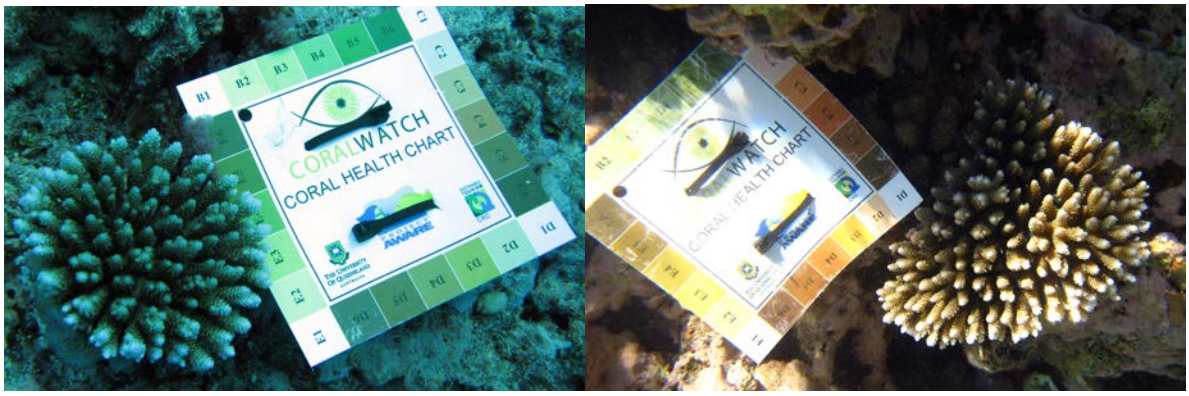
Investigating host and symbiont genetic structure in the reef-building coral *Acropora hyacinthus* from two varying reef environments in French Polynesia

Sarah W. Davies, Ph.D.



Laboratory: CRIOBE, USR3278, CNRS EPHE
Collaboration: University of Texas at Austin, USA
PI: Mikhail V. Matz, University of Texas at Austin, USA
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Research Report August 2014

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Final Report: Investigating Host-Symbiont Genetic Structure in Moorean Corals
Sarah W. Davies M. Sc. Ph.D. University of Texas at Austin
CRIOBE IRCP Tahiti Perles
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I. INTRODUCTION

The ability of a species 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). Coral reefs are increasingly threatened by shifts in the world's climate and by habitat fragmentation due to anthropogenic stressors (Hoegh-Guldberg *et al.* 2007), therefore a thorough understanding of genetic and demographic connectivity among populations remains a priority.

The majority of corals release gametes into the water annually to produce planktonic larvae that are dispersed by ocean currents, representing the coral's only dispersal opportunity (Fig 1). Since directly tracking these larvae has, to date, been impossible (Selkoe & Toonen 2006), an effective method of evaluating coral connectivity uses genetic techniques. Historically, marine pelagic larvae were thought to disperse large distances (i.e. (Vernon 1995)), but with coral populations in massive decline, it is clear that explicit estimates of genetic population structure are essential to managing coral reefs.

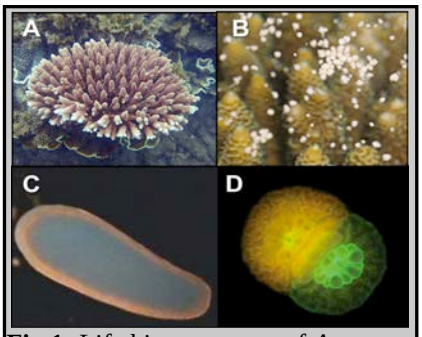

Fig 1: Life history stages of *Acropora spp.* (A) Adult colony; (B) adult colony releasing hermaphroditic gametes during annual spawning event; (C) planula larva; (D) two post-metamorphic recruits.

Corals possess symbiotic algae (zooxanthellae) of the genus *Symbiodinium* and this symbiosis is extremely important to overall fitness of the coral. The process known as coral bleaching occurs when the symbionts are lost or expelled from the host leaving behind the white color of the calcium carbonate skeleton. As symbionts can supply up to 95% of the host's energy requirements (Muscatine 1990), bleaching is very damaging and is an indication of reduced reef health. Bleaching can be induced by many environmental stressors such as change in water temperature, increase in UV, bacterial infections, herbicides, and many others factors, and is considered one of the main threats to coral reefs globally. Research has shown that different strains of *Symbiodinium* confer different thermal tolerance (i.e (Jones & Berkelmans 2010)), so understanding symbiont diversity within and between coral populations is important for

predicting bleaching events (Abrego *et al.* 2009). In the majority of broadcast spawning corals, larvae acquire their symbionts from the local environment rather than directly from their parents. Because of this transmission strategy, we expect increased selection for local adaptation in symbionts resulting in significant population structure, since they are acquired locally and even marginal changes in their performance can have huge fitness consequences.

