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Sarah Whitney Davies

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The Dissertation Committee for Sarah Whitney Davies Certifies that this is the approved version of the following dissertation:

Understanding Coral Dispersal

Committee:

Mikhail V. Matz, Supervisor

Mark Kirkpatrick

Mathew Leibold

Ulrich Mueller

Don Levitan

Understanding Coral Dispersal

by

Sarah Whitney Davies, B.S.; M.S.

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Dedication

I dedicate this dissertation to my amazing parents, Bruce and Terry Davies.

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A dissertation is not a mere reflection of my own personal achievements; instead, it is a document that manifests all of the support I have received from so many great people during my journey here at the University of Texas at Austin. These are the people that have made my dissertation a reality.

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Understanding Coral Dispersal

Sarah Whitney Davies, Ph.D.

The University of Texas at Austin, 2014

Supervisor: Mikhail V. Matz

Abstract: Understanding the factors influencing species ranges and dispersal are becoming increasingly important as climate change alters species distributions worldwide. If species are to persist, life-history strategies must rapidly evolve to accommodate shifting environments. This dissertation assesses the factors modulating dispersal in corals. First, I examined if there were any systematic differences in settlement between Indo-Pacific and Caribbean coral larvae that might explain Caribbean recruitment failures. No differences were observed, however I detected significant divergences in settlement cue preferences among coral species across both the Caribbean (*Diploria strigosa*, and *Montastraea franksi*) and the Indo-Pacific (*Acropora tenuis, A. millepora,* and *Favia lizardensis*), even for coral larvae from the same reef. Secondly, I established the extent of coral dispersal between remote reefs. I evaluated the genetic diversity and divergence across Micronesia for two coral species and investigated if these islands served as a connectivity corridor between the Indo-West-Pacific (Coral Triangle) and the Central Pacific. I found isolation-by-distance patterns whose strength depended on species, suggesting these corals are not panmictic across their ranges and that island stepping-stones facilitate gene flow to remote Pacific reefs. Next, I investigated genetic structure of symbionts in these same corals, to see if horizontally transmitted symbionts are less dispersive than their coral hosts. Symbiont genetic divergence between islands

was an order of magnitude larger than host divergence and both host species and environment modulated symbiont composition. These results suggest that symbiont populations are host-specific and associating with local symbionts might be a mechanism for broadly dispersing corals to adapt locally. Lastly, I estimated heritable variation in dispersal-related traits in coral larvae. I observed strong heritable variation in gene expression, as well as parental effects on two phenotypic traits, settlement and fluorescence. I observed that patterns of differential expression in three-day-old larvae predicted variation in settlement and fluorescence two days later. Correlations between proteoglycan expression and settlement suggest that the larval extracellular matrix plays a role in settlement. Down-regulation of ribosomal proteins and differential expression of oxidative stress genes correlated with increasing fluorescence, possibly indicating reduced growth and increased stress. Overall, this dissertation contributes to our knowledge of factors affecting coral dispersal and the potential for evolution of dispersal-related traits.

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Introduction

As the climate changes and humans continue to modify habitats worldwide, understanding the mechanisms that allow organisms to cope with these changes is imperative. If a species is experiencing stressful conditions in its natal habitat, that species has three choices: 1) remain in the natal habitat but suffer reduced fitness, 2) adapt to the local environment through natural selection on standing genetic variation or 3) disperse to new, more favorable environments. Understanding the factors that influence species ranges and their potential for dispersal to new habitats is becoming increasingly important as the effects of climate change are modifying species distributions worldwide. However, research in marine environments lags behind terrestrial environments. For example, open-ended questions in marine research include: What environmental variables influence dispersal? How far do marine species disperse? Are dispersal related traits heritable? Can these traits evolve in response to climate change? In this dissertation, I use a variety of approaches ranging from traditional field ecology to functional genomics to assess the factors modulating dispersal in corals.

Coral reefs are in decline globally, so understanding dispersal patterns is integral to reef management. Building on my masters work, the first dissertation chapter took a proximate approach to coral recruitment and investigated if there were any systematic differences in settlement cue responsiveness of coral larvae from the Indo-Pacific compared with the Caribbean that might help explain the lack of Caribbean recruitment (Chapter 1). Next, I used molecular genetic methods to predict migration patterns between reefs in the Micronesian Pacific, both for the coral host (Chapter 2) and its symbiont (Chapter 3), helping better inform management of several threatened coral species. In addition to global declines, corals are also expanding their ranges to higher

latitudes in response to climate change. I examined the natural genetic variation in a suite of dispersal-related larval traits to determine if these traits might be targets of natural selection for enhanced dispersal under climate change (Chapter 4). I measured quantitative traits and correlated these traits with gene expression (RNAseq) patterns to understand the molecular mechanisms underlying this variation. My dissertation work has contributed to our understanding of marine dispersal and how dispersal related traits might be selected under climate change.

Across the Caribbean, coral reefs have deteriorated and recruitment rates of broadcast spawning corals are negligible, while Indo-Pacific reefs are healthy and experience high rates of recruitment. I hypothesized that Caribbean recruitment declines might be due to reduced quality of settlement cues available in the environment and/or impaired sensitivity of Caribbean coral larvae to those cues, relative to the Pacific. To test this hypothesis I assembled a collection of crustose coralline algae (CCA) communities, a known settlement inducer for corals, from multiple reefs in both the Caribbean and Indo-Pacific. I meta-barcoded these samples and determined that the majority of these communities were primarily CCA. Over three field seasons, I tested the efficacy of these cues in eliciting a settlement response in various Caribbean and Indo-Pacific coral species. Refuting my hypothesis, I observed no systematic differences in the overall responsiveness of coral larvae from different ocean provinces and no differences in cue effectiveness from different provinces. Interestingly, I did detect species-specific cue preferences and these preferences were even divergent among coral species from the same reef. These results suggest that: 1) Caribbean larvae are capable of settlement, 2) Caribbean settlement cues are intact and elicit larval settlement, 3) a coral's settlement decision is a complex behavior that varies among coral species, and 4) lack of Caribbean

recruitment is likely due to some other environmental factor such as post settlement mortality.

Chapter 2 investigates how isolated reef habitats might influence genetic connectivity in reef-building corals by acting as dispersal stepping-stones. Here, I studied population genetics of two species of Indo-Pacific acroporid corals known to have extensive geographical ranges that span the Coral Triangle. Biophysical models have implicated the Micronesian islands as important dispersal stepping-stones from the Coral Triangle to the central Pacific. To explore genetic connectivity in this region I estimated gene flow patterns using population genetics for both species from Palau to the Marshall Islands and then used a spatially explicit biophysical model to estimate dispersal resistances across the seascape. As expected, I observe genetic divergences that follow a stepping stone model with divergence accumulating and genetic diversity diminishing as distance from the Coral Triangle increases. The biophysical model explained 15-21% more of the variation compared with geographic distance alone and interestingly, our biophysical model suggests that for most islands, stepping-stone dispersal occurs in both directions. Unexpectedly, these two congeneric corals exhibited different magnitudes of divergence, suggesting different migration capabilities. Overall this work demonstrates that 1) broadcast spawning corals possess enormous dispersal potential, however the number of migrants can differ between two closely related species and 2) Micronesia serves as a corridor between the Coral Triangle and the central Pacific, but dispersal likely occurs in both directions. This study represents the most comprehensive analysis of dispersal patterns in Micronesia.

Many broadcast spawning corals exhibit horizontal transmission of symbionts where symbiotic adults produce highly dispersive aposymbiotic larvae. Upon arrival to new habitat, these larvae settle and acquire symbionts from their local environment. This

transmission strategy may be adaptive for highly dispersive corals since associations with local symbiont populations could increase host fitness in its new environment. This hypothesis requires the symbiont populations to diverge on a much finer spatial scale than the host, and the host to be highly promiscuous in its choice of symbionts throughout its range. In Chapter 3, I elucidate *Symbiodinium* population structure in two highly dispersive host species (Chapter 2) across Micronesia. I observe that symbiont structure is an order of magnitude greater than host structure, and that symbionts show greater divergence in the coral host species with higher divergence. Associations with highly divergent *Symbiodnium* types across islands confirm the expectation of host promiscuity, whereas within islands the symbiont genetic diversity is primarily driven by host specificity and, secondarily, by environmental partitioning. These patterns indicate that evolutionary dynamics of host-symbiont associations are governed by continuous evolution of host-specific symbiont strains on local scales, which could play an important role in matching the holobiont physiology to the local environment.

Research has suggested that at least on historical timescales, long-range migration between broadcast-spawning coral populations is substantial enough to maintain gene flow across thousands of kilometers (Chapter 2). This extent of migration raises the question of how dispersal might vary on ecological timescales, depending on the degree of habitat fragmentation, the demographic history of populations, and, most importantly, climate-induced selection favoring emigration from natal habitats. To estimate the degree to which dispersal is variable and evolvable, Chapter 4 examines the additive genetic variation in a suite of behavioral and physiological larval traits typically associated with dispersal potential in the Indo-pacific coral *Acropora millepora*: 1) early responsiveness to settlement cue, 2) red fluorescence, 3) rate of protein/lipid utilization, and 4) gene expression. This study is also the first to investigate heritability of gene expression in

corals and it is the first to uncover expression patterns that predict phenotypic outcomes. I find strong evidence for heritable gene expression in coral larvae, as well as red fluorescence and settlement. I also observed expression profiles involving sensory machinery associated with settlement and stress-related expression correlating with fluorescence. These results clearly demonstrate that significant heritable variation is available within coral populations, which may serve as fuel for natural selection under climate change.

Chapter 1: A cross-ocean comparison of responses to settlement cues in reef-building corals

ABSTRACT

Caribbean coral reefs have deteriorated substantially over the past 30 years, which is broadly attributable to the effects of global climate change. However, Indo-Pacific reefs maintain higher coral cover and typically recover rapidly after disturbances. This difference in reef resilience is largely due to much higher coral recruitment rates in the Pacific. I hypothesized that the lack of Caribbean recruitment might be explained by diminishing quality of settlement cues and/or impaired sensitivity of Caribbean coral larvae to those cues, relative to the Pacific. To evaluate this hypothesis, I assembled a collection of bulk samples of reef encrusting communities, mostly consisting of crustose coralline algae (CCA), from various reefs around the world and tested them as settlement cues for several coral species originating from different ocean provinces. Cue samples were meta-barcoded to evaluate their taxonomic diversity. I observed no systematic differences either in cue potency or in strength of larval responses depending on the ocean province, and no preference of coral larvae towards cues from the same ocean. Instead, I detected significant differences in cue preferences among coral species, even for corals originating from the same reef. I conclude that the region-wide disruption of the settlement process is unlikely to be the major cause of Caribbean reef loss. However, due to their high sensitivity to the effects of climate change, shifts in the composition of CCA-associated communities, combined with pronounced differences in cue preferences among coral species, could substantially influence future coral community structure.[1]

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[1] Considerable portions of this chapter were published as Davies SW, Meyer E, Guermond SM, Matz MV. 2014 A cross-ocean comparison of responses to settlement cues in reef-building corals. PeerJ 2:e333 http://dx.doi.org/10.7717/peerj.333. **Contributions** - Conceived and designed the experiments: SWD EM MVM. Performed the experiments: SWD SMG. Analyzed the data: SWD. Wrote the paper: SWD MVM.

INTRODUCTION

The majority of reef-building corals are broadcast-spawning species that release gametes annually to produce planktonic larvae that are dispersed by ocean currents (Baird, Guest, & Willis, 2009). Reef recovery after disturbances, such as catastrophic bleaching events or hurricanes, is critically dependent on the successful recruitment of these planktonic larvae back to reefs (Buston, Jones, Planes, & Thorrold, 2012). Coral reefs worldwide are declining at accelerating rates, which has been generally attributed to the increase in both global and local anthropogenic stressors (Hoegh-Guldberg et al., 2007). The specific factors driving this decline, including those affecting coral recruitment, are the subject of active ongoing research.

While coral cover has been declining in Indo-Pacific reefs in recent years (Bruno & Selig, 2007; De'ath, Fabricius, Sweatman, & Puotinen, 2012; Wakeford, Done, & Johnson, 2008), their higher biodiversity and range of recruitment and post-recruitment strategies appear to make these reefs more resilient (Adjeroud et al., 2009; Roff & Mumby, 2012). Caribbean reefs exhibit lower resilience than Indo-Pacific reefs, which has been attributed to several factors including recruitment failure (Connell, Hughes, & Wallace, 1997; Roff & Mumby, 2012). Across the Caribbean, recruitment rates of broadcast spawning corals are consistently low (Davies, Matz, & Vize, 2013; Gardner, Cote, Gill, Grant, & Watkinson, 2003; Hughes & Tanner, 2000; Vermeij, 2006), even though large reef builders still dominate coral cover on Caribbean reefs (Kramer, 2003). Instead, brooding genera such as *Agaricia* and *Porites* are the dominant coral species recruiting on Caribbean reefs (Bak & Engel, 1979; Davies, Matz, et al., 2013; Green, 2008). Spectacular recoveries after disturbances are not uncommon on Pacific reefs (i.e (Golbuu et al., 2007), but comparable levels of recovery have not been documented in the Caribbean (but see (Carpenter & Edmunds, 2006; Idjadi et al., 2006). A comparative

study of proximal causes of this difference in coral recruitment among ocean regions could elucidate some of the main drivers of Caribbean recruitment failure.

In principle, low recruitment rates might result from a variety of factors such as reduced coral population sizes, poor spawning synchrony, low fertilization rate, or high mortality (either pre- or post-settlement). Some of these potential explanations are unlikely to apply to the Caribbean-wide recruitment failure. For example, adult population sizes, at least for some Caribbean reefs, are still adequate and spawning remains highly synchronous and prolific (i.e. Flower Garden Banks, (Vize, 2005). High fertilization success is also observed under natural conditions (Levitan et al., 2004). While pre- and post-settlement mortality remains among the main potential causes, it is also possible that the effects of climate change in the Caribbean may have disrupted ecological interactions required for the recruitment process itself (Harrison, 1990), specifically the interaction between coral larvae and natural settlement cues.

Various factors influence coral settlement (Maida, 1994; Mundy & Babcock, 1998; Raimondi & Morse, 2000), however for many corals the biological properties of the reef surface appear to play a pivotal role in this choice (Babcock & Mundy, 1996; Heyward & Negri, 1999; Price, 2010; Ritson-Williams, Paul, Arnold, & Steneck, 2010). Crustose coralline algae (CCA; Rhodophyta, Corallinaceae) and associated communities have been shown to be one of the primary inducers of settlement and metamorphosis in coral larvae (Heyward & Negri, 1999; A. N. C. Morse et al., 1996; D. E. Morse & Morse, 1988). While marine bacteria also influence settlement in coral larvae (Negri, Webster, Hill, & Heyward, 2001; Tebben et al., 2011; Tran & Hadfield, 2011), recent work demonstrates that CCA species known to elicit the strongest settlement responses are also the most affected by the changes in ocean chemistry associated with climate change (Anthony, Kline, Diaz-Pulido, Dove, & Hoegh-Guldberg, 2008; Doropoulos, Ward,

Diaz-Pulido, Hoegh-Guldberg, & Mumby, 2012; Smith, Price, Nelson, & Haas, 2013), suggesting that changes in these CCA communities might be responsible for reduced coral recruitment.

I hypothesized that the correspondence between coral larval preferences and availability/quality of settlement cues (CCA associated communities) on Caribbean reefs may have broken down, resulting in reduced coral recruitment. This mismatch may take two forms: (1) appropriate settlement cues may be present, but larvae have lost the ability to respond to them, or (2) larval responses remain intact, but effective settlement cues are absent. To evaluate these possibilities, I performed reciprocal preference trials for three species of broadcast spawning Caribbean corals (*Montastraea franksi, Diploria strigosa* and *Stephanocoenia intersepta*) and four Indo-Pacific corals (*Acropora millepora, Acropora tenuis, Favia lizardensis* and *Ctenactis echinata*). Larval response of each species was tested against a collection of seven samples of CCA-associated communities from various locations in the Caribbean (n=3) and the Indo-Pacific (n=4). Since I was not interested in characterizing larval responses to particular CCA species but rather wanted to generally evaluate cue presence-absence in the environment, I collected whole encrusting communities from reef top or rubble to better approximate what coral larvae might encounter in nature rather than picking specific CCA species. To evaluate the diversity of the cues tested, their taxonomic composition was characterized post hoc by metabarcoding based on the eukaryotic ribosomal 18S rRNA gene.

MATERIALS AND METHODS

Settlement Cue Collections: Collections of CCA associated communities (which I will refer to as "cue*s" from now on) from a number of locations in the Caribbean and

Pacific was assembled (Table 1). Caribbean locations included the Florida Keys (FF), the Flower Garden Banks (FGB) and Bonaire (B). Pacific locations included Orpheus Island (Great Barrier Reef, Australia: A1, A2), Pohnpei (P) and Guam (G). Samples were stored in seawater at -80°C.

Table 1. Settlement cue panel and metabarcoding statistics
CCA cue information including: name of the cue, site where the cue was collected and the oceanographic region in which the site was located. Metabarcoding statistics including: number of quality-filtered reads, number of operational taxonomic units (OTUs), number of reads uniquely mapping to OTUs, and the mapping efficiency of the reads.

Cue	Site	Region	# of quality-filtered reads	# of OTUs	# of reads uniquely mapping to OTUs	Mapping Efficiency
A1	Orpheus Island (GBR)	Pacific	2760	6	2714	0.983
A2	Orpheus Island (GBR)	Pacific	4906	10	3566	0.727
B	Bonaire	Caribbean	1447	8	1222	0.844
FF	Florida Keys	Caribbean	2762	10	2411	0.873
FGB	Flower Garden Banks	Caribbean	2492	9	2341	0.939
G	Guam	Pacific	4495	11	2963	0.659
P	Pohnpei	Pacific	NA	NA	NA	NA

Caribbean Spawn I

On the evening of August 31, 2010 (eight days after the full moon), during the annual coral spawning event at the Flower Garden Banks National Marine Sanctuary (FGBNMS), gamete bundles were collected with mesh nets directly from three distinct *Montastraea franksi* colonies. Bundles were brought to the surface, cross-fertilized for one hour and then excess sperm was removed by rinsing through 150 μm nylon mesh. Larvae were reared in 1 μm filtered seawater (FSW) in three replicate plastic culture vessels at 5 larvae per ml. Larvae were transferred to the laboratory at the University of Texas at Austin on September 1, 2010. Samples were collected under the FGBNMS permit # FGBNMS-2009-005-A2.

Preliminary competency experiments assayed with several CCA samples determined that *M. franksi* larvae did not reach competence until 14 days post-fertilization, therefore CCA preference trials were started at this age. To quantify the responsiveness of settlement-competent larvae to six different cue samples (Table 1), twenty larvae per well were transferred into 10 ml of FSW in 6-well plates. Cue samples were finely ground with a mortar and pestle shortly before the settlement trials and a single drop of the resulting uniform slurry was added to each well (n=4 well replicates per cue, randomly assigning cues to wells). Four FSW control treatments were also included. The proportion of metamorphosed larvae (visual presence of septa) was quantified after 48 hours using a fluorescent stereomicroscope MZ-FL-III (Leica, Bannockburn, IL, USA) equipped with F/R double-bandpass filter (Chroma no. 51004v2) (Fig. 1b, 1c).

Pacific Spawn I

In November 2010, at Orpheus Island Research Station, Great Barrier Reef, Australia, the same type of experiments as described in the previous section were conducted with the same panel of cues (plus an additional Australian cue, A2). Four species of broadcast spawning corals were tested: *Acropora millepora, A. tenuis, Favia lizardensis,* and *Ctenactis echinata*. Adult corals were collected and maintained in raceways until spawning at which point they were isolated in 20-gallon plastic bins. Following spawning, gametes were collected from several colonies and cross-fertilized as described above. Initial trials to test for larval competency were conducted and final data were collected on 5d-old larvae, although *C. echinata* were never observed to settle over a period of several weeks, even in response to GLWamide (data not shown). Settlement assays were conducted as in the 2010 Caribbean Spawn I described above, the only

differences being inclusion of A2 cue and increase of per-cue replication level to n=6 (Table 1). Samples for Australian fieldwork were collected under Great Barrier Reef Marine Park Authority permit number G10/33943.1.

Caribbean Spawn II

On the evening of August 18, 2011 (eight days after the full moon), gamete bundles from multiple colonies of three broadcast-spawning Caribbean coral species were collected from FGBNMS (*Diploria strigosa, Montastraea franksi* and *Stephanocoenia intersepta*). Gametes were cross-fertilized and maintained in similar conditions as in 2010 and transferred to the laboratory at the University of Texas at Austin on August 21, 2011. Samples were collected under permit FGBNMS-2009-005-A3. Settlement assays were conducted on all species across all cues in the panel including A2 (n=6 per cue). *D. strigosa* trials were conducted on four day old larvae after initial testing for competence and *M. franksi* trials were completed at 21 days old after competence was determined. *S. intersepta* were never observed to settle over a period of two months.

Metabarcoding of cue communities

In order to determine the taxonomic composition of each cue sample, I used deep amplicon sequencing. DNA was isolated from ground-up cue samples as described in (Davies, Rahman, et al., 2013). The conserved 5' portion of the eukaryotic small-subunit ribosomal RNA gene (18S SSU) was amplified via PCR using the SP-F-30 forward primer (5' TCTCAAAGACTAAGCCATGC 3') and the reverse primer SP-R-540 (5' TTACAGAGCTGGAATTACCG 3') (Vidal, Meneses, & Smith, 2002). Each 30 µl polymerase chain reaction (PCR) mixture contained 10 ng of DNA template, 0.1 µM

forward primer, 0.1 μM reverse primer, 0.2 mM dNTP, 3 μl 10X ExTaq buffer, 0.025 U ExTaq Polymerase (Takara Biotechnology) and 0.0125 U Pfu Polymerase (Agilent Technologies), and was amplified using a DNA Engine Tetrad2 Thermal Cycler (Bio-Rad, Hercules, CA, USA) with a cycling profile of 94ºC 5min – (94ºC 40sec - 55ºC 2min - 72ºC 60sec) x N - 72ºC 10min, with N = 17-24 depending on the sample. Amplicons (~550 bp bands) were successfully obtained from 6 out of 7 samples (Pohnpei sample failed to amplify despite increased cycle numbers and repeated attempts). Amplicons were cleaned using PCR clean-up kit (Fermentas), 10 ng of the cleaned product was used as template in a second PCR to incorporate 454-Titanium primers and unique barcodes. Each PCR contained 0.1 μM of the universal Btn-SPR-F forward primer (5' CCTATCCCCTGTGTGCCTTGGC-AGTCTCAGTCTCAAAGACTAAGCCATGC 3', underlined stretch matches SP-F-30 primer) and 0.1 μM of unique reverse primer containing a 4-bp barcode (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG**TACT**TTACAGAGCTGGAATTACCG 3', underlined stretch matches SP-R-540 primer, bold indicates 4 bp barcode). The cycling profile was 95ºC 5min – (95ºC 30sec- 55ºC 30sec - 72ºC 60sec) x4 - 72ºC 5min. Amplicons were gel-purified and pyrosequenced using 454-FLX (Roche) with Titanium chemistry at the Genome Sequencing and Analysis Facility (GSAF) at the University of Texas at Austin. All cue samples were sequenced with the exception of Pohnpei, which I was unable to amplify, even with additional efforts involving modifying DNA template concentration and PCR cycle numbers.

Resulting reads were split by barcode and trimmed using a custom Perl script that removes adaptors, barcodes and low quality read ends. Reads that became shorter than 250 bp after this trimming step were discarded. Reads were then clustered at 97% identity using the program cd-hit-454 (Huang, Niu, Gao, Fu, & Li, 2010). The longest sequences

from clusters containing >1% of the filtered reads were selected as representatives of distinct operational taxonomic units (OTUs) and used as reference sequences for mapping the filtered reads using the runMapping module of Newbler v. 2.6 (Roche) with repeat score threshold (parameter –rst) of 3 (i.e., a read was considered uniquely mapped if its best hit among OTU sequences was different from the next-best hit by 3 or more additionally aligned bases). The proportion of reads uniquely mapping to a particular OTU was taken as a measure of the relative abundance of this OTU in the sample. All OTUs accounting for ≥1% mapped reads were assigned to their most likely taxonomic order based on BLAST matches (Altschul et al., 1997) against nonredundant (nr) NCBI database. The non-metric multidimensional scaling (NMDS) analysis based on Bray-Curtis similarities of relative proportions of observed orders was performed using the vegan package in R (Oksanen et al., 2013).

To evaluate the degree to which our sequencing coverage captured sequence diversity in each sample, I conducted rarefaction analysis. The reads mapping to major OTUs (OTUs comprising ≥1% of each sample) were randomly resampled at various depths to simulate the effects of lower sequencing coverage. For each simulated sequencing depth, I randomly sampled with replacement and counted the number of OTUs identified in the sampled subset. Sampling was performed 1000 times for each simulated sequencing depth to calculate the average number of OTUs detected at each depth.

To further characterize the taxonomic diversity of cue samples, two OTUs accounting for the highest proportion of reads within each sample (together representing 39.4-68.3% of the total mapped reads in a cue sample) were aligned using MAFFT version 7 (Katoh & Standley, 2013) (Table 2). This alignment was then used to construct a neighbor-joining tree in BIONJ (Gascuel, 1997) with 1000 bootstrap replicates. This

tree was downloaded in Newick format and modified for visualization using FigTree V1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

Statistical Analysis

All statistical analyses were implemented in R (R Development Core Team, 2013) using the ANOVA function based on arcsine square root transformed proportions of settled larvae. For all models, two factors were included: cue sample nested within cue origin (Pacific/ Caribbean) and coral species. Significance of factors was evaluated using likelihood ratio tests (LRT). If a factor was found to be significant, a post-hoc Tukey's HSD test was used to evaluate the significance of each pair-wise comparison. All assumptions of parametric testing were validated using diagnostic plots in R.

To visualize coral species-specific cue preferences, both principal components analysis (PCA) and non-metric multidimensional scaling (NMDS) ordination were used. PCA was computed using the cmdscale (R Development Core Team, 2013) and vegan (Oksanen et al., 2013) packages. Bray-Curtis similarity coefficients were used for NMDS analysis using vegan package (Oksanen et al., 2013). The resulting PCA and NMDS scores were visualized in two-dimensional ordination space.

RESULTS

Caribbean Spawn I

Larvae of the only coral species that was obtained, *Montastraea franksi*, exhibited distinct preferences for specific cues in the panel tested (Table 2, P_{LRT}<0.001). Settlement was significantly higher in response to Caribbean cues, although the cue from Pohnpei

was only significantly surpassed by the most preferred Caribbean cue (Florida, FF) (Fig. 1a; Tukey's HSD, p=0.006). No recruits were observed in the control wells.

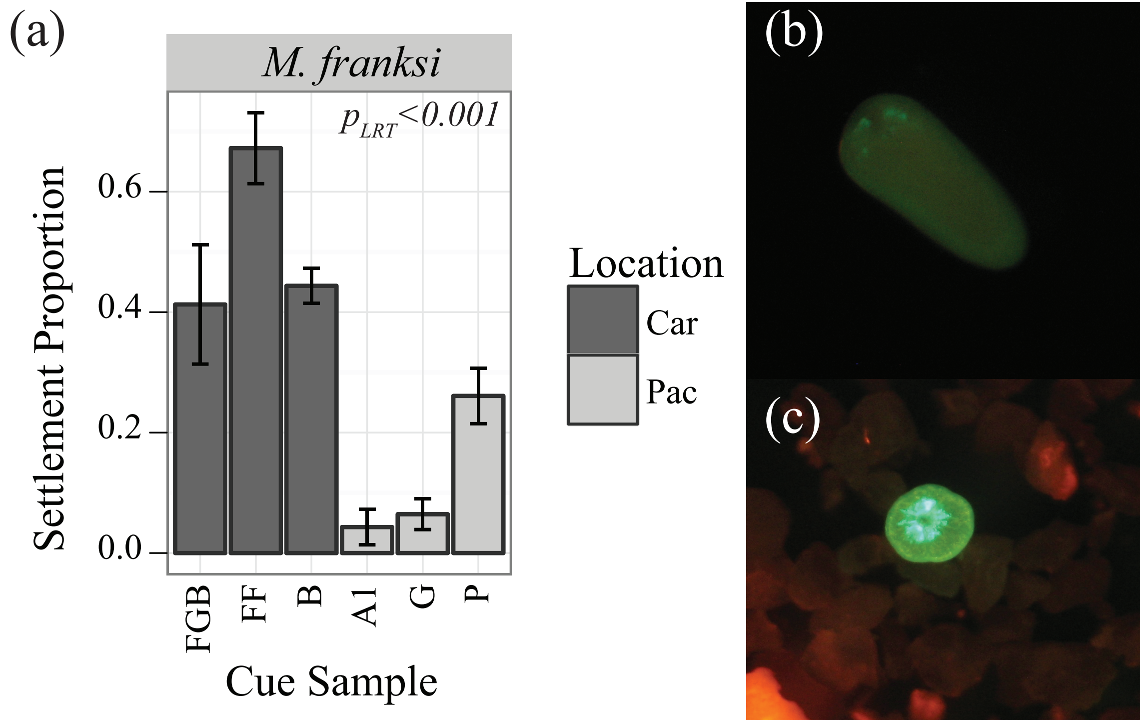


Figure 1: Settlement responses of *M. franksi* in 2010
a) Proportion of coral settlement. Darker bars correspond to Caribbean cues, lighter bars to Pacific cues. b) Fluorescent photograph of *M. franksi* larvae before settlement. c) Fluorescent photograph of *M. franksi* recruit post-settlement.

Table 2: Statistics for CCA settlement response

Likelihood ratio test (LRT) and Tukey's HSD statistics for significant model terms testing the proportion of settlement in response to different CCA cues.

Experiment	Test	Factor	*df*	SS	F	*p*
Caribbean Spawn I						
M. franksi	LRT	Cue	5	1.99	18.34	<0.001
		Residuals	18	0.40	0.02	
	Tukey HSD	B – A1				<0.001
		FF – A1				<0.001
		FGB – A1				<0.001
		P – A1				0.02
		G – B				0.002
		G – FF				<0.001
		P – FF				0.007
		G – FGB				0.003
Pacific Spawn I	LRT	Cue	6	7.89	1.31	<0.001
		Species	2	3.28	1.64	0.012
		Cue * Species	12	2.24	0.19	0.005
		Residuals	104	7.52	0.07	
	Tukey HSD	Species				
		Mil - Liz				<0.001
		Ten - Liz				<0.001
		Cue				
		A2 - A1				<0.001
		B – A2				<0.001
		FF – A2				<0.001
		FGB – A2				<0.001
		G – A2				<0.001
		P – A2				<0.001
		FF – B				0.015
		P – B				0.027
		G – FF				0.002
		P - G				0.003
		Cue* Species				
		Favia Lizardensis				
		None				
		Acropora millepora				
		A2 – A1				<0.001
		A2 – B				<0.001
		A2 – FF				0.011
		A2 – FGB				<0.001
		A2 – G				<0.001
		A2 – P				<0.001
		Acropora tenuis				
		A2 – A1				0.006
		A2 – B				0.004
		A2 – FGB				<0.001
		A2 – G				<0.001
		FF – FGB				0.05
		FF - G				0.03

Table 2 continued

Caribbean Spawn II	LRT	Cue	6	2.17	0.36	<0.001
		Species	1	2.44	2.44	<0.001
		Cue*Species	6	0.55	0.09	0.004
		Residuals	70	2445.07		
	Tukey HSD	Species				
		Fra - Str				<0.001
		Cue				
		A2 – A1				<0.001
		B – A1				0.045
		FF – A1				<0.001
		FGB – A1				<0.001
		A2 – B				0.001
		A2 - G				<0.001
		A2 – P				<0.001
		FF – G				<0.001
		FGB - G				0.003
		Cue * Species				
		Diploria strigosa				
		A2 – A1				0.002
		A2 – G				0.017
		A2 – P				0.018
		B – A1				0.010
		Montastraea franksi				
		A2 – A1				<0.001
		A2 – B				<0.001
		A2 – G				<0.001
		A2 - P				0.05
		FF – A1				0.004
		FF – B				0.014
		FF – G				0.004

Cues: A1 = Australia 1, A2 = Australia 2, B = Bonaire, G = Guam, FF = Florida, FGB = Flower Garden Banks, P = Pohnpei
Species: *Fra = Montastraea franksi, Liz = Favia lizardensis, Mil = Acropora millepora, Str = Diploria strigosa, Ten = Acropora tenuis*

Pacific Spawn I

Both main effects of cue (P_{LRT}<0.001) and coral species (P_{LRT}<0.001) were significant, as well as their interaction (P_{LRT}=0.005), the latter indicating that the coral species differed significantly in their cue preferences (Fig. 2). There were no observable tendencies of Indo-Pacific larvae to prefer cues from either Indo-Pacific or Caribbean. Pairwise comparisons between species in their responses to settlement cues determined that both *A. millepora* and *A. tenuis* were different from *F. lizardensis*, but no significant

difference was observed between these two acroporids (Tukey's HSD, p=0.483) (Table 2). With the exception of *Ctenactis echinata* that failed to respond to any cue, all species exhibited high response to the Australia 2 (A2) cue and also responded to Florida (FF) and Pohnpei (P) cues greater than those cues from Bonaire (B) and Guam (G) (Table 2). *F. lizardensis* responded to all cues; the only suggestion of specificity was a marginal, but insignificant, difference (Tukey's HSD, p=0.063) between A2 (70% settlement) and G (30% settlement). The acroporids were similar in their cue preferences, although *A. tenuis* settled in greater than *A. millepora* and demonstrated no selectivity between Australia 2 (A2) and Florida (FF) or Pohnpei (P). *A. tenuis* also preferred Florida (FF) cue over the Flower Garden Banks (FGB) (Tukey's HSD, p=0.05) and Bonaire (B) (Tukey's HSD, p=0.03) cues. No larvae of any species tested were observed to settle in control conditions.

