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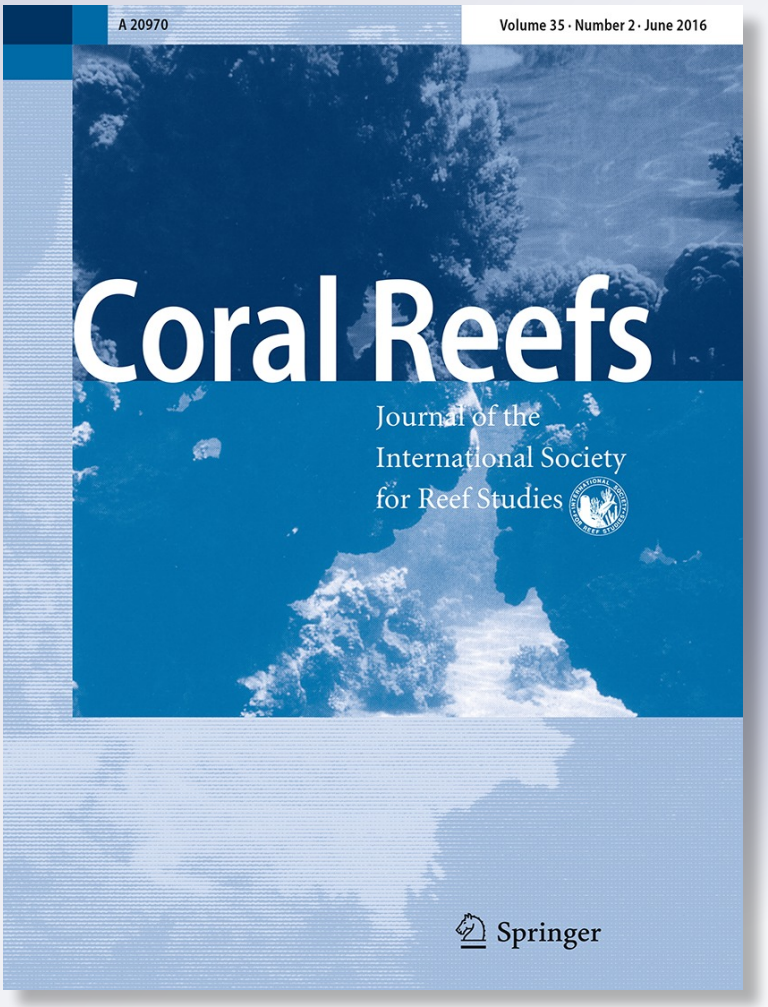

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REPORT

Discordant coral–symbiont structuring: factors shaping geographical variation of *Symbiodinium* communities in a facultative zooxanthellate coral genus, *Oculina*

Karine Posbic Leydet[1] · **Michael E. Hellberg**[1]

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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.

Keywords *Oculina* · *Symbiodinium* · Facultative zooxanthellate · Zooxanthellae · Host–symbiont associations

Introduction

Coral colonies constitute a partnership among many species. The coral animal itself, its endosymbiotic algae, and its resident microbes compose the coral holobiont (Rosenberg et al. 2007; Bourne et al. 2009). Photosynthetic algae of the genus *Symbiodinium* contribute nutritionally to the coral (Muscatine and Porter 1977; Falkowski et al. 1984) 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; Szmant and Gassman 1990; Harvell et al. 2002). 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 and Baums 2014).

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

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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 and LaJeunesse 2011; Prada et al. 2014). Alternatively, the algal symbionts may vary geographically within species (LaJeunesse et al. 2004; Keshavmurthy et al. 2012), 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 (Thornhill et al. 2006b; Jones et al. 2008), anthropogenic thermal stressors (Keshavmurthy et al. 2012), and irradiance variation (Toller et al. 2001; Finney et al. 2010). This is because *Symbiodinium* clades and even types vary in their thermal tolerance (Tchernov et al. 2004; McGinty et al. 2012) and photophysiology (Iglesias-Prieto and 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 and 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) (Stat et al. 2006; Baird et al. 2009). 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 (Stat et al. 2009, 2013; Pettay et al. 2011).

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 (Fine et al. 2001; Brooke and Young 2003, 2005) 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 (Thornhill et al. 2008; LaJeunesse et al. 2012; Rodolfo-Metalpa et al. 2014; Rubio-Portillo et al. 2014c), 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 (Fine et al. 2001; Rosenberg and Ben-Haim 2002; Armoza-Zvuloni et al. 2011; Rubio-Portillo et al. 2014a).

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.

