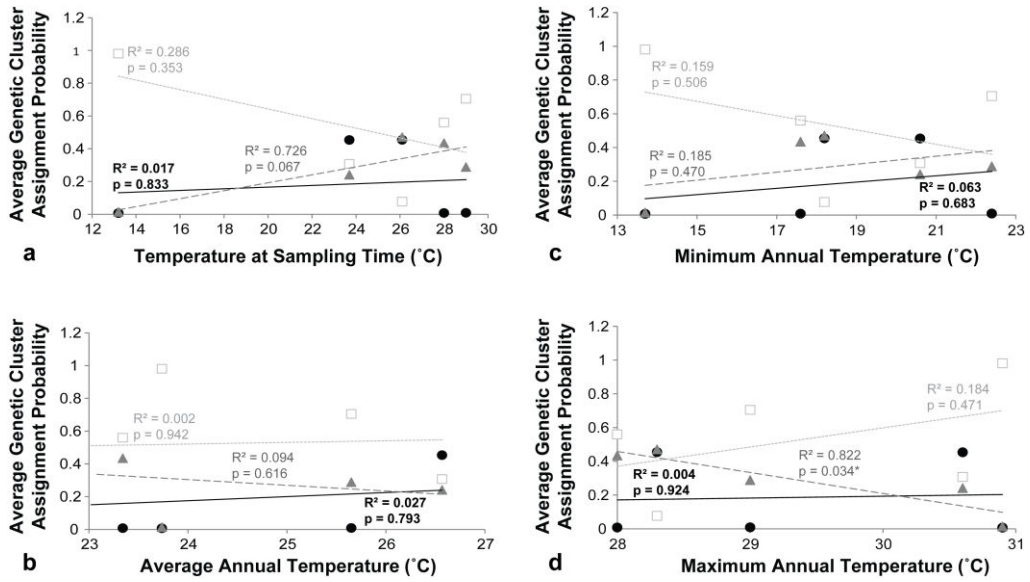
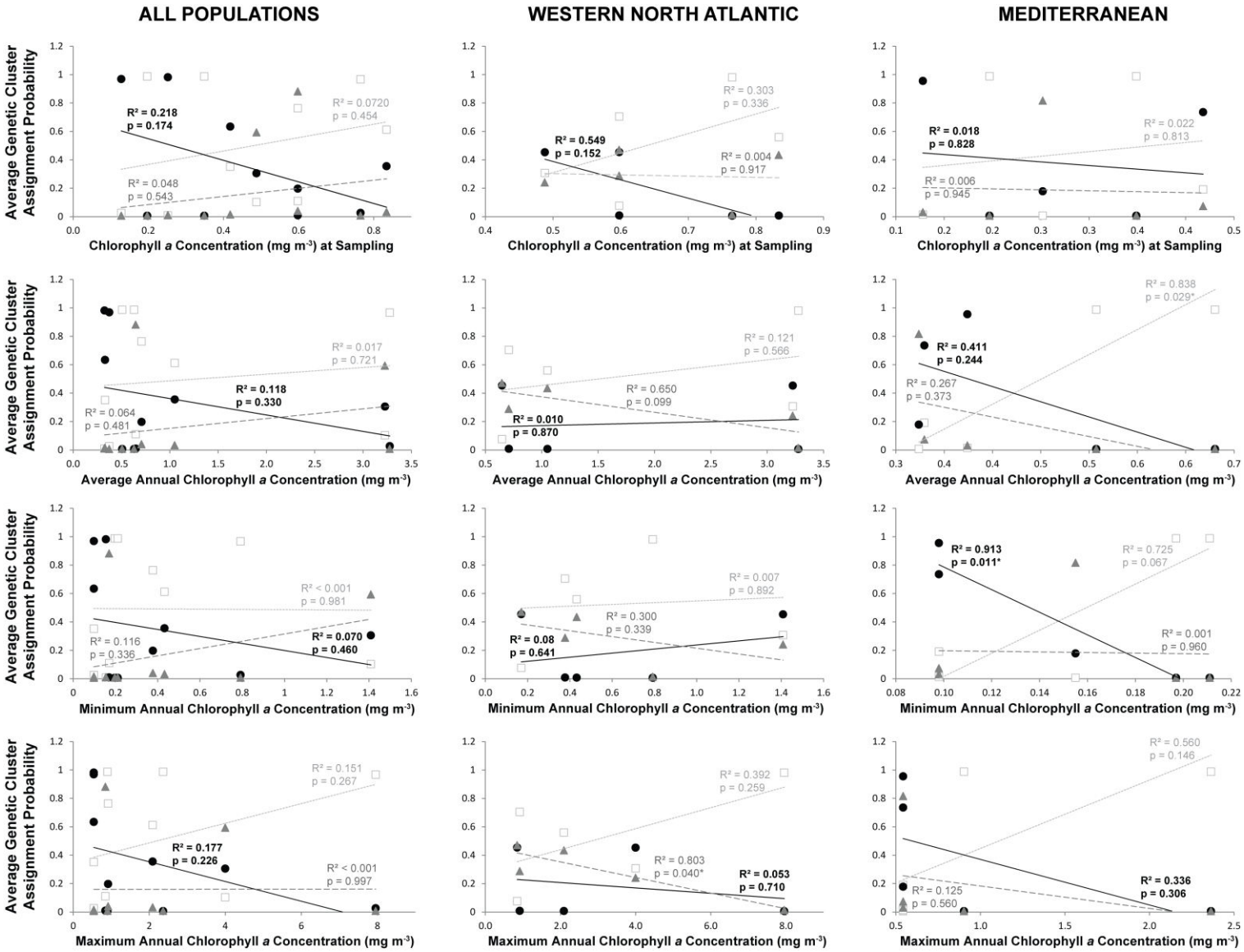