Compared to Australia's Great Barrier Reef and other reefs within the Coral Triangle, few studies have looked at coral host connectivity among remote Pacific reefs, and few have explored potential differences in symbiont genetic structure. Pacific reefs occur as discrete habitats across vast oceanic distances and little is known about the source-sink dynamics occurring between these reefs, however these data are imperative to reef conservation. Studies have projected larval dynamics and possible exchange between reefs (Kool *et al.* 2011; Treml *et al.* 2008), however the value of these models for coral reef management remains uncertain as no genetic data were used in generating these models.

French Polynesian coral reefs are interesting for studying population connectivity because, since the 1980's, these reefs have experienced several major perturbations. These events include outbreaks of corallivorous crown-of-thorns starfish (*Acanthaster planci*), multiple cyclones (i.e. Cyclone Oli), and a number of intense bleaching events (Adjeroud *et al.* 2009; Pratchett *et al.* 2013; Pratchett *et al.* 2008; Pratchett *et al.* 2009). However, even though reef cover during these events has been greatly reduced, especially on the forereef, juvenile coral recruitment has been remarkably high (Adam *et al.* 2011), indicating some level of connectivity between neighboring reefs. One island in French Polynesia, Moorea, is particularly interesting for studying connectivity since it has a unique long term monitoring project that has set up six sites in three different habitats since 2005. Adding a genetic component to this monitoring project will greatly ameliorate our biological understanding, as well as the management of coal reefs.

In this study I have used genetic tools to infer genetic connectivity between discrete populations across Moorea, Tetiaroa, and Tahiti for the reef-building coral *Acropora hyacinthus*. I also analyzed symbiont genetic structure across these islands using next generation metabarcoding techniques, to help substantiate the theory of locally adapted symbionts in broadcast spawning corals. This dataset helps to define the migration limits of acroporid corals

and helps us understand host and symbiont genetic structure and diversity across Moorean reefs to better predict the coral holobiont response to global climate change.

Overarching Hypothesis:

Due to their relatively long pelagic larval durations I hypothesize that Moorean acroporid corals will exhibit large dispersal ranges, while their symbionts will be highly differentiated in comparison to the host.

II. SPECIFIC AIMS

Aim 1. Determine the connectivity patterns and distribution of genetic diversity in *Acropora hyacinthus* on French Polynesian reefs, specifically targeting Moorea.

Aim 2. Determine the connectivity patterns and distribution of genetic diversity in symbionts of the coral host *Acropora hyacinthus* across two different habitats on French Polynesian reefs.

These aims represent complementary approaches to better understand reef connectivity, and will provide evidence for or against the theory of local adaptation in the symbionts of broadcast spawning corals.

III. STUDY SYSTEM

Acroporid corals are the most prominent reef building corals in the Pacific and provide habitat for many commercially important invertebrate and fish species. They provide both ecological and societal services and therefore they remain a top conservation priority. These corals are also especially sensitive to climate-induced coral bleaching so understanding their symbiont population genetics also represents a current gap in knowledge for management. From an evolutionary perspective, studying the connectivity of acroporid corals is interesting since they have some of the largest geographical ranges of any corals (i.e. *A. digitifera* range >100,000km^2) suggesting the potential for long-range dispersal. Since genetic tools are currently the most effective way to measure connectivity in corals, acroporid corals are good study organisms since they have the most genetic information available for any coral genus worldwide. The focal species for this study is the Indo-pacific staghorn coral of the genus *Acropora*, *A. hyacinthus*. ITS sequencing results indicate this species hosts *Symbiodinium* Clade C for which significant

genetic information is also currently available.

IV. MATERIALS AND METHODS

A. Sampling Locations and Methodology

From July 26th-August 9, 2013, my field assistant, Matthew R. Kanke and myself visited 9 reefs on 3 islands in French Polynesia (Fig. 2) and stayed at the CRIOBE station on Moorea. Our goal was to sample different spatial scales: reefs on different islands, reefs on the same islands, and paired reefs (inner/outer barrier) at the same sites. A combination of boats, vehicles and kayaks were used to transport collectors. Snorkeling or scuba were used to sample >25 individuals of our focal coral species (*A. hyacinthus*) per reef, a sample size adequate for population assignment tests (Hellberg 2007). Before sampling, each colony was photographed to determine color, size and for confirmation of correct species identification (when possible). Small (~2cm) coral branch tips were collected, preserved in 96% ethanol, and kept at -20°C. Samples were transported to the University of Texas at Austin under CITES export permit #FP1398700064-E. Table 1 has specific site information along with the number of corals collected at each site. The numbers of corals collected at each site represents the total number of corals collected and are all assumed to be *A. hyacinthus*, however species identification can be difficult and future whole-genome analyses will most likely reveal cryptic diversity (Davies et al., in preparation).