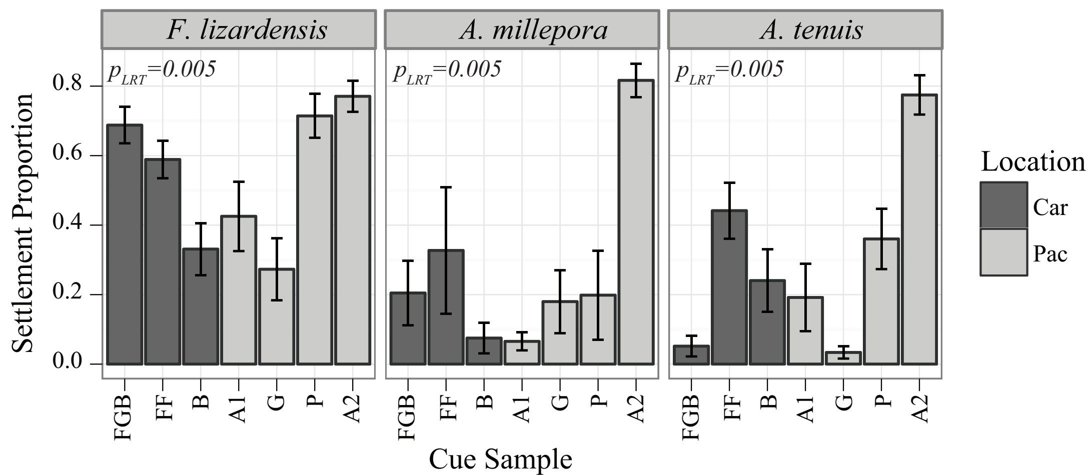


Figure 2: Settlement responses of Pacific corals in 2010
Settlement responses of Pacific corals from Orpheus Island, GBR, Australia. Darker bars correspond to Caribbean cues, lighter bars to Pacific cues.

Caribbean Spawn II

Similarly to the results of the Pacific spawn, there were significant main effects of cue (P_{LRT}<0.001) and species (P_{LRT}<0.001) and a significant interaction term (P_{LRT}=0.004) (Fig. 3, Table 2). The most preferred cue of *D. strigosa* was Australia 2 (A2), followed by all Caribbean cues. The tendency of *M. franksi* larvae to prefer Caribbean cues observed in 2010 was not detected in 2011, as *M.franksi* preferred A2 (which was not included in the 2010 panel) to any other cue in the panel. Compared to *M. franksi, D. strigosa* settled at a higher rate, regardless of cue (Tukey's HSD, p<0.001). No settlement was observed for the gonochoristic broadcaster *Stephanocoenia intersepta* regardless of the cue offered. No *M. franksi* larvae were observed to settle in the control conditions, however; for *D. strigosa*, an average of 3% of larvae spontaneously settled in control conditions (data not shown).

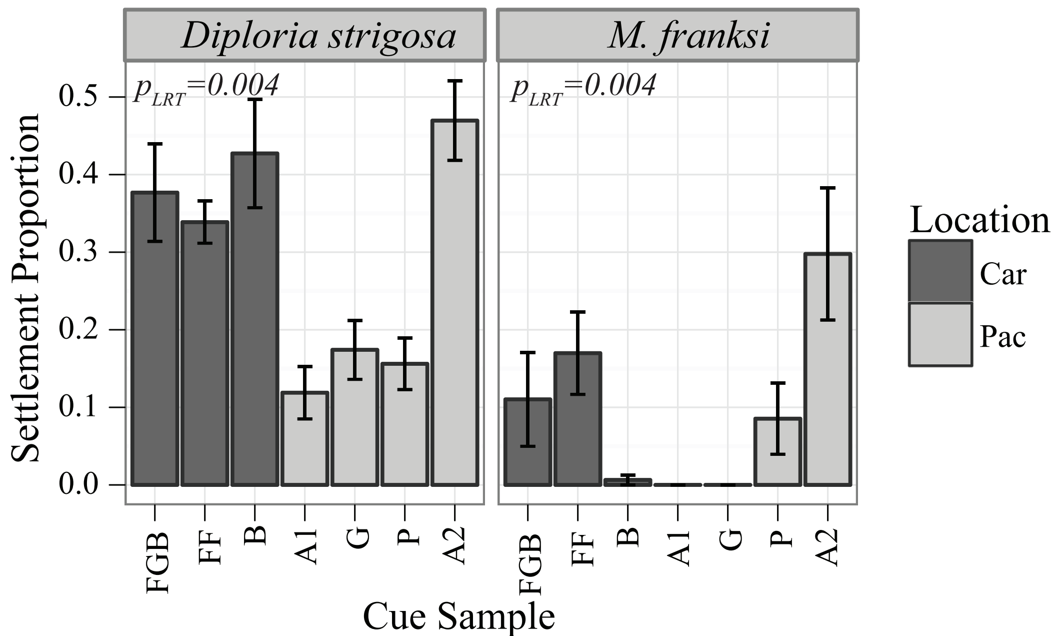


Figure 3: Settlement responses of Caribbean corals in 2011
Settlement responses of Caribbean corals from Flower Garden Banks in 2011. Darker bars correspond to Caribbean cues, lighter bars to Pacific cues.

Metabarcoding of cue samples

From the total 20,872 reads, 18,862 were left after quality filtering (~90%). 15,217 reads mapped to the OTUs derived from 97% similarity clusters containing >1% of the total reads. Mapping efficiencies for each cue sample back to its OTUs was 66-98% with a mean of 81%. Rarefaction analysis indicated that our sequencing coverage efficiently captured sequence diversity in each sequenced sample (Fig. 4). The relative proportions of each taxonomic order differed between cue samples (Fig. 5). Australia 2 (A2), Florida (FF), Guam (G) and Flower Garden Banks (FGB) all contained >50% of the order Corallinales, to which crustose coralline algae (CCA) belong. Both Bonaire (B) and Guam (G) also contained high proportions (>25%) of filamentous red algal orders within the Phylum Rhodophyta (Gelidiales, Gigartinales and Peyssonneliales) (Fig. 5a) Interestingly Australia 1 (A1) contained no Corallinales reads and the majority of its OTUs remained taxonomically unplaced. NMDS also demonstrated the differences between cue communities showing cues with similar proportions of order Corallinales clustering more closely (Fig. 5b).

The neighbor-joining tree constructed using the two most highly represented OTUs from each cue sample was well resolved, with bootstrap scores ranging from 0.54 to 1 (Fig. 5 bottom). Analysis of sequence similarity using BLAST confirmed that all but one (A1) of the successfully sequenced cues predominantly contained Rhodophyta (red algae) sequences. Of these, all but one OTU from Bonaire were from order Corallinales (CCAs). The two main clades in the neighbor-joining tree corresponded to the subfamilies Mastophorideae and Melobesioideae (Table 3). One of the references from FGB was identified to the order Corallinales, but its family remained unresolved.

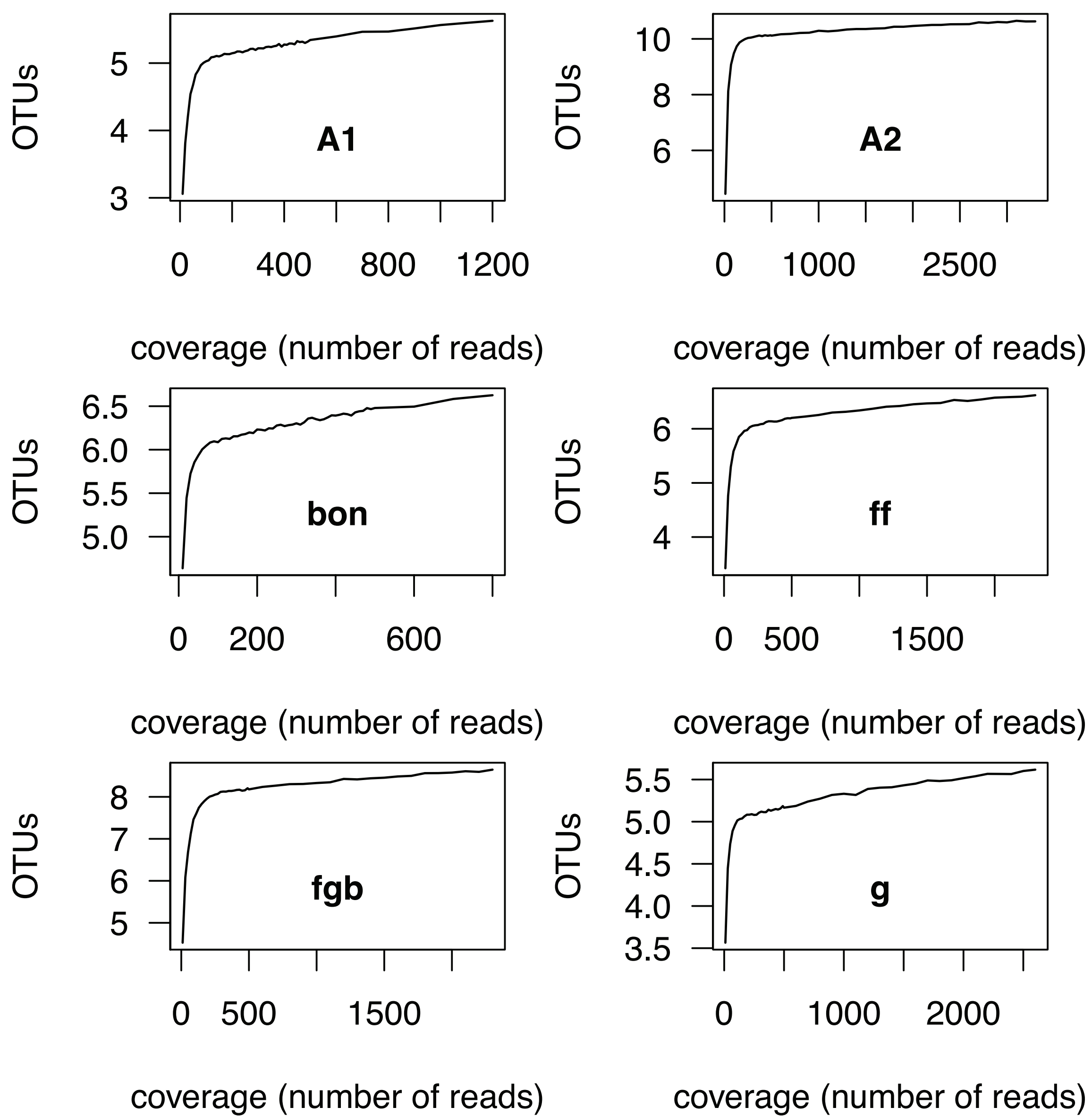


Figure 4: Rarefaction analysis of sequence coverage
Average number of OTUs identified in each cue sample at various coverage depths (number of reads).

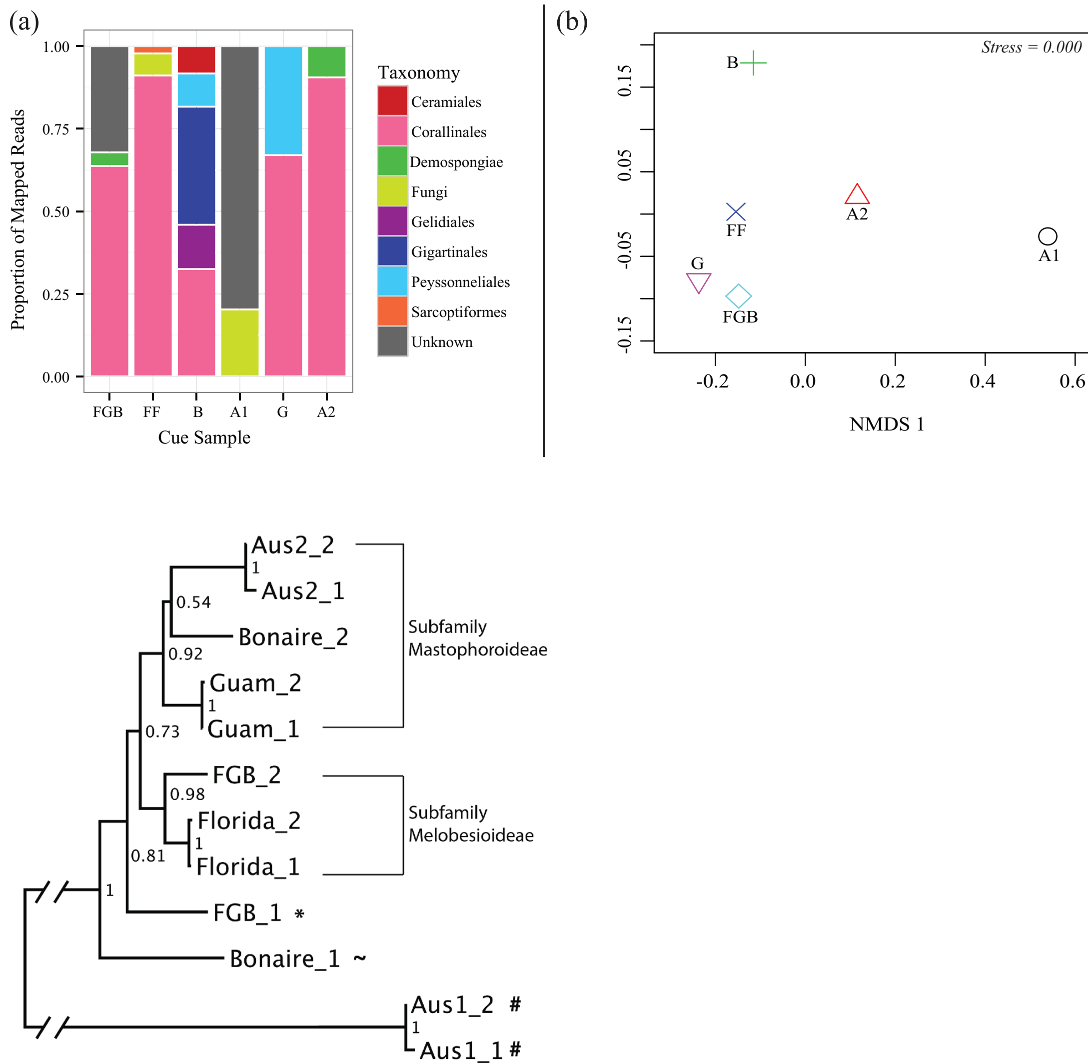


Figure 5: Cue community compositions
a) Relative proportions of mapped reads belonging to various taxonomic groups. b) Non-metric multidimensional scaling (Bray-Curtis nMDS –2 dimensional) based on proportions of taxa in the cue communities. Bottom) Neighbor-joining NJ tree of the two most abundant OTUs in each cue sample. Bootstrap support is shown at each node. Symbol (*) indicates that the reference sequence belongs to order Corallinales, (~) belongs to the Phylum Rhodophyta and (#) indicates that the taxonomic affiliation of the OTU could not be resolved.

Table 3: Characteristics of the top OTUs
Characteristics of the top two most abundant operational taxonomic units (OTUs) in each cue sample including: the OTU name, length of the consensus sequence, percent of the mapped reads that mapped to that OTU, the best NCBI Blast hit for that OTU, if that blast hit was a CCA species, and if that blast hit was in the phylum Rhodophyta.

OTU	Length (bp)	% mapped reads	NCBI Blast Hit	CCA	Rhodophyta	Genbank Accession #
Australia1_1	498	54.2	Uncultured fungus	N	N	KJ609529
Australia1_2	482	14.1	Uncultured fungus	N	N	KJ609530
Australia2_1	514	36.9	Mastophoroideae	Y	Y	KJ609525
Australia2_2	513	6.6	Mastophoroideae	Y	Y	KJ609526
Bonaire_1	528	27.8	Order Gigartinales	N	Y	KJ609527
Bonaire_2	516	15.4	Hydrolithion spp	Y	Y	KJ609528
Florida_1	519	52.0	Subfamily Melobesioideae	Y	Y	KJ609523
Florida_2	519	12.7	Subfamily Melobesioideae	Y	Y	KJ609524
FGB_1	531	27.4	Order Corallinales	Y	Y	KJ609531
FGB_2	520	21.6	Subfamily Melobesioideae	Y	Y	KJ609532
Guam_1	520	26.3	Hydrolithon onkodes	Y	Y	KJ609521
Guam_2	520	13.1	Hydrolithon onkodes	Y	Y	KJ609522

Coral Species-Specific Preferences

Both PCA and NMDS analyses demonstrated that corals exhibit species-specific cue preferences, with the exception of the two *Acropora* species that were similar to each other (Fig. 6). NMDS was superior to PCA at resolving these differences with a low stress value (0.0692) (Fig. 6b). For the PCA (Fig. 6a), component 1 (PCA1) explained 45% of the variation and component 2 (PCA2) explained 15%.

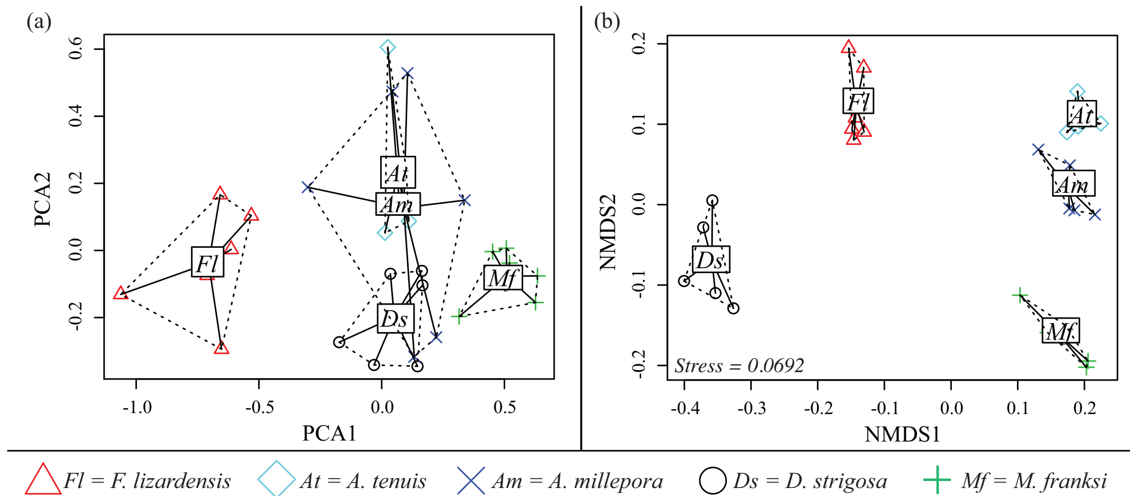


Figure 6: Cue preference differences between corals
Cue preference differences between coral species from the Caribbean and Pacific (see legend), based on proportion of larvae that settled in response to the cue. a) Principle component analysis (PCA) b) Non-metric multidimensional scaling (Bray-Curtis nMDS, 2-dimensional).

DISCUSSION

Caribbean larvae, with the exception of the gonochoric broadcaster *S. intersepta* that failed to respond to any cue, responded to the settlement cues tested in a similar manner to Pacific larvae, suggesting that the lack of recruitment observed in the Caribbean is not due to poor ability of larvae to perceive settlement cue. Furthermore, the panel of Caribbean cues tested here were very successful in inducing settlement of both Caribbean and Indo-Pacific corals tested (Fig. 1-3), demonstrating that effective cues are present on Caribbean reefs and were represented within this collection of cue samples. Previous studies of coral settlement, from both the Caribbean and Indo-Pacific, have demonstrated that coral larvae settle higher in response to certain species of CCAs over others (Arnold, Steneck, & Mumby, 2010; Harrington, Fabricius, De'Ath, & Negri, 2004; Price, 2010; Ritson-Williams et al., 2010). These data confirm these results and further

demonstrate that these preferences can vary substantially among broadcast-spawning coral species, even if these corals are from the same reef environment at the same location. In addition, some species, such as *F. lizardensis*, appear to be less specific overall and settle in high proportions regardless of cue type (at least for the cue panel tested here), while others did not respond to any cues tested (*C. echinata, S. intersepta*).

Preferences of Caribbean corals

Data from the pilot study in the Caribbean (2010) suggested the potential for co-adaptation between larval cue receptors and Caribbean cues, as the larvae of *M. franksi* settled in higher proportions in response to Caribbean cues rather than Pacific cues (Fig. 1). However, results of the second Caribbean spawning season (2011) did not support this hypothesis since both *M. franksi* and *D. strigosa* responded best to the newly introduced Pacific cue (A2). Beyond A2, Caribbean larvae settled well in response to Caribbean cues and even (in case of *D. strigosa*) tended to prefer them (Fig. 3), indicating that the Caribbean corals tested were fully capable of settlement in response to local Caribbean cues. *M. franksi* and *D. strigosa* also demonstrated species-specific cue preferences (Fig. 6). Year-to-year variation in settlement success for *M. franksi* was observed, with settlement in 2011 being less successful than 2010 (Fig. 1 and 3). Although great care was taken to culture larvae in identical conditions, unknown year-to-year variations in culture conditions may have influenced larval settlement. All cues were kept frozen, however each cue was collected at different times so settlement cue age may have altered their effectiveness through time by modifying cue stability. Therefore, the coral responses to the cues were only compared among coral species within the same field season. It is also possible that the year-to-year variation observed in this study reflects the

natural stochasticity of the recruitment process or genetic difference between larval cohorts (Meyer et al., 2009).

Preferences of Pacific corals

No Indo-Pacific-wide trends were ever observed for the corals and cues tested here, but clear differences in cue preferences between coral species were apparent, with the two *Acropora* species exhibiting more specific settlement behavior (Fig. 2 and 6). The strict preferences of *A. millepora* and *A. tenuis* larvae have been reported previously (Harrington et al., 2004), and the similarity of their cue preferences observed in these experiments (Fig. 6) might be attributable to their phylogenetic proximity. *Favia lizardensis* was much less selective and high settlement rates were observed in response to most cues (Fig. 2). This result is similar to observations from its Caribbean congener, *Favia fragum*, which had previously been shown to be relatively indiscriminate in its settlement behavior (Nugues & Szmant, 2006), although it must be noted that *F. fragum* is a brooding rather than broadcast-spawning species. While these data do not formally allow drawing taxonomy-related conclusions, the similarity of cue preferences in congeneric coral species across this cue panel is notable and might reflect the general pattern of cue preference evolution.

Corals that would not settle: *Ctenactis echinata* and *Stephanocoenia intersepta*

Both species demonstrated complete lack of settlement response to the same cue panel that successfully induced metamorphosis in other corals, and therefore these species represent the most extreme demonstration of divergent cue preferences among the corals tested. While *C. echinata* was only tested at five days post fertilization, leaving open a possibility that the culture had not yet reached competency, *S. intersepta* was

assayed for settlement for approximately two months and was still never observed to settle for any cue. Interestingly, these species are from different oceans but share one key life history trait: they are both gonochoric (i.e., have separate sexes) whereas all other coral species tested were hermaphroditic. It is tempting to speculate that this shared life history trait underlies their lack of response in settlement trials. Previous work on a gonorchoric, broadcast-spawning gorgonian coral demonstrated that adult proximity to conspecifics had a large effect on reproductive success (Coffroth & Lasker, 1998), one of the possibilities being that gonochoric corals might need additional cues from conspecifics to ensure close proximity and efficient fertilization during spawning (Tamburri, Zimmer, & Zimmer, 2007). While I cannot discount that these corals were unresponsive because they had not reached competence or they were not offered appropriate cues, I believe that this hypothesis merits detailed investigation in the future.

Composition of the cue communities

Each cue community differed in its relative proportions of taxa; however, most cues that were effective at inducing settlement in the corals tested here contained >50% order Corallinales, the order that contains CCAs (Fig. 5). Notably, one cue (A1) yielded no Corallinales reads yet still induced settlement, although it was among the least effective. Two major CCA sub-families were represented in the cue communities: Mastophorideae and Melobesioideae (Fig. 5 bottom). These taxonomic groups have previously been shown to be strong larval settlement inducers (Harrington et al., 2004; Heyward & Negri, 1999; Ritson-Williams et al., 2010), indicating that these cue collections efforts were, in fact, at least taxonomically-related to previously established settlement cues for corals. While I could only discriminate taxa to the order or family level, this is the first study to create a sequence database of natural coral settlement cues.

Possible consequences of coral species-specific cue preferences

Settlement choice has been shown to strongly influence post-settlement survival, illustrating the consequences of larval selectivity (Babcock & Mundy, 1996; Harrington et al., 2004). Divergent larval settlement preferences correlating with cue availability in the adults' natural habitat have been previously demonstrated for two coral species from Guam, *Stylaraea punctata* and *Goniastrea retiformis* (Golbuu & Richmond, 2007). However, divergent preferences between these species were expected since they do not co-occur in the same reef environment; moreover, *S. punctata* is a brooder while *G. retiformis* is a broadcast spawner. This study is the first to document species-specific preferences in a panel of settlement cues among broadcast-spawning corals from the same reef community for both the Indo-Pacific and the Caribbean (Fig. 6), and it is tempting to speculate that these preferences might play a role in coral community assembly. While this study did not, by any means, exhaust all potential cues available for corals arriving to reefs, it did demonstrate that some coral species are considerably more "choosy". This finding is especially concerning given ongoing climate change, since CCA are among the most sensitive reef organisms to both warming and acidification (Doropoulos & Diaz-Pulido, 2013; Ragazzola et al., 2012; Webster, Soo, Cobb, & Negri, 2011; Webster, Uthicke, Botte, Flores, & Negri, 2013). Diminishing CCA abundances and effectiveness as settlement inducers might be accompanied by a reduction in CCA diversity, which in turn could lead to coral community shifts in favor of less selective coral species that do not require particular settlement cues.

CONCLUSION

This research demonstrates that Caribbean coral larvae can respond to the local settlement cues on par with Indo-Pacific larvae, suggesting that, at least in the lab, interactions between corals and cues on Caribbean reefs have not been compromised

relative to the Indo-Pacific. However, it is clear that other processes are causing region-wide Caribbean recruitment failure, and identifying these processes should remain a research priority.

Chapter 2: Micronesia facilitates genetic exchange between the Coral Triangle and the central Pacific, but only for the most dispersive coral species

ABSTRACT

The Coral Triangle, which is located in the Indo-West Pacific, is a marine biodiversity hotspot that may be an important source of genetic diversity for remote reefs of the Pacific. Simulation studies highlight Micronesia, a scattering of hundreds of small islands situated within the North Equatorial Counter Current as an important migration corridor facilitating this genetic exchange. To test this hypothesis, I characterized the population genetic structure of two ecologically important congeneric species of reef-building corals across greater Micronesia, from Palau to the Marshall Islands. Genetic divergences between islands followed an isolation-by-distance pattern, with *Acropora hyacinthus* exhibiting more than two-fold greater divergence than *Acropora digitifera* across the same distance, suggesting either different migration capabilities or different effective population sizes for these closely related species. Dispersal distance inferred from a biophysical larval transport model explained an additional 15-21% of genetic variation compared to between-island geographic distance alone. For both species, genetic divergence accumulates and genetic diversity diminishes with distance from the Coral Triangle, supporting the hypothesis that Micronesian islands act as important stepping-stones connecting the central Pacific with the species rich Coral Triangle. However, for *A. hyacinthus*, the species with lower genetic connectivity, at the eastern-most islands studied here the immigration from the sub-equatorial Pacific begins to play a larger role in shaping genetic diversity than the input from the Coral Triangle. This work highlights the enormous dispersal potential of broadcast-spawning corals and identifies the biological and physical drivers that shape coral genetic diversity on a regional scale.

INTRODUCTION

Waters of the Indo-West Pacific (also termed "Coral Triangle") support the greatest tropical marine biodiversity on the planet (Briggs, 1987; Hoeksema, 2007; Hughes, Bellwood, & Connolly, 2002; Vernon, 1995; Veron et al., 2009). The processes responsible for generating and redistributing this diversity have significant consequences for the persistence, speciation, and extinction of numerous marine taxa. As human activities continue to affect biodiversity on an unprecedented scale, a thorough understanding of how biodiversity is maintained is essential (Burrows et al., 2011; Hoegh-Guldberg et al., 2007; Parmesan & Yohe, 2003; Pinsky, Worm, Fogarty, Sarmiento, & Levin, 2013). Establishing dispersal and gene flow patterns will improve predictions of how diversity patterns might change in the near future, which is invaluable both for prioritization of management efforts and basic understanding of evolution in the ocean.

Several models of seascape connectivity between the Coral Triangle and its surrounding oceans have been developed (Kool, Paris, Barber, & Cowen, 2011; Treml, Halpin, Urban, & Pratson, 2008; Wood, Paris, Ridgwell, & Hendy, 2014). Most recently, Wood et al. (Wood et al., 2014) inferred little direct migration of corals from the Coral Triangle to the central Pacific, but instead proposed that the Coral Triangle supplies larvae to Micronesia, which in turn serves as a source for the central Pacific via the North Equatorial Counter Current (NECC, Fig. 7A). Micronesia is therefore hypothesized to be a corridor to the Pacific for many Coral Triangle genotypes. If these larvae are incapable of dispersing throughout this entire range, differentiation in gene frequencies should build up as distance between populations increases (termed "Isolation by distance (IBD)) (Slatkin, 1993; Wright, 1943) and such a process should result in a 'stepping stone' population structure across Micronesian islands over evolutionary time (M. Kimura &

Weiss, 1964). This prediction of can be explicitly tested through population-genetics studies across the region.

Only a few studies have explored genetic connectivity within Micronesia and between Micronesia and the rest of the Pacific, and thus far dispersal patterns remain unresolved. A study of the yellow tang fish (*Zebrasoma flavescens*) found that migration rates from Hawaii westward to the Central Pacific (Pohnpei) was supported (Eble et al., 2011), contrasting with the eastward migration patterns suggested in studies of other marine species (Priest, Halford, & McIlwain, 2012; Timmers, Bird, Skillings, Smouse, & Toonen, 2012). Lack of consistency for dispersal patterns among taxa across the Pacific might be due to variations in species life history, dispersal capabilities, or spawning characteristics. Regardless, the group of organisms that serve as the foundation for these ecosystems, reef-building corals (phylum Cnidaria, class Anthozoa, order Scleractinia), remain understudied in the Pacific (Keyse et al., 2014).

Coral reefs shape global marine biodiversity patterns, determine physical and ecological characteristics of coastlines, and are globally threatened (Hughes et al., 2003; Pandolfi et al., 2003), which is why understanding coral migration limits remains a high conservation priority. Coral dispersal is potentially extensive because most major reef-building species reproduce by releasing gametes into the water column, resulting in pelagic larvae that disperse broadly with ocean currents (Baird et al., 2009). Many of these coral larvae can survive for months in the absence of settlement cues (Graham, Baird, & Connolly, 2008; Graham, Baird, Connolly, Sewell, & Willis, 2013), and different species are capable of variable pelagic larval durations (PLD) (Connolly & Baird, 2010). Coral population genetics studies have revealed gene flow on scales ranging from tens to hundreds of kilometers (Ayre & Hughes, 2000, 2004; Underwood, Smith, van Oppen, & Gilmour, 2009), with evidence for long-distance dispersal (Baums,

Miller, & Hellberg, 2005; Severance & Karl, 2006; van Oppen, Peplow, Kininmonth, & Berkelmans, 2011), resulting in high genetic connectivity and large geographic ranges for many coral species. However, studies are increasingly suggesting that marine dispersal distances are less than previously assumed, revealing high potential for self-recruitment where individuals remain in the same population that they originated in (Figueiredo, Baird, & Connolly, 2013), which suggests that species with large geographical ranges must rely on stepping stones for gene flow. Given the extensive ranges for most coral species, it is reasonable to predict that Micronesian islands serve as effective stepping-stones, even though these islands are small, remote, and separated by large expanses of open ocean.

Here, I implemented a seascape genetics study of two coral species of the genus *Acropora*, the most species-rich and ecologically important genus with some of the largest geographical ranges (i.e. *A. digitifera* range >100,000km^2). I sampled *A. digitifera* and *A. hyacinthus* over the entire range of greater Micronesia, in addition to one subequatorial Pacific location to evaluate (i) dispersal limits for a marine invertebrate with a long pelagic larval duration (PLD) and a large geographical range; (ii) whether the distribution of genetic diversity in the region is consistent with a stepping-stone model; (iii) the extent to which biological-physical modeled estimates of larval dispersal explain patterns found for genetic differentiation; (iv) whether two closely related species sharing the same reproductive strategy exhibit similar genetic connectivity; and (v) whether Micronesia serves as a dispersal corridor between the Coral Triangle and the central Pacific.

