**Identifying compatibility between *Montastraea faveolata* individuals using larvae ratios**

*Introduction:*

Coral reefs play a large role in maintaining the health of marine ecosystems. However, many species of corals are now endangered due to recent human impacts. According to Tom C. L. Bridge, over 80% of coral reefs in Chinese waters have been depleted due to coastal development and overfishing. World-wide, coral reef coverage has decreased roughly 60% (1). To help restore coral reefs, restoration efforts have been made by creating coral nurseries. In these nurseries, coral individuals are grown for a year and then planted in a reef in the wild (2). In order for these corals to perpetuate in the wild, they must be able to reproduce which requires out planting of compatible individuals back into the wild together.

Unfortunately, there has yet to be discovered a genetic feature of compatibility between coral individuals. In humans, MHC alleles have a role in compatibility, and in plants it is the S allele locus (3). However, through making batch cultures of equal amounts of gametes from each coral parent and genotyping the offspring we can determine whether the parents contributed equally to the batch or not. In August 2012, batch cultures were made using 4 different *Montastraea faveolata* colonies for each batch and the offspring from 1 of the batch cultures was genotyped. The null hypothesis is that there would be equal ratios of parental contribution, meaning that all parents were equally compatible. Alternatively, some coral colonies will be more compatible than others.

*Materials and Methods:*

Four different *Montastraea faveolata* from the Grecian Rocks Florida Keys were mated for a batch culture. In total there were 3 batch culture made. From this batch of larvae, four subsamples were taken at the 24 hours post fertilization. The parental DNA was extracted from egg and sperm samples using the Nucleon Phenotype Genomic DNA Extraction method with the following modifications. An attempt was made to extract sperm cells from the parental samples. The sperm were expected to be located in the white mass in the same tube of the eggs cell tubes. The white mass was spun down to separate the sperm from the white mass, but no sperm pelleted. Because of the failure to extract DNA from sperm, only DNA from the egg in the corresponding tubes were extracted and analyzed. Another modification to the Nucleon Phenotype Genomic DNA Extraction method was the length of incubation. The prepared samples were incubated overnight after the reagents had been added. In regard to the larvae, DNA was extracted from single larva using the Chelex method. In this experiment, 5% Chelex solution was used. In summary, 11 parental samples were extracted and 185 individual larvae.

*Table 1.* *M. faveolata* sample data from the Grecian Rock taken at 24 hours

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Date | Batch | Parent Colony | Time Budles release | Time Gametes | Fertilization duration | Tube | Egg volume | Sperm Volume | Notes |
| 27-Aug | 1 | Mfav1 (GR) | 23:28 | 1:41 | 1 hr | LF2021 | 2.5 | 42 | Survived well and settled! |
| 27-Aug | 1 | Mfav2 (GR) | 23:28 | LF2020 | 2.5 | 40 |
| 27-Aug | 1 | Mfav3(GR) | 23:37 | LF2022 | 2.5 | 42 |
| 27-Aug | 1 | Mfav5B(GR) | 23:33 | LF2017 | 2.3 | 41 |
|  |  |  |  |  |  |  |  |  |  |
| 27-Aug | 2 | Mfav4 (GR) | ? | 1:48 | 1 hr | LF2019 | 2.5 | 42.5 | Crashed on the first day; genotype samples may or may not contain live larvae |
| 27-Aug | 2 | Mfav5(GR) | 23:33 | LF2018 | 4 | 41 |
| 27-Aug | 2 | Mfav6(GR) | 23:33 | LF2016 | 3 | 42 |
| 27-Aug | 2 | Mfav7(GR) | 23:33 | LF2015 | 2 | 43 |
|  |  |  |  |  |  |  |  |  |  |
| 27-Aug | 3 | Mfav8(HS) | ? | 2:55 | 1 hr | F023 | 3 | 41 | Crashed on the first day; genotype samples may or may not contain live larvae |
| 27-Aug | 3 | Mfav9(HS) | ? | LF2011 | 6 | 39 |
| 27-Aug | 3 | Mfav10(HS) | ? | LF2012 | 4 | 40 |
| 27-Aug | 3 | Mfav11(HS) | ? | LF2013 | 5 | 37 |