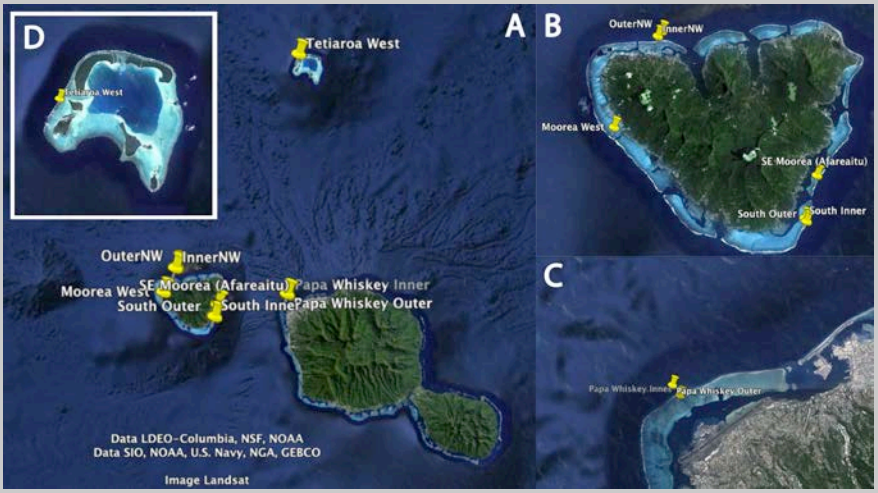

Fig 2: French Polynesia island locations where *A. hyacinthus* were sampled In August 2013. A. Overall map of collections in French Polynesia. B. Inset of Moorea collection sites. C. Inset of Tahiti collection sites. and D. Inset of Tetiaroa collection sites. All collection sites are marked with a yellow pin.

Table 1: Site information including GSP coordinates, site description and number of corals sampled for all French Polynesian sites collected at in August 2013.

Site	GPS	Description	Number of Corals
Moorea NW Inner	17°29'6.02S; 149°52'59.87W	Inner Reef Off Papetoai	54
Moorea NW Outer	17°28'55.57S; 149°52'59.87W	Outer Reef Off Papetoai	51
Moorea E Inner	17°33'59.68S; 149°47'5.78W	Inner Reef Off Central Afareaitu	50
Tetiaroa W Inner	17°0'34.44S; 149°35'40.77W	West Inner Reef Off Tetiaroa	75
Moorea SE Inner	17°35'28.12"S; 149°47'35.98"W	Inner Reef Off South Afareaitu	98
Moorea SE Outer	17°35'34.66"S; 149°47'31.75"W	Outer Reef Off South Afareaitu	49
Moorea W Inner	17°32'18.69"S; 149°54'40.39"W	Inner Reef Off Central Haapiti	65
Tahiti NW Inner	17°32'44.80"S; 149°37'16.58"W	Inner Reef Off Airport	55
Tahiti NW Outer	17°32'34.58"S; 149°37'24.47"W	Outer Reef Off Airport	50

B. Coral Host Population Genetics Using SSR Loci

I. Laboratory Procedures: With the help of four personally trained undergraduate assistants, DNA was isolated from all 547 coral samples using a customized DNA extraction protocol (Davies *et al.* 2013). DNA was then quantified using a NanoDrop 1000 Spectophotometer (Thermo Scientific) and diluted to 10ng/µl. A multiplexed assay of twelve microsatellite loci (modified from (Wang *et al.* 2009)) was established and loci were amplified in 10 µl polymerase chain reactions (PCR). Multiplex and primer information are located in Table 2. 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 499 DNA samples were successfully genotyped across the microsatellite panel.

Table 2: Summary of twelve microsatellite loci transferable from *A. millepora* SSR markers (Modified from (Wang *et al.* 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.

II. Genetic diversity and Population Differentiation: 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). To determine population genetic subdivision between all sites and islands (sites within islands pooled), pairwise F_{ST}'s and unbiased Nei's genetic distances were calculated also using GENALEX v6.5 (Peakall & Smouse 2006). Pairwise Nei's unbiased genetic distances were applied to create two-dimensional Principle Coordinate Analysis (PCoA) plots for islands and sites to visualize the divergences between populations.

C. Holobiont divergence across habitats: To better understand how the host and symbiont was structured between inshore and offshore reefs, we only analyzed a subset of the sampling sites. We used holobiont samples from all sites where we had sampled both the inshore and the offshore site (Moorea NW, Moorea S, and Tahiti). We also only included holobiont samples that we were confident of species identification (based on photographs). For the host genetics, we used a total of 121 individuals (Moorea NW Inner: 16, Moorea NW Outer: 22, Moorea S Inner:

24, Moorea S Outer: 17, Tahiti Inner: 19, Tahiti Outer: 23) and for the *Symbiodinium* genetics we used N=16 per site, which were all subset of the individuals used in the host genetics.