Figure 3.S3. Correlation between *Symbiodinium* community composition and temperature for western North Atlantic populations only. The white squares, gray triangles, and black circles represent the white, gray, and black genetic clusters, respectively, obtained from STRUCTURE analyses (Figure 3.3b). Trend lines are as follow: white= short dashed line, gray= large dashed line, black= solid line. Significant correlation is indicated with an asterisk; however, it did not survive a multiple comparisons correction.

Figure 3.S4. Correlation between *Symbiodinium* community composition and chlorophyll *a* concentration. The white squares, gray triangles, and black circles represent the white, gray, and black genetic clusters, respectively, obtained from STRUCTURE analyses (Figure 3.2 for all populations; Figure 3.3b for western North Atlantic; Figure 3.3d for Mediterranean). Trend lines are as follow: white= short dashed line, gray= large dashed line, black= solid line. Significant correlations are indicated with an asterisk; however, only the correlation between the 'black' genetic cluster and minimum annual concentration in the Mediterranean survived a multiple comparisons correction.


ALL POPULATIONS
WESTERN NORTH ATLANTIC
MEDITERRANEAN
Average Genetic Cluster Assignment Probability
Chlorophyll a Concentration (mg m-3) at Sampling
Average Annual Chlorophyll a Concentration (mg m-3)
Minimum Annual Chlorophyll a Concentration (mg m-3)
Maximum Annual Chlorophyll a Concentration (mg m-3)
R² = 0.218 p = 0.174
R² = 0.0720 p = 0.454
R² = 0.048 p = 0.543
R² = 0.549 p = 0.152
R² = 0.303 p = 0.336
R² = 0.004 p = 0.917
R² = 0.018 p = 0.828
R² = 0.022 p = 0.813
R² = 0.006 p = 0.945
R² = 0.118 p = 0.330
R² = 0.017 p = 0.721
R² = 0.064 p = 0.481
R² = 0.010 p = 0.870
R² = 0.121 p = 0.566
R² = 0.650 p = 0.099
R² = 0.411 p = 0.244
R² = 0.838 p = 0.029*
R² = 0.267 p = 0.373
R² = 0.070 p = 0.460
R² < 0.001 p = 0.981
R² = 0.116 p = 0.336
R² = 0.08 p = 0.641
R² = 0.007 p = 0.892
R² = 0.300 p = 0.339
R² = 0.913 p = 0.011*
R² = 0.725 p = 0.067
R² = 0.001 p = 0.960
R² = 0.177 p = 0.226
R² = 0.151 p = 0.267
R² < 0.001 p = 0.997
R² = 0.053 p = 0.710
R² = 0.392 p = 0.259
R² = 0.803 p = 0.040*
R² = 0.336 p = 0.306
R² = 0.560 p = 0.146
R² = 0.125 p = 0.560