Methods

Sampling Locations and Methodology

From 2009 to 2011, 23 reef sites on ten islands throughout Micronesia were visited (Fig. 7) with the goal of sampling different spatial scales: reefs on the same island, within the same island group, and between island groups separated by various distances. Site information is given in Table 4. Snorkeling or scuba (3-7m depth) were used to sample approximately fifty unique colonies (>2m apart) of each of the two focal coral species (*Acropora hyacinthus* and *A. digitifera*) per reef, a sample size adequate for population assignment tests (Hellberg, 2007). Prior to sampling, colonies were photographed for confirmation of species identification and a small (~2cm), randomly chosen branch tip was collected, preserved in 96% ethanol, and stored at -20ºC until DNA isolation. Fragments were collected from approximately 2300 individuals. An additional site south of the equator (Phoenix Islands N=19) was later included to test specific dispersal hypotheses for *A. hyacinthus*. All the necessary collection and export permits were obtained prior to sampling.

Table 4. Reef Site Collections.

GPS coordinates, main island group, number of *A. digitifera* and *A. hyacinthus* genotyped. Site letter corresponds to Figure 7 island insets.

Site	Island	GPS	*A. digitifera*	*A. hyacinthus*
a. West Channel Reef	Palau	7°31'55.7 N, 134°29'42.8 E	39	44
b. Lighthouse Reef	Palau	7°16'62.4 N, 134°27'61.9 E	49	50
c. Ngulu	Ngulu Atoll	8°18'12.0 N, 137°29'18.7 E	0[2]	46
d. South Tip Reef	Yap	9°26'05.4 N, 138°02'10.4 E	45	48
e. West Outer Reef	Yap	9°33'47.3 N, 138°05'71.5 E	46	50
f. Goofnuw Channel	Yap	9°34'26.4 N, 138°12'19.2 E	49	37
g. Pago Bay	Guam	13°25'66.6 N, 144°47'94.3 E	45	0*
h. Tanguisson	Guam	13°32'61.1 N, 144°48'52.6 E	50	0*
i. West Polle	Chuuk	7°19'69.7 N, 151°33'21.1E	45[1]	39
j. Aroche Patch Reef	Chuuk	7°14'42.0 N, 151°53'95.4 E	0[2]	49
k. South East Pass	Chuuk	7°14'60.3 N, 152°01'29.1 E	0[2]	49
l. Ant Atoll (South)	Pohnpei	6°45'05.9 N, 157°59'23.3 E	0[2]	48
m. Ant Atoll (East)	Pohnpei	6°47'42.3 N, 158°01'20.7 E	47	47
n. Roj	Pohnpei	6°46'37.7 N, 158°12'24.1 E	50	43
o. Coral Garden	Kosrae	5°18'47.2 N, 162°53'01.8 E	46	44
p. Hiroshi Point	Kosrae	5°15'88.0 N, 162°59'01.8 E	41	46
q. Nell Pass	Kwajalein	9°6'58.9 N, 167°18'71.7 E	21	0*
r. Carlson Reef	Kwajalein	8°44'95.7 N, 167°40'70.0 E	47	0*
s. North Point	Kwajalein	8°44'63.4 N, 167°44'11.5 E	48	0*
t. Laura Cove	Majuro	7°07'92.8 N, 171°02'64.7 E	46	16[1]
u. Army School	Majuro	7°07'40.5 N, 171°03'10.3 E	47	0*
v. Arno	Arno Atoll	7°2'96.7 N, 171°33'92.2 E	45	0*
w. Ine	Arno Atoll	6°58'98.1 N, 171°41'84.5 E	40	0*
Kiribati	Phoenix Islands	4°27'18.6 S, 171°14'36.3 W	0[2]	17
TOTAL			**846**	**673**

* indicates that no individuals of this species were found

1 indicates all individuals from this island group were pooled for analyses

2 indicates that individuals were not collected from this site but are likely present

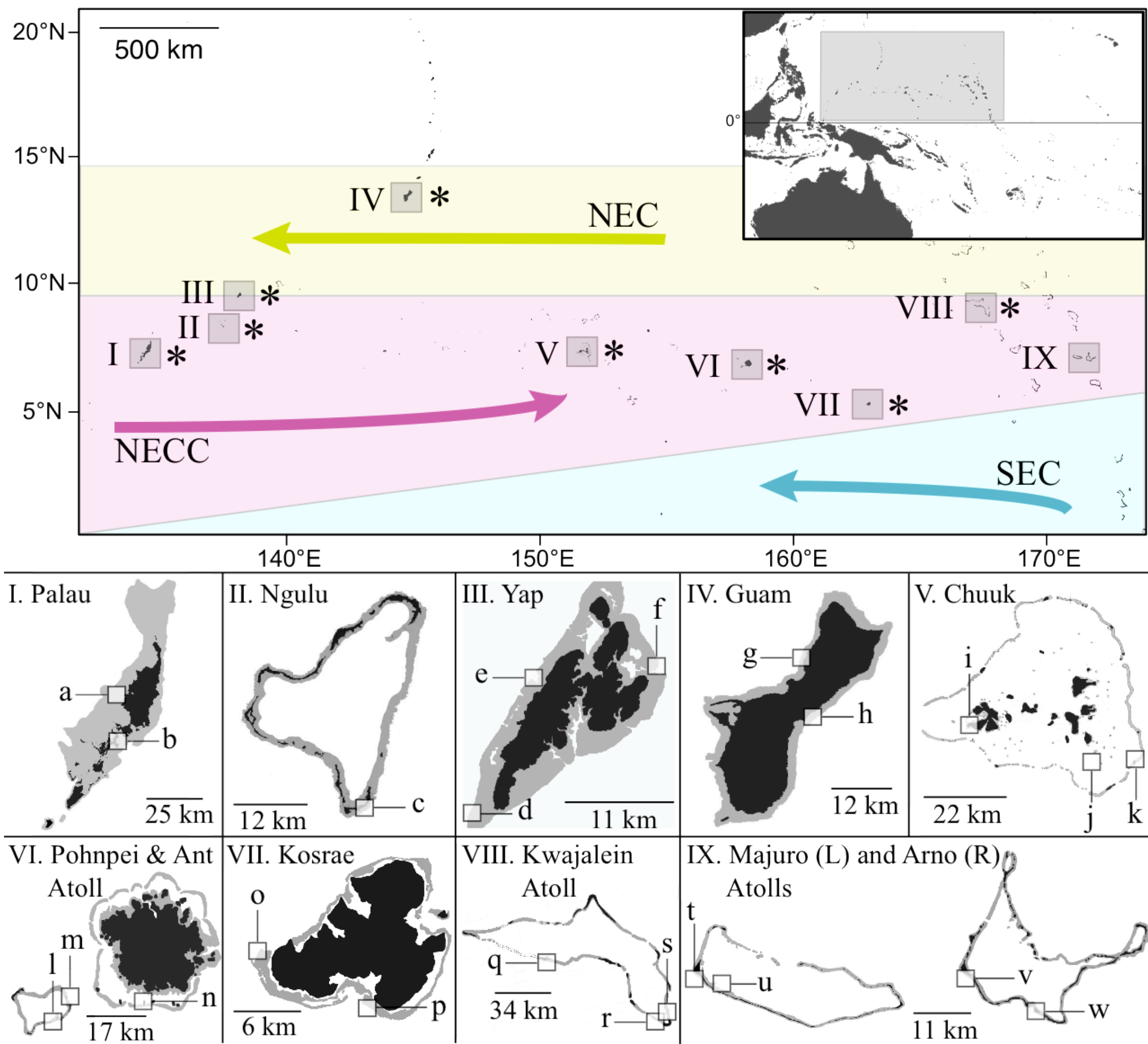


Figure 7: Micronesian sampling sites
Geographic location of the Micronesian islands where *A. hyacinthus* and *A. digitifera* corals were collected. Top: Map of the Micronesian Pacific with an inset of the Pacific Ocean for reference. Islands where samples were collected are designated with grey boxes. The subset of islands included in the biophysical model are marked with an (*). Colored blocks are estimates of dominant current patterns (Bonjean & Lagerloef, 2002). Yellow designates the North Equatorial Current (NEC). Pink designates the North Equatorial Counter-current (NECC). Blue represents the South Equatorial Current (SEC). Arrows in each quadrant represent current direction. Bottom: Enlarged regional maps for each island with sampling sites shown in boxes. Detailed information on sampling sites is located in Table 4.

Laboratory Procedures

DNA was isolated from a total of 1762 coral samples following (Davies, Rahman, et al., 2013), quantified using a NanoDrop 1000 Spectophotometer (Thermo Scientific) and diluted to 10ng/μl. A multiplexed assay of twelve microsatellite loci (modified from (Wang, Zhang, & Matz, 2009)) was established and loci were amplified in 10 μl polymerase chain reactions (PCR). Multiplex and primer information are located in Table 5. PCR mixtures contained 10 ng of DNA template, 0.1 μM of each forward primer, 0.1 μM of each reverse primer, 0.2 mM dNTP, 1 μl 10X *ExTaq* buffer, 0.025 U *ExTaq* Polymerase (Takara Biotechnology) and 0.0125 U *Pfu* Polymerase (Agilent Technologies). Amplifications were performed using a DNA Engine Tetrad2 Thermal Cycler (Bio-Rad, Hercules CA). Cycling began at 94°C for 5 min, followed by 35 cycles of 94°C for 40 s, 60°C for 60 s, and 72°C for 60 s and then a 10 minute extension period at 72°C. Amplicon sizes were analyzed using the ABI 3130XL capillary sequencer with an in-house ROX-labeled size standard. Allele sizes were scored from raw electrophoregrams using GeneMarker software (Soft Genetics LLC). A custom script (FragBin.pl) was used to bin the data into unique allele sizes. Individuals failing to amplify at ≥4 loci were excluded from analyses. A total of 1744 DNA samples were successfully genotyped.

Table 5: Summary of twelve microsatellite loci
Microsatellite loci transferable from *A. millepora* SSR markers (Modified from (Wang, Zhang, & Matz, 2009)) and their corresponding multiplexing groups.

Family	PCR Mulitplex	Locus (Repeat)	Primer Sequence 5'-3'	Repeat
A	A1	*EST007*	F: FAM-tgcaatggttctgttgcagtca R: gatctctttaccgatttacagca	$(TTTC)_5$
	A1	*WGS112*	F: HEX-actccactcagtcctattacca R: acacttccaagagtccctaca	$(AAT)_9$
	A2	*EST062*	F: NED[a]-cgagttagtctgttaagatggt R: ctctaagtccgatcttcttcca	$(GAT)_9$
B	B1	*EST032*	F: FAM-aggcacaagaaagtggaaaacaa R: tgaagggatgtgaagcatggt	$(TTA)_{21}$
	B1	*WGS153*	F: HEX-tttccaagttgctgtgagtaca R: cgggtgctaagcttgctcaa	$(AATC)_7$
	B2	*EST254*	F: ggtgaccaatcagagtcttga R: NED[a]-tacacttgctatagtaacttgct	$(CA)_{10}$
C	C1	*EST097*	F: FAM-tgacaacgacatcaatcatggt R: acagcaggagctgtcagcact	$(TGA)_7$
	C1	*WGS189*	F: HEX-aaatgagcgcctgtgcacga R: gagcatgaaactctgagtagca	$(ATCT)_7$
	C2	*EST016*	F: NED[a]-ctatctgtgtatgatcaggacta R: tccatctgttgtggaaactggt	$(AAC)_7$
D	D1	*EST181*	F: FAM-tgattgctgagaaagctagagat R: gcctcaccttgccttgtaca	$(ATG)_{10}$
	D1	*WGS092*	F: HEX-ctgggcaaatattaccacttga R: aagacaggtatgtatgcaatgat	$(ATT)_{12}$
	D2	*EST121*	F: NED[a]-acagttgcaggccttgcaga R: gtgggaattgcgacaggcat	$(ATGCCG)_4$

[a] NED-labeled primers were indirectly labeled in each PCR reaction with an additional NED labeled tag sequence: NED-tgtagcgtgaagacgacagaa.

Data Analysis

Species identification

Morphological species identification remains difficult for Pacific acroporid corals. Here, I employed a conservative approach to species identification. I pooled all data (omitting locus WGS153 that failed to consistently amplify for *A. digitifera*) and applied the Bayesian approach implemented in STRUCTURE v2.3.3 (Pritchard, Stephens, & Donnelly, 2000) to validate species designations. STRUCTURE uses a Monte Carlo Markov chain (MCMC) clustering algorithm to assign individuals with similar multilocus genotypes to distinct populations. Mean and variance of log likelihood values of the number of populations for K (1-4) were inferred by STRUCTURE with 10^6 iterations (burn in = 300,000 iterations) in four replicate runs. An admixture model was employed with no priors. K=3 was chosen to distinguish (1) *A. hyacinthus,* (2) *A. digitifera* and (3) incorrect collections. The mean membership (q) for each sample was used to determine the likelihood of each individual belonging to each of these populations. All individuals with a q>0.5 for (3) were removed, which was corroborated by photographic evidence, leaving 875 *A. digitifera* and 663 *A. hyacinthus* samples (Fig. 8). Although great care was taken to avoid sampling identical individuals, Genalex 6.5 (Peakall & Smouse, 2006) identified clone mates, which were then removed. Eight clones were detected for *A. hyacinthus* and 29 clones for *A. digitifera*, yielding 846 *A. digitifera* and 655 *A. hyacinthus* in all subsequent analyses (Table 4). For several sites, incorrect collections resulted in a marked reduction in sample size, and in these cases all reef sites from an island were considered one population (Chuuk: *A. digitifera*; Marshall Islands: *A. hyacinthus*).

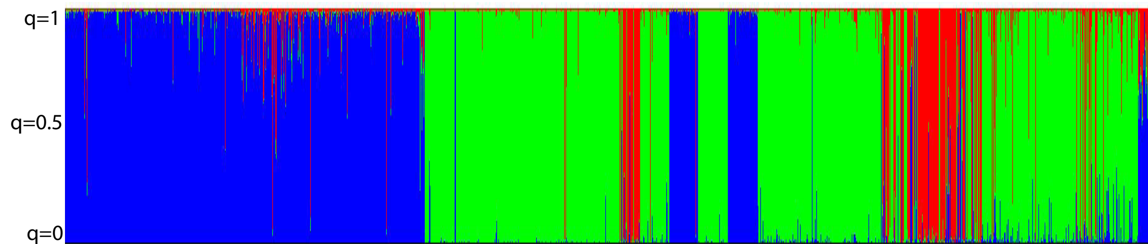


Figure 8: STRUCTURE population assignment for *Acropora* corals
STRUCTURE population assignment for all coral DNA samples that were isolated and amplified across the range of Micronesia sampled from Palau to the Phoenix Islands. The results are for population number (K) of 3. Individuals that were assigned to the blue population with a q value >0.5 were kept in the *A. hyacinthus* dataset, individuals with a q-value >0.5 for the green population were kept in the *A. digitifera* dataset. All red individuals were removed and corroborated well with photographic evidence of incorrect collections.

Genetic diversity

GENEPOP v4.2 (Raymond & Rousset, 1995) tested for heterozygote deficits with 5000 dememorizations, 1000 batches and 5000 iterations per batch. Observed (H_o) and expected (H_e) heterozygosities, number of alleles (N_a), number of private alleles, and Shannon's diversity index (SHa) were calculated using GENALEX version 6.5 (Peakall & Smouse, 2006) and regressions against both Euclidean and biophysical distances were computed. Statistics were calculated for each site and each island; only the island-wise results are presented here.

Population Differentiation and Isolation By Distance (IBD)

Pairwise F_{ST} and unbiased Nei's genetic distances were calculated in GENALEX v6.5 (Peakall & Smouse, 2006) to determine population genetic subdivision between all sites and islands. Pairwise F_{ST} values were used to test for isolation by distance (IBD) (described below) and pairwise Nei's unbiased genetic distances were applied to create

two-dimensional Principle Coordinate Analysis (PCoA) plots for each species. Mantel's tests (Mantel, 1967) determined whether significant IBD (negative correlations between geographic (Euclidean) and normalized pairwise genetic distances (F_{ST} /(1- F_{ST})) was observed. Tests were performed for each species independently using MantelTest function in the *ecodist* package in R (Goslee & Urban, 2007) with 10000 permutations. Slopes of these relationships were calculated using a linear model and a likelihood ratio test was used to determine whether the difference between the slopes (i.e., species by distance interaction term) was significant.

Bayesian Clustering and Genetic Structure Analysis

Log-likelihood values for each K (number of inferred populations) (1–5) were computed by running STRUCTURE separately on genetic data for *A. hyacinthus* (12 loci) and *A. digitifera* (11 loci). Ten replicate runs were computed with 10^6 iterations (burnin = 300,000) for each K. An admixture model was implemented with collection site as a location prior. Following the recommendations of (Evanno, Regnaut, & Goudet, 2005), the ad hoc statistic ΔK was calculated based on the rate of change of the log-likelihood between consecutive K values, which is implemented in the program STRUCTURE Harvester (Earl & Vonholdt, 2012). CLUMPP (Jakobsson & Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004) were used to produce all graphics. Once initial clusters were identified, additional hierarchical analyses on data subsets were conducted to investigate potential within-cluster structure. Subclusters were analyzed separately using the same models described above. Hierarchical analyses were performed until individuals showed equal membership to each cluster as identified in the optimum K, however only the first two iterations are visualized here.

Biophysical Model

A spatially-explicit biophysical modeling framework was used to predict the dispersal potential between coral reefs/islands of Micronesia (Starred islands in Fig. 7), thereby revealing the location, strength, and structure of a species' potential population connectivity (Treml, 2012). Model resolution of 10 km coincides with the best-available hydrodynamic data for this region and results in tractable solutions capturing local and regional dynamics. Coral reef habitat data were derived from the Global Distribution of Coral Reefs product from UNEP-WCMC (data.unep-wcmc.org/datasets/13) (Table 6A). Land/sea boundaries were extracted from the Global Self-consistent, Hierarchical, and High-resolution Shoreline (GSHHS) databases (Wessel & Smith, 1996). To capture broad-scale variability we used the HYCOM + NCODA Global 1/12deg analysis product available at daily resolution from 2004 to 2012 (Chassignet et al., 2009).

Table 6: Biophysical Model information
A. Biophysical model information for reef data and release times. B. Biophysical model information for spawning time for Palau (left) and the rest of Micronesia (right). C. Details on the biological parameters specified in the biophysical model.
A.

Site Code	Model ID	Relative Reef Area	Release Times
PAL	2	7.79	Palau Release
NGO	3	1.09	Greater Micronesia Release
YAP	4	1.37	Greater Micronesia Release
GUA	10	0.65	Greater Micronesia Release
CHU	41	8.09	Greater Micronesia Release
POH	50	3.69	Greater Micronesia Release
KOS	54	0.38	Greater Micronesia Release
KWA	65	4.1	Greater Micronesia Release

Table 6 continued

B. Palau Releases (5 days after full moon)				Greater Micronesia Releases (5 days after full moon)			
Release#	**Year**	**Month**	**Day**	**Release#**	**Year**	**Month**	**Day**
1	2004	4	10	1	2004	6	8
2	2004	5	9	2	2004	7	7
3	2005	4	29	3	2005	6	27
4	2005	5	28	4	2005	7	28
5	2006	4	18	5	2006	6	16
6	2006	5	18	6	2006	7	16
7	2007	4	7	7	2007	6	6
8	2007	5	7	8	2007	7	4
9	2008	4	25	9	2008	6	23
10	2008	5	25	10	2008	7	23
11	2009	4	14	11	2009	6	12
12	2009	5	14	12	2009	7	12
13	2010	5	3	13	2010	7	1
14	2010	6	2	14	2010	8	31
15	2011	4	23	15	2011	6	20
16	2011	5	22	16	2011	7	20
17	2012	4	11	17	2012	6	9
18	2012	5	11	18	2012	7	8

C. **Parameter**	**Value**
Maximum PLD (days)	90 days 65 days 45 days
Simulation Time-step	30 minutes
Competency (Gamma CDF shape parameter)[1]	12
Competency (Gamma CDF scale parameter)[1]	0.5
Behaviour	passive
Diffusivity (m^2s^{-1})	100
Survival model (Weibull λ or scale parameter)[2]	0.043
Survival model (Weibull v or shape parameter)[2]	0.57
Migration rate threshold[3]	1/100,000

1) Parameters for 50% competent at 6 days (~1% at day 3)

2) (Connolly & Baird, 2010), *A. millepora*, Table 6

3) Lower probability threshold, below which no migration was inferred (Treml et al., 2012)

The species-specific biological parameters included were larval release timing (5 days after full moon, see Table 6B), periodicity (annual), maximum dispersal duration (45, 65, & 90 day PLD), pre-competency (~3 days) and competency periods (onwards from 3 to 6 days), passive larval behavior (no swimming, and no homing capabilities), and larval mortality (Table 6B). Pre-settlement larval mortality was implemented using a Weibull survivorship curve for corals ((Connolly & Baird, 2010), *A. millepora* Weibull parameters, Table 6C). All biological parameters were maintained with the exception of maximum PLD, which varied between the three models. The spatially explicit dispersal simulations model the dispersal kernel (2-D surface) as a 'cloud' of larvae as it evolves through time and space, allowing it to be concentrated and/or dispersed as defined by the biophysical parameters. An advection transport algorithm was used for transporting larvae within the flow fields (Smolarkiewicz & Margolin, 1998). This modeling approach provides the high precision and computational efficiency required to investigate connectivity across life history profiles and across the entire seascape.

Simulations were carried out by releasing a cloud of larvae into the model seascape at all individual reef sites and allowing larvae to be transported downstream with currents. Ocean current velocities and turbulent diffusion ($100 m^2 s^{-1}$) were the main factors that moved larvae through the seascape at each time-step, whereas larval competency and mortality determined when and what proportions of larvae settled in habitat cells at each time step. When habitat was encountered, the concentration of larvae settling within the habitat cell is recorded at that time-step. Simulation data were saved in the form of a 3-D dispersal matrix representing the cumulative quantity of larvae released from each source patch that have settled in each destination patch through time. From

these larval settlement matrices, two final matrices were produced: 1) The connectivity probability matrix quantifies the likelihood that a larva released from each habitat patch survives to settle on another patch (natal or downstream sites) in any year (diagonal of this matrix is the probability of local retention), and 2) the migration matrix representing the proportion of settlers at a reef patch that came from a particular larval source (the diagonal of this matrix is proportion of self-recruitment). See (Treml et al., 2012) for model details and sensitivity analysis. The migration matrix, M, was converted to 'oceanographic dispersal distance' using log (M^{-1}) to transform the values to be the same rank-order as geographic distance (high proportion of settlers then have a short 'dispersal distance') required for some network-based algorithms. Pathways through this dispersal distance matrix were used as a proxy for stepping-stone migration distance, and is referred to as such throughout the manuscript (Crandall et al., 2014). For each pair of islands, the dispersal simulations generated two dispersal distances, e.g., from Palau to Chuuk, as well as from Chuuk to Palau, capturing the asymmetries in dispersal strength and direction. I explored the correlation between genetic divergence and minimum, maximum and mean dispersal distance for all pairs of islands for three PLD values (45, 65, 90 days) for each species.

RESULTS

Heterozygote deficits

After controlling for false discovery rates, some loci exhibited significant heterozygote deficiencies within islands. For *A. digitifera,* 27/99 tests resulted in significant heterozygote deficiencies (F_{IS}) (Table 7) after Bonferroni correction ($p < 0.00051$) and locus WGS189 deviated at all but one island. Data for *A. hyacinthus*

showed significant heterozygote deficiencies for 19/84 tests and one locus (EST007) was monomorphic in two populations (Table 8, Bonferroni correction $p < 0.0006$). Since departures from HWE are not generally bases for locus removal (Selkoe & Toonen, 2006), all loci remained in downstream analyses. Locus GST189 showed significant heterozygote deficits across multiple islands, so preliminary analyses were run with and without this locus to confirm that the results would not change (data not shown). Results did not differ substantially and all eleven loci were retained in subsequent analyses.

Linkage Disequilibrium

For *A. hyacinthus,* 5/528 pairwise LD tests were significant after multiple test correction ($p<0.0001$). For *A. digitifera,* 36/495 pairwise tests showed significant linkage disequilibrium after Bonferroni correction ($p<0.0001$). However, all loci (with the exception of EST097) have previously been mapped onto linkage groups for the congener *A. millepora* (Wang, Zhang, Meyer, & Matz, 2009) and loci sharing common linkage groups never expressed significant LD for either species, suggesting that the observed LD is not due to physical linkage. All loci remained in downstream analyses.

Table 7. Summary statistics for SSR loci for *Acropora digitifera*
Genetic summary statistics of eleven microsatellite loci from nine islands for *A. digitifera*. Fis estimates showing significant heterozygote deficiencies are indicated with bold text highlighted in grey. Palau (Pal), Guam (Gua), Chuuk (Chu), Pohnpei (Poh), Kosrae (Kos), Kwajalein (Kwa), Majuro (Maj).

Island		FAM7	H112	N62	F32	N254	N16	F97	H189	F181	H92	N121
Pal	**N**	85	85	83	86	85	84	85	88	88	88	87
	Na	9	13	9	23	18	5	31	6	15	8	3
	Ho	0.271	0.882	0.386	0.907	0.706	0.179	0.741	0.625	0.784	0.727	0.195
	He	0.349	0.883	0.798	0.926	0.866	0.439	0.940	0.578	0.851	0.660	0.180
	Fis	0.022	0.026	**0.000**	0.155	**0.000**	0.145	**0.000**	**0.000**	0.0036	0.041	1.000
	sHa	0.817	2.303	1.750	2.794	2.311	0.826	3.069	1.093	2.157	1.307	0.379
Yap	**N**	139	138	139	140	140	139	137	139	140	139	137
	Na	6	11	8	21	16	6	32	5	16	7	4
	Ho	0.144	0.862	0.576	0.871	0.814	0.273	0.540	0.424	0.757	0.849	0.153
	He	0.258	0.846	0.707	0.920	0.881	0.416	0.919	0.413	0.875	0.744	0.175
	Fis	**0.000**	0.080	0.009	**0.000**	0.005	0.482	0.007	**0.000**	**0.000**	0.748	0.106
	sHa	0.554	2.054	1.480	2.700	2.314	0.811	2.970	0.812	2.280	1.534	0.379
Gua	**N**	92	94	94	94	94	95	92	90	95	95	95
	Na	5	9	7	16	12	5	23	5	13	6	3
	Ho	0.293	0.713	0.745	0.936	0.723	0.263	0.652	0.522	0.811	0.705	0.168
	He	0.273	0.751	0.736	0.883	0.794	0.350	0.922	0.520	0.803	0.714	0.155
	HWE	0.011	0.236	0.691	0.004	**0.000**	0.015	0.002	**0.000**	0.105	0.503	1.000
	sHa	0.537	1.682	1.554	2.360	1.890	0.712	2.797	0.887	1.847	1.463	0.309
Chu	**N**	42	44	45	44	44	45	43	44	45	45	44
	Na	5	10	7	17	13	6	23	3	13	6	4
	Ho	0.357	0.864	0.422	0.864	0.773	0.133	0.721	0.409	0.800	0.867	0.091
	He	0.385	0.790	0.687	0.903	0.871	0.310	0.920	0.346	0.843	0.768	0.150
	Fis	0.045	0.745	**0.000**	0.061	0.010	1.000	0.004	**0.000**	0.126	0.952	0.018
	sHa	0.819	1.822	1.391	2.525	2.213	0.668	2.837	0.647	2.070	1.568	0.354
Poh	**N**	97	96	97	97	95	97	97	97	97	97	97
	Na	3	9	6	20	15	4	31	5	13	5	3
	Ho	0.196	0.844	0.536	1.000	0.747	0.124	0.649	0.361	0.629	0.825	0.124
	He	0.213	0.823	0.703	0.922	0.890	0.322	0.938	0.358	0.805	0.747	0.126
	Fis	**0.000**	0.863	0.005	1.000	**0.000**	0.281	0.005	**0.000**	0.001	0.948	0.351
	sHa	0.419	1.902	1.360	2.680	2.364	0.610	3.078	0.703	1.852	1.482	0.264

Table 7 continued

Kos	**N**	86	86	85	84	86	87	83	87	87	87	86
	Na	5	8	9	17	13	5	20	3	25	5	3
	Ho	0.105	0.698	0.435	0.940	0.674	0.253	0.711	0.149	0.724	0.655	0.140
	He	0.144	0.769	0.718	0.895	0.822	0.248	0.899	0.160	0.834	0.684	0.212
	Fis	0.002	0.002	**0.000**	0.473	0.018	0.309	0.007	**0.000**	0.048	0.158	0.001
	sHa	0.364	1.626	1.558	2.467	2.001	0.539	2.599	0.344	2.284	1.279	0.435
Kwaj	**N**	116	115	113	112	116	116	112	115	115	115	115
	Na	4	8	5	18	12	5	24	3	19	6	3
	Ho	0.078	0.817	0.504	0.964	0.612	0.129	0.723	0.217	0.591	0.739	0.209
	He	0.084	0.791	0.703	0.899	0.811	0.191	0.926	0.208	0.830	0.699	0.221
	Fis	0.183	0.742	0.002	0.973	**0.000**	0.796	**0.000**	**0.000**	**0.000**	0.004	0.210
	sHa	0.223	1.681	1.323	2.485	1.952	0.436	2.808	0.380	2.134	1.327	0.450
Maj	**N**	91	92	91	89	92	93	93	92	90	92	92
	Na	2	8	5	16	10	4	22	2	18	7	3
	Ho	0.088	0.685	0.440	0.910	0.652	0.151	0.796	0.272	0.722	0.641	0.185
	He	0.084	0.738	0.578	0.891	0.776	0.214	0.922	0.294	0.847	0.659	0.169
	Fis	1.000	0.014	**0.000**	0.800	0.002	0.316	**0.000**	**0.000**	0.074	0.002	1.000
	sHa	0.180	1.495	1.075	2.405	1.792	0.440	2.742	0.470	2.210	1.293	0.342
Arno	**N**	83	83	84	84	85	83	85	83	85	84	85
	Na	8	5	16	11	4	23	3	20	4	3	4
	Ho	0.819	0.337	0.952	0.655	0.200	0.783	0.271	0.795	0.765	0.155	0.129
	He	0.759	0.587	0.893	0.804	0.299	0.919	0.302	0.873	0.710	0.145	0.145
	Fis	0.145	0.928	**0.000**	0.958	**0.000**	0.003	**0.000**	0.003	0.023	0.904	1.000
	sHa	0.347	1.576	1.133	2.413	1.918	0.612	2.738	0.519	2.365	1.289	0.314

Table 8. Summary statistics for SSR loci for *Acropora hyacinthus*

Genetic summary statistics of eleven microsatellite loci from seven islands for *A. hyacinthus*. Fis estimates showing significant heterozygote deficiencies are indicated with bold text highlighted in grey. Palau (Pal), Ngulu (Ngu), Chuuk (Chu), Pohnpei (Poh), Kosrae (Kos), Majuro (Maj), Phoenix (Pho).