I. Host: Bayesian Clustering and Genetic Structure Analysis: Results from the SSR data described above were used as the input data into STRUCTURE. Log-likelihood values for each K (number of inferred populations) (1–5) were computed by running STRUCTURE on the data subset for *A. hyacinthus* (12 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 *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.

II. Symbiont: Genetic Structure Using Next-Generation Sequencing of ITS Locus

a. Amplicon Sequencing Laboratory Procedures: Symbiont DNA was amplified from the same coral holobiont samples used for host genotyping (N=96). The first step was determining the concentration of *Symbiodinium* present in the holobiont extraction. qPCR (protocol described in (Kenkel *et al.* 2013)) of the ITS2 fragment was used to assess *Symbiodinium* DNA concentrations to determine the optimal number of PCR cycles for each of the samples to avoid overamplification. Note: Cycle checking using qPCR is only efficient when only one *Symbiodinium* clade is present in your sample, if more than one are present (you will know from the present of multiple melt peaks), cycle checking must be done by eye using gel electrophoresis. For the sample subset used here, the cycle number ranged from 19-24 across our holobiont samples and all samples only hosted Clade C.

Once optimal cycle numbers was established, the first step in the amplicon sequencing protocol is amplifying the ITS2 region via PCR using 0.33 uM modified forward *its-dino* (5' TCGTCGGCAGCGTC AGATGTGTATAAGAGACAG **GTGAATTGCAGAACTCCGTG** 3') and 0.33 uM reverse *its2rev2* (5' GTCTCGTGGGCTCGG AGATGTGTATAAGAGACAG **CCTCCGCTTACTTATATGCTT** 3') primers where the underlined portion of the primer corresponds to the adapter linker, the bold bases correspond to the previously published primer

sequences for ITS2 (Pochon *et al.* 2001), and the extra bases are for PCR stability. 15 ul PCR mixtures contained 50 ng of DNA template, 0.33 μM of each of the two described primers, 0.2 mM dNTP, 1.5 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 95°C for 5 min, followed by 19-24 cycles of 95°C for 40 s, 59°C for 120 s, and 72°C for 60 s and then a 10 minute extension period at 72°C. Each resulting PCR was then cleaned with the ThermoFisher Gene Jet PCR clean up kit and eluted in 25ul of elusion buffer. Next, Miseq Illumina barcoded adapters hybrids were added to both ends of the product using 0.15 uM forward (5' AATGATACGGCGACCAC CGAGATCTACAC *NNNNNN* **TCGTCGGCAGCGTC** 3') and 0.15 uM reverse (5' CAAGCAGAAGACGGCATAC GAGAT *NNNNNN* **GTCTCGTGGGCTCGG** 3') primers. Here, bold sequences correspond to the adapter linker, underlined bases correspond to the Miseq adapter, and each forward and reverse Miseq adapter also has an incorporated unique six base pair barcode (delineated as *NNNNNN* here). PCR conditions were the same as the previous reaction only different primers were used, the reaction volume was 20 ul, 1.5 ul of the PCR-cleaned product from the first PCR was used as template, and only 5 cycles were completed (95°C for 5 min, followed by 19-24 cycles of 95°C for 40 s, 63°C for 120 s, and 72°C for 60 s and then a 10 minute extension period at 72°C).

All samples were verified on a gel and 10 μl of each barcoded sample was pooled for the Miseq sequencing run based on visually assessed band intensity. The pooled sample was cleaned via ethanol precipitation and re-suspended in 25 μl milli-Q water. 10 μl of this cleaned product was run on a 1% Agarose gel stained with SYBR Green (Invitrogen) for 45 min at 100V. The gel was visualized on a blue-light box and the target band excised using a clean razor blade and placed in 25 μl milli-Q water for overnight incubation at 4°C. The resulting supernatant was then submitted for Miseq Illumina sequencing.

b. Miseq Data Analysis: In total, sequences were obtained for 96 individuals: N=16 at each inner and outer reef from 3 sites (NW Moorea, S Moorea, and Tahiti). First, all sequences in the '.fastq' Illumina output files were renamed to include sample designation. All '.fastq' files were concatenated into a single file for OTU identification. cd-hit-otu-illumina-0.0.1 was used for OTU identification using the cd-hit-otu-all-pair.pl command for paired-end Illumina data

clustering at 100% due to high sequence similarity amongst samples (Quigley *et al.* 2014). The total number of reads mapping across samples was 350,088 with a mean of 3,842 sequences per sample across 139 OTUs.