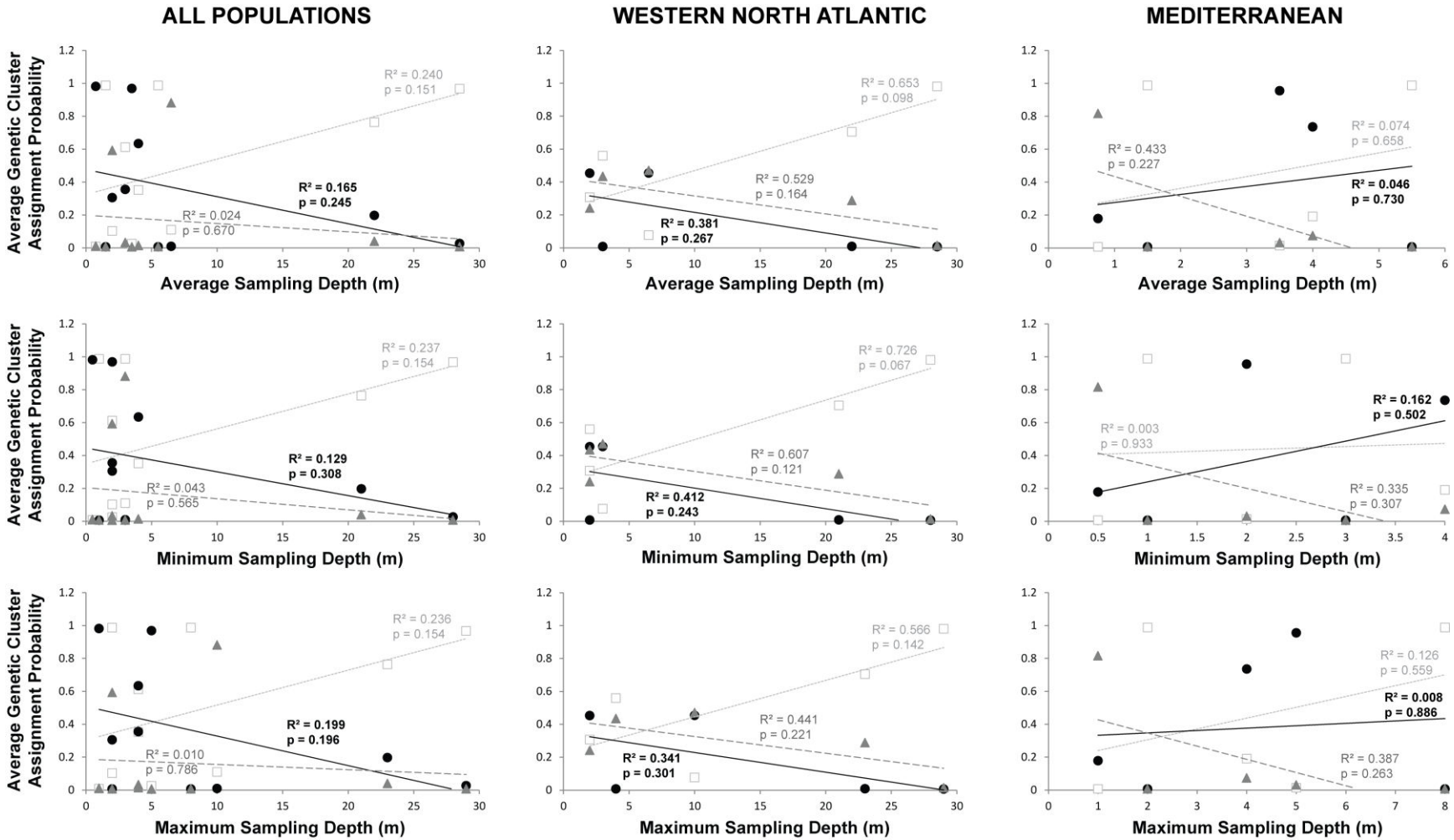


Figure 3.S5. Correlation between *Symbiodinium* community composition and depth (Appendix B, Table 3.S1). The white squares, gray triangles, and black circles represent the white, gray, and black genetic clusters, respectively, obtained from STRUCTURE analyses (Figure 3.2 for all populations; Figure 3.3b for western North Atlantic; Figure 3.3d for Mediterranean). Trend lines are as follow: white= short dashed line, gray= large dashed line, black= solid line. None of the correlations were significant.

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APPENDIX C.
SUPPLEMENTARY MATERIAL FOR CHAPTER 4

Table 4.S1. Effective sample sizes for selection tests performed in BayeScEnv used to access auto-correlation. a. along the westward expansion, and b. along the northward expansion.

a.	Longitude	Temperature
logL	877548	491103
Fst1	992702	996030
Fst2	993258	992499
Fst3	999999	991468
Fst4	995844	982067
Fst5	995191	992295
Fst6	996562	999999

b.	Latitude	Temperature	Substrate
logL	711681	256648	259055
Fst1	989712	991013	993002
Fst2	988618	992447	977795
Fst3	992260	995751	962350