Island		FAM7	H112	N62	F32	H153	N254	N16	F97	H189	F181	H92	N121
Pal	**N**	91	91	89	91	92	90	92	91	92	92	91	92
	Na	5	10	6	19	8	15	4	15	8	16	22	4
	Ho	0.110	0.593	0.416	0.912	0.576	0.644	0.598	0.813	0.739	0.761	0.582	0.500
	He	0.209	0.788	0.477	0.898	0.712	0.863	0.578	0.855	0.776	0.807	0.920	0.533
	Fis	**0.000**	0.002	0.048	0.761	0.009	**0.000**	0.233	0.049	0.065	0.200	**0.000**	0.154
	sHa	0.430	1.815	0.952	2.533	1.497	2.243	0.981	2.823	1.653	2.092	2.757	0.944
Ngu	**N**	45	43	43	43	43	44	45	42	44	45	44	45
	Na	4	7	8	15	9	12	3	10	6	12	18	4
	Ho	0.178	0.512	0.605	0.837	0.465	0.682	0.556	0.595	0.727	0.644	0.727	0.511
	He	0.240	0.763	0.689	0.910	0.786	0.877	0.509	0.724	0.763	0.770	0.890	0.541
	Fis	0.008	0.008	0.049	0.090	**0.000**	0.001	0.737	**0.000**	0.156	0.005	**0.000**	0.075
	sHa	0.525	1.616	1.489	2.535	1.756	2.202	0.822	2.083	1.543	1.896	2.500	0.940
Yap	**N**	130	130	123	128	126	128	134	131	133	132	132	133
	Na	5	8	7	17	11	16	4	15	7	14	20	5
	Ho	0.192	0.415	0.553	0.883	0.532	0.641	0.537	0.695	0.729	0.727	0.818	0.571
	He	0.261	0.799	0.543	0.916	0.819	0.864	0.573	0.734	0.773	0.779	0.898	0.553
	Fis	**0.000**	**0.000**	0.171	0.065	**0.000**	**0.000**	0.039	0.005	0.163	0.031	0.001	0.249
	sHa	0.510	1.762	1.125	2.595	1.911	2.234	0.965	2.338	1.609	1.912	2.524	0.941
Chu	**N**	137	135	134	135	134	134	137	136	137	137	137	137
	Na	2	9	11	18	10	14	6	14	7	14	25	4
	Ho	0.007	0.504	0.276	0.896	0.784	0.381	0.577	0.699	0.547	0.715	0.956	0.482
	He	0.007	0.773	0.255	0.899	0.835	0.753	0.563	0.755	0.642	0.686	0.938	0.486
	Fis	*	**0.000**	1.000	0.420	**0.000**	**0.000**	0.075	0.005	0.001	0.754	0.883	0.019
	sHa	0.024	1.711	0.697	2.468	1.916	1.677	0.970	2.469	1.316	1.558	2.915	0.789
Poh	**N**	132	133	134	133	134	133	133	131	132	132	132	132
	Na	2	8	5	16	12	12	5	14	8	16	22	4
	Ho	0.008	0.436	0.112	0.932	0.821	0.383	0.594	0.740	0.561	0.652	0.932	0.508
	He	0.008	0.796	0.108	0.885	0.816	0.676	0.580	0.839	0.601	0.690	0.930	0.488
	Fis	1.000	**0.000**	1.000	0.960	0.042	**0.000**	0.137	**0.000**	0.016	0.081	0.760	0.003
	sHa	0.049	1.772	0.286	2.391	1.908	1.566	0.988	2.754	1.253	1.673	2.805	0.850

Table 8 continued

Kos	**N**	90	89	88	90	89	90	90	90	90	89	90	90
	Na	2	8	8	20	15	13	4	15	8	10	20	3
	Ho	0.078	0.438	0.227	0.933	0.798	0.500	0.578	0.733	0.644	0.573	0.900	0.489
	He	0.134	0.774	0.209	0.919	0.850	0.758	0.514	0.773	0.769	0.549	0.902	0.504
	Fis	0.004	**0.000**	1.000	0.685	0.065	**0.000**	0.889	0.293	0.003	0.515	0.429	0.410
	sHa	0.259	1.715	0.506	2.665	2.215	1.666	0.789	2.362	1.687	1.280	2.587	0.722
Maj	**N**	16	16	16	15	16	16	16	15	16	16	16	16
	Na	1	9	5	13	6	9	2	12	6	10	15	3
	Ho	0.688	0.438	0.867	0.500	0.500	0.250	0.867	0.750	0.875	0.875	0.438	0.688
	He	0.824	0.445	0.907	0.598	0.830	0.468	0.818	0.715	0.879	0.912	0.600	0.824
	Fis	*	0.130	0.430	0.123	0.232	0.001	0.068	0.584	0.568	0.339	0.183	0.174
	sHa	0.000	1.940	0.876	2.453	1.235	1.941	0.662	2.044	1.455	2.186	2.550	0.981
Pho	**N**	17	17	14	17	17	17	17	17	17	17	17	17
	Na	2	8	4	13	8	8	4	8	4	13	12	4
	Ho	0.353	0.529	0.214	0.941	0.706	0.588	0.471	0.765	0.706	0.824	0.882	0.353
	He	0.291	0.841	0.314	0.901	0.758	0.739	0.559	0.780	0.645	0.900	0.894	0.559
	Fis	1.000	**0.000**	0.073	0.517	0.249	0.003	0.260	0.423	0.059	0.117	0.490	0.032
	sHa	0.466	1.936	0.658	2.421	1.683	1.623	0.990	1.746	1.171	2.423	2.356	1.041

Genetic Diversity Within Populations

Within-island diversity was measured in four ways: observed heterozygosity (H_o), allelic richness (N_a), Shannon diversity index (sHa), and the number of private alleles (Tables 7, 8). Diversity estimates for both species were negatively correlated with island distance from Palau, and on average, *A. hyacinthus* had higher diversity across its range than *A. digitifera* (Fig. 9B). Shannon genetic diversity estimates significantly decreased with island distance (km) from west to east for both *A. hyacinthus* (r^2_{adj} = 0.79, p=0.005) and *A. digitifera* (r^2_{adj} = 0.82, p=0.0005 (Fig. 9B), however the slope for *A. digitifera* was 1.47 times greater indicating that *A. digitifera* diversity dropped more quickly than *A. hyacinthus* and this difference was significant (p_{LRT} = 0.017). For *A. digitifera,* mean number of private alleles per island ranged from 0.09 to 1.27 and there was a significant decrease with distance from Palau (r^2_{adj} = 0.55, p=0.013). The mean number of private

alleles for *A. hyacinthus* ranged from 0.08 to 0.42 and there was no effect of distance on the private allele number for this species (Fig. 10).

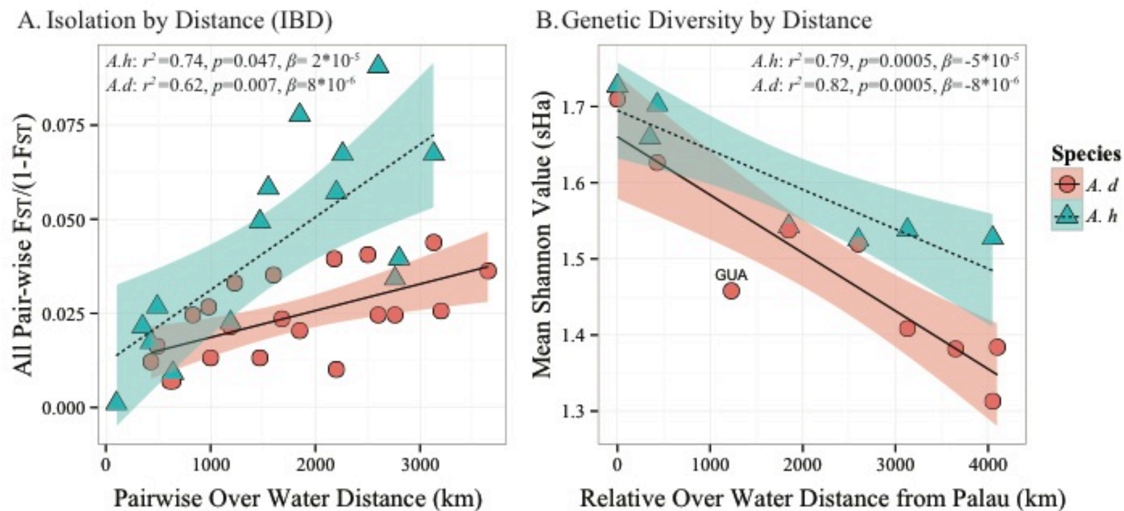


Figure 9: Isolation By Distance (IBD) and Genetic Diversity
IBD and genetic diversity observed for *A. digitifera* and *A. hyacinthus*. A. Pairwise genetic differentiation [(F_{ST} /(1-F_{ST})] of two species of *Acropora* coral between geographic distance between islands (Km). B. Mean Shannon diversity estimates (sHa) for each sampled island relative to over water distance from Palau (western-most island) to the Marshall Islands.

Population Differentiation

Global F_{ST} values were significant for both species (*A. digitifera*: 0.023; *A. hyacinthus*: 0.042), however differentiation was nearly doubled in *A. hyacinthus*. All *A. digitifera* pairwise F_{ST} values between islands were significant, but relatively low, ranging from 0.003 (Majuro-Arno) to 0.042 (Kosrae-Palau) (Table 9A). For *A. hyacinthus* one pairwise F_{ST} value was not significant (Yap-Ngulu) but the remainder were and ranged from 0.009 (Chuuk-Pohnpei) to 0.127 (Pohnpei-Phoenix) (Table 9B). Strong isolation by distance (IBD) patterns were observed for both species (*A. digitifera* Mantel's r^2: 0.616,

p=0.007; *A. hyacinthus* Mantel's r^2: 0.740, p=0.047), supporting the stepping stone model of isolation by distance. Notably, the IBD slope for *A. hyacinthus* was 2.73 times greater than for *A. digitifera* and this difference between species was significant (p_{LRT} = 0.021, Fig. 9A). PCoA results based on pairwise genetic distances explain 58% of the variation for *A. digitifera* and 79% for *A. hyacinthus* and data for both species nearly recapitulate island geographical configuration (Fig. 11). Based on these population differentiation results, the null model of panmixia can be rejected.

Table 9. F_{ST} values between all island pairs
Summary of pairwise F_{ST} values between all island pairs. Permutations were run 999 times. All significant comparisons are shaded in grey. Kwajalein (Kwaj).

	Palau	**Yap**	**Guam**	**Chuuk**	**Pohnpei**	**Kosrae**	**Kwaj**	**Majuro**	**Arno**
Palau	0.000	***	***	***	***	***	***	***	***
Yap	0.012	0.000	***	***	***	***	***	***	***
Guam	0.032	0.024	0.000	***	***	***	***	***	***
Chuuk	0.020	0.013	0.026	0.000	***	***	***	***	***
Pohnpei	0.024	0.010	0.034	0.007	0.000	***	***	***	***
Kosrae	0.042	0.024	0.038	0.021	0.016	0.000	***	***	***
Kwajalein	0.035	0.025	0.039	0.023	0.013	0.007	0.000	***	***
Majuro	0.038	0.025	0.034	0.026	0.024	0.015	0.014	0.000	***
Arno	0.036	0.019	0.038	0.027	0.019	0.011	0.012	0.003	0.000

B. *A. hyacinthus*

	Palau	**Ngulu**	**Yap**	**Chuuk**	**Pohnpei**	**Kosrae**	**Majuro**	**Phoenix**
Palau	0.000	***	***	***	***	***	***	***
Ngulu	0.021	0.000	**0.177**	***	***	***	***	***
Yap	0.017	**0.001**	0.000	***	***	***	***	***
Chuuk	0.072	0.055	0.047	0.000	***	***	***	***
Pohnpei	0.083	0.063	0.054	0.009	0.000	***	***	***
Kosrae	0.063	0.038	0.033	0.022	0.026	0.000	***	***
Majuro	0.051	0.036	0.030	0.062	0.066	0.054	0.000	***
Phoenix	0.072	0.085	0.075	0.115	0.127	0.104	0.086	0.000

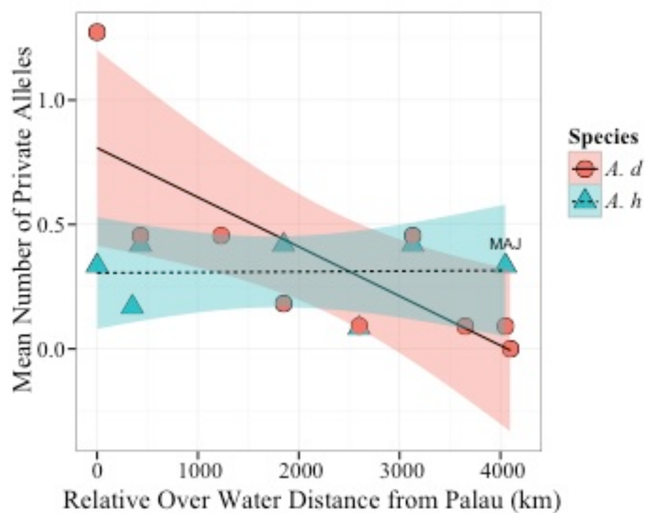


Figure 10: Private allele richness
Private allele richness for *Acropora hyacinthus* and *A. digitifera*. Mean number of private alleles for each sampled island relative to over water distance from Palau (most westerly island) to the Marshall Islands.

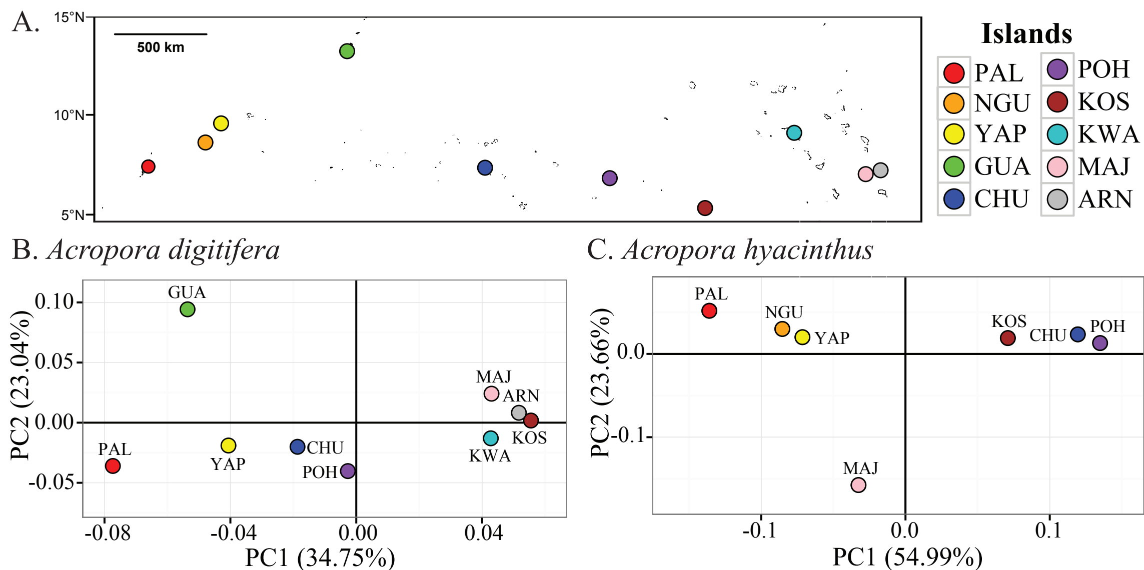


Figure 11: Principal Coordinate Analysis (PCoA)
PCoA of genetic relationships (Nei's genetic distances) among Micronesian populations of *Acropora digitifera* and *Acropora hyacinthus*. A. Actual geographical map of island configuration. B. PCoA for *A. digitifera* explaining ~58% of the variation. C. PCoA for *A. hyacinthus* explaining ~78% of the variation.

Bayesian Analysis of Genetic Structure

A. digitifera

STRUCTURE analysis corroborated patterns observed in F_{ST} values and suggested that significant genetic differentiation builds up across the region as populations increasingly diverge with distance (Fig. 12A). When all *A. digitifera* individuals ($n = 846$) were analyzed, an optimal solution of K=3 clusters was found using the calculation of ΔK. One cluster (yellow) assigned strongly to only individuals from Guam (Fig. 12A), and all other populations followed IBD patterns with the orange cluster being replaced by burgundy as collections moved from west to east. Further STRUCTURE analyses were performed on each of major cluster and data were split with west to Pohnpei (strongly orange assignments) and Kosrae to the east (strongly burgundy assignments) run independently. Within the western islands ($n = 370$ individuals), K=2 optimal subclusters were identified (Fig. 12A) and within the eastern islands ($n = 381$ individuals), K=4 was the optimal solution. All individual membership assignments for both east and west islands groups supported the presence of IBD expected under the stepping stone migration model.

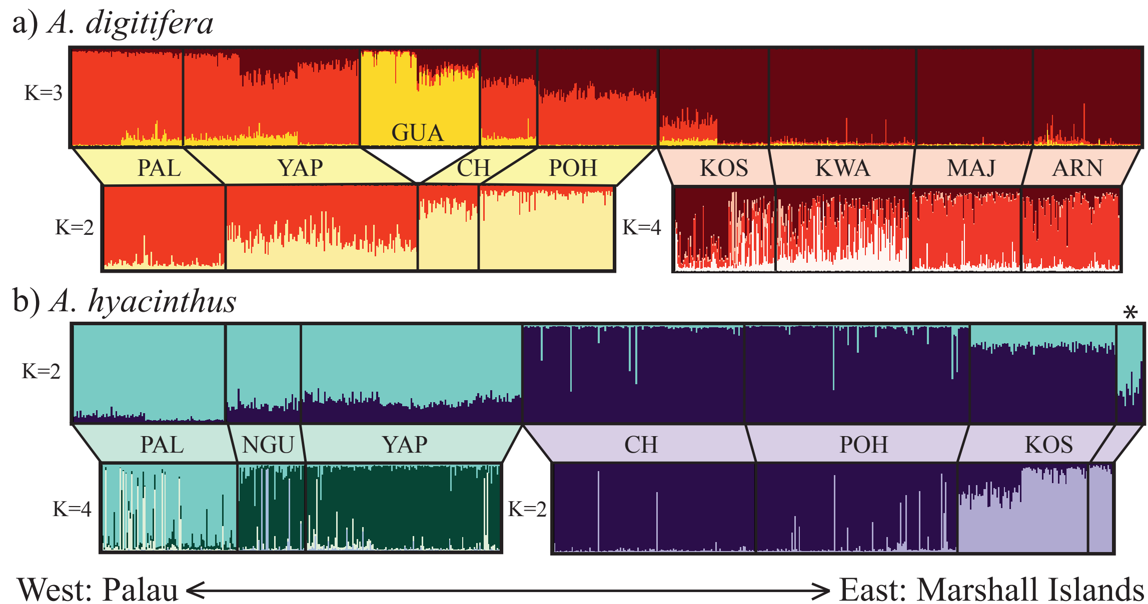


Figure 12: STRUCTURE analysis across greater Micronesia
STRUCTURE population assignment for two species of *Acropora* across greater Micronesia. A. The top panel shows the results for all *A. digitifera* individuals at an optimal population number (K) of 3. The bottom panels show population assignments when the dataset was split for Palau-Pohnpei (K=2) and Kosrae-Arno (K=4) and Guam was removed. B. The top panel shows the results for all *A. hyacinthus* individuals at an optimal population number (K) of 2. The bottom panels show population assignments when the dataset was split for Palau-Yap (K=4) and Chuuk to the Marshall Islands (K=2). The asterisk (*) designates the Marshall Island samples.

A. hyacinthus

When all *A. hyacinthus* ($n = 656$ individuals) data were analyzed, an optimal solution of K=2 clusters was found. Unlike *A. digitifera,* the visual signature of IBD was not as strong, although Mantel's test was highly significant. Instead, there was a genetic break between western Micronesia (Palau, Ngulu and Yap) and eastern Micronesia (Chuuk, Pohnpei and Kosrae) (Fig. 12B). Curiously, Majuro appeared more similar to western islands than to the rest of Micronesia. When further STRUCTURE analyses were performed on two subsets of the data 1) within western (Palau-Yap) and 2) within eastern (Chuuk-Majuro) Micronesia, western islands ($n = 275$ individuals) had K=4 optimal

subclusters and for the eastern islands ($n = 381$ individuals), K=2 was the optimal solution. Additional breaks were detected both in western and eastern Micronesia. There was a clear delineation between Palau and Ngulu-Yap, which is corroborated by the non-significant F_{ST} value between Ngulu and Yap (Table 9B). Results from the eastern islands demonstrate that Chuuk and Pohnpei are assigned to the same cluster ($F_{ST} = 0.009$), while Kosrae and the Marshalls exhibited more divergence ($F_{ST} = 0.054$). I hypothesized that the Marshall Islands might be receiving *A. hyacinthus* immigrants from subequatorial locations via the Southern Equatorial Current (SEC, Fig. 7a), which could also be the source of immigrants for western Micronesia. To test this hypothesis, samples from the Phoenix Islands were included in additional STRUCTURE analyses, which confirmed that *A. hyacinthus* from the Phoenix islands are indeed more closely related to both Marshall Islands and western Micronesia populations than to other central Micronesian islands (Fig 13).

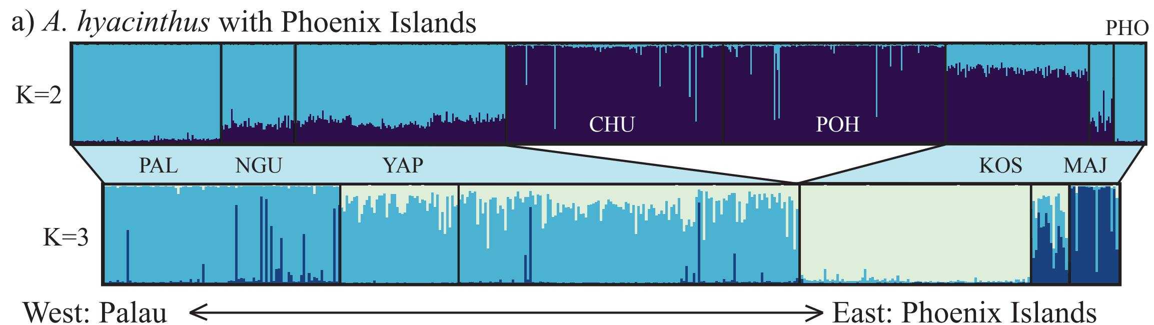


Figure 13: STRUCTURE analysis with Phoenix Islands
STRUCTURE population assignment for *Acropora hyacinthus* in Micronesia with the addition of the Phoenix Islands (a sub-equatorial Central Pacific location). The top panel shows the results for all individuals at an optimal population number (K) of 2. The bottom panel shows population assignments for all individuals when Chuuk and Pohnpei were removed and a K of 3.

Biophysical Modeling

On average, dispersal distances generated by the biophysical larval dispersal model were more strongly correlated with genetic divergence when compared to Euclidean distances and, when the best-fit matrix was chosen, 15-21% more variation was explained. The best-fit model for *A. hyacinthus* was 65 day PLD minimum dispersal distance (when comparing both possible directions of migration) between all pairs of sampled islands (Fig. 14B, Mantel's r^2: 0.89, p=0.014). For *A. digitifera*, 90 day PLD with the maximum of the two oceanographic dispersal distances between islands showed the strongest correlation (Fig. 14A, Mantel's r^2: 0.83, p<0.001). The biophysical model also did a superior job explaining variation in genetic diversity when best-fit models for each species were tested (Fig. 14C). The best-fit model for *A. digitifera* increased the correlation by 13% and explained nearly all variation in genetic diversity observed across islands (Mantel's r^2: 0.95, p<0.001). For *A. hyacinthus*, the model explained 7% more variation in genetic diversity (Fig. 14C, Mantel's r^2: 0.86, p=0.005). For all PLDs tested, the modeled migration in westerly and easterly directions were very similar (near 1:1 line), however the two islands for which the most asymmetric migration was predicted were Guam and Kosrae. For island pairs involving Guam (except Guam-Palau pair) westerly migration was preferred, whereas island pairs involving Kosrae (except Kosrae-Guam and Kosrae-Kwajalein) showed a strong preference for easterly migration (Fig. 14D).

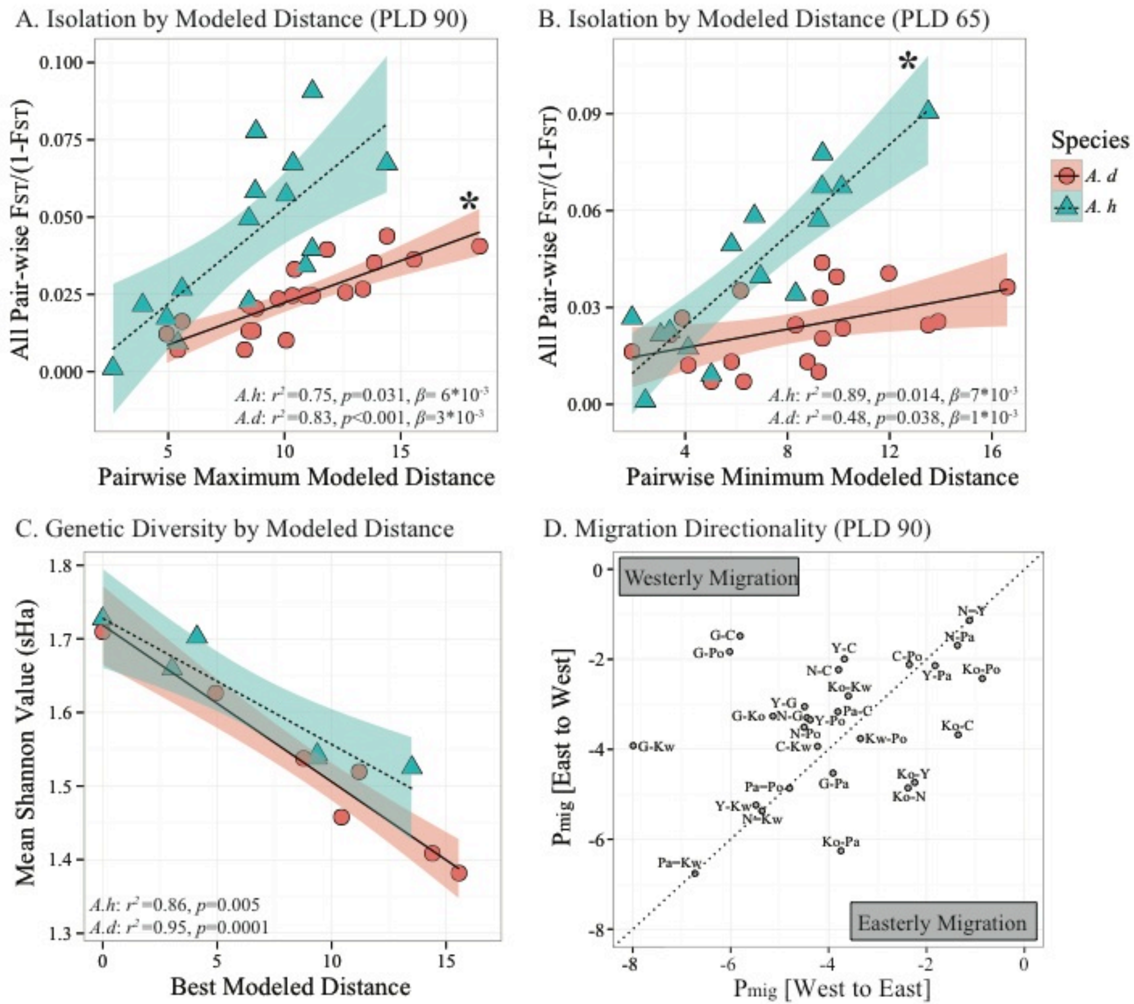


Figure 14: Biophysical dispersal modeling results

A. Pairwise genetic differentiation [(F_{ST} /(1-F_{ST})] of two species of *Acropora* coral between maximum pairwise modeled distance distances (90 day maximum PLD). This model was the best fit for *A. digitifera* explaining 83% of the variation. B. Pairwise genetic differentiation between minimum pairwise distances at a PLD of 65 days. This model was the best fit for *A hyacinthus* explaining 89% of the variation. C. Mean genetic diversity estimates (sHa) for each sampled island relative to the best-modeled distance for each species. D. Migration directionality as estimated from the model. Islands falling along the 1:1 line (dashed line) are projected to have equal migration between island pairs. Islands falling above the line exhibit easterly migration preference and those falling below exhibit preferences towards westerly migration.

DISCUSSION

Stepping stone migration of *Acropora*

Assuming equilibria between migration and other evolutionary forces, theory predicts that, unless individuals are able to disperse a distance equivalent to their range, differentiation in gene frequencies will be negatively correlated with population distances, yielding an isolation-by-distance (IBD) pattern (Slatkin, 1993; Wright, 1943). Across over 4000km of ocean, the two acroporid corals examined here show only 4-5% genetic divergence (F_{ST}), most likely suggesting that gene flow across this region is pervasive, however historical processes cannot be discounted since these populations may not be at equilibrium. Still, I show strong evidence that island stepping-stones are important gene flow facilitators for remote Pacific reefs: between-island distance alone explains between 62-74% of the estimated pairwise genetic divergences (F_{ST}) (Fig. 9A) and for both species genetic distances closely recapitulate island configuration in principle coordinate space (Fig. 11). These genetic patterns strongly associating with geography in broadcast-spawning corals is especially noteworthy given that previous genetic work on these highly dispersive broadcast-spawning corals rarely find compelling geographic trends (Ayre & Hughes, 2004; Baums, Johnson, Devlin-Durante, & Miller, 2010; Baums et al., 2005; Magalon, Adjeroud, & Veuille, 2005; Nakajima, Nishikawa, Isomura, Iguchi, & Sakai, 2009). But there are exceptions: *Porites lobata* in the Hawaiian islands (IBD=37%, (Polato, Concepcion, Toonen, & Baums, 2010)), a Caribbean sea fan *Gorgonia ventali* (IBD=17%, (Andras, Rypien, & Harvell, 2013)), and *Acropora millepora* along the Great Barrier Reef (IBD=54%, (van Oppen et al., 2011)) all exhibited significant population divergence as geographical distances increased (Isolation by distance (IBD)). However, this study has revealed the strongest evidence for IBD in broadcast spawning corals to date, perhaps due to sampling efforts across a large

geographical range and perhaps I gained increased resolution by using twelve microsatellite loci.

The IBD pattern indicative of stepping stone dispersal, consisting in gradual accumulation of genetic divergence with increasing distance, is evident in *A. digitifera* F_{ST} and STRUCTURE results (Fig. 9 and Fig. 12). However, its congener, *A. hyacinthus,* exhibits stronger population breaks (Fig. 12) not unlike the rabbitfish *Siganus sapidus*, in which the populations from eastern Micronesia are strongly divergent from western Micronesia (Priest et al., 2012). When dispersal distances derived from the biophysical model are considered instead of Euclidean distances, a more convincing pattern of IBD pattern emerges for *A. hyacinthus* (Fig. 14B), indicating that the genetic breaks detected in the STRUCTURE analysis could in fact be a result of sampling bias across a relatively smooth, although steep, IBD gradient.

Differences in population genetic structure among congeneric coral species

There are two distinct sexual reproductive modes in corals, 1) broadcast spawning, where corals synchronously release gametes into the water column for fertilization and development, and 2) brooding, where eggs are fertilized internally, larvae develop within the colony and the larvae are competent to settle within hours of release (Harrison, 1990). Previous research has demonstrated stark contrasts between brooding and broadcast spawning corals in pelagic larval duration (PLD), concomitant with more pronounced genetic structure in brooders (Ayre & Hughes, 2000; Hellberg, 1996; Underwood et al., 2009). Here I observe strong differences in genetic divergence between two congeneric coral species that share the same life history strategy (Fig. 9A) and are found next to each other in the same reef environment. *Both* species are

hermaphroditic, broadcast-spawning corals that participate in annual synchronous spawning events (Baird et al., 2009) and would be expected, at least under most management regimes, to disperse in similar ways. However, *A. hyacinthus* was more than two times more genetically structured than *A. digitifera* (P_{LRT} = 0.021), suggesting reduced dispersal potential in *A. hyacinthus*. Other studies of closely related species both on land and in the ocean have similarly observed considerable variation among species in both the magnitude of genetic variation and in the size and strength of IBD (Moyle, 2006; Zayed et al., 2005). While studies have explored variations in population genetics across phylogenetically related coral species (i.e (Ayre & Hughes, 2000; Severance & Karl, 2006)), no study has yet demonstrated significantly different IBD strengths in congeneric corals.

Genetic connectivity differences between these species could have several underlying causes. The most obvious cause could be differences in effective population sizes (N_e) between species. Since F_{ST} is influenced by $N_{e,}$ it would lead to differences in the absolute number of migrants (Wright, 1951). At the moment there is no reliable data on the relative abundances of the two species in Micronesia to evaluate this possibility. Increased structure in *A. hyacinthus* may also be due to the unintentional sampling of cryptic species since this species has specifically been shown to exhibit such cryptic diversity(Ladner & Palumbi, 2012). Alternatively, the differences could be attributed to the parameters of larval biology. Competency, defined as the settlement responsiveness of coral larvae through time, has been shown to vary even between closely related *Acropora* species (Ayre & Hughes, 2000; Connolly & Baird, 2010) and therefore might explain at least some of the variation I observe. *Acropora* larvae exhibit similar pre-competency periods of 4–6 days (Harrison, 1990), so pre-competency is unlikely to be the cause of between-species differences in dispersal. PLDs for these study species have

been previously estimated in the laboratory at >45 days for *A. digitifera* (Graham et al., 2008; Nishikawa & Sakai, 2005) and >91 days for *A. hyacinthus* (Graham et al., 2008). This study provided an opportunity to verify these estimates by investigating which PLD is more compatible with observed genetic divergences in a natural reef system, given the biophysical model of larval exchange and assuming equal effective population sizes for the two species. This analysis suggests that *A. digitifera* should have a longer PLD than *A. hyacinthus*: 65-day PLD best predicts genetic divergence for *A. hyacinthus* while the 90-day PLD best predicts divergence in *A. digitifera* (Fig. 14A, B). The disagreement with previously published results is hardly surprising because lab-based conditions for estimating PLD are unlikely to mirror all important aspects of life for planktonic coral larvae in the ocean.

Even closely-related species can have dramatically different range sizes (J. H. Brown, 1984; J. H. Brown, Stevens, & Kaufman, 1996), and *A. digitifera* exhibits a broader geographic range than *A. hyacinthus* (Veron, 2000). Specifically within this study, Guam was located outside the range of *A. hyacinthus*, but *A. digitifera* was prolific there. This larger geographical range aligns well with the higher dispersal capability we have inferred for *A. digitifera*. These results suggest that congeneric species may have very different migration patterns, generating challenging consequences for management.