The output table from cd-hit-otu was then used as the input file for the *MCMC.OTU* statistical package (Green *et al.* 2014) implemented in R (R Development Core Team 2013). This package first identified outlier samples (low sequence coverage) and OTUs that do not pass the frequency cut-off of 0.001 (Quigley *et al.* 2014). The package was then used to identify significant OTU differences between inner and outer reefs as well as differences between islands regardless of reef type. Data were then visualized using Principle Component Analysis (PCA) using the package *vegan* implemented in R (Oksanen *et al.* 2013). Consensus sequences from the cd-hit-otu output was then used as a query to blast against the GenBank (NCBI) nucleotide collection to determine which clades were present among OTUs.

V. FINAL RESULTS

A. Host Population Genetics

I. Genetic Diversity Among Islands: Within-island Shannon diversity estimates (sHa) ranged from 1.691 (Tetiaroa) to 1.783 (Moorea) with no significant differences observed between islands. Private allele number (Pa) per island ranged from 0.083 to 2.00 with Moorea having the largest value, however Moorea also was the most densely sampled island as well. Mean allele number per island ranged from 11.44-15 (Table 3).

Table 3. Genetic summary statistics of twelve microsatellite loci from three islands for *A. hyacinthus.*

Island	N	Na	Ho	He	Fis	sHa	Pa
Moorea	332	15	0.588	0.709	0.182	1.783	2.000
Tetiaroa	73	11.33	0.595	0.699	0.160	1.691	0.083
Tahiti	94	12.25	0.595	0.723	0.189	1.764	0.667

*II. **Genetic Diversity Among Sites Within Islands:*** Shannon diversity estimates (sHa) between all sites ranged from 1.497 (Moorea SE Inner) to 1.809 (Moorea W Inner) with no large differences between sites. Private allele number (Pa) per island ranged from 0.083 to 0.333 with both Tahitian sites having the largest values. Mean allele number per site ranged from 9.58-11.75 (Table 4).

Table 4. Genetic summary statistics of twelve microsatellite loci across nine sites on three islands for *A. hyacinthus.*

Island	Site	N	Na	Ho	He	Fis	sHa	Pa
Moorea	**NW Inner**	36	10.25	0.594	0.709	0.155	1.711	0.083
	NW Outer	48	9.58	0.581	0.639	0.116	1.512	0.167
	SE Inner	49	9.83	0.521	0.622	0.137	1.497	0.083
	S Outer	37	9.83	0.621	0.758	0.190	1.780	0.167
	S Inner	98	11.75	0.583	0.649	0.098	1.563	0.167
	W Inner	64	11.25	0.629	0.748	0.162	1.809	0.083
Tetiaroa	**Inner**	73	11.33	0.595	0.699	0.160	1.691	0.083
Tahiti	**Outer**	46	10.58	0.600	0.738	0.190	1.764	0.333
	Inner	48	10.25	0.591	0.696	0.177	1.648	0.250

III. Population Subdivision Among Islands: Significant global F_{ST} values were observed between islands (0.021), however differentiation between Moorea and Tetiaroa was an order of magnitude less (0.006) than the differentiation between Moorea and Tahiti (0.028) and Tahiti and Tetiroa (0.039) suggesting that Moorea and Tetiaroa are more connected than the other sites. Overall, however, all between island pairwise F_{ST} values were significant (Table 5). Principal Coordinate Analysis (PCoA) based on Nei's genetic distances explained 100% of the variation and nicely recapitulated island geographical configuration (Fig. 3).

Table 5. Summary of pairwise F_{ST} values between all island pairs. Permutations were run 999 times. All significant comparisons are shaded in grey.

	Moorea	Tetiaroa	Tahiti
Moorea	0.000	**	***
Tetiaroa	0.006	0.000	***
Tahiti	0.038	0.039	0.000

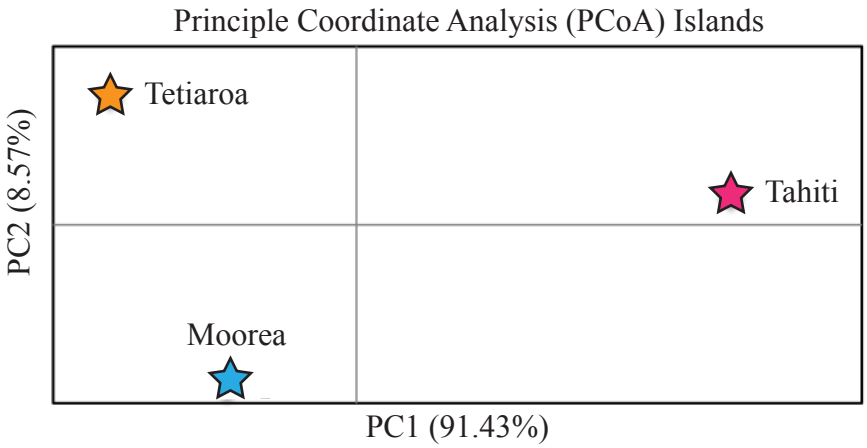

Figure 3: Principal Coordinate Analysis (PCoA) of genetic relationships (Nei's genetic distances) among *Acropora hyacinthus* from three different islands. PCoA explains 100% of the variation in these data.