Methods

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 and Hellberg 2015). *Oculina* spp. colonies were sampled from five localities in the western North Atlantic (Fig. 1; Electronic Supplementary Material, ESM Table 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). *Oculina patagonica* samples were collected from five localities spanning their Mediterranean distribution (Fig. 1; ESM Table 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.

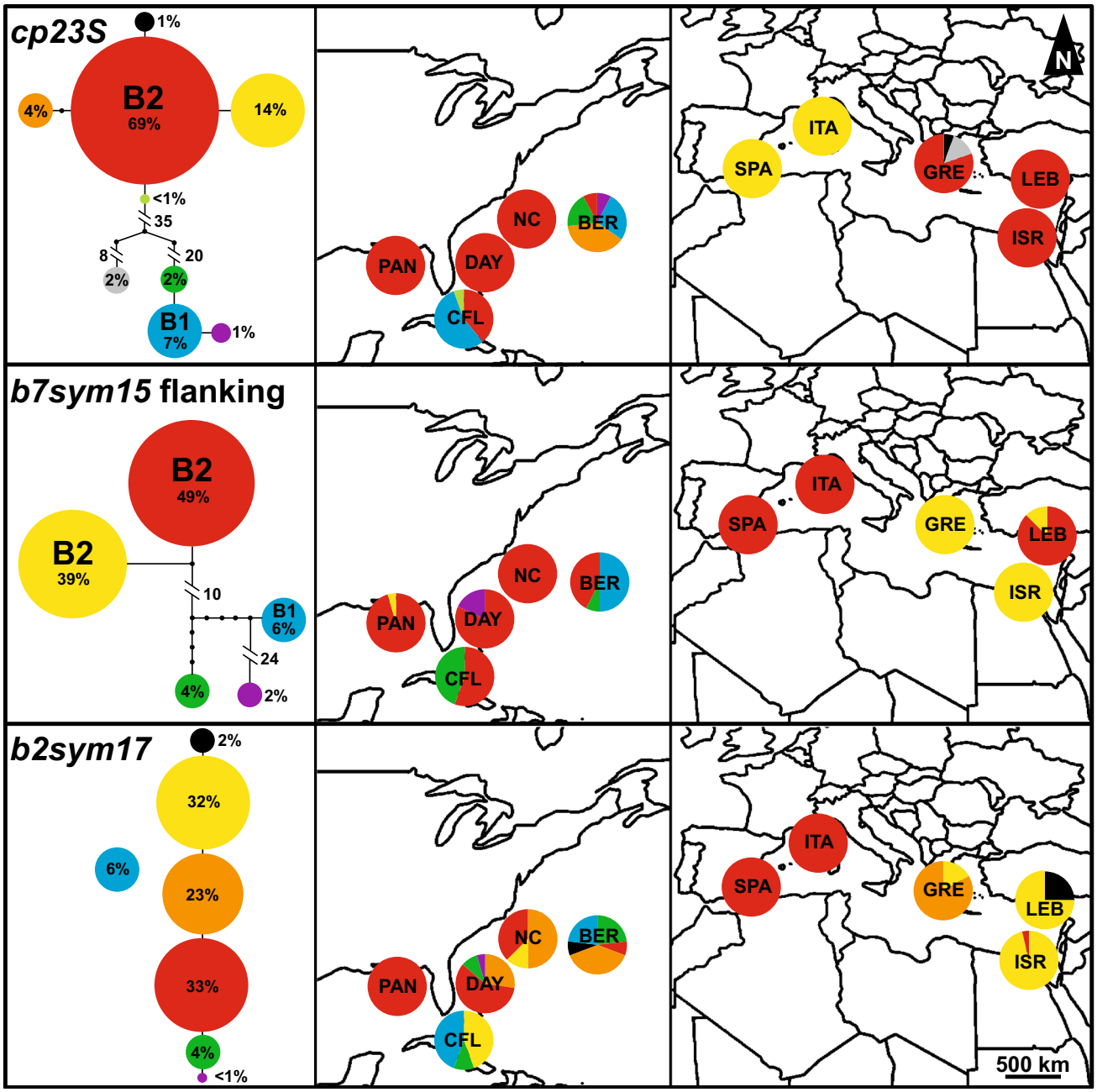

Fig. 1 Haplotype maps showing the distribution and proportion of haplotypes across all populations for the three variable markers used in this study. Populations include North Carolina (NC, $n = 8$), Daytona Beach (DAY, $n = 11$), Cape Florida (CFL, $n = 9$), Panama City (PAN, $n = 11$), Bermuda (BER, $n = 13$), Spain (SPA, $n = 14$), Italy (ITA, $n = 2$), Greece (GRE, $n = 18$), Lebanon (LEB, $n = 4$), and Israel (ISR, $n = 27$) (ESM Table 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 ESM Table S2 and S3 for all BLAST results)