Seascape resistance and connectivity for Micronesian corals

In the marine environment, ocean currents are important dispersal agents, but determining the degree and directionality of migration in these environments remains a fundamental problem (Botsford et al., 2009; Palumbi, 1997; Warner & Cowen, 2002). Seascape genetic models have previously been employed on local and regional scales to

elucidate spatial patterns of genetic differentiation. These models use empirical estimates of oceanographic features to predict spatial patterns of genetic differentiation. Here, I compare empirical population genetic divergence estimates observed for *A. hyacinthus* and *A. digitifera* to the biophysical model of larval dispersal to determine if gene flow is better predicted by the biophysical model when compared to Euclidean distances alone. Genetic differentiations of both species of *Acropora* were considerably more correlated with modeled migration stepping-stone distances than by Euclidean distances (compare Fig. 9 and Fig. 14 A, B), demonstrating that ocean currents play an important role in structuring coral populations. This result has been observed previously for other marine systems ranging from corals (Foster et al., 2012; Galindo, Olson, & Palumbi, 2006) to mussels (Gilg & Hilbish, 2003). The substantial improvement in correlation for *A. digitifera* was likely due to the presence of *A. digitifera* at Guam, which is located within the North Equatorial Current (NEC Fig. 7A) and exhibits strong bias towards westerly migration (Treml et al., 2008) that can not be accounted for by Euclidean distance alone (Fig. 14D). Previous work on reef fish has also demonstrated strong subdivision between Guam and other Pacific islands (Priest et al., 2012).

Overall, results from the biophysical model confirm that Micronesia can serve as a dispersal corridor between the Coral Triangle and the central Pacific. However, I find evidence that dispersal in Micronesia is more complex than the unidirectional easterly flow predicted earlier (Wood et al., 2014) and involves bi-directional exchange between most islands. However, at the island closest to the equator (Kosrae) predominantly eastward migration is predicted, while at the northernmost Guam a bias towards westerly migration is predicted (Fig. 14). This complexity is likely due to the latitudinal fluctuations of the North Equatorial Countercurrent (NECC), variability of its strength

during El Nino and La Nina years, and proximity of the westerly South Equatorial Current (Fig. 7A (Bonjean & Lagerloef, 2002)).

Factors influencing genetic diversity of *Acropora spp.* across Micronesia

Allelic diversities (sHa) for both coral species and the mean number of private alleles for *A. digitifera* were significantly correlated with island distance from Palau, with islands closer to the Coral Triangle exhibiting higher genetic diversities (Fig. 9B, 14C). These observations corroborate evidence of species diversity declines with longitudinal distance from the Coral Triangle (Veron, 2000; Veron et al., 2009). This decrease in diversity could be a consequence of biased easterly dispersal out of the Coral Triangle (Connolly, Bellwood, & Hughes, 2003; Jokiel & Martinelli, 1992; Treml et al., 2008; Wood et al., 2014). However, lower diversities may also reflect diminishing effective population sizes since the combination of isolation and genetic drift associated with low effective population sizes is expected to result in reduced genetic diversity, especially at the edges of species ranges (Hoffmann & Blows, 1994).

Lower diversities may also be the result of reef age, with the expectation that more diversity would develop in older systems. However, island age is unlikely to explain the observed genetic diversity gradient since Micronesian reefs are expected to be of similar age corresponding to the time since last glaciation. Experimentally estimated reef ages for Palau (~8k years) and the Marshall Islands (~6-8k years) are remarkably similar between islands (Montaggioni, 2005), contrary to genetic diversity estimates. In agreement with a source/sink dynamic driven by the prevailing North Equatorial Countercurrent (NECC), *A. digitifera* from the Marshall Islands, the farthest sampled island from the Coral Triangle, had among the lowest values of genetic diversity (Table

7; Fig. 9B) and this pattern held for private allelic richness (Fig. 10). In contrast, the less dispersive species, *A. hyacinthus*, had enriched private alleles in the eastern-most Micronesian islands (Kosrae and the Marshall Islands) compared to central Micronesia (Chuuk and Pohnpei) (Table 8; Fig. 10). This seemingly paradoxical observation is explained by results from the STRUCTURE analysis involving *A. hyacinthus* from the Phoenix Islands (Fig. 13), which indicates that genetic diversity of eastern populations of *A. hyacinthus* is influenced more by the genetic exchange from south of the equator than by the diminishing trickle of Coral Triangle genotypes through the Micronesian islands. A connection between the Marshall Islands and the Phoenix Islands through the Gilbert Islands and Tuvalu has been previously suggested (Treml et al., 2008) for species exhibiting pelagic larval durations (PLDs) exceeding 30 days, which is likely the case for *A. hyacinthus* (Fig. 14A, 14B). Notably, the shared genetic influence of the sub-equatorial gene pool, possibly via the South Equatorial Current (SEC), could also explain why the eastern-most *A. hyacinthus* populations are more genetically similar to the western Micronesian islands than to central Micronesian islands (Fig. 11C; 12B). The young age of these Micronesian coral populations (~6-8k years) prompts a cautionary note, however: assuming the mean age of reproductive maturity for *Acropora* species is 3–8 years (Wallace, 1999), only about 1,000 generations have passed since the populations became established. This time is most likely considerably less than the time needed for the population to reach genetic equilibrium yet and therefore we caution that the genetic diversity patterns discussed here might to some degree reflect historical bottlenecks and migration patterns at the time of initial island colonization. A comprehensive sampling scheme involving more coral species and broader geographic range would be required to rigorously investigate this possibility.

CONCLUSIONS

Populations of two congeneric species of acroporid corals maintain genetic connectivity over thousands of kilometers by using small Micronesian islands as stepping-stones. In addition to distance, current speed and direction clearly affect genetic connectivity. The diversity patterns differ significantly between the two species investigated here despite their close phylogenetic relatedness, ecological similarity, and the use of the same reproductive strategy, which may reflect differences in effective population sizes and/or larval biology. Generally, the results from this study corroborate previous simulation models suggesting that Micronesia serves as a migration corridor from the Coral Triangle to the more remote islands of the central Pacific. However, this hypothesis is well supported only for the more dispersive of the two coral species examined here, *A. digitifera*. Future work should aim to understand the biological factors that differentiate potential connectivity from realized connectivity, as this study suggests.

Chapter 3: Host and environmental drivers of *Symbiodinium* diversity in reef-building corals: Evidence for host specialization and ecological partitioning

ABSTRACT

Reef-building corals, like many other marine invertebrates, rely on an obligate symbiosis with dinoflagellate algae in the genus *Symbiodinium*. Many coral species exhibit a horizontal transmission strategy where the coral disperses great distances as symbiont-free larvae and then acquires its symbionts upon recruitment. This association with a locally-adapted symbiont could help maximize coral-host fitness across diverse environments, but only if the symbionts adapt locally. This 'global host - local symbiont' hypothesis assumed that the coral host should maintain high promiscuity to successfully establish symbiosis throughout its range, while symbionts must evolve host-specificity to outcompete other sympatric strains. Here, I investigated the interplay between these factors by contrasting genetic structures of host and symbiont across different spatial scales in two species of *Acropora* corals in Micronesia. As expected, population genetic structure of the symbiont is much more pronounced and is an order of magnitude greater than host population genetic structure. However, symbiont genetic structure is also greater in the host species that has greater divergence. In at least one location (Palau) both host species associate with a highly divergent local *Symbiodinium* genotype, which confirms that the host is capable of high promiscuity. Throughout the region, *Symbiodinium* exhibits strong host-specificity accounting for 50-66% of total within-island genetic variation, but also shows divergence between reef locations at nearly every island, accounting for 5-14% of variation. Overall, these patterns support the view that association with locally adapted *Symbiodinium* could provide a mechanism to improve fitness in the coral holobiont, indicating that the variation in coral-*Symbiodinium*

associations across the seascape is driven by the continuous evolution of host-specific *Symbiodinium* strains on a local scale.

INTRODUCTION

Symbioses are ubiquitous across all environments, including marine systems. Interactions between symbiotic partners have been implicated in the evolutionary diversification of many eukaryotes (Brucker & Bordenstein, 2012; Moran, 2006; Thornhill, Lewis, Wham, & LaJeunesse, 2014). Symbionts can enhance the host's ability to acquire nutrients from its environment or provide organic compounds necessary for synthesis or catalysis pathways (Yellowlees, Rees, & Leggat, 2008). Arguably the most well known marine symbiosis is the association between cnidarian hosts and their photosynthetic dinoflagellate algae in the genus *Symbiodinium* (M.P Lesser, Stat, & Gates, 2013). This symbiosis is obligatory for the host and enhances cnidarian calcification and supports metabolism by supplying photosynthetic products to the host while providing inorganic nutrients and residence for symbionts (Muscatine, 1990; Muscatine & Cernichiari, 1969; Trench & Blank, 1987). During stressful conditions, a functional loss of symbiosis occurs in a physiological process termed coral bleaching (Glynn, 1993; Hoegh-Guldberg, 1999; Hoegh-Guldberg & Smith, 1989). Bleaching episodes fueled by ecological pressures from climate change can incur considerable coral mortality and have become more frequent in recent decades (Harvell et al., 1999; Hoegh-Guldberg et al., 2007; M.P. Lesser, 2007), highlighting the need for a more comprehensive understanding of coral-*Symbiodinium* associations in the face of environmental change.

The coral symbiosis, like many other ecologically important symbioses, is endosymbiotic (occur within cells) and can establish by two fundamentally different modes of transmission: vertical (symbiont inheritance from mother) and horizontal (symbiont from environmental, free-living sources) (Harrison, 1990). Vertically-transmitting corals guarantee the maintenance of symbiosis in their offspring; however, if

larvae encounter novel environments, their symbiont composition may be suboptimal, resulting in reduced fitness (Byler, Carmi-Veal, Fine, & Goulet, 2013; Douglas, 1998). During horizontal transmission, aposymbiotic (without symbionts) larvae can have flexibility in symbiont acquisition, so upon arrival to new environments they can uptake novel symbionts not present in parental populations (Abrego, MJ, & Willis, 2009; Abrego, van Oppen, & Willis, 2009; Gómez-Cabrera, Ortiz, Loh, Ward, & Hoegh-Guldberg, 2008; Little, van Oppen, & Willis, 2004), perhaps providing some fitness advantage by enabling acclimatization on an individual level (Byler et al., 2013; Howells et al., 2012; Rowan & Knowlton, 1995).

The majority of host transmission in cnidarian/algal symbioses (~85%) is horizontal (Fadlallah, 1983; Harrison, 1990) and patterns for both host-specificity and environmental partitioning have been observed in symbionts of horizontally transmitting corals. Host-specificity has been suggested as the primary ecological driver in variations in *Symbiodinium* populations (Thornhill et al., 2014); indeed, convincing evidence exists for strong host associations (Ackerly, Schwilk, & Webb, 2006; Coffroth, Santos, & Goulet, 2001; Finney et al., 2010; Lajeunesse, 2005; LaJeunesse et al., 2010; Rodriguez-Lanetty, Krupp, & Weis, 2004; Thornhill, Xiang, Fitt, & Santos, 2009; Weis, Reynolds, deBoer, & Krupp, 2001). However, considerable evidence also suggests environmental partitioning of *Symbiodinium* variation in response to abiotic factors (Baker, 2003; Howells et al., 2012; Iglesias-Prieto, Beltran, LaJeunesse, Reyes-Bonilla, & Thome, 2004; Rowan & Knowlton, 1995; Thornhill et al., 2014; Ulstrup & Van Oppen, 2003). In fact, Howells et al. (Howells et al., 2012) demonstrated that, within a horizontally-transmitting host species, functional variation exists in *Symbiodinium* thermal tolerance among populations, consistent with local adaptation.

In an evolutionary context, horizontal transmission strategies should lead to more parasitic characteristics of the symbiont (Sachs & Wilcox, 2006). If hosts are highly dispersive, selection should promote promiscuity in symbiont uptake, as this indiscriminate behavior would be beneficial from the perspective of host adaptation. Conversely, selection on symbionts should require host-specialization in order to outcompete other sympatric symbiont strains and secure host acquisition, resulting in symbiont diversification. Indeed, *Symbiodinium* genetic diversity is remarkably great (Coffroth & Santos, 2005; LaJeunesse & Thornhill, 2011). However, this diversity of symbionts is rarely investigated within the context of host genetics, and estimates of symbiont diversity partitioning across host species and environments remain scarce (but see (LaJeunesse et al., 2010; Thornhill et al., 2014; Thornhill et al., 2009; Wirshing, Feldheim, & Baker, 2013). This information is particularly lacking for the Indo-Pacific region, which comprises the overwhelming majority of coral biodiversity. In the Indo-Pacific, clade C *Symbiodinium* are particularly diverse and associate with a wide variety of host species. Recent advancements in high-resolution population genetic loci for this clade (Bay, Howells, & van Oppen, 2009; Howells, van Oppen, & Bay, 2009; Wham, Carmichael, & LaJeunesse, 2014) offer the chance to gain detailed insights into how genetic variation in clade C *Symbiodinium* is partitioned among host species, reefs, and reef environments. In Chapter 2 I identified dispersal patterns of two coral host species (*Acropora digitifera* and *A. hyacinthus*) over the entire range of greater Micronesia (>4000km) and I found that migration capabilities of both host species were extensive, although not equal. Here I present a study elucidating the partitioning of *Symbiodinium* genotypic variation relative to host genotypic variation across different spatial scales.

METHODS

Sampling Locations and Methodology

From 2009 to 2011, coral holobiont samples were collected from thirteen reef sites on seven islands throughout the Micronesian Pacific (Table 10, Fig. 15). Study samples were a subset of the host genetic dataset previously described in Chapter 2. Twenty-five individuals of each coral host species (*Acropora hyacinthus* and *Acropora digitifera*) were analyzed per reef site and two reef sites per island, with the exception of Ngulu (the only species collected was *A. hyacinthus*) and Guam (no *A. hyacinthus* were found and only *A. digitifera* was collected).

Table 10. *Symbiodinium* reef site collections
GPS coordinates, main island group, and number of *A. digitifera* and *A. hyacinthus* hosts genotyped. Site letter corresponds to island insets in Figure 15.

Site	Island	GPS	*A. digitifera*	*A. hyacinthus*
a. West Channel Reef	Palau	7°31’55.7 N, 134°29'42.8 E	24	25
b. Lighthouse Reef	Palau	7°16'62.4 N, 134°27'61.9 E	23	24
c. Ngulu	Ngulu Atoll	8°18’12.0 N, 137°29’18.7 E	0[1]	39
d. South Tip Reef	Yap	9°26’05.4 N, 138°02’10.4 E	25	25
e. Goofnuw Channel	Yap	9°34’26.4 N, 138°12’19.2 E	24	25
f. Pago Bay	Guam	13°25’66.6 N, 144°47’94.3 E	26	0*
g. Tanguisson	Guam	13°32’61.1 N, 144°48’52.6 E	21	0*
h. West Polle	Chuuk	7°19’69.7 N, 151°33’21.1E	15	24
i. South East Pass	Chuuk	7°14’60.3 N, 152°01’29.1 E	21	22
j. Ant Atoll (East)	Pohnpei	6°47’42.3 N, 158°01’20.7 E	24	22
k. Roj	Pohnpei	6°46’37.7 N, 158°12’24.1 E	24	23
l. Coral Garden	Kosrae	5°18’47.2 N, 162°53’01.8 E	25	24
m. Hiroshi Point	Kosrae	5°15’88.0 N, 162°59’01.8 E	25	25
TOTAL			**277**	**278**

* indicates that no individuals of this species were found
[1] indicates that individuals were not collected from this site but are likely present

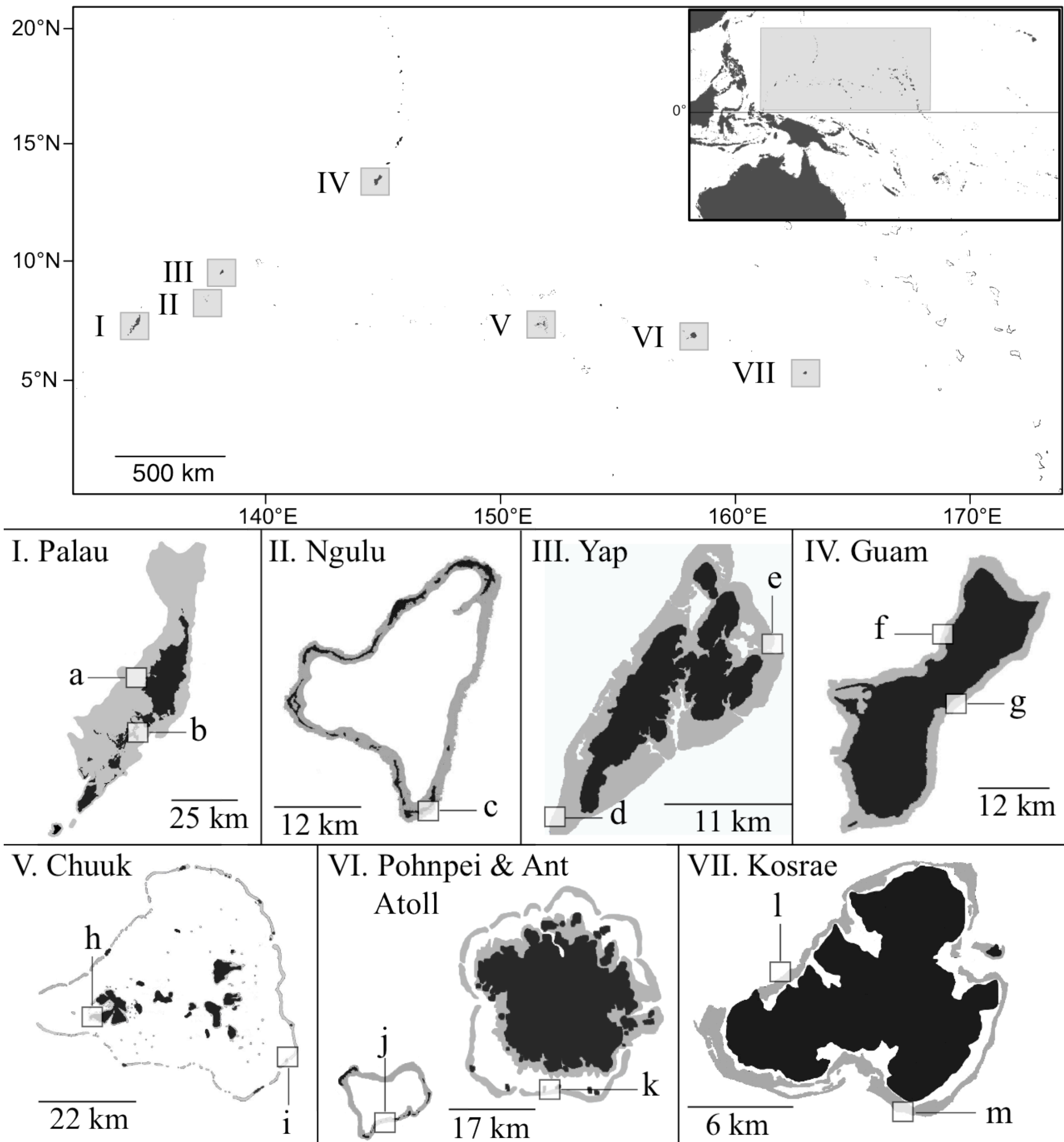


Figure 15: Geographic locations where coral hosts were collected
Top: Map of the Micronesian Pacific with an inset of the Pacific Ocean for reference. Islands where samples were collected and analyzed for *Symbiodnium* genetics are designated with grey boxes. Detailed information on each sampling site is located in Table 10.

Laboratory Procedures

Holobiont DNA was isolated following (Davies, Rahman, et al., 2013). Microsatellite primers for this study consisted of six previously published clade C loci (Bay, Howells, et al., 2009; Howells et al., 2009; Wham et al., 2014) and two novel loci that were mined using Msatcommander (Faircloth, 2008) from nucleotide EST data for *Symbiodinium* sp. clade C3 in GenBank (Leggat, Hoegh-Guldberg, Dove, & Yellowlees, 2007) (Table 11). Loci were multiplexed according to annealing temperatures and fragment sizes. Each 20 μl polymerase chain reaction (PCR) mixture contained 10 ng of DNA template, 0.1 μM of each forward primer, 0.1 μM of each reverse primer, 0.2 mM dNTP, 1 μl 10X *ExTaq* buffer, 0.025 U *ExTaq* Polymerase (Takara Biotechnology) and 0.0125 U *Pfu* Polymerase (Agilent Technologies). Amplifications were performed using a DNA Engine Tetrad2 Thermal Cycler (Bio-Rad, Hercules CA). Cycling began at 94°C for 5 min, followed by 35 cycles of 94°C for 40 s, annealing temperature for 120 s, and 72°C for 60 s and a 10 minute extension period at 72°C. Molecular weights were analyzed using the ABI 3130XL capillary sequencer with an in-house ROX-labeled size standard. Data were binned as previously described in Chapter 2 and individuals failing to amplify at ≥3 loci were excluded from analyses.

Table 11: Summary of clade C *Symbiodinium* microsatellite loci
Eight polymorphic *Symbiodinium* microsatellite loci used to assess genetic variation *Symbiodinium* clade C hosted by *A. hyacinthus* and *A. digitifera* and their corresponding multiplexing groups.

Multiplex Group	Locus (Repeat)	Primer Sequence 5'-3'	Repeat	Annealing Temperature	Source
A	*SgrSpl_30*	F: FAM-ccgaactacctttggtcaac R: aaaagacaaggacatctcgg	TA	53	Wham et al., 2013
B	*SgrSpl_78*	F: FAM-tgaaattcggtgttcattgt R:ctcagatgtttccgacgagt	TA	54	Wham et al., 2013
	Sgr_21	F: HEX-tgctgagtggcgtgtatatc R: tgatggtacttgatggtg	TCA	54	Wham et al., 2013
	Spl_33	F: HEX-acttgcaaagtccaagatcg R: gaacggtgaaaggaaaatga	CAT	54	Wham et al., 2013
C	*C124*	F: Fadp-agcctttgtttgtggaggaac R: agcaacgacaggcacaatac	AAC	60	This Study
	C784	F: Hadp-ctccttaggactggactcgc R: agaagtcaaatcgtcaccatcg	ATC	60	This Study
D	*C105*	F: FAM-tttcgttgttggacattgttatg R: ggactgaaaggtgcttgagg	complex	55	Bay *et al.*, 2009
	C304	F: FAM- aaacaaatgaggtggatggga R: ttaatgtgacggtgattgtggtg	complex	55	Howells et al., 2009

Fadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter tag sequence: FAM: agcagcgaactcagtacaaca
Hadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter tag sequence: HEX: tcgtcgcttgagtcatcgtta

Data Analysis

Symbiodinium clade C microsatellite data were treated as haploid based on available information on the ploidy of these organisms (Howells et al., 2012; Santos & Coffroth, 2003). Since each sample potentially contained a population of genetically distinct individuals, multilocus genotypes could not be constructed and instead, data were coded as binary with presence or absence of alleles within each host sample. Partitioning of *Symbiodinium* genetic variation among species, islands, and sites was investigated using analyses of molecular variance for binary haploid data (Phi_{PT}) (AMOVA, 9999 permutations in GENALEX v6.5, (Peakall & Smouse, 2006)) (Tables 12-14). Pairwise differentiations and pairwise island distances were compared to test for isolation by distance. Symbiont differentiations between coral hosts and between hosts were also

compared using Wilcoxon signed ranks tests for all pairwise island divergences. Allelic diversity and number of private alleles were also calculated in GENALEX v6.5 (Table 15). As a first pass, non-metric multidimensional scaling (NMDS) analysis was computed based on Bray-Curtis similarities of binary genetic information using the *vegan* package in R (Oksanen et al., 2013). To determine the optimal number of dimensions (k), stress values in response to different dimensions (k=1-13) were calculated (Fig. 16). Ten dimensions were found to be optimal. To visualize how these data clustered between islands and between coral host species, data were coded by island or by species and plotted in two-dimensional ordination space.

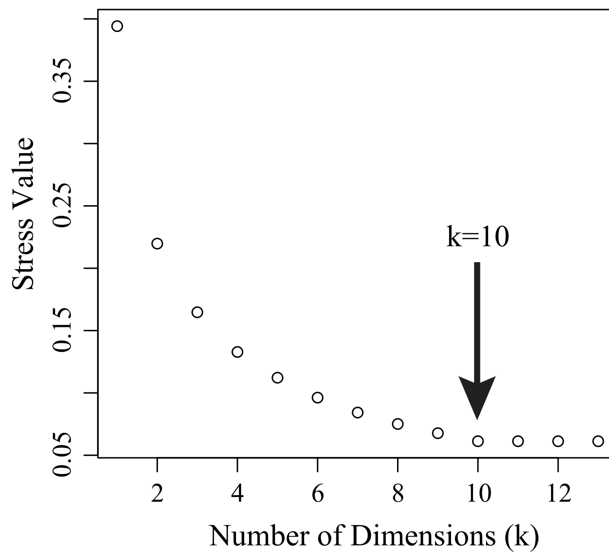


Figure 16: Optimal number of dimensions for NMDS analysis
Optimal values indicated by the plateauing stress value. k=10 was chosen here.

Next, I applied a Bayesian approach implemented in STRUCTURE v2.3.3 (Pritchard et al., 2000). STRUCTURE uses a Monte Carlo Markov chain (MCMC) clustering algorithm to assign individuals with similar genotypes to populations. Mean and variance

of log likelihood values of the number of populations K (1-10) were inferred by STRUCTURE with 10^6 iterations (burn in = 300,000 iterations) in ten replicate runs for each K. An admixture model was implemented with collection site as a location prior. Following the recommendations of (Evanno et al., 2005), the ad hoc statistic ΔK was calculated based on the rate of change of the log-likelihood between consecutive K values, which is implemented in the program STRUCTURE Harvester (Earl & Vonholdt, 2012). CLUMPP (Jakobsson & Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004) were used to produce graphics. Then data were split by host species to visualize island differences within hosts. Instead of using the ΔK statistic, I presented the K that maximized the mean of the estimated probability of data while minimizing the standard deviation. These K's were K=6 for *A. digitifera* and K=8 for *A. hyacinthus* (Fig. 17).

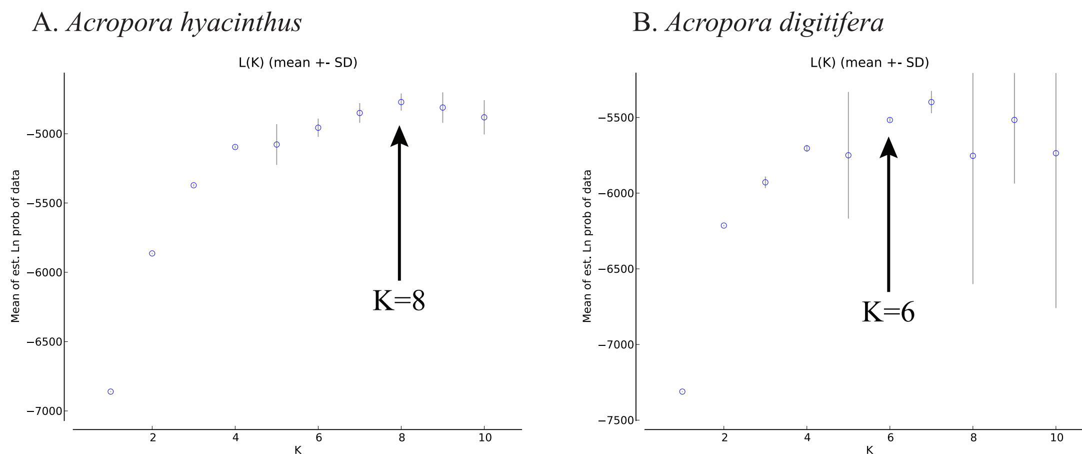


Figure 17: Number of populations (K) for *Symbiodinium* STRUCTURE analyses
K for each host species was chosen based on the maximized mean of the estimated probability of data while minimizing the standard deviation. These K's were K=6 for *A. digitifera* and K=8 for *A. hyacinthus*.

To resolve differences within each species and within each island, assignment of samples to genetic clusters using discriminant analysis of principal components (DAPC) was performed in R (R Development Core Team, 2013) using the ADEGENET package (Jombart, 2008; Jombart, Devillard, & Balloux, 2010). Here, *Symbiodinium* binary data were converted into 50 principle components for host species analyses (discriminations of islands within species) and 25 principle components for island analyses (discriminations of sites and species within islands) and then a-scores were used to determine trade-offs between power of discrimination and over-fitting the models. Relationships were examined by DAPC, which optimizes variation among clusters by minimizing variation within clusters, while retaining the optimal number of principle components and maximum number of discriminant functions. All information on DAPC model parameters and results are contained in Table 16. Cluster assignment patterns were compared within each species among islands and within each island among species and sites.

RESULTS

Using eight microsatellite loci, a total of 149 alleles were discovered from *Symbiodinium* clade C populations from thirteen sites on seven islands across two coral host species in Micronesia (Fig. 15; Table 10).

All host data

Genetic variation predominantly occurred between islands (18%, Table 12A, Fig. 18), with an additional 2% explained by sites within islands (20%). Host species constituted the second axis of variation explaining 16% of the variation (Table 12A, Fig.

18A). NMDS analysis also partitioned islands on the first axis and host species on the second axis, with a low stress value of 0.0612 with k=10 dimensions (Fig. 16), indicating a good model fit (Fig. 18A, B). However, island differences along NMDS axis 1 were predominantly differentiating Palau and Ngulu apart from all other islands, while NMDS axis 2 cleanly separated host species (Fig. 18B). STRUCTURE analysis corroborated these results with an optimal ΔK value of 3. The most highly differentiated genotype clusters (Fig. 18A and C) were the Palau, Ngulu, and east Micronesia clusters. The Palau cluster comprised both host species from Palau, the majority of *A. digitifera* from Goofnuw Channel reef in Yap, and a few *A. digitifera* individuals from Chuuk and Kosrae. The Ngulu cluster appears to be intermediate between Palau and eastern Micronesia clusters and only comprises *A. hyacinthus* from Ngulu (no *A. digitifera* sampled in Ngulu). The east Micronesia cluster included samples from Yap, Guam, Chuuk, Pohnpei and Kosrae and this cluster was strongly differentiated with respect to host species (Fig. 18B and C); it should be noted that the same host-related differentiation is also detected for the Palau cluster using NMDS (Fig. 18A and B) and DAPC analysis (see below), although it is not revealed in the STRUCTURE plot due to high divergence of Palau from the rest of the islands.

Table 12. Clade C *Symbiodinium* genetic divergence
Analysis of molecular variance (AMOVA) of binary microsatellite allele data for A. Symbiont genetic variation partitioned by host species, islands, and sites within *Acropora digitifera* and *A. hyacinthus* hosts in Micronesia. B. *Symbiondinium* clade C data for *A. digitifera* hosts partitioned by island and site. C. *Symbiondinium* clade C data for *A. hyacinthus* hosts partitioned by island and site. All divergence p-values in bold were significant.

Source of Variation	**df**	**SS**	**MS**	**Est Var**	**% Var**	***Phi*$_{PT}$**	**P-value**
A. All Data							
Phi$_{PT:}$ Host Species	1	572.192	572.192	2.024	16	0.161	**0.001**
Phi$_{PT:}$ Island	6	1076.722	179.454	2.173	18	0.182	**0.001**
Phi$_{PT:}$ Site	12	1331.889	110.991	2.389	20	0.203	**0.001**
B. *A. digitifera*							
PhiPT: Island	5	534.549	106.901	2.093	17	0.166	**0.001**
PhiPT: Site	11	769.078	69.916	2.606	21	0.209	**0.001**
C. *A. hyacinthus*							
PhiPT: Island	5	672.951	134.590	2.767	30	0.296	**0.001**
PhiPT: Site	10	799.564	79.956	2.927	32	0.320	**0.001**

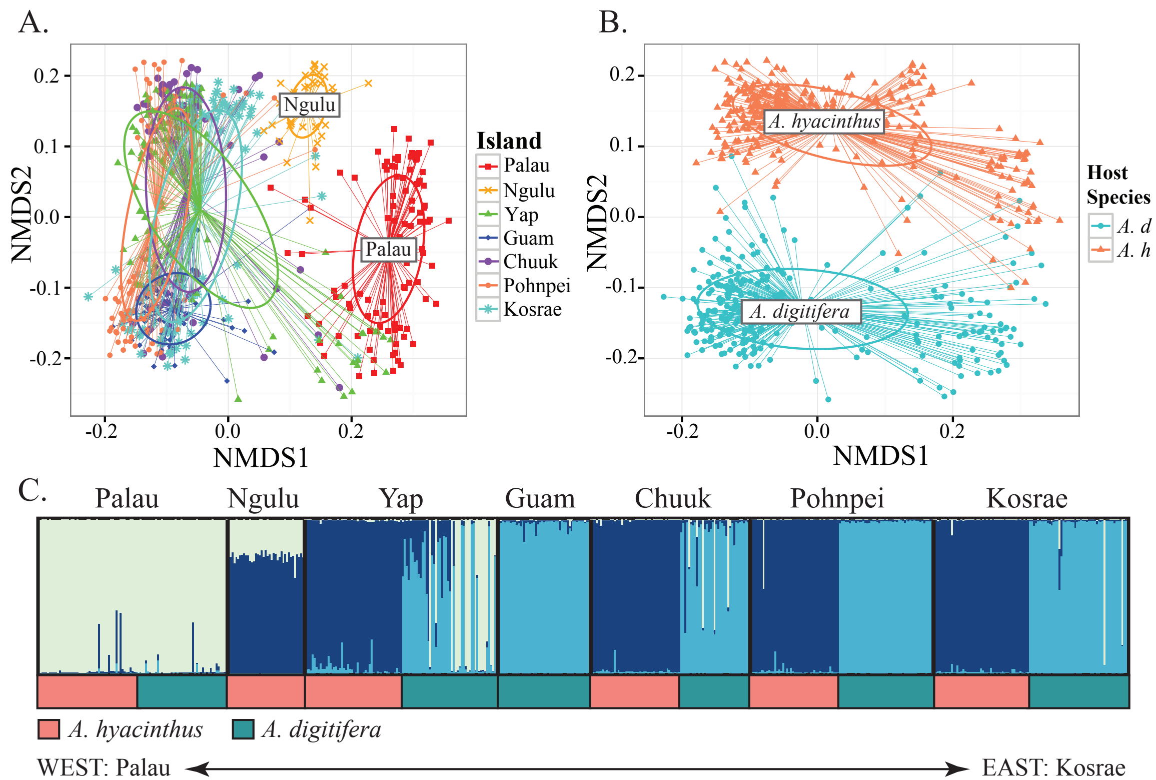


Figure 18: Analyses of all *Symbiodinium* clade C2 data
Symbiodinium hosted by *Acropora hyacinthus* and *Acropora digitifera* at thirteen sites across seven islands in Micronesia, using a data set of the presence and absence of all microsatellite alleles within samples. A. Non-metric multidimensional scaling (Bray-Curtis nMDS – k=10 dimensions) analysis of *Symbiodinium* clustered by islands. B. Non-metric multidimensional scaling (Bray-Curtis nMDS – k=10 dimensions) analysis of *Symbiodinium* clustered by host species. C. STRUCTURE population assignment for *Symbiodinium* from two *Acropora* host species across greater Micronesia at an optimal population number K=3. Colors in the bottom panels correspond to host species.