Fst4	1000108	995973	990028
Fst5	986966	991158	
Fst6	999999	999999	
Fst7	996640	995313	
Fst8	992133	987196	
Fst9	999999	999999	
Fst10	994734	999999	
Fst11	991774	990571	

Table 4.S2. Top Blastx matches of the loci found to be fixed for a single SNP allele in SOE (3 loci), and NOE (10 loci). These 13 loci were polymorphic in ALB and CAT.

Zone	Contig ID	Top Blastx match	putative gene ID	E-value
SOE	51818	XP015748588	52 kDa repressor of the inhibitor of the protein kinase-like	6e-05
	11502	XP015758989	leucine-rich repeat-containing protein 40-like	0.017
	6703	XP015770793	tetratricopeptide repeat protein 7B-like	7.0
NOE	16614	no matches	–	–
	1888	XP015752860	daf-12-interacting protein 1-like	1e-11
	35333	XP015773152	homeobox protein prophet of Pit-1-like isoform X1	7.8
	2413	no matches	–	–
	74814	XP015753452	exonuclease 1-like	0.29
	45449	XP015752302	uncharacterized protein LOC107332086	4e-19
	26338	XP015756712	uncharacterized protein LOC107336159	1e-24
	5097	XP015776996	E3 ubiquitin-protein ligase MARCH6-like	6.1
	35112	XP015767167	transmembrane prolyl 4-hydroxylase-like	0.17
	845	XP015754954	uncharacterized protein LOC107334529	2e-27

