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Identifying Genotypes of *Acropora cervicornis* that are Resilient to White Band Disease

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ABSTRACT

White band disease in the Caribbean, which targets framework-building stony corals like *Acropora cervicornis* (staghorn coral), has become commonplace on reefs in the Florida Keys. This increase in white band disease has resulted in significant loss of *Acropora* species. To combat this rapid decline, *A. cervicornis* is grown in nurseries in situ and transplanted onto affected reefs. In order for transplanting efforts to be the most successful, the transplanted corals should be resilient to disease outbreaks. To propagate resilient corals in nurseries, scientists should first determine whether varying genotypes differ in disease susceptibility. An experimental laboratory manipulation was conducted to test whether nine genotypes from an in situ nursery on Summerland Key varied in disease susceptibility. The corals were arranged in three distances from a diseased individual to test for genotypic resilience to white band disease. Though the evidence suggests there is variation among genotypic susceptibility, the data was not significant. However, the B/O genotype was able to withstand contracting white band disease in all but one individual, suggesting this genotype may be more resilient than others. There was also no difference in susceptibility among distances from the diseased coral, although there was a trend of higher rates of disease infection at the closest distance. These results suggest that there may indeed be differences in susceptibility among genotypes of *A. cervicornis*, although further study with higher replication is needed.

1 INTRODUCTION

As the primary reef-building corals, stony corals are essential to reef function. Without them, coral cover begins to decline substantially and the 4,000 species of fish and hundreds of other organisms that rely upon stony corals specifically for sustenance or shelter are forced to relocate or die. Not only do these corals serve an important purpose in ocean ecosystems, they are also vital on shore to the local eco-tourism industries. Coral reefs attract several million people worldwide on a yearly basis and have an estimated annual worth of 375 billion dollars (Constanza et al., 1997). To commercial fishing industries that rely on reefs for profit, income can exceed 100 million dollars per year. Medicinal research incorporates coral reef organisms into medications as possible cures for cancer, arthritis, viruses, and other diseases. With so much potential, it is essential to learn as much as we can about white band disease to prevent substantial coral losses from disease in the future.

In 2014, the National Oceanic and Atmospheric Administration (NOAA) announced 20 new coral species as "threatened" under the Endangered Species Act (ESA) including Staghorn coral. *A. cervicornis* made the list in the initial 2006 naming phase and continues to remain on the top of the list despite nearly eight years since initial protection by the ESA (50 C.F.R. 223, 2014). The inability of *A. cervicornis* to overcome setbacks can be attributed to many factors including ocean acidification, warming water temperatures, pollution, and disease, specifically white band (Ault et al., 2001).

White band disease is a prevalent disease amongst Caribbean *Acropora* corals and comes in two forms: type I—caused by possible bacterial pathogens throughout the Caribbean (Kline & Vollmer, 2011)—and type II—predominantly found in the Bahamas and distinguished by a band of bleached tissue proceeding the dead tissue (Kline & Vollmer, 2011; Aronson & Precht, 2001). As an aggressive coral disease, white band has the potential to kill coral tissue, composed of thousands of individual polyps, at several centimeters per day (Kline & Vollmer, 2011; Jordán-Garza et al., 2010). Spreading quickly, white band can cause mortality in an entire colony within several days of initial infection (Jordán-Garza et al., 2010). Based on the observed rapid loss of stony corals to white band disease in coral reefs worldwide (Aronson & Precht, 2001), it is imperative that scientists find a cure or preventative measures to combat white band disease. In order to better understand the disease, it is important to quantify the natural capabilities of *A. cervicornis* to resist white band infection (Reed et al., 2010). If a specific genotype harbors a higher capability to withstand white band, then that genotype can be grown in nurseries and transplanted onto dying reefs to help rebuild the stony coral population with a disease-resistant coral.

To mimic natural conditions, experiment tanks were set up radially, with a diseased coral individual at the forefront. From here, two main hypotheses were tested. First, that the genotype of each coral will significantly influence disease susceptibility. Second, it is hypothesized that distance will not significantly influence disease susceptibility. If the hypotheses are supported then resistant genotypes should have a reduced infection rate regardless of their distance from the diseased coral.