*Table 2*. *M. faveolata* batch and number samples taken from each subsample

|  |  |
| --- | --- |
| *M. faveolata* larvae batch – number of subsample | Number of samples taken |
| Mfav1 - 1 | 35 |
| Mfav1 - 2 | 50 |
| Mfav1 - 3 | 50 |
| Mfav1 - 4 | 50 |

DNA in the samples were selectively amplified using 6 previously published microsatellite primers (4). The six published microsatellite primers used in the PCR were 5, 11, 12, 28 (added to PCR multiplex I), 4, and 8 (added to PCR multiplex II). PCR products were visualized using an ABI 3730 automated sequencer with an internal size standard for accurate sizing. Electropherograms were analyzed with GeneMapper Software 4.0 (AppliedBiosystems). Then program Cervus was supposed to be used to compare allele frequencies and identify the most likely parents for each individual larvae, but proved unnecessary.

*Results*

Table 3. Parental Alleles at Primers

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cross | Sample |  | 4 |  | 5 |  | 8 |  | 11 |  | 12 |  | 28 |  |
| + CT |  |  | 318 | 318 | 320 | 324 | 201 | 201 | 321 | 321 | 250 | 252 | 191 | 191 |
| Batch |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | 11660 | MFAV4 | 308 | 318 | 309 | 315 | 193 | 212 | 321 | 321 | 252 | 254 | 194 | 212 |
| 2 | 11661 | MFAV5 | 308 | 318 | 309 | 315 | 198 | 198 | 321 | 321 | 252 | 254 | 194 | 212 |
| 2 | 11662 | MFAV6 | 308 | 318 | 309 | 315 | 191 | 201 | 321 | 321 | 252 | 254 | 194 | 212 |
| 2 | 11663 | MFAV7 | 308 | 318 | 309 | 315 | 191 | 201 | 321 | 321 | 252 | 254 | 194 | 212 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 3 | 11664 | MFAV8 | 308 | 311 | 320 | 333 | 191 | 201 | 321 | 321 | 252 | 252 | 191 | 191 |
| 3 | 11665 | MFAV9 | 308 | 311 | 320 | 333 | 191 | 201 | 321 | 321 | 252 | 252 | 191 | 191 |
| 3 | 11666 | MFAV10 | 253 | 298 | 318 | 320 | 193 | 193 | 310 | 310 | 248 | 262 | 197 | 211 |
| 3 | 11667 | MFAV11 | 315 | 315 | 318 | 324 | 198 | 212 | 321 | 321 | 282 | 282 | 194 | 194 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 1 | 11657 | MFAV1 | 315 | 318 | 315 | 315 | 191 | 191 | 318 | 321 | 250 | 250 | 191 | 202 |
| 1 | 11658 | MFAV2 | 315 | 318 | 315 | 324 | 193 | 212 | 318 | 321 | 250 | 250 | 191 | 202 |
| 1 | 11659 | MFAV3 | 315 | 318 | 315 | 324 | 193 | 212 | 318 | 321 | 250 | 250 | 191 | 202 |
| 1 | 11661 | MFAV5 | 308 | 318 | 309 | 315 | 198 | 198 | 321 | 321 | 252 | 254 | 194 | 212 |

Table 4. Observed Larvae Allele Ratios

|  |  |
| --- | --- |
| Row Labels | Count of Sample Name |
|  |  |
| 198198 | 1 |
| 198201 | 63 |
| 198203 | 51 |
| 201201 | 3 |
| 201203 | 2 |
| 203203 | 1 |
| Failed | 64 |
| Grand Total | 185 |