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 1). All polymerase chain reaction (PCR) amplifications were conducted in 25 µl reactions consisting of 2.5 µl of 10 × buffer (containing 15 mM $MgCl_2$), 2 µl of dNTPs (2.5 mM), 1 µl of each primer (10 µM), 0.2 µl of One Taq DNA polymerase (New England Biolabs Inc.), and 1 µl 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 s, annealing for 1 min, and 72 °C for 1 min 15 s, 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 *cp23S* amplification failed, we used alternative primers (Santos et al. 2002; annealing temperature 55 °C) that amplify a larger portion of the *cp23S–rDNA domain V*. Annealing temperature for the flanking region of the microsatellite marker *b7sym15* (Pettay and 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′ GGCAACAATCATATTGACTA GGCC 3′) to amplify *b2sym17* for individuals that failed under the above conditions.

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 (ESM Table 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 ESM Tables S2, S3 for all BLAST results).

Table 1 Markers used to genotype *Symbiodinium* associated with *Oculina* spp. in this study

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

[a] Final cropped alignment

[b] Cropped to length of *cp23S-rDNA domain V* (areas a and b)

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 and 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 and 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 Markov chain Monte Carlo 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.

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 and 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 and 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 and 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 ESM Fig. S1 for additional information).

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 the *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; Fig. 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 (ESM Table 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; Fig. 3c, d).

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 corrected for multiple comparisons according to the method described by Benjamini and Hochberg (1995).

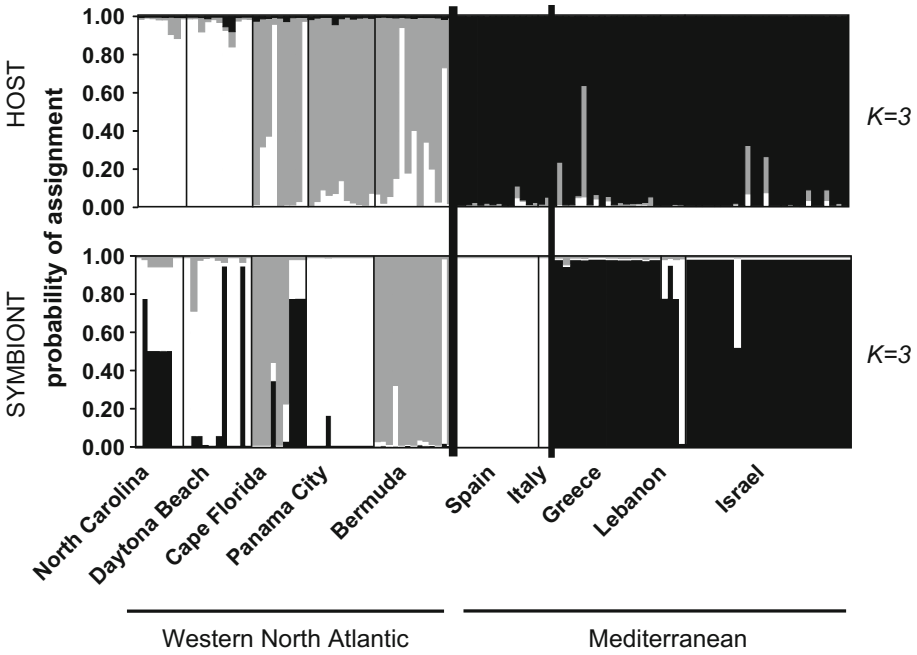

Fig. 2 STRUCTURE *bar plots* for the coral host (*top*) and their respective algal symbiont (*bottom*) when all populations were analyzed together. Individuals (*bars*) are grouped by populations along the *x*-axis, with the probability of assignment to a particular genetic cluster (represented by *different 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*