IV. Population Subdivision Among Sites: Significant global F_{ST} values were observed between sites (0.042), and the only non-significant pairwise comparison was between Moorea NW Outer and Moorea SE Inner. Overall, all between site pairwise F_{ST} values were significant and ranged from 0-0.09, with the largest differentiation observed between Moorea W Inner and Moorea NW Outer (Table 6). Principal Coordinate Analysis (PCoA) based on Nei's genetic distances explained 79.3% of the variation and separated sites into four groups: 1. Tahitian sites, 2. Moorea S outer with Moorea W inner, 3. Moorea NW Inner with Tetiaroa, and 4. Moorea NW outer, SE inner and S Inner (Fig. 4).

Table 6. Summary of pairwise F_{ST} values between all site pairs. Permutations were run 999 times. All significant comparisons are shaded in grey. M=Moorea, Tet=Tetiaroa, Tah=Tahiti, I=inner, O=outer.

	M-NW-I	**M-NW-O**	**M-SE-I**	**M-S-O**	**M-S-I**	**M-W-I**	**Tet-I**	**Tah-O**	**Tah-I**
M-NW-I	0.000	***	***	***	***	***	***	***	***
M-NW-O	0.026	0.000	0.550	***	**	***	***	***	***
M-SE-I	0.023	0.000	0.000	***	***	***	***	***	***
M-S-O	0.028	0.078	0.073	0.000	***	***	***	***	***
M-S-I	0.032	0.005	0.011	0.077	0.000	***	***	***	***
M-W-I	0.031	0.090	0.082	0.021	0.084	0.000	***	***	***
Tet-I	0.009	0.023	0.016	0.039	0.022	0.040	0.000	***	***
Tah-O	0.015	0.059	0.058	0.014	0.055	0.022	0.031	0.000	***
Tah-I	0.038	0.074	0.081	0.040	0.069	0.038	0.052	0.010	0.000

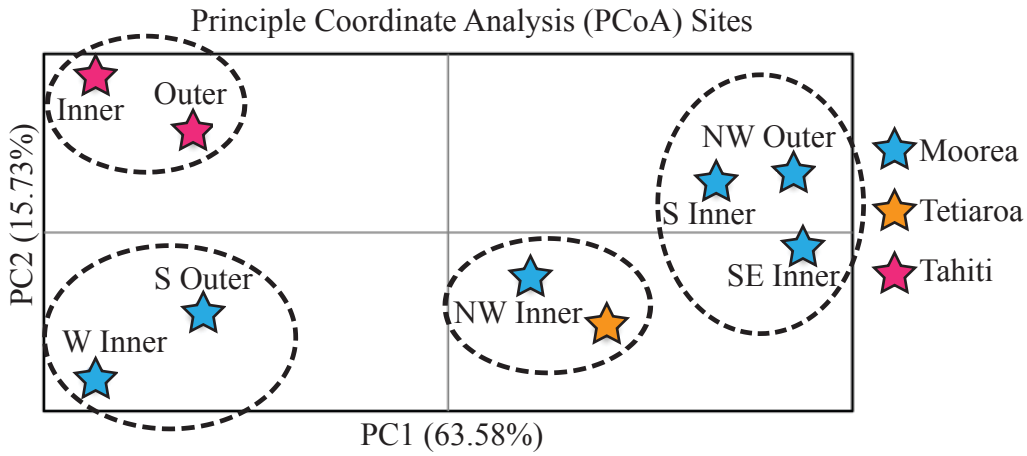

Figure 4: Principal Coordinate Analysis (PCoA) of genetic relationships (Nei's genetic distances) among *Acropora hyacinthus* from nine different sites across three different islands. PCoA explains 79.3% of the variation in these data.

B. Bayesian Analysis of Host Genetic Structure Between Habitats: STRUCTURE uses a Monte Carlo Markov chain (MCMC) clustering algorithm to assign individuals with similar multilocus genotypes to distinct populations. An optimal solution of K=2 clusters was used based on the ΔK statistic. STRUCTURE analysis demonstrated that individuals from Tahiti assigned strongly to the mint cluster only (Fig. 5), while Moorea populations were mixed with a strong divergence observed between Moorea South inner and outer. Moorea SE outer strongly assigned to the mint cluster, suggesting that this population is more related to the Tahitian populations than to other Moorean populations. Some individuals from Moorea NW inner also assigned strongly to the mint cluster, suggesting migration events between these populations or the presence of cryptic species. But overall most Moorean individuals, with the exception of SE outer corals, assigned to the navy cluster.