***Symbiondinium* genetic structure in *A. digitifera* coral hosts**

17% of the genetic variation in *Symbiodinium* in *A. digitifera* hosts was explained by island differences with an additional 4% explained by sites within islands (21%, Table 12B). All pairwise island Phi_{PT} values were also significant and ranged from 0.024 to 0.291 (Table 13A). Pairwise Phi_{PT} values between sites, however, were not all significant. For example, no significant differentiation was detected between sites within Palau or

Goofnuw Channel in Yap and Palau sites. Also pairwise comparisons within Guam and between some sites amongst Guam, Chuuk, and Pohnpei were also not significant after multiple test correction (Table 14A). No difference in the number of alleles at each island was observed, with numbers ranging from 54-68, slightly higher than those observed in *A. hyacinthus* (Table 15). Number of private alleles did vary, however, with Palau having 15 private alleles while all other islands had < 8 private alleles (Table 15). Discriminant analysis of principal components (DAPC) clustered by island explained 71% of the variation in the data, significantly clustering Palau and Kosrae from other islands (Table 16, Fig. 19A). Assignment of individual samples to model clusters using DAPC was consistent with STRUCTURE analysis. For example, some samples from Yap clearly assign to the Palau cluster, which also corroborates STRUCTURE results where Palau and some Goofnuw Channel individuals from Yap assign to the same population (Fig. 18C). For some islands, strong separation between sites is observed (i.e. Yap and Kosrae) and these assignments coincide with strong between-site Phi_{PT} values (Table 14A).

Table 13. Summary of pairwise Phi_{PT} values between all islands
Permutations were run 9999 times. All significant comparisons are shaded in grey.

A. *A. digitifera*

	PAL	YAP	GUA	CHU	POH	KOS
PAL	0.000	***	***	***	***	***
YAP	0.045	0.000	***	***	***	***
GUA	0.207	0.227	0.000	***	***	***
CHU	0.084	0.066	0.098	0.000	***	***
POH	0.230	0.257	0.086	0.155	0.000	***
KOS	0.264	0.291	0.084	0.165	0.024	0.000

B. *A. hyacinthus*

	PAL	NGU	YAP	CHU	POH	KOS
PAL	0.000	***	***	***	***	***
NGU	0.360	0.000	***	***	***	***
YAP	0.418	0.351	0.000	***	***	***
CHU	0.381	0.305	0.121	0.000	***	***
POH	0.439	0.396	0.085	0.069	0.000	***
KOS	0.392	0.286	0.182	0.186	0.218	0.000

Table 14. Summary of pairwise Phi_{PT} values between all sites
Permutations were run 9999 times. All significant comparisons are shaded in grey.

A. *A. digitifera*

	PA1	PA2	Y1	Y2	G1	G2	C1	C2	PO1	PO2	K1	K2
PA1	0.000	**0.062**	***	**0.004**	***	***	**	***	***	***	***	***
PA2	**0.045**	0.000	***	**0.002**	***	***	***	***	***	***	***	***
Y1	0.207	0.227	0.000	***	**	**	**	***	***	***	***	***
Y2	**0.084**	**0.066**	0.098	0.000	***	***	***	***	***	***	***	***
G1	0.230	0.257	0.086	0.155	0.000	**0.112**	**0.007**	***	***	***	***	***
G2	0.264	0.291	0.084	0.165	**0.024**	0.000	**0.038**	***	***	**0.082**	***	***
C1	0.171	0.247	0.106	0.147	**0.088**	**0.055**	0.000	***	***	**	***	***
C2	0.314	0.343	0.167	0.201	0.213	0.149	0.121	0.000	***	***	***	***
PO1	0.371	0.393	0.158	0.232	0.163	0.150	0.207	0.278	0.000	***	***	***
PO2	0.328	0.359	0.095	0.189	0.093	**0.028**	0.127	0.201	0.138	0.000	***	***
K1	0.308	0.314	0.197	0.217	0.199	0.161	0.182	0.285	0.275	0.209	0.000	***
K2	0.373	0.393	0.217	0.238	0.166	0.156	0.246	0.249	0.258	0.180	0.290	0.000

B. *A. hyacinthus*

	PA1	PA2	N1	Y1	Y2	C1	C2	PO1	PO2	K1	K2
PA1	0.000	**0.002**	***	***	***	***	***	***	***	***	***
PA2	**0.037**	0.000	***	***	***	***	***	***	***	***	***
N1	0.360	0.368	0.000	***	***	***	***	***	***	***	***
Y1	0.412	0.430	0.346	0.000	***	***	***	***	***	***	***
Y2	0.436	0.455	0.369	0.054	0.000	***	***	***	***	***	***
C1	0.366	0.392	0.318	0.126	0.093	0.000	***	***	***	***	***
C2	0.450	0.475	0.371	0.233	0.276	0.165	0.000	***	***	***	***
PO1	0.433	0.459	0.402	0.168	0.113	0.056	0.234	0.000	***	***	***
PO2	0.454	0.492	0.397	0.093	0.108	0.080	0.221	0.106	0.000	***	***
K1	0.404	0.405	0.314	0.279	0.257	0.248	0.342	0.314	0.284	0.000	***
K2	0.455	0.461	0.330	0.191	0.221	0.192	0.301	0.293	0.249	0.196	0.000

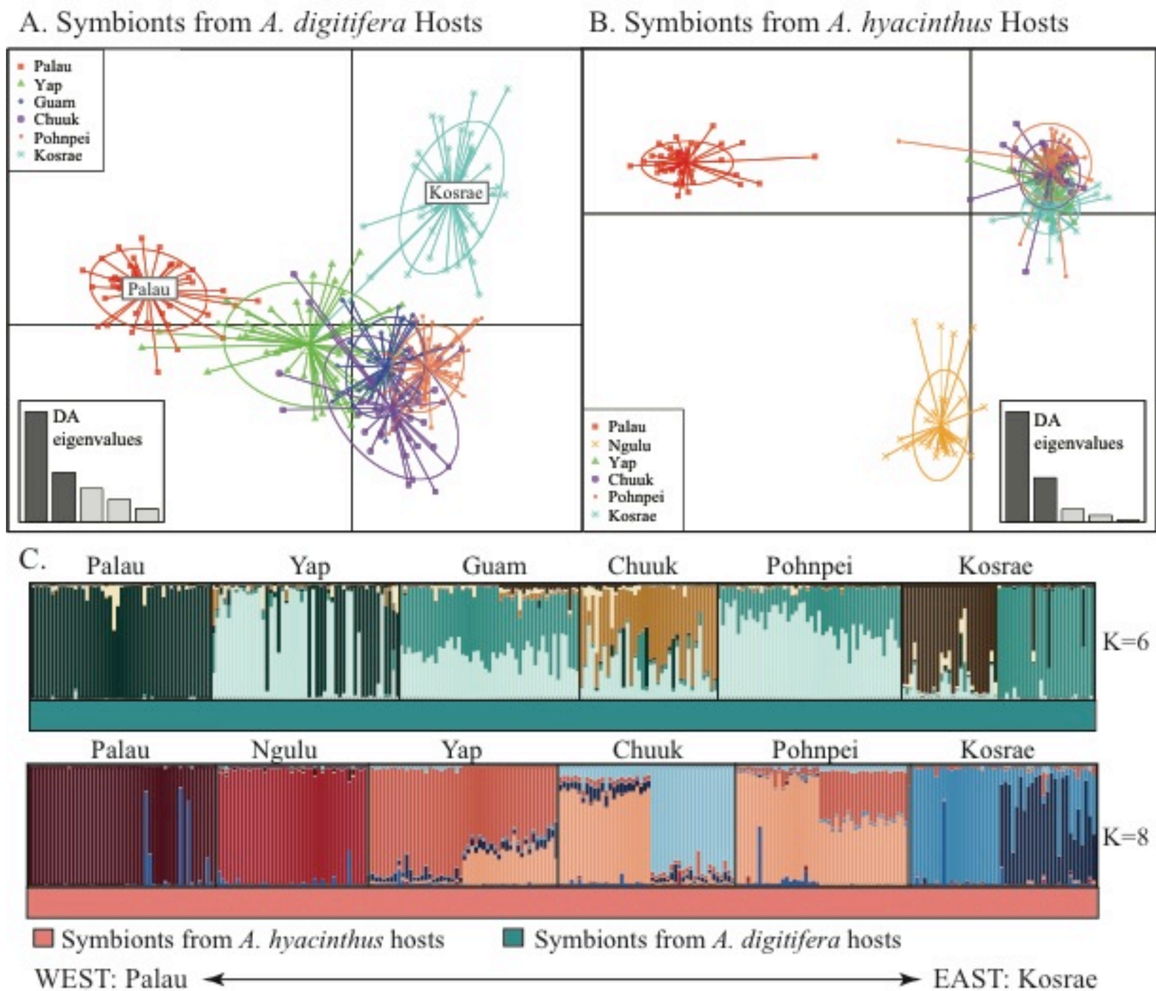


Figure 19: *Symbiodinium* structure within coral host species
Discriminant analysis of principal components (DAPC) of microsatellite data for *Symbiodinium* C2 hosted by *Acropora hyacinthus* and *Acropora digitifera* at thirteen sites across seven islands in Micronesia, using a data set of the presence and absence of all microsatellite alleles within samples. A. Scatter plot of individual samples from *A. digitifera* hosts (represented by colored dots corresponding to island) that were clustered by island. The analysis accounted for 50% of the genetic variation in the data set. B. Scatter plot of individual samples from *A. hyacinthus* hosts (represented by colored dots corresponding to island) that were clustered by island. The analysis accounted for 48% of the genetic variation in the data set. C. STRUCTURE population assignment for *Symbiodinium* run independently for each *Acropora* host species across greater Micronesia at an optimal population number of K=6 for *A. digitifera* and K=8 for *A. hyacinthus*. Colors below each panel correspond to host species.

Symbiondinium genetic structure in _A. hyacinthus_ coral hosts

Higher between-island genetic divergence was observed in *Symbiodinium* hosted by *A. hyacinthus* than for *A. digitifera* hosts (Table 12C; Fig. 19B). Additional genetic variation was explained by sites within islands (32%), however this site effect was nested within islands, similar to *A. digitifera* (Table 12C). All pairwise island Phi_{PT} values were significant and ranged from 0.069 to 0.439, significantly greater than values observed for *A. digitifera* (Wilcoxin's p=0.037, Table 13B; Fig. 19B). All pairwise Phi_{PT} values between sites were also significant, with the exception of sites within Palau (Table 14B). Again, no difference in the allele number at each island was observed with numbers ranging from 47-59, lower than those observed in *A. digitifera* (Table 15). Number of private alleles also did not vary in any obvious pattern and ranged from 2-7 (Table 15). DAPC analysis clustered by island explained 48% of the variation in *Symbiodinium* hosted by *A. hyacinthus* and significantly clusters Palau and Ngulu from all other sites (Table 13B, Fig. 19B). DAPC individual assignments to model clusters was not as informative as hierarchical analysis. In STRUCTURE results I detect strong population assignments for Palau, Ngulu, and sites within Kosrae (Fig. 19C) and I observe site assignment differences within each island, which are validated by strong pairwise Phi_{PT} values (Table 13B).

Table 15: Diversity and private alleles of *Symbiodinium*
The total number of alleles and number of private alleles observed at each island for each host species.

	Host Species	Palau	Ngulu	Yap	Guam	Chuuk	Pohnpei	Kosrae
Number of Alleles	*A. digitifera*	64	NA	68	56	66	54	67
	A. hyacinthus	56	47	55	NA	52	59	57
Number of Private alleles	*A. digitifera*	15	NA	6	8	7	2	3
	A. hyacinthus	6	2	4	NA	3	6	7

Table 16: DAPC model information
Discriminant analysis of principle component (DAPC) model information includes the number of principle components ("PC") and discriminant functions ("DF") retained, the variation explained by the clustering model ("var"), the percent of total variation explained by the first two eigenvalues ("eig") and Wilk's p-value indicating model significance ("Wilk's p"). A. DAPC information for *Symbiodinium* from *A. digitifera* hosts. B. DAPC information for *Symbiodinium* for *A. hyacinthus* hosts. C. Models within each island where 'spp' corresponds to the amount of variation explained by eigenvalue 1 (x-axis: Species axis) and 'site' corresponds to the amount of variation explained by eigenvalue 2 (y-axis: Site axis).

Model Information

A. *A. digitifera*	**PC**	**DF**	**var**	**eig**		**Wilk's p**
Island	16	5	0.706	0.503		< 2.2e-16 ***
Site	21	11	0.777	0.462		< 2.2e-16 ***
B. *A. hyacinthus*	**PC**	**DF**	**var**	**eig**		**Wilk's p**
Island	8	5	0.538	0.479		< 2.2e-16 ***
Site	15	10	0.704	0.553		< 2.2e-16 ***
C. Within Island	**PC**	**DF**	**var**	**spp**	**site**	
Palau	10	3	0.591	0.501	0.080	
Yap	19	3	0.809	0.661	0.103	
Chuuk	14	3	0.736	0.557	0.145	
Pohnpei	8	3	0.614	0.565	0.046	
Kosrae	9	3	0.665	0.536	0.108	

***Symbiodinium* structure within islands between coral hosts**

Symbiont genetic differentiation was clearly driven by host species, islands and also by sites within islands. Nearly all pairwise Phi_{PT} values between sites within islands were significant for *Symbiodinium* hosted by both *A. digitifera* and *A. hyacinthus* (Table 13). Palau was the only island where genetic differentiation of *Symbiodinium* between sites was not statistically significant (post multiple testing correction) for either host species. DAPC analysis was used within each island to cluster *Symbiondinium* data by host species and site (Table 16). Consistently across Micronesia, the first eigenvalue of *Symbiodinium* genetic variation within each island corresponds to host species difference and accounts for 50-66% of the variation, while the second eigenvalue corresponds to the difference due to sites within islands (environmental variation) and is responsible for 5-14% of variation (Fig. 20).

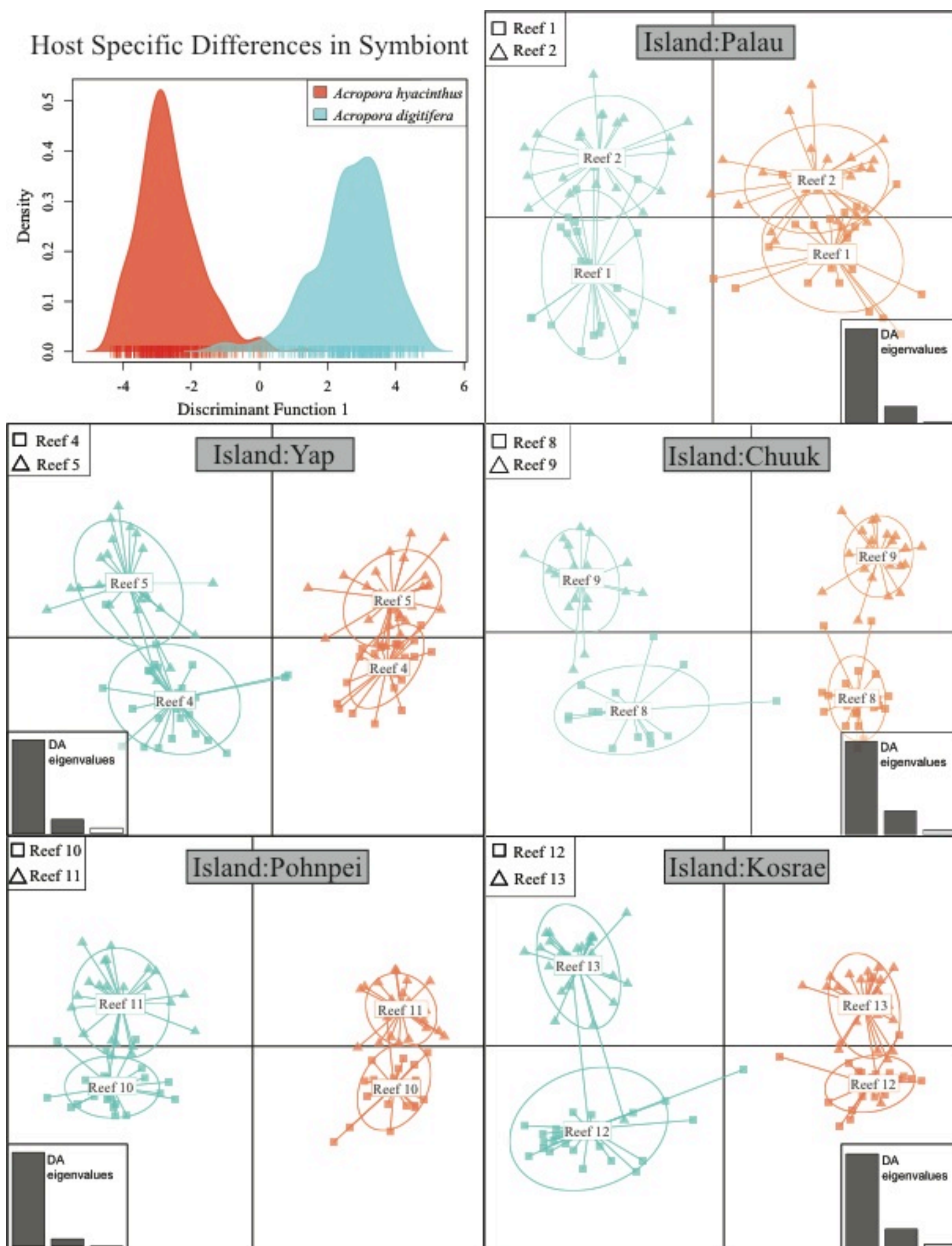


Figure 20: DAPC on *Symbiodinium* within islands
Discriminant analysis of principal components (DAPC) of microsatellite data for C2 hosted by *Acropora hyacinthus* and *Acropora digitifera* for five islands for which two sites were sampled. Top left: Analysis on two discriminant functions demonstrating strong host effects across all islands. All other panels, scatter plots represent *Symbiodinium* from both hosts at each island clustered by species and sites within islands. Information on the variation explained by each axis can be found in Table 16.

Symbiodinium divergence compared with host divergence

Symbiont genetic divergence was an order of magnitude greater than divergence observed in the host (Fig. 21). I also detected no evidence for isolation by distance (IBD, correlation between genetic divergence and island distances) for *Symbiodinium* from either host species (Fig. 21B), even though both hosts exhibited IBD patterns (Fig. 21A). Most interestingly, I observed significantly stronger between-island differentiation in *Symbiodinium* hosted by *A. hyacinthus* when compared to *A. digitifera* (Wilcoxon signed rank test p=0.037), following the overall significantly higher host divergence in *A. hyacinthus* (Wilcoxon signed rank test p=0.0019, Fig. 21).

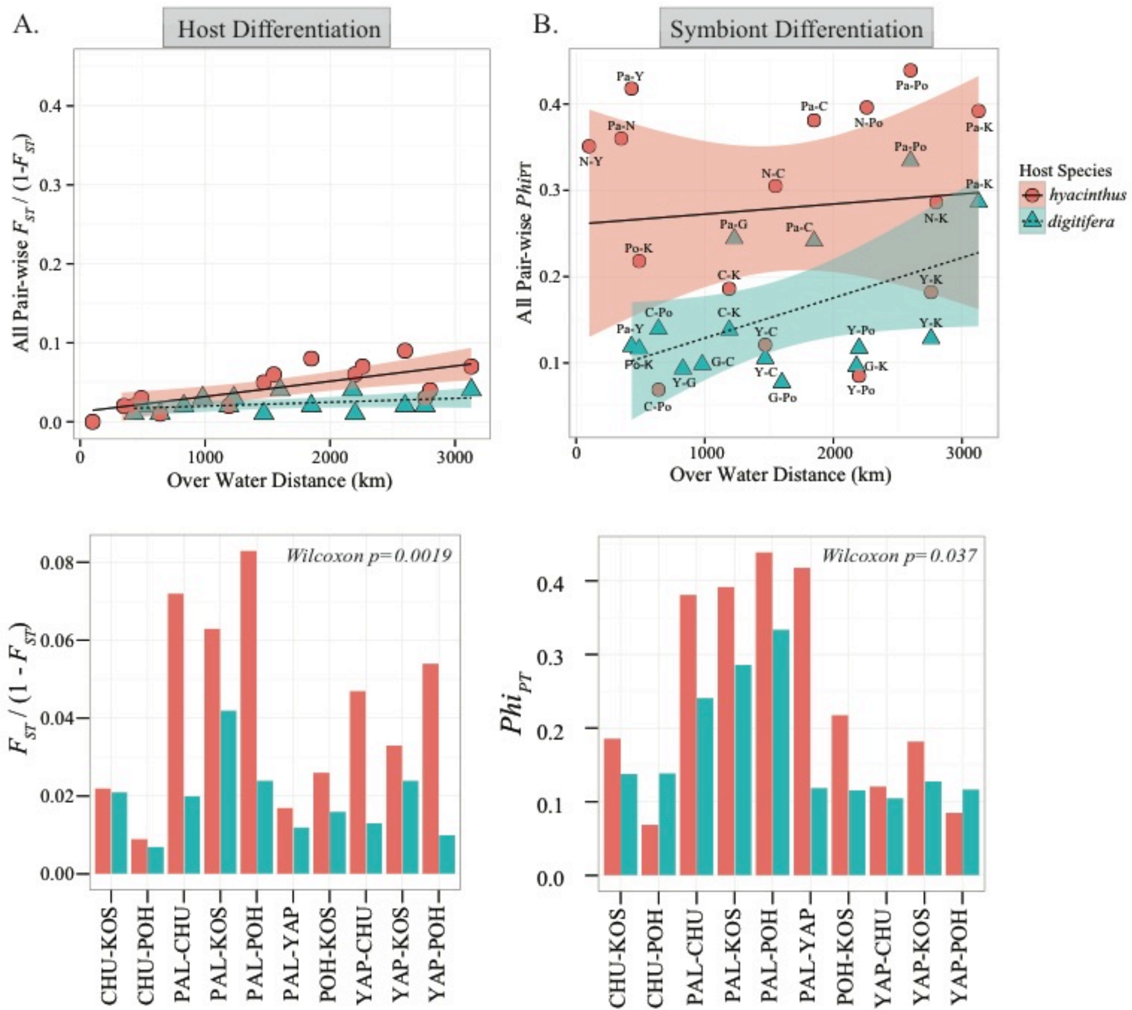


Figure 21: Comparison of host and *Symbiodinium* divergence
A. Top: Pairwise genetic differentiation [(F_{ST} /(1-F_{ST})] of two species of *Acropora* coral across linear distances (Km) demonstrating significant isolation by distance. Bottom: Host divergence observed for each pair of islands across both host species. B. Top: Pairwise genetic differentiation (Phi_{PT}) of *Symbiodinium* from two host species across linear distances (Km) demonstrating no isolation by distance. Bottom: Symbiont divergence observed for each pair of islands across *Symbiodinium* from both host species.

DISCUSSION

Throughout Micronesia, two *Acropora* reef-building corals both hosted clade C *Symbiodinium*, but genetic diversity was highly structured within this clade between host species, between islands, and between sites within islands. The use of microsatellites loci in this study (Bay, Howells, et al., 2009; Howells et al., 2009; Wham et al., 2014) facilitated the investigation of genetic structure of symbiont populations on a much finer scale than traditional ITS genotyping (Andras et al., 2013; Howells et al., 2009; Pettay, Wham, Pinzon, & LaJeunesse, 2011; Thornhill et al., 2014). I find divergence patterns of *Symbiodinium* consistent with previous findings of genetic structure on the scale of tens to hundreds of km (e.g., (Andras et al., 2013; Howells et al., 2009; Thornhill et al., 2009)). Overall, the observed *Symbiodinium* divergence illustrates strong host species specificity at each island as well as divergence between reefs within islands, all on the backdrop of occasional pronounced divergence between islands. These results indicate that coral hosts remain highly promiscuous in symbiont acquisition across their connectivity ranges while their symbionts are specializing on particular host species within each location. Divergences of host-specific *Symbiodinium* populations (Fig. 21B) are an order of magnitude greater than host genetic divergences (Fig. 21A). Notably, even between the same pairs of locations, *Symbiodinium* divergence is significantly greater within the more divergent host (*A. hyacinthus*), indicating that the process of host specialization in *Symbiodinium* populations contributes to their divergence among locations. Essentially, these data suggest that, as hosts diverge, symbionts codiversify in parallel to reflect this divergence (Thornhill et al., 2014; Thornhill et al., 2009). Overall, this study suggests that the variation in coral-*Symbiodinium* associations across the seascape is driven by the continuous evolution of host-specific *Symbiodinium* strains on local scales.

Symbiodinium Diversity

Clade C *Symbiodinium* are considered to be the most derived lineage within the genus *Symbiodinium* and exhibit significantly higher within-clade diversity when compared to other, more basal, clades (M.P Lesser et al., 2013; Pochon & Gates, 2010; Pochon, Montoya-Burgos, Stadelmann, & Pawlowski, 2006). Clade C *Symbiodinium* are also the dominant symbiont type found in Indo-Pacific reef-building corals, presumably because this clade sustains greater rates of carbon fixation (Stat, Morris, & Gates, 2008) and increased carbon translocation to hosts, which positively influences host fitness (growth) when compared to other clades (A. Jones & Berkelmans, 2010; Mieog et al., 2009; Stat et al., 2008). Here, high levels of clade C diversity across Micronesia were observed with a total number of 149 unique alleles across eight microsatellite loci and high allelic diversities were observed across the range (Table 15). Previous studies on clade C that have implemented microsatellite loci have also found similar levels of high genetic diversity (Howells et al., 2012; Howells et al., 2009; Thornhill et al., 2014), suggesting limited dispersal in these symbionts. However, this study is the first to demonstrate how this diversity is partitioned across islands, host species and sites within islands, all relative to genetic structure of hosts (Fig. 21).

Limited connectivity of Symbiodinium populations: Island Structure

All pairwise Phi_{PT} values between islands for *Symbiodinium* from both coral hosts were significant (Table 13) suggesting low genetic connectivity between *Symbiodinium* populations across islands (Fig. 19). Similarly high levels of genetic structure have previously been shown for other *Symbiodinium* populations (e.g (Andras et al., 2013; Howells et al., 2009)). Strong *Symbiodinium* differentiation is probable given their life history involves little active dispersal of free-living forms (Fitt, Chang, & Trench, 1981;

Fitt & Trench, 1983; Yacobovitch, Benayahu, & Weis, 2004) since this form largely exists in the benthos where dispersal by ocean currents is limited (Littman, van Oppen, & Willis, 2008) and once symbiosis is established, *Symbiodinium* live an endosymbiotic existence within the sedentary host. Strong genetic structure implies either limited dispersal capacities of clade C *Symbiondinium*, strong local selection on *Symbiodinium* along with high migration, or the presence of some other barrier preventing migrants from successful host infection in novel habitats already dominated by other *Symbiodinium* genotypes (reviewed in (Thornhill et al., 2009)).

The major NMDS axis of *Symbiodinium* population structure separrated Palau and Ngulu from the rest of Micronesia (Fig. 18A), suggesting that symbionts in western Micronesia are highly divergent from symbionts on other islands. Interestingly, signatures of these western Micronesian genotypes can be identified in one population of *A. digitifera* in Yap and to a lesser degree at other islands (white population, Fig. 18C). I hypothesize that western Micronesian *Symbiodinium* from Palau are dispersing to other islands and successfully infecting *A. digitifera* at low rates, however dispersal was not directly estimated here. Strong signatures of this Palau-type *Symbiodinium* in some eastern Micronesian corals (white bars, Fig. 18C) suggest that recent *Symbiodinium* immigrants from Palau infect these corals, or that the Palau-type *Symbiodinium* are reproductively isolated from symbionts in eastern Micronesia. It has been argued that *Symbiodinium* migration could be facilitated by some vertically-transmitting hosts through the vectored dispersal of symbionts within their larvae, which could shuttle novel *Symbiodinium* from parental reefs into new environments (Wirshing et al., 2013). However, a recent meta-analysis suggests that symbionts exhibit specificity for transmission mode, and specific symbiont types are associated with either horizontal or vertical transmission, with few symbiont generalists associated across both transmission

strategies ((Fabina, Putnam, Franklin, Stat, & Gates, 2012), reviewed in (M.P Lesser et al., 2013)). These results indicate strong host-specificity even among horizontally-transmitting species, which would make vectored dispersal of symbionts by other host species even less likely. This host-specificity of the putative Palau migrants is highlighted by the fact that they infect *A. digitifera* but not *A. hyacinthus* from the same locations.

Host Specificity

There is strong evidence that specific *Symbiodinium* populations preferentially associate with specific host species (Fig. 18B, C, Fig. 20). When each island is explored independently, host species accounts for 50-66% of the variation, suggesting that within islands, evolution of host specificity is the primary driver of symbiont diversity (Fig. 20). Host specificity has long been suggested as a mechanism for diversification in *Symbiodinium,* with divergent selection acting on standing genetic variation within symbiont populations to favor adaptations that increase symbiont fitness in their habitat, which in this case is defined by the host's intracellular environment (Dieckmann & Doebeli, 1999; Schluter, 2001). After initial divergence, assortative mating between same-host genotypes could drive further diversification of symbionts inhabiting different hosts, with disruptive selection reinforcing reproductive isolation (Dieckmann & Doebeli, 1999; Schluter, 2001, 2009). Indeed, using evidence from recombination events, Thornhill et al. (Thornhill et al., 2014) demonstrated that symbionts within host lineages were sexually recombining but were highly reproductively isolated from other lineages inhabiting different host species within the same environment, indicating that host specialization drives *Symbiodinium* diversification.

Evidence for *Symbiodinium* coral host specificity is compelling (i.e (Fabina, Putnam, Franklin, Stat, & Gates, 2013; Rodriguez-Lanetty et al., 2004; Thornhill et al., 2014; Weis et al., 2001)) and these specific associations can even be maintained during temporary environmental shifts favoring more stress-tolerant symbionts (McGinley et al., 2012). However, other studies have suggested that this specificity can be much more flexible (Putnam, Stat, Pochon, & Gates, 2012; Silverstein, Correa, & Baker, 2012). Interestingly, studies of symbiosis specificity during early ontogeny suggest that these associations are initially very flexible and coral juveniles can be infected with various symbiont strains (Abrego, van Oppen, et al., 2009; Andras et al., 2013; Coffroth et al., 2001; Little et al., 2004), which can provide an adaptive advantage during recruitment to novel habitats. In the majority of cases, however, over time the *Symbiodinium* composition in the juveniles shift to match the adults of the same species at the same location (Abrego, van Oppen, et al., 2009; Coffroth et al., 2001).

It is not surprising that I observe host specificity in this study because tight associations have been demonstrated for a wide variety of symbioses. For example, Paulsrud et al. (Paulsrud, Rikkinen, & Lindblad, 2000) found that many different cyanolichen species that grow in close physical contact to other cyanolichen species housed different photobiont strains, suggesting that it was lichen species, not locality, that determined cyanobiont identity (reviewed in (Rikkinen, 2013)). Specificity for host species has also been observed in the diversification of phytophagous insects in association with host plants (Funk, Filchak, & Feder, 2002). One example is symbiotic aphids in the genus *Hyalopterus,* which exhibit strong host-associated differentiation for various tree species (Lozier, Foottit, Miller, Mills, & Roderick, 2008; Lozier, Roderick, & Mills, 2007), whereas the parasotoid species of this aphid exhibited no host differentiation (Lozier, Roderick, & Mills, 2009). Some parasitic microfungi of the genus

Escovopsis that infect gardens of *Apterostigma* fungus-growing ants have also demonstrated some host specificity, where closely related *Escovopsis* infect closely related ant hosts (Gerardo, Mueller, & Currie, 2006). Mehdiabadi et al. (Mehdiabadi, Mueller, Brady, Himler, & Schultz, 2012) also demonstrated that ants in the *Cyphomyrmex wheeleri* species group showed strong species specificity and that each ant species exclusively associated with a single fungal cultivar, even though alternative cultivars were available in their environment. Clearly, host specificity is ubiquitous across symbioses and these strict associations may play a role in symbiont diversification.