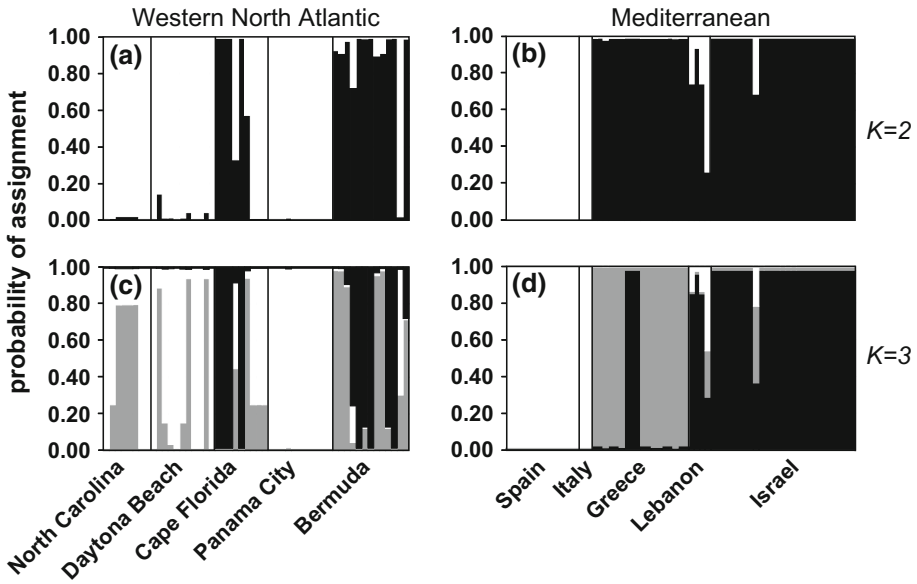

Fig. 3 STRUCTURE *bar plots* for the algal symbiont when western North Atlantic (*left*) and Mediterranean (*right*) populations were analyzed separately. Individuals (*bars*) are grouped by populations along the *x*-axis, with the probability of assignment to a particular genetic cluster (represented by *different shades*) along the *y*-axis. The number of genetic clusters or populations (*K*) is shown for each analysis

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) (Fig. 1; ESM Table S2 and S3).

Genetic diversity

Oculina spp. colonies from the western North Atlantic harbored greater *Symbiodinium* diversity both within and between populations than did the Mediterranean populations (Fig. 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 (Fig. 1). All Spanish and Italian colonies ($n = 16$) harbored a single *Symbiodinium* genotype, whereas those from 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.

Geographical differentiation

We used haplotype networks (Fig. 1), AMOVAs (Table 2), and STRUCTURE (Figs. 2, 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 2a). In contrast to their coral host (Leydet and Hellberg 2015), algal populations from the western North Atlantic were not significantly subdivided from Mediterranean populations. However, when populations from 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 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 2b).

When all populations were analyzed together using STRUCTURE, the most likely K was three (Fig. 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 (Fig. 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 (Fig. 3a). When $K = 3$, Cape Florida and Bermuda were comprised of two admixed genetic clusters and harbored the most diversity (Fig. 3c). 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 (Fig. 3b). When $K = 3$, most individuals from Greece fell into a distinct cluster (Fig. 3d).

Table 2 Analysis of molecular variance performed for all populations (a) and the western North Atlantic and Mediterranean populations separately (b)

(a)	Western North Atlantic versus 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

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 (Fig. 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 ESM Fig. S1 for additional information).

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 (ESM Fig. 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 (ESM Fig. 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 (Fig. 4; ESM Fig. 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) (ESM Fig. S3). Although the 'gray' genetic cluster was significantly negatively correlated with increased maximum temperature ($p = 0.034$) (ESM Fig. 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) (Fig. 4; ESM Fig. 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 (ESM Fig. 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 %, $p = 0.011$) (ESM Fig. S4).