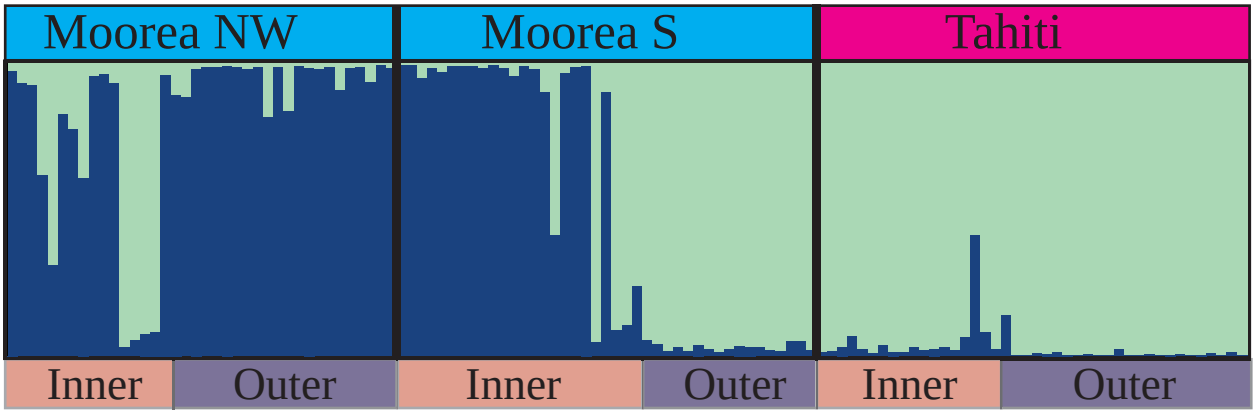

Figure 5: STRUCTURE population assignment for *Acropora hyacinthus* across inner and outer reefs on Moorea and Tahiti. The results are for an optimal population number (K) of 2. Each column is an individual coral and the color of the color corresponds to the population that it belongs to.

C. Analysis of *Symbiodinium* Structure Between Habitats

A total number of 540,474 sequences were generated by Miseq and of these 520,629 passed quality filtering. Clustering at 100%, 139 OTUs were detected and of these, 27 passed the frequency cutoff of 0.001. Three of the 96 samples were removed from analyses (76, 87, 513) due to low read numbers. Of the 27 OTUs that passed stringent cut-offs six were significantly different between inner and outer reefs with outer reefs hosting less diverse communities (Fig. 6A). Inner reefs had lower proportions of the dominant OTU (OTU2), however these corals had higher proportions of many of the background OTUs (21,23, 27). Six OTUs also significantly differed between islands; however only OTU23 and OTU27 were common among the OTUs differing between reef locations. For islands, several OTUs were island specific. For example, OTU14 was only found on Tahiti, while OTU23 and OTU27 were only present in Moorea (Fig. 6B). When data are viewed in principle coordinate space, we observe strong differences between Moorea inner and outer reefs, and fewer differences between Tahitian reef environments (Fig. 6C). When OTUs were blasted, all OTUs were found to be *Symbiodinium* Clade C.