2 METHODS

To test for resilient genotypes of *A. cervicornis* coral to white band disease, nine differing genotypes from the Mote Marine Laboratory in situ coral nursery (Summerland Key) were collected, mounted on PVC pucks, and marked with differing colored bands to distinguish them (reference Table 1 for corresponding genotype colors and numbers. Genotypes will henceforth be referred to by color). Genotypes were previously determined by Dr. Iliana Baums using five microsatellite markers.

Five $50.8\,\text{cm} \times 25.4\,\text{cm}$ tanks were set up in a bath with dimensions of $137.16\,\text{cm} \times 78.74\,\text{cm} \times 38.1\,\text{cm}$ with a flow tube

providing constant ambient water and one 2 W power head in each tank. Each power head pointed towards one diseased coral in a corner with nine corals—representing three different genotypes—at three different distances (5 cm, 10 cm and 15 cm) from the diseased coral (Fig. 1).

Band Color	Genotype Number
Blue	5
Pink	34
Yellow	4
Orange	10
Clear	58
Black	56
None	54
P/Y	31
B/O	38

Table 1. Corresponding genotype number from the Summerland Key in situ coral nursery and the colored band they received.

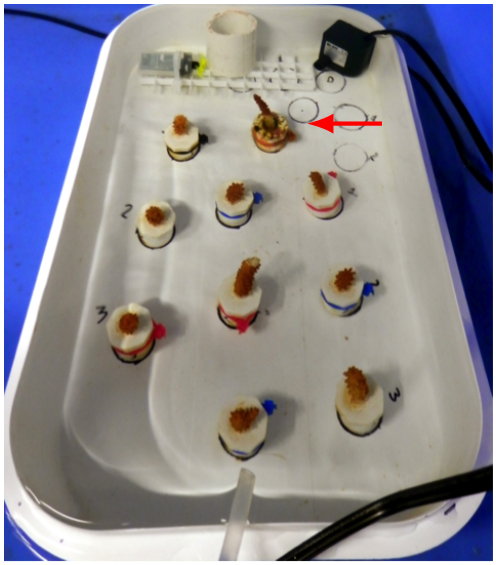

Figure 1. Experimental tank set up with diseased coral indicated by red arrow.

Four tanks were used to incorporate all nine genotypes and a diseased coral. The fifth tank served as a control tank replacing the diseased coral with a healthy coral. This set up was repeated three times for replication among genotypes and also for distance between the diseased coral and each genotype, creating nine total tanks. The date of first exposure, the number of days exposed, the date of the first sign of white band, the date of death, and the number of days with white band were recorded for each coral, as well as the rate of tissue loss per day when disease occurred. Upon succumbing to white band disease, the disease progression was measured and recorded each day and the disease progression between days was calculated. Using the changes from day one to day two, from day two to day three, etc. for each coral in each genotype, the average rate of tissue loss per genotype was calculated.

	Degrees Freedom	F-value	P-value
Genotype	8	1.09	0.388
Distance	1	2.02	0.364
Genotype×Distance	8	1.133	0.363

Table 2. Two-way Analysis of Variance (ANOVA) analyzing the correlation between genotype, distance from the diseased coral (cm), and the days exposed before signs of disease.

For each trial, the independent variables were the genotype and the distance from the disease, while the dependent variable was the number of days until visible disease infection. The R programming language (www.r-project.org) was utilized to test for differences among genotypes, distances from diseased coral, and the interaction of the two independent variables using a 2-way analysis of variance (ANOVA) test. Parametric assumptions were met. Excel was then used to create bar graphs of the data.

3 RESULTS & DISCUSSION

Genotypic Variation

There were observable trends in genotypic susceptibility to disease. For example, genotype Blue was the most susceptible to white band disease (5 out of 6 or 83.33%) and B/O had the smallest proportion (1 out of 6 or 16.67%) (Fig. 2). However, neither the genotype nor the distance was found to have a significant affect on the pattern of disease distribution among corals, nor was there a significant interaction effect (Table 2).