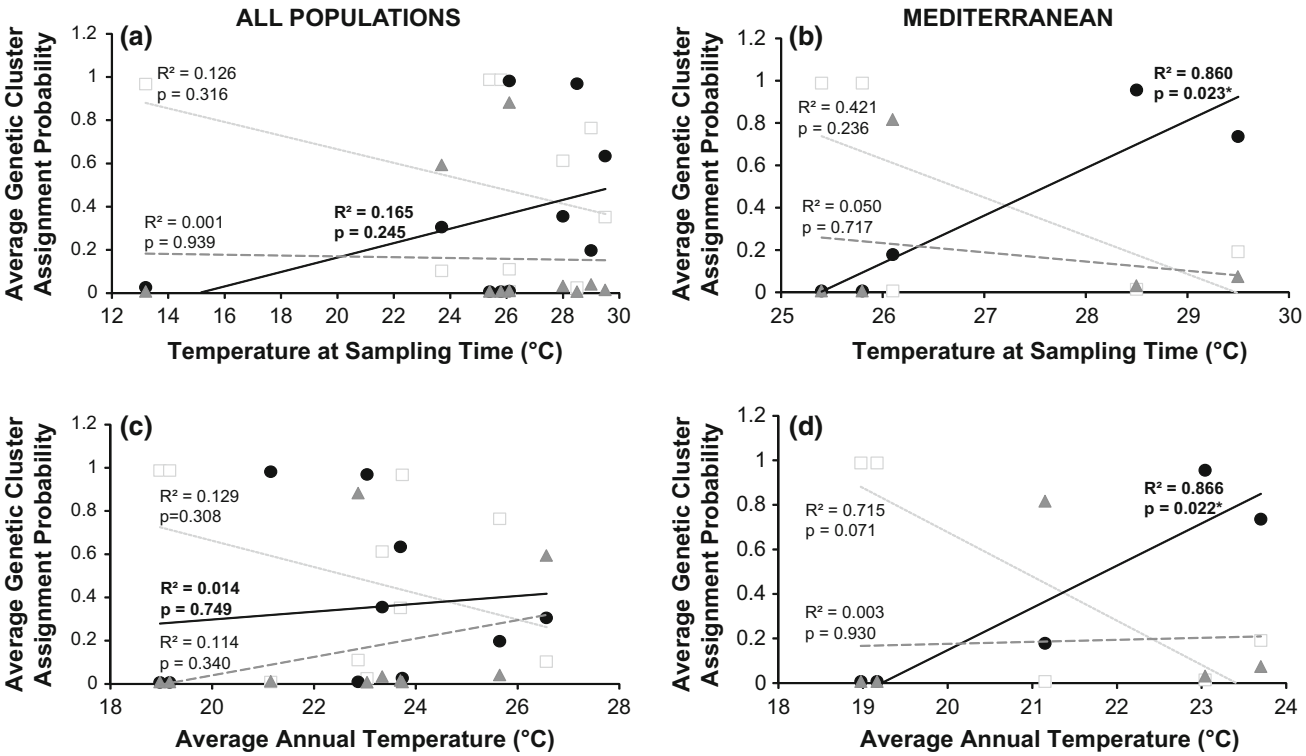

Fig. 4 Correlation between *Symbiodinium* community composition and temperature (at sampling time and average annual) for all populations (**a**, **c**) and Mediterranean populations only (**b**, **d**). The *white squares*, *gray triangles*, and *black circles* represent the *white*, *gray*, and *black genetic clusters*, respectively, obtained from STRUCTURE analyses (Fig. 2 for all populations; Fig. 3d for Mediterranean). Trend lines are as follows: *white short dashed line*, *gray large dashed line*, and *black solid line*. Significant correlations are indicated with an *asterisk*

Depth was the worst predictor variable. It explained at best 73 % ($p = 0.067$) of the *Symbiodinium* communities across all analyses (ESM Fig. 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 (ESM Fig. 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.

Discussion

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 (Thornhill et al. 2008; LaJeunesse et al. 2012; Rodolfo-Metalpa et al. 2014; Rubio-Portillo et al. 2014c). The majority of the colonies harbored type B2, again consistent with earlier studies (Thornhill et al. 2008; Rodolfo-Metalpa et al. 2014; Rubio-Portillo et al. 2014c). 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 historical 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; Thornhill et al. 2013; Parkinson et al. 2015). 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, b). 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. 2006; Shenkar et al. 2006; Rodolfo-Metalpa et al. 2008). 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.

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 (Figs. 1, 2). Within the western North Atlantic, Cape Florida and Bermuda harbor *Symbiodinium* communities genetically distinct from all other populations (Fig. 3a), likely because these populations possess two *Symbiodinium* types (Fig. 1).

A stronger pattern of subdivision is evident within the Mediterranean, where we found a clear west versus east division (Fig. 3b). 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 and Loya 1995; Fine et al. 2001; Rosenberg and Ben-Haim 2002), while bleaching events in western populations have only been reported in the last 5 years (Rubio-Portillo et al. 2014a). 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 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 (Rowan et al. 1997; Fabricius et al. 2004). Future work is needed to elucidate the factors driving these differences in diversity between regions.

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 (Fig. 2). *Oculina* spp. populations in the western North Atlantic and the Mediterranean show a clear genetic break (Fig. 2; ESM Fig. S1; Leydet and 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 (Fig. 2; ESM Fig. S1; Leydet and 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; Symula et al. 2011; Prada et al. 2014). However, even some obligate coral–algal symbioses show discordant geographical variation between partners (Baums et al. 2010; Pettay et al. 2011; Keshavmurthy et al. 2012; Baums et al. 2014), 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 the corals. Overall, temperature explained *Symbiodinium* communities better than chlorophyll *a* concentration and depth, in terms of both significance and consistency of the observed trends (Fig. 4). We therefore focus our discussion on temperature, while recognizing that environmental variables often covary and that testing for causal 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 and 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 and 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 and 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. Simple 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, although 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.

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