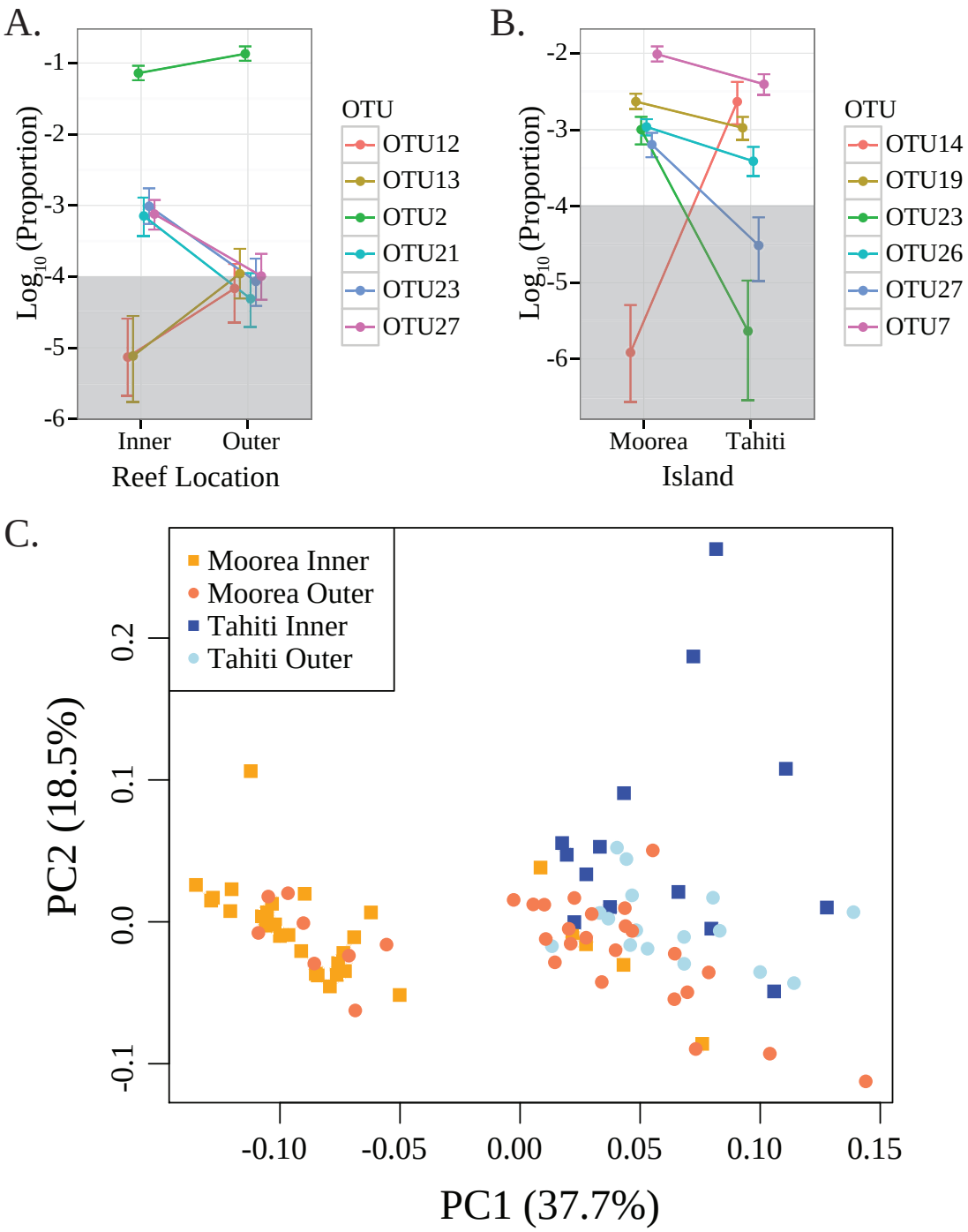

Figure 6: *Symbiodinium* population differences between reef types (inner and outer reefs) and islands (Moorea and Tahiti). A. Log_{10} proportions of the OTUs differing between reef environments. Data points falling within the grey box are considered not present due to the frequency cut-off of 0.001 (Quigley et al., 2014). B. Proportions of the OTUs that significantly differed between islands. C. Principle coordinate analysis (PCA) of Log_{10} proportions of OTUs color coded by island and reef type within islands. PCA explains 56.2% of the variation.

VI. PERSPECTIVES AND CONTINUING RESEARCH

This study has demonstrated the genetic connectivity of the recovering coral *Acropora hyacinthus* and its symbiotic partner in French Polynesia. We find that the coral hosts between Moorea, Tetiaroa, and Tahiti are highly connected, however some divergence exists, suggesting that these corals do not disperse the great distances that their con-specifics do elsewhere in the world, such a the Great Barrier Reef (van Oppen *et al.* 2011). For the coral symbionts, we find that all of the corals genotyped maintain symbiosis with the Clade C *Symbiodinium*. However, within Clade C we find significant differences in the relative proportions of different ITS2 variants between islands and between reef types (inner and outer reefs), perhaps suggesting the potential for local adaptation of the symbiont to its environment. The final results presented here are currently being prepared for manuscript submission to the journal of Molecular Ecology, a highly popular and well-cited journal for these types of data. We expect this study to be published by early 2015 and IRCP will be included in the author affiliations as well as in the acknowledgements.

An additional project has also stemmed from this IRCP grant. We have begun population genomic analyses of the inner and outer reefs (three population pairs of inner and outer reefs) using the Matz lab's 2bRAD genotyping method (Wang *et al.* 2012). 2bRAD genotyping resequences ~1% of the genome allowing for the detection of loci under selection in the different environments. Here our different environments are the inner and outer reefs, which are very ecologically different, so we expect different selection pressures to be on the corals in these different habitats. 96 of the 150 total sequencing libraries for this project have already been prepared, which were part of a genomics workshop taught by Sarah Davies and Mikhail Matz this past summer 2014. We are currently seeking additional funding to pay for the costs of sequencing these samples on Illumina and preparing the additional 54 libraries for the samples from Tahiti. For more information on the course, please see the link below:

http://www.bio.utexas.edu/research/matz_lab/matzlab/2bRAD.html

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