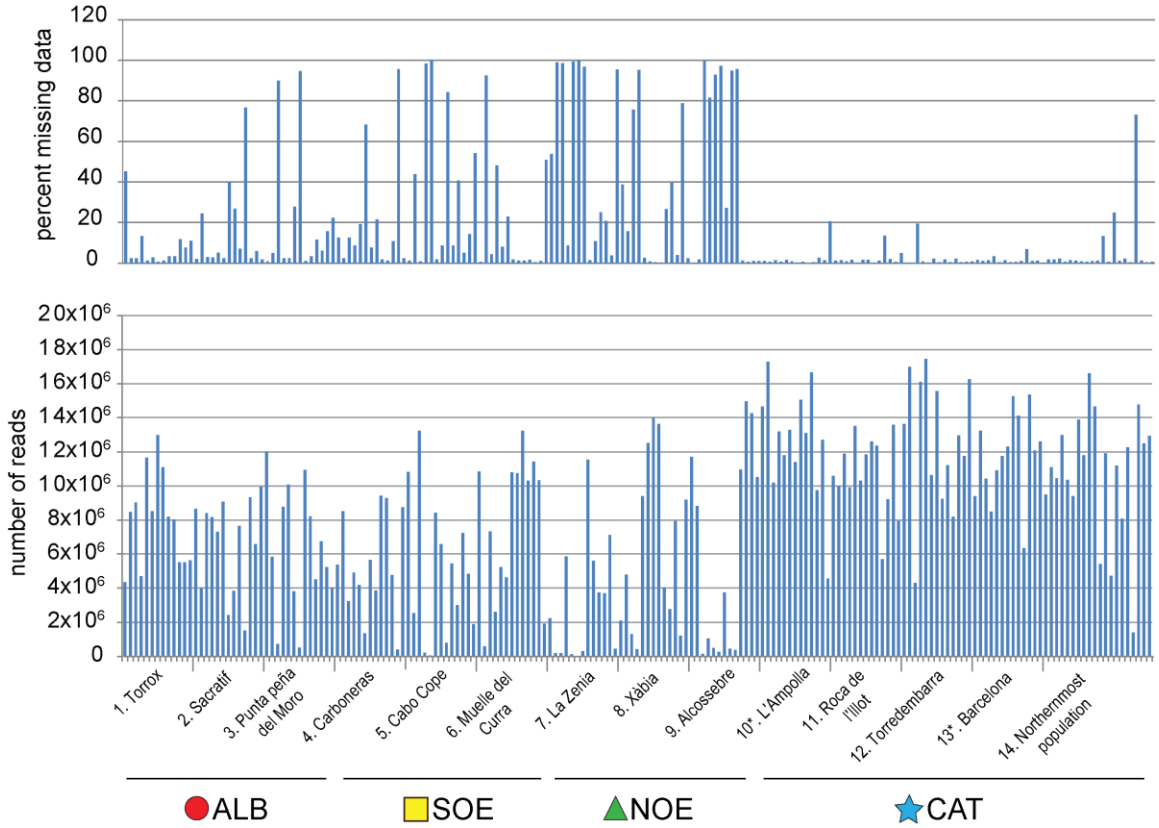

Figure 4.S1. Bar graphs showing the percent missing data (top) and number of reads that passed quality control (bottom) for all 189 individuals sequenced. 28 individuals with >50% missing data were excluded from further analyses.