Environmental Partitioning

Within most islands, I observed differentiation between sites along similar secondary axes (Eigenvalue 2; Fig. 20), which I posit is suggestive of locally adapted symbionts. Small-scale environmental partitioning has been previously observed in corals. Howells et al. (Howells et al., 2009) demonstrated genetic differentiation in populations of *Symbiodinium* hosted by *Sinularia flexibilis* separated by as little as 16 km along the Great Barrier Reef. Several studies on Caribbean gorgonian corals have also shown that populations of *Symbiodinium* are differentiated across tens of kilometers in the Bahamas (Santos, Gutierrez-Rodriguez, Lasker, & Coffroth, 2003) and in the Florida Keys (Kirk, Andras, Harvell, Santos, & Coffroth, 2009). Studies on *Symbiodinium* structure in scleractinian corals have demonstrated that haplotypes can be endemic to single reefs (Thornhill et al., 2009), colonies can host different symbiont types across a colony and these proportions can vary with depth (Rowan & Knowlton, 1995), and unique habitat-specific *Symbiodinium* types are observed in temperate environments along range margins of coral distributions (Lien, Fukami, & Yamashita, 2013), all

suggestive of habitat-specific environmental partitioning of *Symbiodinium*. While (Thornhill et al., 2014) demonstrated strong host associations in *Symbiodinium* clade C, in concordance with this study, they also determined that host-specific populations contained geographically segregated symbionts, implying that their secondary axis of *Symbiodinium* divergence is spatial isolation. Clearly, substantial genetic diversity due to habitat specificity exists within *Symbiodinium,* providing additional ecological explanations for *Symbiodinium* diversity.

While this study does not directly demonstrate local adaptation in the symbionts, previous research suggests that this is likely to be the case. Firstly, it is well known that corals can host more than one clade and more than one genotype within a clade (Pochon & Gates, 2010; Pochon et al., 2006), which generates the possibility for physiological plasticity through symbiont ‘‘shuffling’’ to better suit environmental conditions (Baker, 2003; Trench & Blank, 1987). Secondly, good evidence exists that symbiont genetic diversity between clades reflects functional diversity. For example, *Symbiodnium* clade D are more thermally resistant to bleaching under elevated temperatures (Berkelmans & van Oppen, 2006; A. M. Jones, Berkelmans, van Oppen, Mieog, & Sinclair, 2008), however this resistance comes with a cost of reduced growth and reproduction (A. Jones & Berkelmans, 2010). Clade C *Symbiodinium*, while not as thermal tolerant as clade D, generally express higher rates of carbon fixation that increase host growth compared to other clades (A. Jones & Berkelmans, 2010; Mieog et al., 2009; Stat et al., 2008). Third, studies have found functional variation within *Symbiodinium* clades. Using active chlorophyll fluorescent measurements to quantify functional PSII units (Tchernov et al., 2004) identified heat resistant genotypes within multiple clades of *Symbiodinium* demonstrating that ample within-clade variation exists for selection to act on. Probably the most elegant example explicitly demonstrating symbiont local adaptation comes from

(Howells et al., 2012). In Howells' study, photochemical performance and survivorship of *Symbiodinium* from two thermal environments were measured in response to elevated temperatures both in symbiosis and in culture (Howells et al., 2012). Their results convincingly demonstrate variation in thermal tolerance and that this variation is consistent with local adaptation (Howells et al., 2012).

In this study, not all pairwise site differences within islands were significant: the symbionts were not significantly differentiated among reef sites for both host species in Palau and for *A. digitifera* at Guam. This lack of differentiation across sites could be due to symbiont migration between sites, lack of environmental variation between these sites, or perhaps the biophysical environments on these islands mediating high connectivity. However, regardless of these exceptions, the grand majority of islands demonstrate significant within island site variation for both host species. I acknowledge that the limited connectivity I observed among *Symbiodinium* populations in this study could easily be due to some combination of limited *Symbiodinium* dispersal or biogeographic barriers. Nevertheless, I would also like to suggest that local conditions and environmental variation between reefs may be selecting for locally adapted *Symbiodinium* populations because the direction of change within each reef is identical for both host species on all islands (eigenvalue 2, Fig. 20).

Correlations in host-symbiont structure

With horizontally-transmitting corals, theory predicts independent diversification of coral hosts and their algal symbionts. Indeed, I observe no correlation between host genetic structure (F_{ST}) and the structure of *Symbiodinium* (Phi_{PT}) for either host species investigated, whereas I do observe that more differentiated hosts establish symbiosis with

more divergent symbionts (Fig. 21). Although only two host species were studied here, this result suggests that *Symbiodinium* diversification in *Acropora* may not be completely independent of the host and host divergence may drive symbiont diversification. However, host/symbiont patterns were not all correlated. For example, host genetic subdivision was subtle, followed isolation by distance (IBD) patterns, and no significant differentiation was ever observed between sites within islands (Chapter 2). Conversely, *Symbiodinium* populations exhibit genetic differentiation that is an order of magnitude larger (pairwise island Phi_{PT} 0.05-0.44) than values observed for the host (pairwise island F_{ST} 0-0.08) and nearly all pairwise differences between sites within islands were significant (Table 14). Similarly to a study of the Caribbean seafan *Gorgonia ventalina* and its symbionts (Andras, Kirk, & Harvell, 2011; Andras et al., 2013), I observe uncorrelated host/symbiont genetics patterns for both coral host species studied here. However, isolation by distance (IBD) was demonstrated for *Symbiodinium* of *G. ventalina,* which is also presumed to be a horizontally transmitting host (Andras et al., 2011). Contrarily, I detect no IBD in *Symbiodinium* for these horizontally transmitting corals (Fig. 19), even though both hosts exhibit strong evidence for IBD across the same spatial scale (Chapter 2). Perhaps since I see such strong differentiation in *Symbiodinium* within these islands, the lack of IBD patterns is simply due to the scale in which I sampled. Clade C *Symbiodinium* are the most speciose and evolutionarily successful clade, having undergone many adaptive radiations, so future sampling at finer scales may elucidate subtle IBD patterns (Thornhill et al., 2014).

Differences in genetic structure between the host and symbiont may reflect differences in dispersal capabilities and differences in adaptive capacities of each partner in the symbiosis. According to the cost-of-complexity hypothesis*, Symbiodinium* are expected to adapt faster than the coral host due to their reduced complexity, faster

generation times (Orr, 2000; Welch & Waxman, 2003), and their largely endosymbiont behavior limiting migration opportunities. For coral species that display horizontal symbiont acquisition, this reduced dispersal and increased adaptive capacity of their symbiont may provide fitness benefits for the holobiont by allowing acclimatization to new reef environments on the level of the individual.

CONCLUSION

This study demonstrates that population genetic divergences of *Symbiodinium* populations are much more pronounced and an order of magnitude greater than genetic divergences observed for the coral host. Interestingly, *Symbiodinium* divergence is also greater in the host species with the higher divergence, perhaps suggesting co-diversification of the coral host and the symbiont. Across the range of Micronesia, both coral hosts associate with highly divergent local *Symbiodinium* genotypes, confirming host promiscuity in symbiont uptake. *Symbiodinium* exhibit strong host-specificity as well as divergences between reef locations within islands. These patterns support the view that association with locally adapted *Symbiodinium* could provide a mechanism to improve fitness in the coral holobiont.

Chapter 4: Heritability of dispersal-related traits and associated gene expression in a reef-building coral

ABSTRACT

Reef-building corals are in a state of unprecedented decline as a result of both direct and indirect anthropogenic influences, including elevated sea-surface temperatures due to climate change. Range shifts can be one mechanism by which corals can escape the adverse effects of climate change. Because most reef-building corals release gametes into the water that develop into planktonic larvae, selection for dispersal potential therefore may optimize larval traits and can be investigated through classical quantitative genetics and functional genomics using laboratory-reared larvae. To determine if climate change can select for genotypes capable of longer-range dispersal, I aimed to quantify the additive genetic variation in a suite of dispersal-related traits in the reef-building coral, *Acropora millepora*. Twenty full-sib larval families were established and four phenotypic traits relevant to larval dispersal potential were quantified: early responsiveness to settlement cue (the dispersive stage ends when the larva decides to settle), rate of lipid loss, rate of protein loss, and red fluorescence. Significant variation between families was observed only for early settlement and red fluorescence, with mean broad-sense heritability > 0.45. Tag-based RNA-seq was used to identify genes whose expression was associated with these traits and to assess the overall heritability of gene expression. 577 genes showed differential expression across sires; expression of these genes was so consistent with respect to paternal genotype that I was able to infer that some of the sires were clone mates, which I then confirmed by genotyping. The most pronounced parental effects were observed in genes implicated in genome stability and stress response, which is likely to be the consequence of heritable variation in overall physiological condition. Expression profiles associated with variation in settlement included receptor activity and

cell surface/extracellular matrix components, pointing towards the possible role(s) of heritable variation in sensory machinery. Gene expression associated with larval red fluorescence indicated that redder larvae grew less, developed slower, and exhibited differential regulation of stress response genes. This result suggests that the previously documented diminished settlement response in red fluorescent larvae might not be an indication of long-range dispersal potential, but rather a consequence of inherited stressed condition. Overall these data demonstrate that significant heritable variation is available within coral populations that may serve as fuel for natural selection shaping novel adaptations under climate change.

INTRODUCTION

With climate change modifying habitats worldwide, understanding how species adapt to these changes is imperative. If a species is to escape unfavorable conditions, it must adapt locally or disperse to more favorable habitats. A species' ability to disperse determines its potential to escape adverse conditions, re-colonize disturbed habitats, colonize novel habitats, and spread beneficial alleles between populations (Ritson-Williams et al., 2009). Dispersal also allows a species to persist globally despite local extinction by allowing organisms to track environmental conditions (Ronce, 2007). Global climate change increases selection pressure on dispersal mechanisms for many species when unfavorable environmental conditions are induced within historical species ranges, influencing species distributions worldwide (Burrows et al., 2011; Hoegh-Guldberg et al., 2007; Parmesan & Yohe, 2003; Pinsky et al., 2013).

Reef-building corals are in a state of unprecedented decline due to a number of direct and indirect anthropogenic influences, including elevated sea surface temperatures due to climate change (Hoegh-Guldberg et al., 2007; Munday, Warner, Monro, Pandolfi, & Marshall, 2013). Understanding how corals will cope with these environmental challenges is the prime subject of ongoing coral research. Range shifts have been suggested as one possible coping-mechanism, and latitudinal shifts have been observed in corals during past climatic changes (Greenstein & Pandolfi, 2008). Range shifts have also recently been detected in response to contemporary change (Baird, Sommer, & Madin, 2012; Yamano, Sugihara, & Nomura, 2011). However, very little is understood about how selection might act on dispersal-related traits and if corals can evolve to increase their dispersal capacity.

The majority of corals, like many other marine invertebrates, release gametes into the water annually that develop into planktonic larvae that are dispersed by ocean

currents, representing the coral's only dispersal opportunity (Baird et al., 2009). These pelagic larvae have the opportunity to travel great distances with surface currents before settling on a reef but once the larva settles in a location, it will remain at that location for the duration of its life. The settlement choice therefore is the most crucial decision in a coral's life, critical to the survival of the species and for replenishment of coral populations on neighboring reefs. Selection for dispersal potential is limited to optimizing larval traits, which can be investigated through classical quantitative genetics (e.g. (Meyer et al., 2009)) and functional genomics (e.g. (Meyer, Aglyamova, & Matz, 2011)).

Dispersal of coral larvae depends on many factors, including the age at which the larva is physiologically capable to settle (larval competency), metabolic rate of maternally inherited lipid and protein reserves, and responsiveness to settlement cue (Babcock & Mundy, 1996; Connolly & Baird, 2010; Cowen & Sponaugle, 2009; Figueiredo et al., 2012; Graham, Baird, Connolly, et al., 2013; Graham, Baird, Willis, & Connolly, 2013; Tay, Guest, Chou, & Todd, 2011; Vermeij, Fogarty, & Miller, 2006)). Two measures, the time at which larvae become competent and lipid/protein metabolism, are considered particularly reliable proxies of dispersal potential (Richmond, 1987). Lipid metabolism genes have been shown to be under strong positive selection in corals (Voolstra et al., 2011). However, even phenotypic traits such as larval fluorescence have also been correlated with dispersal potential (Kenkel, Traylor, Wiedenmann, Salih, & Matz, 2011).

Previous work has suggested that heritable variation exists for a variety of traits across many marine organisms (Foo, Dworjanyn, Poore, & Byrne, 2012; Johnson, Christie, & Moye, 2010; Kelly, Padilla-Gamino, & Hofmann, 2013; Lobon, Acuna, Lopez-Alvarez, & Capitanio, 2011; McKenzie, Brooks, & Johnston, 2011; Parsons, 1997), including corals (Kenkel et al., 2011; Meyer et al., 2009). In fact, previous studies

have indicated that heritable variation exists for nearly any trait measured in corals (Carlon, Budd, Lippe, & Andrew, 2011; Kenkel et al., 2011; Meyer et al., 2011; Meyer et al., 2009), but see Csaszar et al. (Csaszar, Ralph, Frankham, Berkelmans, & van Oppen, 2010). An ecologically important life-history trait such as larval dispersal is therefore also likely to exhibit heritable variation; however, in order to predict the potential for reef-building corals to adapt to warming temperatures through range-shifts, it is necessary to estimate the amount of natural genetic variation in dispersal-related traits. If the heritable variation is large, selection can act, potentially facilitating adaptive evolution. To date, no study has established the extent of heritable variation in coral dispersal traits, precluding predictions regarding how likely corals are to adapt to climate change through dispersal to new habitats.

Likewise, no study has ever explored gene expression patterns associated with dispersal related traits. Global gene expression profiling is a powerful hypothesis-forming tool that can help us understand the molecular underpinnings of phenotypic traits and physiological responses. Previous research in corals has shown that specific transcriptional regulation occurs in the emerging coral genomic model, *Acropora millepora* larvae in response to various environmental stimuli, including presentation of a settlement cue (Meyer et al., 2011; Moya et al., 2012). Here, I aim to identify gene expression patterns associated with heritable dispersal trait variation in *A. millepora*. Knowledge of the genes correlating with dispersal potential will provide tools to trace ongoing adaptation in real time, which is valuable, both for prioritization of management efforts and for basic understanding of evolution in the ocean.

Methods

Sample collection and crossing design

In November 2011, prior to the annual coral spawning event, twelve colonies of *A. millepora* were collected from the Little Pioneer Bay, Orpheus Island, Australia. (18°36’06.30 S, 146°29'11.82 E). Colonies were kept at the Orpheus Island Research Station in an outdoor raceway with natural seawater flow-through until the night of spawning, when colonies were individually isolated in independent tubs filled with 1 μm filtered seawater (FSW). After spawning, gamete bundles from each colony were collected by surface scooping, broken by gentle stirring, and sperm and eggs were separated by sieving through a 300 μm nylon mesh. Twenty crosses were established (Table 17), using eggs from two colonies (A and C) and sperm from the other ten colonies (P to Z), corresponding to a classic line-by-tester design to maximize the accuracy of characterizing additive (sire-related) genetic effects (Kempthorne, 1955). There was no detectable self-fertilization in egg batches that were not mixed with sperm from other colonies. Two N=25 and one N=50 egg samples were collected for each dam for lipid and protein analysis. Also, small fragments of each parental colony were collected and preserved in 95% ethanol. All parents were later genotyped at 12 microsatellite loci (Wang, Zhang, & Matz, 2009) to identify clonal colonies.

Two hours were allotted for fertilization and excess sperm were then removed by gentle sieving through 300 μm mesh and rinsing with FSW three times. Fertilization success (>80% for all crosses) was estimated by counting dividing embryos under a stereomicroscope. Two replicate cultures per cross were stocked into FSW in plastic culture vessels at the density of 1 embryo ml^{-1} (Table 17). Culture seawater (FSW) was changed daily in the first two days after fertilization to remove unfertilized eggs and cell

debris and once every two days afterwards, by gently sieving the embryos and replacing the water.

Table 17. *Acropora millepora* crossing design
Names of 20 larval families produced by crossing two dam colonies (rows) and 10 sire colonies (columns) with the number after the cross representing the culture replicate. Parental genotyping later indicated that two sires were clonemates (T=W, Q=Y).

	Sire Colonies								
Dam Colonies	**Q[2]**	**R**	**S**	**T[1]**	**V**	**W[1]**	**X**	**Y[2]**	**Z**
A	AQ1	AR1	AS1	AT1	AV1	AW1	AX1	AY1	AZ1
	AQ2	AR2	AS2	AT2	AV2	AW2	AX2	AY2	AZ2
C	CQ1	CR1	CS1	CT1	CV1	CW1	CX1	CY1	CZ1
	CQ2	CR2	CS2	CT2	CV2	CW2	CX2	CY2	CZ2

[1] Indicated clones T, W
[2] Indicated clones Q, Y

Larval sampling

Three days post fertilization N=50 larvae were collected from each culture and were fixed in RNALater (Ambion, Life Technologies) for subsequent RNAseq analysis. Samples were stored at -20ºC on Orpheus Island and were transported to The University of Texas at Austin on blue ice and stored at -20ºC upon arrival. Five days post fertilization, larvae were sampled for lipid (1 @ N=50 larvae/ culture) and protein (2 @ N=25 /culture), flash frozen in liquid nitrogen, and stored at -80ºC until processing.

Evaluating larval early response to settlement cue

To quantify the early responsiveness of competent larvae to a natural settlement cue, crustose coralline algae (CCA) (Heyward & Negri, 1999), samples of 20 to 30 larvae from each culture vessel were transferred into 10 ml of FSW in sterile 6-well plates. Five-day-old larvae from cultures were used in settlement trials, which were all performed at

ambient temperatures in the absence of light. CCAs were collected from the local reef environment and samples were chosen that were known to elicit settlement responses (Davies, Chapter 1). These samples were finely ground with a mortar and pestle, autoclaved, and a drop of the resulting uniform slurry was distributed to each well (N=6 wells per culture replicate). The proportion of metamorphosed larvae (visual presence of septa) was quantified after 48 hours using a fluorescent stereomicroscope MZ-FL-III (Leica, Bannockburn, IL, USA) equipped with F/R double-bandpass filter (Chroma no. 51004v2). The arcsine square-root transformed proportions of settled larvae were analyzed using a linear mixed model with dam, sire, dam:sire interaction, and plate as random effects, and no fixed effects, using MCMCglmm package in R (Hadfield, 2010). Mean and 95% credible intervals were calculated based on the samples from the posterior to estimate the variance explained by 1) maternal effects (dam minus sire effect), 2) narrow sense heritability (h^2) (2 x sire effect), 3) interaction (dam:sire), and 4) broad sense heritability (H^2) (Parental effects: dam+sire+dam:sire).

Model:

MCMCglmm (asin(sqrt(prop)) ~ 1, random = ~ plate + sire + dam + dam:sire, family = "gaussian", data=t48, nitt = 30000)

Quantifying larval fluorescence

Larval fluorescence was quantified similar to procedures in (Kenkel et al., 2011) with a few modifications. Briefly, larvae were imaged using a fluorescent stereomicroscope MZ FL-III (Leica, Bannockburn, IL, USA) equipped with a double-bandpass F/R filter (Chroma no. 51004v2) and a Canon G6 camera. The larvae were killed in 0.04% paraformaldehyde and photographed immediately. Approximately 20

larvae were photographed from each culture and only larvae oriented laterally were quantified. Lateral larvae were chosen since larvae in this position result in the most robust fluorescence estimate. Photo processing used the program IMAGEJ (W. Rashband, NIMH, Bethesda, MD, USA). For all laterally-positioned larvae, red, blue, and green raw integrated density color values were recorded for the entire size of each individual larva. To control for differences in background intensity, blank circles of dark background of uniform size were measured for each photograph. Blank values were subtracted from each larval reading. Relative red fluorescence was then quantified by the color value for red divided by the color values for red, green and blue, normalized to the background. Parental effects on red fluorescence were determined using linear mixed model approach as described in the previous section.

Model:

MCMCglmm (red fluorescence ~ 1, random = ~ sire + dam + dam:sire, family = "gaussian", data=fluor, nitt = 30000)

Measurement of protein and lipid loss

To quantify variation in protein and lipid metabolism between families, the protein and lipid content five days post fertilization was quantified and subtracted from the quantity in the eggs. Since larvae of *A. millepora* are lecitrophic (non-feeding), these calculations are a good proxy for larval metabolic rate.

Protein content of larvae was measured throughout development (eggs to 5 d post-fertilization) using the RED 660 protein colorimetric assay. Twenty-five eggs from each dam and two samples of twenty-five larvae from each culture were homogenized in 500ml of extraction buffer (100nM Tris HCl with 0.05mM DTT pH 7.8) and stored on ice. 20 µl of resulting homogenate was combined with 180 µl of Protein Red 660 in a 96-

well flat-bottom plate. Each plate included duplicate dilutions of a standard curve prepared from bovine serum albumin (BSA), ranging from 2mg/µl to 0.03 mg/µl per well. Protein content was calculated based on the absorbance at 660 nm using a SpectraMax M2 plate reader (Molecular Devices) and comparison to the standard curve. Multiple replicates (n = 3) were measured from each homogenate. Mean protein loss during development from eggs to five days post fertilization was calculated for each culture vessel as the difference between initial and final contents across replicates.

Larval lipid content was also measured throughout development (eggs to 5 d post-fertilization). 50 eggs from each dam and 50 5 d post-fertilization larvae from each culture were outsourced to the Chemistry and Biochemistry Core at the Boston University School of Medicine (http://www.bumc.bu.edu/phys-biophys/facilities/chem_biochem). In brief, lipids were homogenized and extracted in 300 µl of water and suspended in 1 ml of chloroform:methanol (1:1) following the (Folch, Lees, & Stanley, 1957) extraction procedure. Dry weights were then quantified for all samples. Because initial data suggested that larvae consisted mostly of wax esters, only total lipid (dry weight) was calculated. Parental effects on protein and lipid loss were modeled using the linear mixed model approach as described above.

Protein and Lipid Models:

MCMCglmm (loss ~ 1, random = ~sire + dam + dam:sire + culture = "gaussian", data=df, nitt=30000)

Tag-based RNA-seq Preparation

Total RNA from N=50 3 d post-fertilization larvae was extracted using RNAqueous kits (Ambion, Life Technologies) and samples were then DNAse treated as in (Meyer et al., 2011). RNA quality was evaluated through gel electrophoresis and

assessed based on the presence of ribosomal RNA bands. 100-700 ng of RNA per sample was prepared for tag-based RNA-seq following (Meyer et al., 2011), with modifications for Illumina sequencing:
(Protocol: http://www.bio.utexas.edu/research/matz_lab/matzlab/Methods.html).

RNA-seq and bioinformatic analyses

40 RNAseq libraries were prepared from larvae from each culture replicate. Samples were sequenced using Illumina HiSeq at The University of Texas at Austin's Genome Sequencing and Analysis Facility (GSAF). The Full pipeline for the bioinformatics analysis used here can be found at
http://www.bio.utexas.edu/research/matz_lab/matzlab/Methods.html.

In brief, 286.3 million raw reads were generated, with individual sample counts ranging from 2.1 to 41.9 million per sample (median = 8.6 million reads). Raw reads without the 5'-Illumina leader sequence were discarded, and if this leader was present it was trimmed from the reads. We used *fastx_toolkit* to trim the reads after adaptor or a homopolymer run of 'A' ≥ 8 bases was encountered, retain reads with minimum sequence length of 20 bases, and quality filter them requiring PHRED quality of at least 20 over 90% of the sequence.. After filtering, 1.3 to 27.6 million reads per sample (median = 3.6 million reads) remained. Filtered reads were then mapped to the *Acropora millepora* reference transcriptome (Moya et al., 2012) using the gmapper command in SHRiMP 2.1.1 (David, Dzamba, Lister, Ilie, & Brudno, 2011; Rumble et al., 2009), with flags –strata to return only the best-scoring alignments of identical quality, and -local to perform local alignment (i.e. not requiring the ends of the read to match). Read counts were then assembled according to isogroup (a collection of contigs representing splice

and assembly variants of the same gene) using a custom perl script that discards PCR duplicates, which were defined as reads mapping to the same starting position in the reference and aligning with 100% identity along the length of the shorter read. Reads mapping to multiple isogroups were disregarded. In total, 555 to 1.1 million unique reads per sample (median=416,278 reads) mapped to 43,985 isogroups.

Parental effects on gene expression

Analyses were conducted in the R statistical environment (R Development Core Team, 2013). Five libraries out of forty were identified as outliers using the *arrayQualityMetrics* package (Kauffmann, Gentleman, & Huber, 2009), and these samples corresponded to samples with low RNA yields. These samples were removed from all downstream analyses. All but one of these libraries corresponded with the lowest read depth, likely due to low RNA quality or quantity. Count data for the remaining 35 samples were analyzed using the package *DESeq* (Anders & Huber, 2010). Raw counts were used to estimate dispersions by maximizing the Cox-Reid adjusted profile likelihood (method="pooled-CR") for a model that specified dam and sire effects for each sample. Empirical dispersion values were retained for each gene and low-expressed genes were excluded from subsequent analyses by removing isogroups whose read count standard deviations fell in the bottom 50% quantile, leaving 21,879 highly expressed isogroups. Expression differences in these isogroups were then evaluated with respect to dam, sire and the dam:sire interaction using a series of generalized linear models implemented in the function *fitNbinomGLMs*. The significance of each of these terms for each gene was assessed using likelihood ratio tests. Significance values were adjusted for multiple testing and are reported for 5% and 10% false discovery rates following

(Benjamini & Hochberg, 1995). Heatmaps and principle component analyses (PCA) were used to visualize the top 100 differentially expressed genes corresponding to dam and sire using the package pheatmap (Kolde, 2012).

To visualize heritability of gene expression, we took all genes exhibiting adjusted p values of <0.005 for sire effect and used pheatmap (Kolde, 2012) to cluster samples based on Pearson correlations. To identify over-represented functional groups within these genes, Mann-Whitney-U (MWU) tests on p-values generated by the GLMs for the effect of sire were used in functional enrichment analyses based on Gene Ontology, as described in (Voolstra et al., 2011) (the scripts and instructions can be downloaded from http://www.bio.utexas.edu/research/matz_lab/matzlab/Methods.html).

Differential expression associated with phenotypic traits

To identify gene expression patterns in three-day-old larvae that predict the phenotypic outcome of the quantitative traits which demonstrated significant broad-sense heritability, we correlated gene expression patterns with settlement and fluorescence using *DESeq* (Anders & Huber, 2010). The same samples that were previously identified above as outliers were removed here and an additional two samples were removed for the fluorescence analysis since phenotypic data were not available for those samples. RNAseq analysis followed the same methods described above with the following modification. Isogroups whose read count standard deviations fell in the bottom 40% were removed for settlement analyses and the bottom 52% were removed for fluorescence analyses, leaving 26,365 and 21,102 highly expressed isogroups respectively. Generalized linear models using phenotypic values were then used to detect expression differences. To identify over-represented functional groups within these

genes, MWU tests were again implemented (Voolstra et al., 2011). To visualize these expression differences, significant genes within a GO category were plotted as a heatmap ordered by the ranked phenotypic value (settlement or fluorescence) to demonstrate the up or down regulation of expression that predicts a phenotypic outcome.

Because the red fluorescence phenotype is the outcome of red fluorescent protein (RFP) expression, additional analyses were conducted on the most highly expressed RFP gene. Relative red fluorescence was first correlated with RFP expression using a linear model, and then expression of this gene was plotted with respect to dam and sire to visualize trends.

RESULTS

Parental effects on phenotypic traits

Narrow-sense heritabilities (h^2) were not significantly different from zero for any phenotypic trait measured here, however significant broad-sense heritability (parental effects, including maternal effects) was observed for two traits: settlement and red fluorescence. I observe a significant effect of settlement in response to cue between genetic backgrounds (H^2) (Table 18, Fig. 22A,E), and the model suggests that 45% of the variance is explained by parental effects. I also detected strong parental effects (H^2) on larval red fluorescence, explaining 74% of the variation (Table 18, Fig. 22B,E). Interestingly, I also observed a significant effect of dam (maternal effect) for larval red fluorescence, which explains 61% of the variation, suggesting that this trait is largely maternally inherited in larvae tested here and could relate to egg quality. No significant parental effects were observed for protein or lipid loss, perhaps due to low replication within parents and low technical replication within cultures (Fig. 22C, D).

Table 18: MCMCglmm models for parental effects on phenotypic traits
Data include phenotypic trait, component of the model, mean variance explained and 95% credible intervals. Significant components are shaded in grey.

Phenotype	Component	Variance Explained	Lower 2.5%	Upper 97.5%
Settlement	Dam	0.22	~ 0.0	0.98
	Sire (h^2)	0.13	~ 0.0	0.50
	Interaction	0.10	~ 0.0	0.46
	Parental (H^2)	0.45	0.13	0.98
Protein	Dam	0.05	~ 0.0	0.70
	Sire (h^2)	0.02	~ 0.0	0.27
	Interaction	0.02	~ 0.0	0.26
	Parental (H^2)	0.09	~ 0.0	0.72
Lipid	Dam	0.03	~ 0.0	0.44
	Sire (h^2)	0.01	~ 0.0	0.12
	Interaction	0.02	~ 0.0	0.22
	Parental (H^2)	0.06	~ 0.0	0.52
Fluorescence	Dam	0.61	0.09	0.99
	Sire (h^2)	0.11	~ 0.0	0.41
	Interaction	0.02	~ 0.0	0.25
	Parental (H^2)	0.74	0.34	0.99

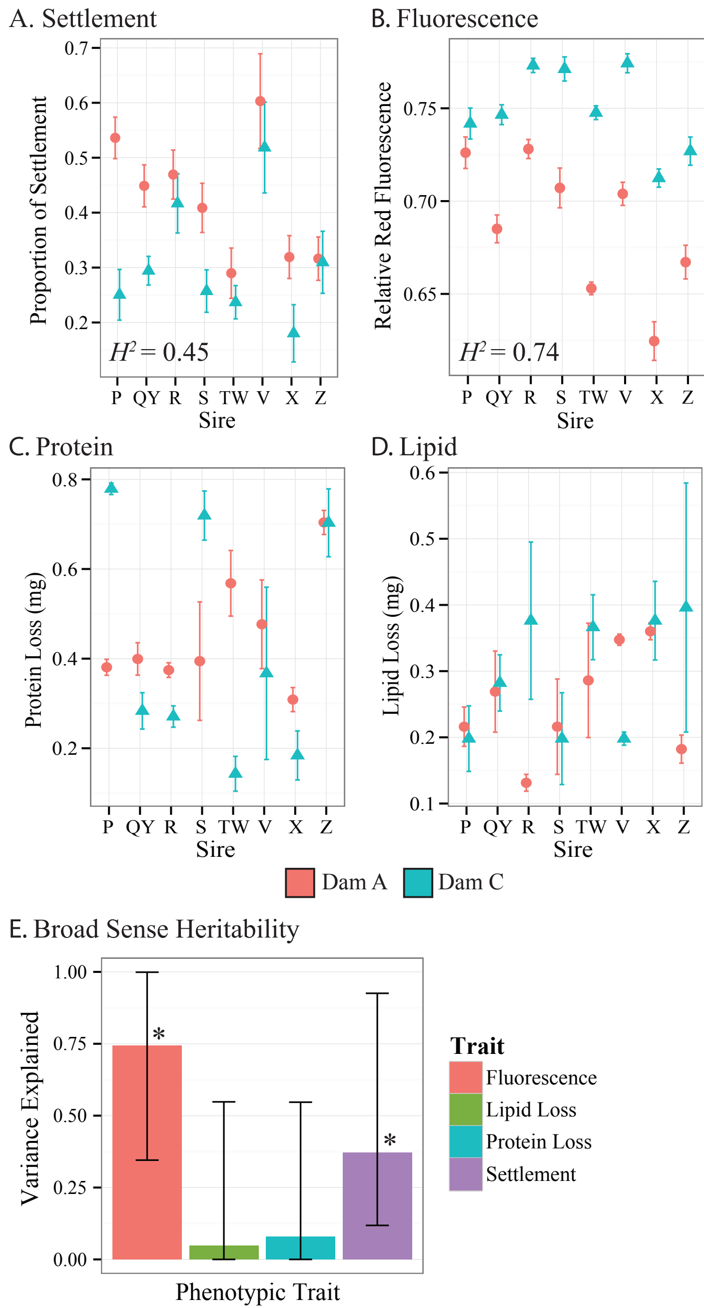


Figure 22: Heritability of dispersal-related traits

Trait means ± SE by family (A-D) and mean heritability estimates ± 95%CI (E) of dispersal-related traits across families. A. Proportion of settlement (broad-sense heritabiliy (H^2) = 0.45). B. Relative red fluorescence (H^2 = 0.74). C. Protein loss. D. Lipid loss. E. Amount of phenotypic variance explained in each trait attributable to genetic variance (H^2).