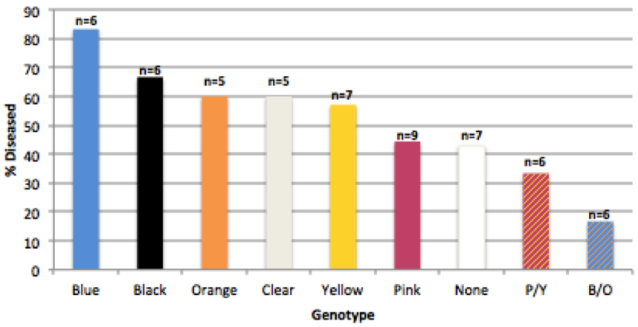

Figure 2. Percent of each genotype that succumbed to white band disease. Total disease percentages were calculated for all genotypes, with Blue having the largest proportion succumb to white band (5 out of 6) and B/O having the smallest proportion (1 out of 5).

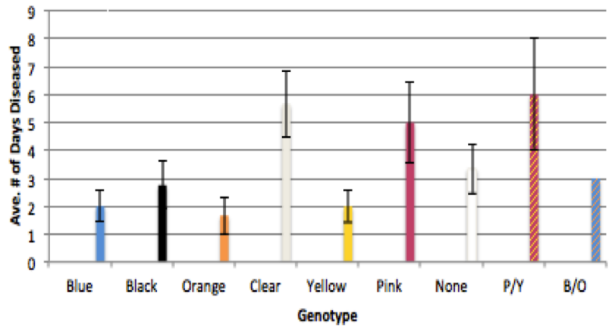

Figure 3. Average number of days with white band disease. Post-disease lifespan varied widely between genotypes, however, orange genotype corals exhibited the shortest post-disease lifespan whereas P/Y exhibited the longest, surviving as long as 8 days with white band.

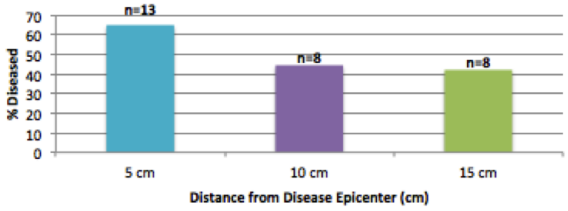

Figure 4. Percent of coral at each distance (cm) from diseased coral that contracted white band disease. The percentage of each distance that had individuals contract disease favored the first row only 5 cm away from the diseased coral (65%). Little difference in disease percentage is exhibited between row 2 (10 cm, 44%) and row 3 (15 cm, 42%).

Since each genotype was equally spaced in varying distance from the diseased coral, this would suggest a genotypic variation in disease susceptibility with B/O as the least susceptible genotype. It was observed that orange genotype corals had the shortest post-disease lifespan ranging anywhere from one to three days, whereas P/Y had the longest, surviving up to 8 days with disease (Fig. 3).

Distance from Disease

The percentage of each distance that had individuals contract disease was higher when experimental corals were 5 cm away from the diseased coral and decreased with increasing distance (Fig. 4). However, these results were not statistically significant.

The total number of disease cases observed in each row showed that out of the 29 total instances of disease, 13 disease cases were located 5 cm away from the diseased coral and 8 cases were located 10 and 15 cm away (Fig. 5). In addition, all but genotype B/O had at least one coral 5 cm away contract disease, whereas several genotypes did not contract disease when they were 10 or 15 cm away. This is indicative of a slight bias with row one being only 5 cm from the diseased coral, but there is no observable difference between the latter two rows, suggesting genotypic resilience may be more important than distance when determining which individuals will become diseased. Had distance been the more prominent factor, it would be expected for the second row 10 cm away from the diseased coral to display a total number of diseased corals between 13 (row 1, 5 cm away) and 8 (row 3, 15 cm away).

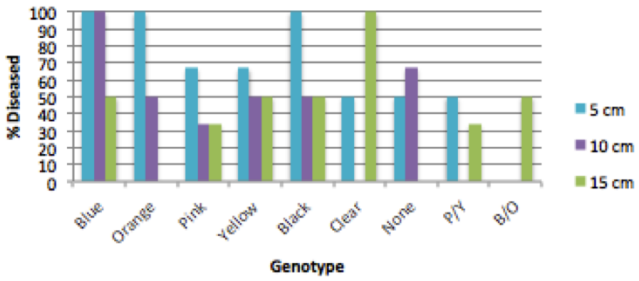

Figure 5. Disease prevalence based on distance (cm) from the diseased coral. Out of the 29 total instances of disease, 13 disease cases were located in row one (5 cm from the diseased coral) and 8 cases located in rows two and three (10 and 15 cm respectively).