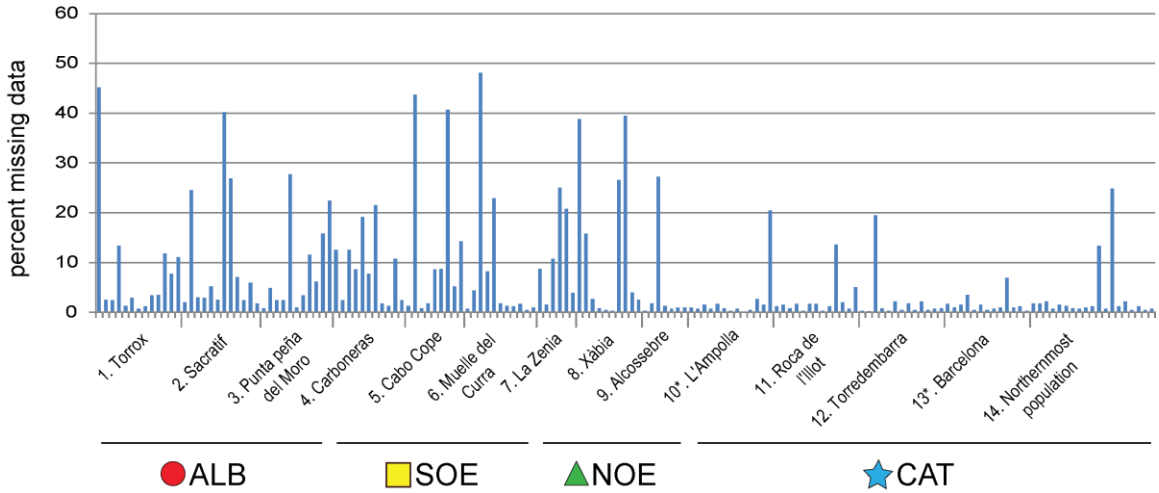

Figure 4.S2. Bar graph showing the percent missing data for 161 individuals with <50% missing data, and included in subsequent genetic analyses.

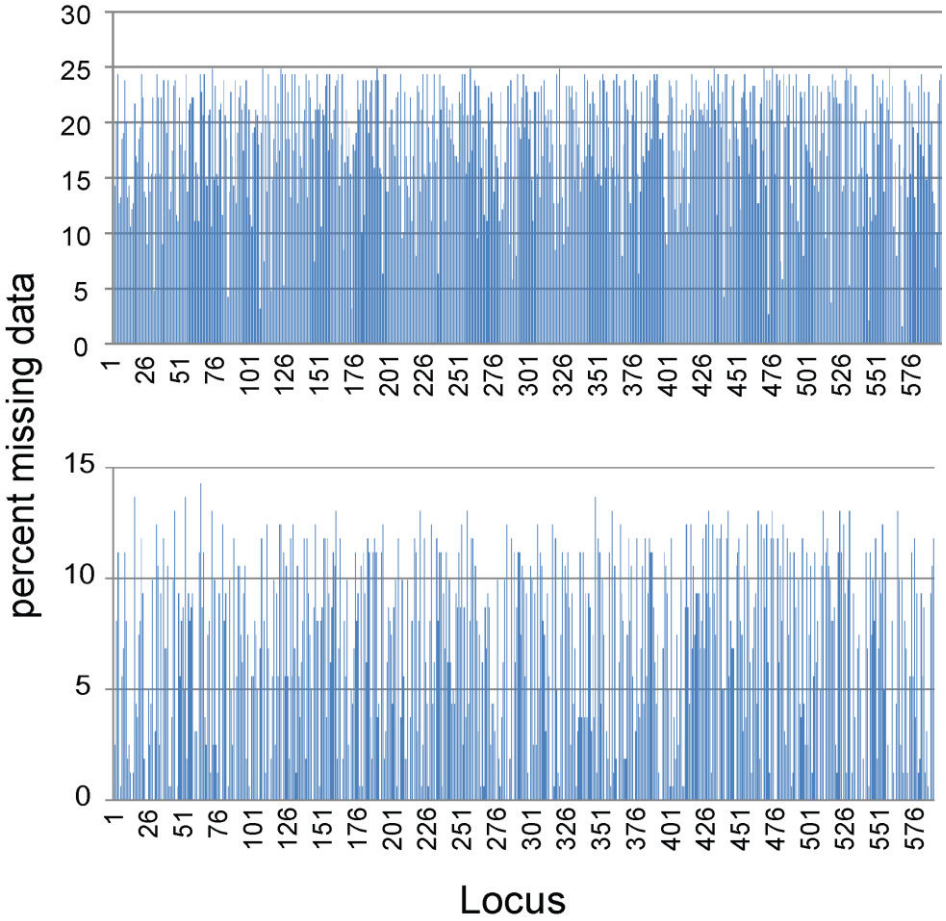

Figure 4.S3. Bar graphs showing the percent missing data for all 595 loci for all 189 sequenced individuals (top), and the 161 individuals with <50% missing data (bottom).