Parental effects of gene expression

Of the 24095 genes that remained after low-expressed genes were removed, *DESeq* identified 1216 differentially expressed genes (DEGs) with respect to dam after FDR correction of 0.1 (909 FDR 0.05) (Fig. 23A). 577 DEGs were found with respect to sire (466 FDR 0.05). Once DEGs were identified, pheatmap visualized the top 100 DEG dam and sire genes, clearly demonstrating strong dam and sire effects on gene expression (Fig. 23C,D). When principle component analysis (PCA) was used to decompose the variation in top 100 dam and sire genes we see that PC1 explains 31% of the variation and differentiates dams (pink arrow) and PC2 explains 12% of the variation and differentiates between sires (blue arrow) (Fig. 23B). Interestingly, we also observed certain sire pairs (TW, QY) clustering together in the PCA (Fig. 23B) and heatmap (Fig. 23D). To determine sire relatedness a panel of twelve SSR loci was assayed across all sires. Alleles across all twelve loci for sires TW and QY were identical, indicating that these individuals were clones and were treated as the same genotype for all analyses (Fig. 23B).

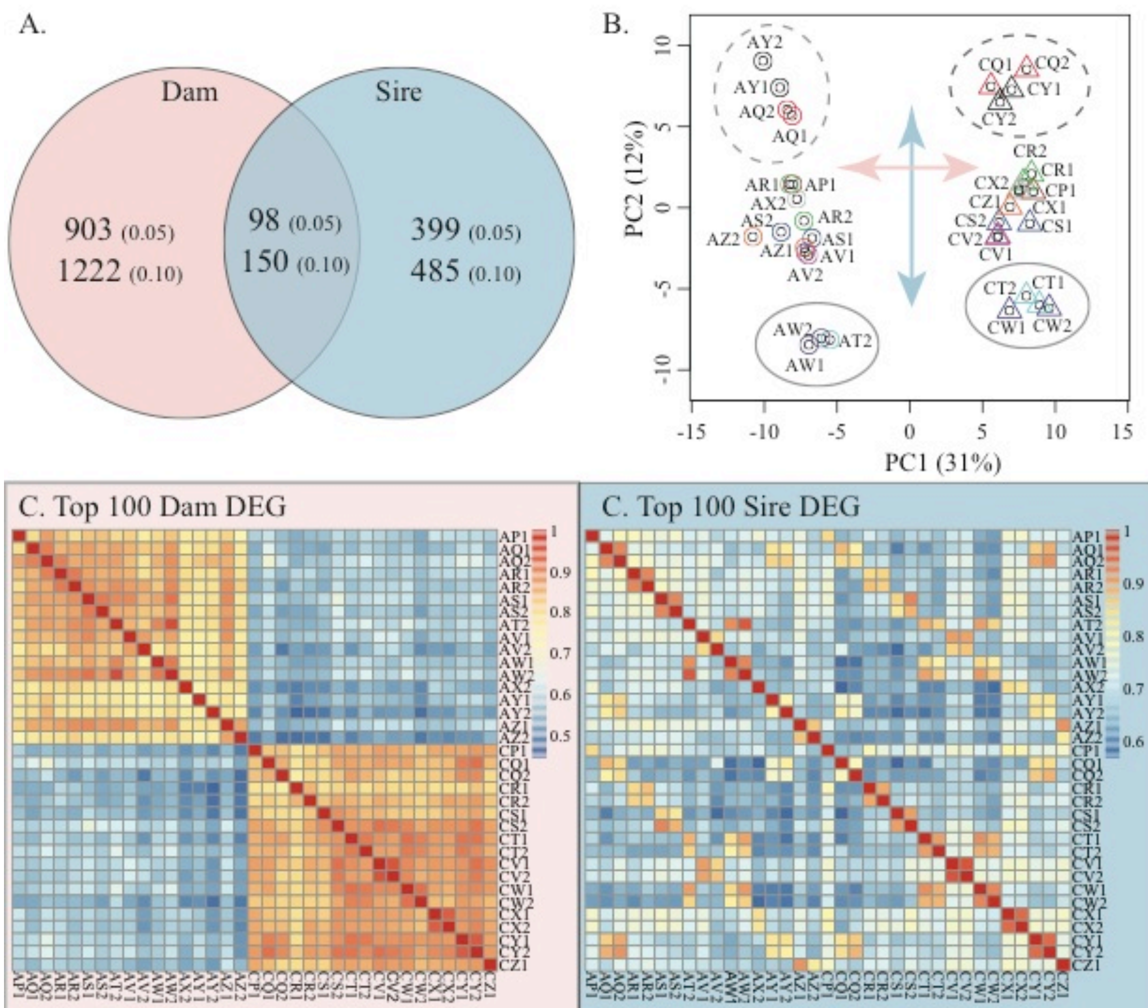
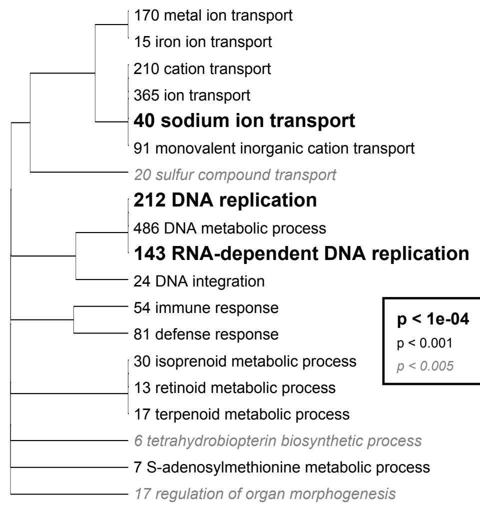
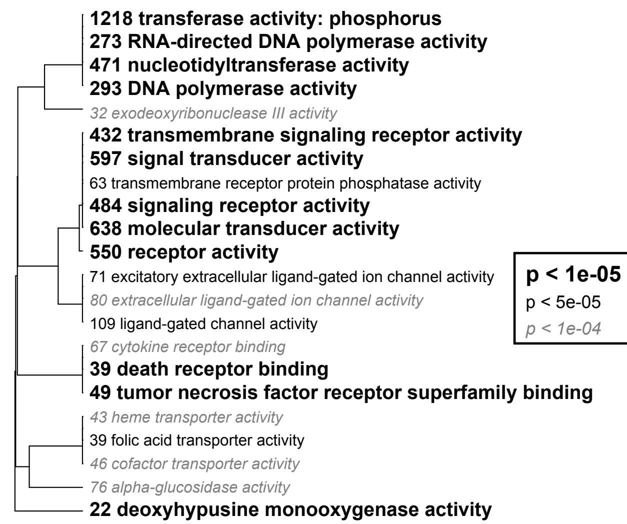


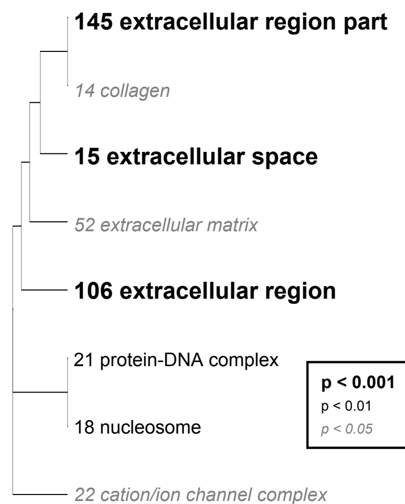
Figure 23: Tag-based RNAseq results
A. Venn diagram showing the number of differentially expressed genes (DEGs) with respect to dam and sire (FDR correction value in brackets). B. Principle component analysis (PCA) for the top 100 DEGs overall. PC1 explains 31% of the variation across samples, which is primarily driven by an effect of dam (pink arrow), while PC2 explains an additional 12% of the variation across samples and reflects the influence of the sire (blue arrow). Clones identified through genotyping are circled in grey. C. Heatmap clustering samples based on gene expression of the top 100 DEGs by dam. D. Heatmap clustering samples based on gene expression of the top 100 DEGs by sire.

Heritability of gene expression and functional enrichment

Expression of the larval families measured in the present study differed between sires (577 DEGs FDR 0.1), indicating heritability of gene expression in coral larvae (Fig. 23D). Gene Ontology (GO) analysis for sire expression indicated that the top GO terms for Biological Processes were "RNA-dependent DNA polymerase activity' (GO:0006278), 'DNA replication' (GO:0006260), and sodium ion transport' (GO:0006814) (Fig. 24A). Top terms for Molecular Function were 'DNA polymerase activity' (GO:0034061), 'RNA-directed DNA polymerase activity (GO:0003964), and 'signaling receptor activity' (GO:0038023) (Fig. 24B). For cellular components, weaker associations were observed (Fig. 24C). When expression variation among sires is visualized, the top FDR corrected (0.005) genes cluster according to sire, again demonstrating strong paternal effects on gene expression (Fig. 24D).







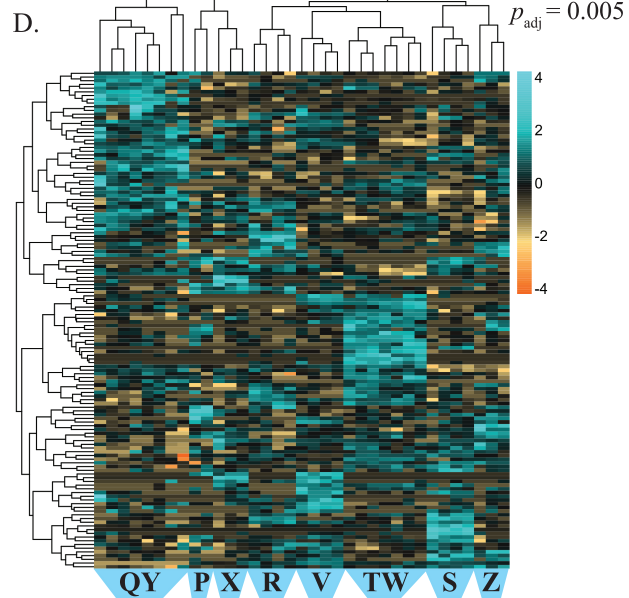


Figure 24: GO analysis of DEGs with respect to sire

A. Significant Gene Ontology (GO) terms for biological processes. B. Significant GO terms for molecular function. C. Significant GO terms for cellular component. D. Top DEGs after FDR correction (p<0.005) demonstrating strong heritable variation in gene expression.

Predictive gene expression for settlement and functional enrichment

When expression patterns were correlated with settlement, expression of 21 genes in three-day-old larvae significantly predicted settlement outcomes for five-day-old larvae (21 DEGs FDR 0.1). Of these 21 genes, only eight genes are annotated, including 'heparan sulfate proteoglycan 2 (perlecan)'. GO analysis for settlement determined that the top GO terms for Cellular Components were 'intrinsic to membrane' (GO:0031224) and 'extracellular region' (GO:0005576) and among the top terms for Molecular Function were 'sulfotransferase activity' (GO:0008146) and 'receptor activity' (GO:0004872) (Fig. 25A,B). For Biological Processes, less strong associations were observed (data not shown). When gene expression patterns ranked by settlement are visualized for specific GO categories, I observe that as settlement increases, so does expression of genes within these GO categories indicating that larvae up regulating these genes are predicted to settle in higher proportions (Fig. 25C).

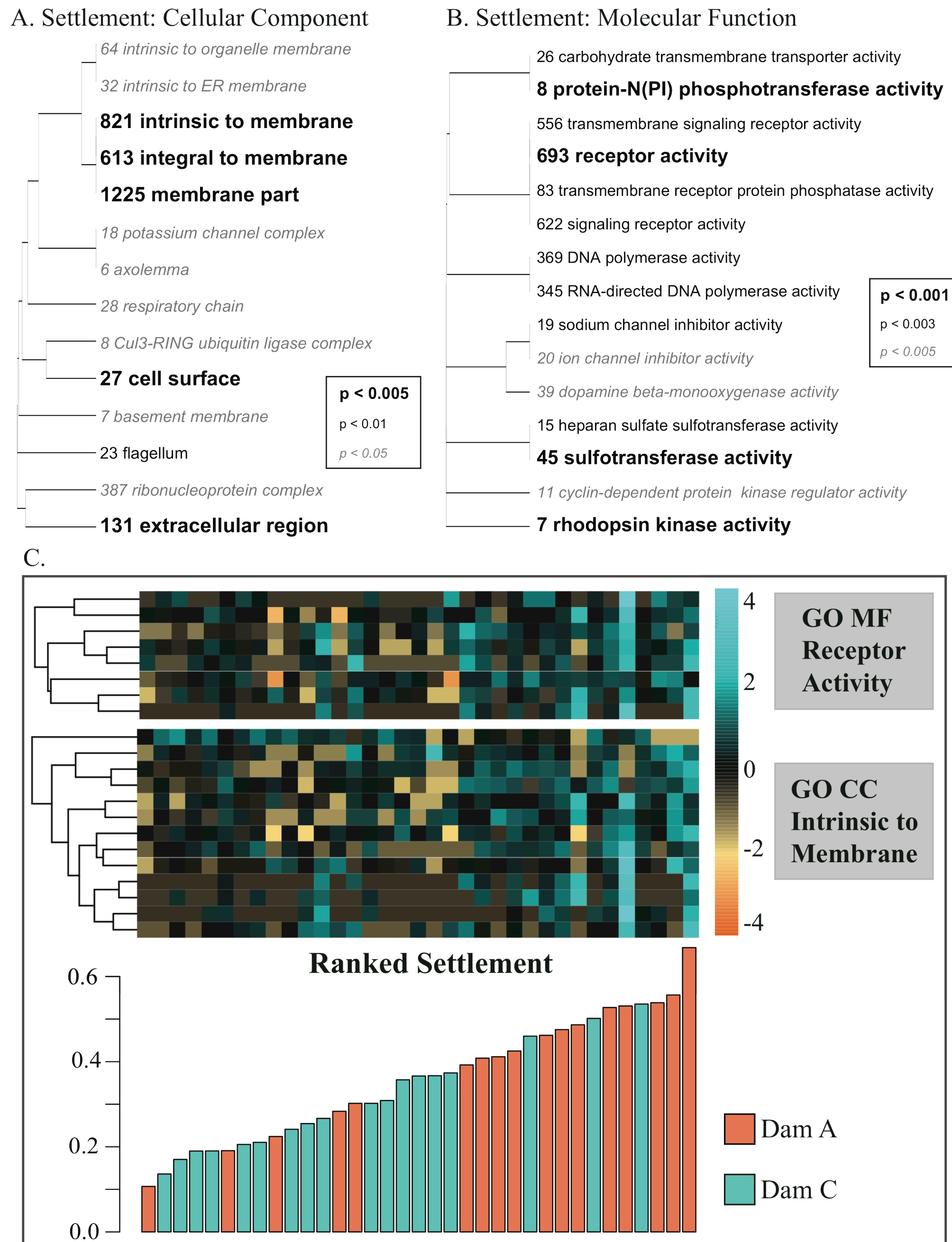


Figure 25: GO analysis for DEGs with respect to settlement
A. Significant Gene Ontoloty (GO) terms for cellular component. B. Significant GO terms for molecular function. C. Differentially expressed genes (DEG) in two of the top GO categories demonstrating the strong positive relationship between larval gene expression at Day 3 and settlement propensity at Day 5.

Gene expression associated with variation in fluorescence

When red fluorescence was correlated with gene expression, 489 genes in three-day-old larvae significantly predicted fluorescence for five-day-old larvae (489 DEGs FDR 0.1). Of these 489 genes, the top DEG was red fluorescent protein (RFP). When RFP expression is correlated with red fluorescence measured for each culture, a strong and highly significant correlation is observed (p-value<0.001) and 60% of the variation in red fluorescence is explained by RFP expression (Fig. 26D top). RFP expression also varies significantly with sire and dam, indicating that inheritance of RFP expression is additive (Fig. 26D bottom). GO analysis for fluorescence determined that top GO terms for Biological Processes were 'reactive oxygen species metabolic process' (GO:0072593), 'hydrogen peroxide metabolic process' (GO:0042743), and 'translation' (GO:0006412) (Fig. 26A). Top GO terms for Cellular Components were 'ribosome' (GO:0005840) and 'mitochondrial part' (GO:0044429") and among the top terms for Molecular Function were 'structural constituent of ribosome' (GO:0003735) and 'oxidoreductase activity' (GO:0016491) (Fig. 26C). When gene expression patterns ranked by fluorescence are visualized for specific GO categories, we observe that for some GO categories (mitochondria, translation, structural constituent of the ribosome), as red fluorescence increases, gene expression decreases. This suggests that larvae down regulating genes in these categories are predicted to have increased red fluorescence (Fig. 26E,F). For other GO categories (ribosome, reactive oxygen species metabolic process, and oxidoreductase activity) we observed differential gene expression (Fig. 26E,F).

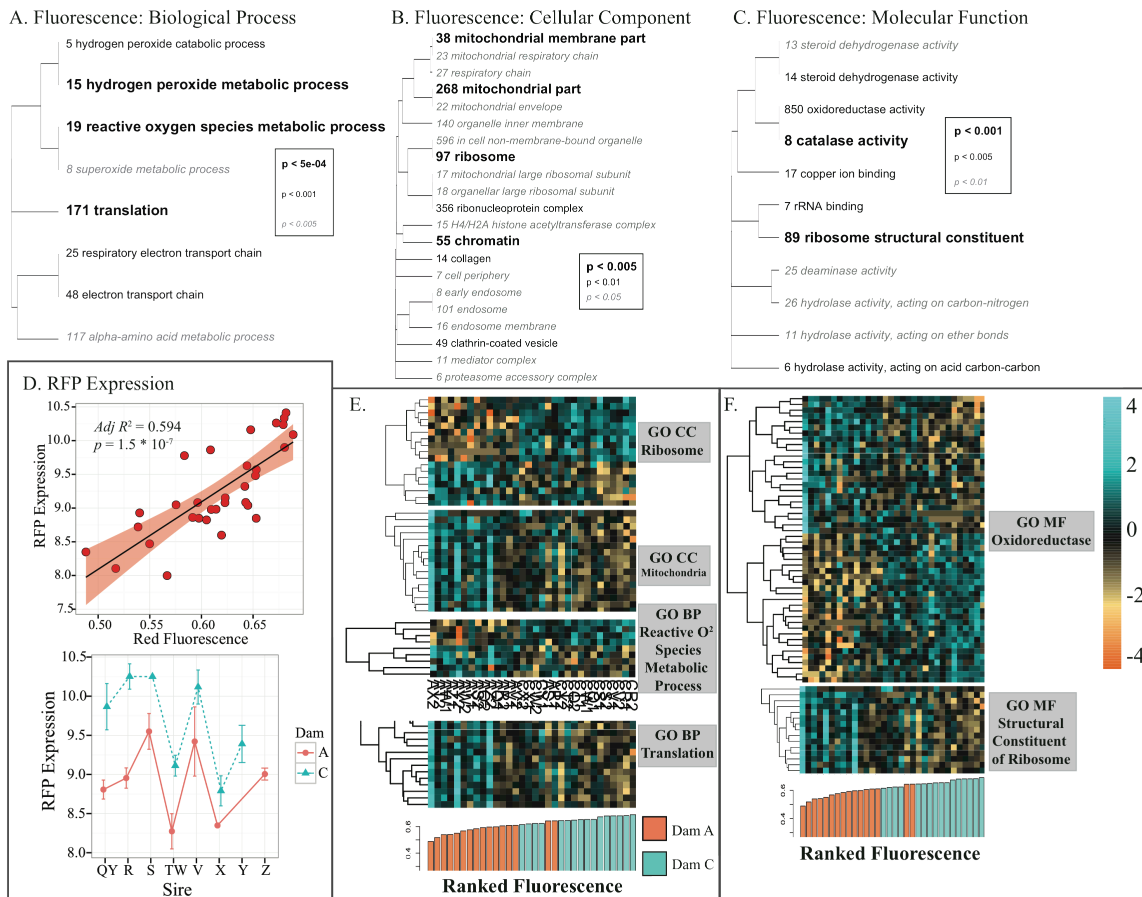


Figure 26: GO analysis for DEGs with respect to fluorescence
A. Significant Gene Ontology (GO) terms for biological processes. B. Significant GO terms for cellular component. C. Significant GO terms for molecular function. D. Expression of a red fluorescent protein (RFP) is correlated with red fluorescence phenotype (top) and illustrates additive genetic effects with respect to dam and sire (bottom). E. Top DEGs in six of the top GO categories demonstrating differential expression with respect to fluorescence phenotype.

DISCUSSION

Heritability of dispersal-related traits

Here we present a study that investigates the heritable variation in four larval dispersal related traits of a reef-building coral to better understand how coral dispersal

might evolve under climate change. The response of a trait to selection depends on the strength of selection and the narrow-sense heritability of that trait, defined as the proportion of phenotypic variance due to additive genetic effects (Lynch & Walsh, 1998). Of the four traits measured, none demonstrated significant narrow-sense heritability (h^2), however significant broad-sense heritability (H^2) was detected for settlement and fluorescence. Because estimates for H^2 include the contribution of dam, sire, and their interaction, I cannot discount the effects of maternal environment (maternal effects, such as egg quality) on these estimates. However in light of previous work and these gene expression results, I propose that this variation has a heritable component and that selection could act on some proportion of this variation.

Responsiveness of coral larvae to settlement cues could be an important determinant of dispersal potential, because larvae that respond early would be less likely to disperse far (Kenkel et al., 2011; Miller & Mundy, 2003). Here we observe H^2 for settlement to be 0.45 (Fig. 22A). While the credible intervals on this estimate are large, this number closely matches a previous estimate of h^2 of 0.49 for corals from the same population under the same conditions (Meyer et al., 2009). Fluorescence was found to be highly heritable and parental effects explained 74% of the variation (mean H^2=0.74) indicating that this trait could respond rapidly to selection (Fig. 22B). (Kenkel et al., 2011) previously documented parental effects on fluorescence (H^2 18%), however this estimate is about four times larger (74%). (Kenkel et al., 2011) also documented a significant correlation between larval fluorescence and settlement, where redder larvae were less likely to settle. In this study I observed no such correlation (Fig. 22A,B). The contrast between these findings and previous studies on larvae of *A. millepora* could be due to environmental or genetically determined differences among corals used in this study. Regardless, the magnitude of the phenotypic variation for settlement and

fluorescence observed here suggests that genetically controlled variation in these traits could be an important determinant of dispersal potential in some coral populations, with potential implications for range shifts during climate change.

Dispersal potential in corals is likely to be correlated with the time spent in the plankton (Hellberg, 2007); however, this planktonic time is expected to be constrained by energetic reserves allocated by maternal colonies and the rate at which these reserves are depleted (Harii, Nadaoka, Yamamoto, & Iwao, 2007; Richmond, 1987). Larvae of *A. millepora* are lecithotrophic (non-feeding) so metabolic rate of their reserves was hypothesized to play a role in dispersal, especially because these genes have been previously demonstrated to be under positive selection in corals (Voolstra et al., 2011). The experiments executed here were unable to detect any heritable signal in lipid or protein depletion rates. We observed substantial variation between families (Fig. 22C, D), however this variation was also quite high among culture replicates. We believe that the lack of signal here does not necessarily rule out heritability of these traits, especially because previous research has suggested a heritable signal in larval protein loss in *A. millepora* (Meyer et al., 2009). In the future more thorough replication schemes with higher technical replicates might reduce the noise and be able to disentangle small, but significant variation with respect to genetic background.

Coral gene expression is heritable

While much coral transcriptomic research has been dedicated to understanding how reef-building corals might respond to environmental stressors (e.g. (Barshis et al., 2013; Kenkel, Meyer, & Matz, 2013; Polato, Voolstra, et al., 2010b)), little is known about how heritable variation in such expression might be. Only three studies have

explicitly investigated heritability in expression and two cases only used a targeted gene approach (Csaszar et al., 2010; Meyer et al., 2009). Results from these studies were inconclusive: Meyer et al. (Meyer et al., 2009) found genetic variation in expression, while Csaszar et al. (Csaszar et al., 2010) detected no heritable component. However, in a paper describing the RNAseq method employed here, Meyer et al (Meyer et al., 2011) provided preliminary evidence for family specific expression levels. Heritable expression is expected in corals because it is highly heritable in many other systems across the phylogeny ranging from yeast to humans (yeast: (Brem, Yvert, Clinton, & Kruglyak, 2002), *Drosophila:* (Jin et al., 2001), butterflies, (Kvist et al., 2013), maize: (Schadt et al., 2003), and human cell lines, (Cheung et al., 2003)). In this study, I demonstrate strong evidence for heritable gene expression in *A. millepora* larvae. 577 genes were differentially expressed with respect to sire (Fig. 23A, D) and these expression patterns were so consistent that we were able to identify adult clones through differential expression in their offspring (Fig. 23B). This heritable variation in transcription likely serves as the raw material for evolution (Whitehead & Crawford, 2006).

Gene Ontology (GO) analysis of gene expression patterns with respect to sire demonstrate up-regulation of RNA-dependent DNA replication, perhaps suggesting variation in transposable element (TEs) activation and overall genome instability (Fig. 26A). Up-regulation of this category is generally a common feature of the eukaryote stress response (e.g. *Drosophila* (Ratner, Zabanov, Kolesnikova, & Vasilyeva, 1992), silkworm (R. H. Kimura, Choudary, Stone, & Schmid, 2001), black tiger shrimp (de la Vega, Degnan, Hall, & Wilson, 2007)) and has also been observed in the broadcast-spawning coral *Orcibella (Montastrea) faveolata* in response to stress (DeSalvo et al., 2008). GO categories 'immune response' and 'tumor necrosis factor (TNF) receptor superfamily binding' were also significant among sires (Fig. 26A,B). TNFs have been

shown to play important functions in immunity, inflammation, differentiation, control of cell proliferation and apoptosis (Shen & Pervaiz, 2006). Specifically, TNF receptors are known to regulate the immune system by binding to TNFs thereby triggering the release of active NF-kB, which activates genes involved in cell survival and inflammation (Shen & Pervaiz, 2006). In *A. millepora's* congener *A. hyacinthus*, TNFs display both differential expression under heat stress and significant "frontloading" (constitutively higher expression) in populations from more variable environments, implying that higher constitutive expression of these genes confers physiological resilience (Barshis et al., 2013). The fact that we see heritability of these immunological pathways in these data suggests that variation in immune response exists within populations of *A. millepora* even among corals on the same reef.

Overall, these data demonstrate strong evidence for heritability of gene expression, which is a significant finding in itself because even minor evolutionary changes in gene expression are likely to be biologically significant (Crawford & Oleksiak, 2007). These data also indicate that *A. millepora* coral larvae demonstrate considerable genetic variation even within the same reef. I also offer preliminary evidence that larval expression differences among sires correspond to differences in immune capabilities and genome instability, suggesting heritable variation in overall physiological condition. In agreement with Meyer et al. (Meyer et al., 2011), these results suggest that bulk-culturing larvae from multiple parents might obscure potentially important variation in expression.

Genes predicting settlement

Here I correlated gene expression patterns of three-day-old larvae with phenotypic variations in settlement observed in five-day-old larvae. This study is the first to investigate gene expression prior to settlement in a predictive context. Several studies have examined transcriptomic profiles of larvae exposed to both natural inducers (CCA) (Grasso et al., 2011; Meyer et al., 2011) and synthetic peptide (GLW-amide) (Meyer et al., 2011). While these response pathways inform physiological responses to known inducers, it is perhaps more interesting to identify genes whose expression predicts the magnitude of these responses, potentially affecting dispersal.

Some of the most enriched GO categories associated with settlement are 'extracellular region' and 'intrinsic to membrane'. In corals, three extracellular matrices are known to be involved in spatially organizing cells according to function, however in larvae, only one of these matrices is relevant: the organic extracellular matrix (Helman et al., 2008). I observe that gene expression in GO categories such as 'receptor activity' and 'membrane signaling' positively correlate with settlement suggesting that cell-to-cell signaling and genes involved in sensing the extracellular environment modulate settlement. Involvement of these signaling and communication genes in settlement is interesting because previous research has shown that the decision to settle is complex, species specific (Davies, Chapter 1), and this decision ultimately determines larval success (Harrington et al., 2004).

Enrichment of gene expression in 'extracellular region', coupled with enrichment of 'sulfotransferase activity', also suggests variation in of extracellular matrix (ECM) production. In fact, one of the top DEGs correlated with settlement is 'heparan sulfate proteoglycan 2 (perlecan)'. Proteoglycans are known to be a component of the ECM in both invertebrates and vertebrates (Czaker, 2000; Har-el & Tanzer, 1993). Specifically in

corals, these proteins have been shown to mediate cell-cell and cell-substratum adhesion (Helman et al., 2008). This information coupled with gene expression results suggest that ECM proteins may be directly involved in settlement. ECM production in calcium-secreting life stages of corals has been shown to respond to changing environmental conditions (B. E. Brown & Bythell, 2005; Jatkar, 2009) and genes in this GO category are enriched in *A. millepora* recruits responding to pCO_2 elevation (Moya et al., 2012). These data suggest that the coral ECM has multiple functions and/or these functions may vary between life stages.

Genes associated with fluorescence

Across coral gene expression studies, one common theme is the differential expression of fluorescent proteins (FPs) in response to environmental perturbations. FP down-regulation in response to transplantation or temperature stress have been consistently observed (Bay, Ulstrup, et al., 2009; DeSalvo et al., 2008; Rodriguez-Lanetty, Harii, & Hoegh-Guldberg, 2009; Smith-Keune & Dove, 2008) as well as FP up-regulation in response to elevated light or infection (Palmer, Modi, & Mydlarz, 2009; Seneca et al., 2010). Consistent expression differences across species suggest a functional role for these proteins; however, the exact function is unknown. In addition to FP expression differences in response to environment, genetic effects on FP expression (Meyer et al., 2011) and phenotype (Kenkel et al., 2011) have also been shown. Not surprisingly, red fluorescent protein (RFP) is one of the top DEGs, and, in agreement with previous work, we observe evidence for additive genetic variation in RFP expression with dam A consistently expressing more RFP than dam C, and sires modulating the magnitude of expression (Fig. 26D lower). RFP was also the top DEG

predicting fluorescence phenotype and expression three days post fertilization explained 60% of the variation in phenotypic red fluorescence five days post fertilization (Fig. 26D upper).

GO analysis of expression correlating with red fluorescence suggests that redder larvae could be metabolically suppressed and experiencing stress. I observe that larvae with increased red fluorescence are down-regulating expression for GO terms associated with translation, mitochondria, and ribosome, all consistent with suppressed growth and development (Fig. 26). Metabolic suppression can be accomplished by minimizing processes such as protein synthesis, which could be adaptive for short-term survival since substantial bioenergetics savings are achieved (Hand & Hardewig, 1996; Lopez-Maury, Marguerat, & Bahler, 2008), however suppression is likely to reduce fitness if maintained long term. Other studies have observed down-regulation of ribosomal genes and metabolic suppression in response to stress (Meyer et al., 2011; Moya et al., 2012; Polato, Voolstra, et al., 2010a; Voolstra et al., 2011), so it is tempting to speculate that redder larvae are indeed experiencing more stress. Further evidence that red fluorescence is correlated with stress is enrichment of the GO terms 'oxidoreductase' and 'reactive oxygen species metabolic process', both known to be associated with stress response in corals (DeSalvo et al., 2010; Lohelaid, Teder, Toldsepp, Ekins, & Samel, 2014).

Because the majority of variation in fluorescence in this experiment was due to difference between just two dams, the possibility remains that the association of red fluorescence with stress phenotype is purely coincidental. Still, mounting evidence suggests that red fluorescence imparts some sort of fitness consequence (Roth & Deheyn, 2013). If so, it is necessary to consider how it might be maintained in a population if fitness consequences are substantial and the trait is heritable ((Kenkel et al., 2011); this study). One mechanism for maintenance of red fluorescence is the presence of ontogentic

shifts in selection coefficients. Tradeoffs in life-history traits across life history stages have been observed for the *Eda* locus in sticklebacks. In this system, ancestral armored plates are selected as larvae but derived alleles (reduced plates) are selected in later life stages because plate reduction allows for increased growth (Barrett, Rogers, & Schluter, 2008). For *A. millepora,* surveys of adult colonies on the Great Barrier Reef determined that across adult populations, red color morphs are consistently the most abundant corals on the reef, perhaps providing some evidence for ontogenetic shifts in selection on fluorescence (Paley & Bay, 2012).

CONCLUSIONS

Data presented here provide the first whole-transcriptome analysis of heritability of gene expression in coral larvae. This study is also the first to investigate how gene expression patterns predict phenotypic outcomes. These findings support the conclusion that additive genetic variance exists for some dispersal-related traits (gene expression) and these traits are expected to have consequences under climate change. Although only eight sires were measured, a number quite small for estimating variance components with confidence or for making robust inferences about population-level heritability, this analysis provides strong evidence for heritable expression differences between sires. I also observed insightful expression patterns predicting phenotypic traits including up regulation of membrane proteins correlating with increased settlement and reduction of growth and increased stress correlating with red fluorescence. While response to selection depends upon effective population sizes and generation times, the magnitude of variation observed here, within a single reef, provides evidence that variation in dispersal-related traits exists and might be selected for under climate change.

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