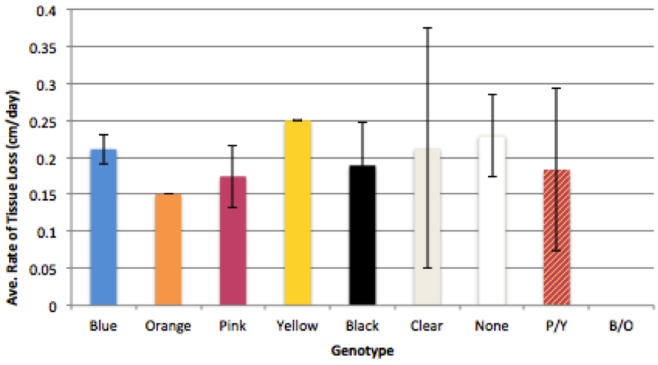

Figure 6. Average rate of tissue loss (cm/day) per genotype due to disease progression. The average rate varies widely with some days exhibiting as much as 0.36 cm difference and other days with no difference.

The average rate of tissue loss varied widely within and among genotypes, with some days exhibiting as much as 0.375 cm difference and other days with 0.05 cm difference or no difference at all (Fig. 6). Even though each distance produced relatively the same number of diseased individuals, when compared with the average rate of tissue loss per distance (Fig. 7), the rate at which the tissue was lost from the corals 10 and 15 cm from the diseased coral was much greater than at 5 cm. This is similar to the highly variable rates of tissue loss observed by Williams & Miller (2005) at White Bank Dry Rocks in the Florida Keys.

4 CONCLUSION

Statistical analyses showed that neither the genotype nor the distance was found to have a significant effect on the pattern of

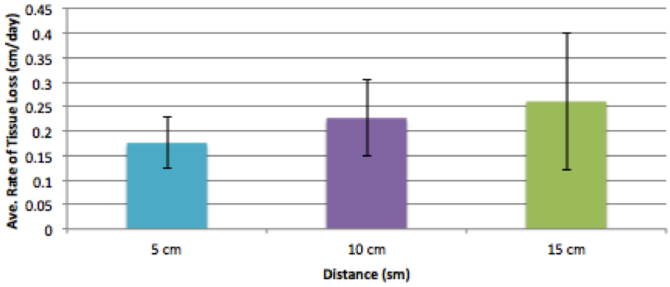

Figure 7. Average rate of tissue loss (cm/day) per distance due to disease progression. The rate at which the tissue was lost from the corals in the latter two rows (10 and 15 cm was much higher the longer the coral stayed diseased, whereas the individuals who succumbed most quickly once contracting white band were often found in row 1 (5 cm), suggesting some kind of distance dependence.

disease distribution among corals. However, interesting trends have emerged suggesting that there may be variation in disease resilience among genotypes and that distance from diseased individual also plays a role in susceptibility. Likely both variables can influence disease prevalence. In fact, Fig. 2 shows there is a large amount of variance among differing genotypes and the percentage that succumbed to white band. Therefore, high variation in all results, likely from low sample sizes, suggests that further studies should be conducted. These studies should include higher sample sizes, to identify which factor has the most influence over disease susceptibility, and identify disease resilient genotypes. The results complement other studies showing transmissibility of white band disease and genetic resilience to disease infection (Vollmer & Kline, 2008; Gignoux-Wolfsohn et al., 2012). In Vollmer & Kline's study, the first evidence of host disease resistance in scleractinian corals occurred, with six percent showing disease resistance. Four years later, Gignoux-Wolfsohn et al. demonstrated waterborne transmission of white band disease to injured staghorn corals, explaining localized spreading. Understanding disease transmission and genotypic resilience will significantly influence the ability to prevent disease outbreaks in the future. Additionally, knowing the variables that affect transmissibility of white band disease, whether distance related or influenced by genetic susceptibility, will also help guide best practices for coral reef restoration.

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