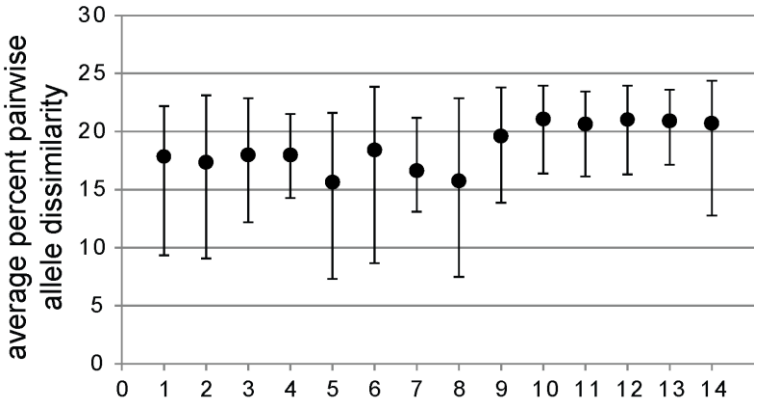

Figure 4.S4. The average percent of pairwise allele dissimilarity between individuals within populations. Bars represent the minimum and maximum percent. Population site IDs are along the x-axis.

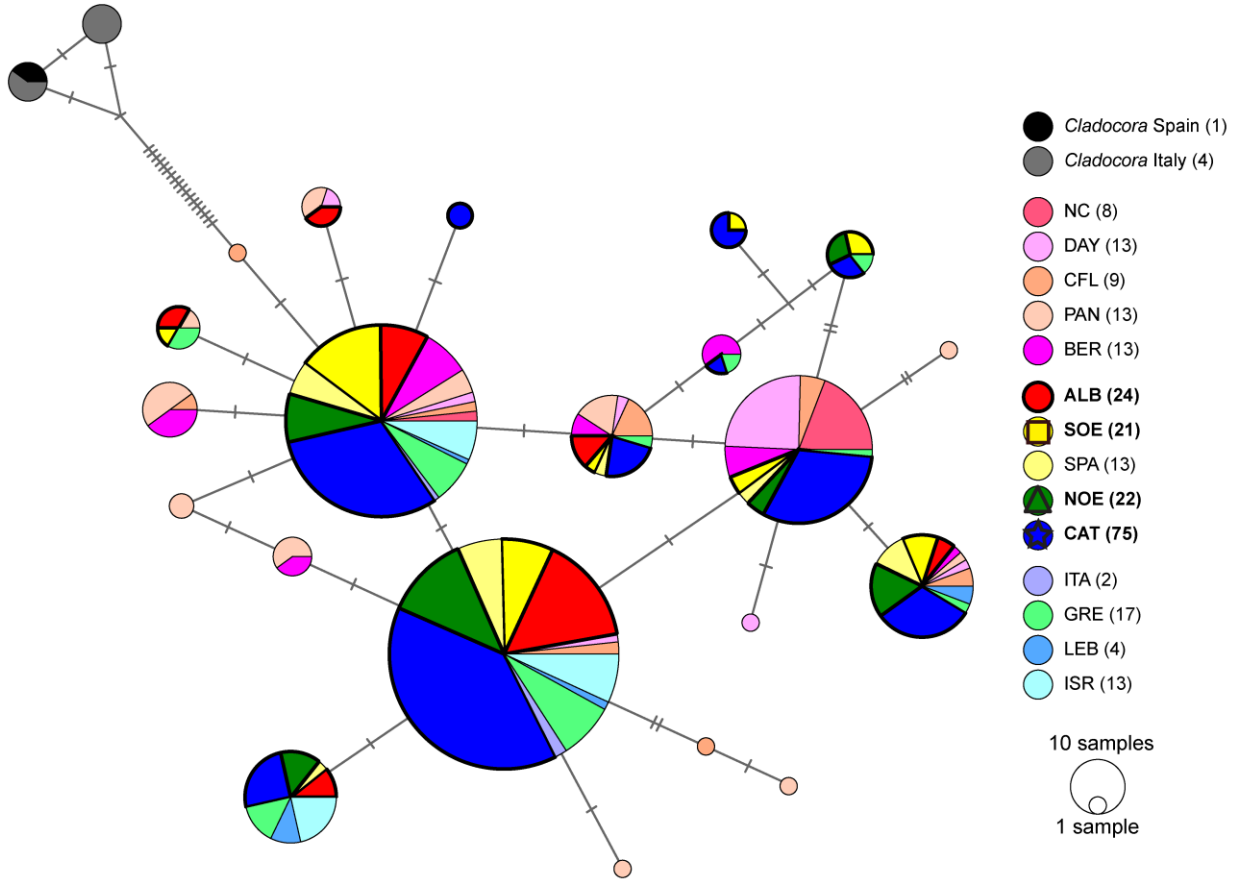

Figure 4.S5. Haplotype network for a variable nuclear gene (p14: Fatty acid elongase; 206 bp) constructed using statistical parsimony (Clement *et al.* 2000) within the program PopART 1.7 (http://popart.otago.ac.nz). We used a Bayesian statistical method implemented in PHASE 2.1 (Stephens *et al.* 2001, Stephens & Donnelly 2003, Stephens & Scheet 2005) to resolve alleles in heterozygous individuals. Nine individuals with alleles that could not be phased to a probability >90% were excluded from the network, although including them (as most likely alleles) did not significantly alter the resulting network (not shown). Each pie graph represents an allele and the shades represent the proportion of individuals from the different populations that share that particular allele. Small dashed lines along line segments connecting alleles represent the number of mutation steps separating the alleles. Spanish *Oculina patagonica* populations included in this study are in bold and colored according to Figure 4.1. *Oculina* spp. from the western North Atlantic (Eytan *et al.* 2009, Leydet & Hellberg 2015) are shades of pink and orange, while *O. patagonica* populations from the central and eastern Mediterranean (Leydet & Hellberg 2015) are shades of light blue and green. *Cladocora caespitosa* from Spain was collected along the Catalan coast. Italian *C. caespitosa* were collected from Pantelleria Island. Numbers in parentheses are the number of individuals from each population, totaling 252 individuals.

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APPENDIX D.
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Karine Eliane Posbic Leydet (born Karine Eliane Posbic) was born in France in 1985 to Jean and Chantal Posbic. She has two siblings, Aline and Vincent Posbic. In 1987, she moved with her family to Maryland where she grew up in a multicultural home, traveling overseas often to visit friends and family. She attended The Visitation Academy (K–8) and Saint John's College Prep High School (formally Saint John's at Prospect Hall) (9–12). In high school, she was an active member of the choir and drama club.

Karine attended Mount Saint Mary's University from 2003–2007, where she majored in Biology and minored in Environmental Studies. She was an undergraduate research assistant in the lab of Dr. Rosie Bolen examining the role of trichome hairs in herbivory defense. In the summer of 2006, she spent a couple of weeks in the rainforests of Gamboa, Panama at the Smithsonian Tropical Research Institute studying female mating preferences in túngara frogs, alongside Dr. Ryan Taylor. She graduated *summa cum laude*.

Upon graduation, Karine worked during the summer of 2007 at the National Cancer Institute at Fort Detrick in Frederick, Maryland as a research technician under a training award. She began her Master's degree at The George Washington University in Washington, District of Columbia in August 2008 under the advisement of Dr. Sheri Church, where she studied salamander migration and dispersal routes. She began her third and final degree at Louisiana State University in August 2010 in the lab of Dr. Michael Hellberg. In July 2014, she married Brian F. Leydet, Jr. (LSU SVM Ph.D. class of 2014). She defended her dissertation on May 9, 2016.