In August 2013, samples from three different batches of were taken from the Florida Keys. The parents were genotyped at six loci, 4, 5, 8, 11, 12 and 28 (Table 3). Batch 2 shows a clone of MFAV 6 and MFAV 7. These parents had exact matches at all loci. In the next batch 3, there was another pair of clones, MFAV 8 and MFAV 9. In this study, we analyzed the larvae of the final parental batch 1 (labeled MFAV1) containing parental colonies MFAV 1, 2, 3, and 5. Parents MFAV 2 and 3 are clones, having the exact same alleles at every marker. Fortunately at locus 8 all parent colonies in batch MFAV1 has unique alleles (highlighted in yellow), therefore only that locus was necessary for genotyping the offspring. MFAV 1 had the alleles 191 and 191. MFAV 2 and its clone MFAV 3 had the alleles 193 and 212. MFAV 5 had the alleles 198 and 198. Genotyping results of the 185 offspring at locus 8 resulted in 6 different allele combinations of 3 unique alleles 198, 201, and 203 (Table 4). The predominant allele pairings were 198/201 at 52% and 198/203 at 42%. Sixty four of the 185 larvae samples, about 34.6% of the samples failed to be detected.

Table 5. Data on the three batches of four parent crosses

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Date** | **Batch** | **Parent Colony** | **Time Budles release** | **Time Gametes** | **Fertilization duration** | **Tube** | **Egg volume** | **Sperm Volume** | **Notes** |
| **27-Aug** | 1 | Mfav1 (GR) | 23:28 | 1:41 | 1 hr | LF2021 | 2.5 | 42 | Survived well and settled! |
| **27-Aug** | 1 | Mfav2 (GR) | 23:28 | LF2020 | 2.5 | 40 |
| **27-Aug** | 1 | Mfav3(GR) | 23:37 | LF2022 | 2.5 | 42 |
| **27-Aug** | 1 | Mfav5B(GR) | 23:33 | LF2017 | 2.3 | 41 |
| **27-Aug** | 2 | Mfav4 (GR) | ? | 1:48 | 1 hr | LF2019 | 2.5 | 42.5 | Crashed on the first day; genotype samples may or may not contain live larvae |
| **27-Aug** | 2 | Mfav5(GR) | 23:33 | LF2018 | 4 | 41 |
| **27-Aug** | 2 | Mfav6(GR) | 23:33 | LF2016 | 3 | 42 |
| **27-Aug** | 2 | Mfav7(GR) | 23:33 | LF2015 | 2 | 43 |
| **27-Aug** | 3 | Mfav8(HS) | ? | 2:55 | 1 hr | F023 | 3 | 41 | Crashed on the first day; genotype samples may or may not contain live larvae |
| **27-Aug** | 3 | Mfav9(HS) | ? | LF2011 | 6 | 39 |
| **27-Aug** | 3 | Mfav10(HS) | ? | LF2012 | 4 | 40 |
| **27-Aug** | 3 | Mfav11(HS) | ? | LF2013 | 5 | 37 |

 Of the three batches, it was found that many of the parents in the crosses were clones of each other. The color corresponds to the genotype. For instance, the parent colonies that are colored in orange have the same genotype and are clones of each other. Thus, the first batch was a single cross between three clones and one unique parent. Therefore, the larvae ratios could not indicate much about compatibility. The parents in the second batch were all the same genotype, thus the cross was a single cross and could not lend any information about compatibility.

*Discussion*

 The results of the this study failed to indicate individual compatibility between the parents MFAV 1, clones MFAV 2 and MFAV 3, and MFAV 5. These parents had the following alleles at locus 8: 191/191, 193/212, 193/212, and 198/198, respectively. It was expected that each parent would contribute to one fourth of the larvae’s alleles. Thus, ¼ of the larvae would have 191 as one of their alleles, ½ of the larvae would have 193 or 212 as one of their alleles (due to two samples being clonemates), and ¼ of the larvae would have 198 as one of their alleles. However, the larvae displayed highly deviating results. Two of the alleles in the larvae, 201 and 203, did not correspond to any of the possible parental alleles at locus 8. It is possible that contamination from another coral colony could have occurred at some point at spawning. It is likely that the parent MFAV 5 mated with a nearby individual that had contaminated the culture. It is also possible that marker 8 could have involved the alleles of the coral symbiont instead of the parental coral’s alleles. New markers have been ordered for the *Montastraea faveolata* coral to reanalyze the alleles of the parents. It was then found that the parents in that cross were actually composed of mainly clones, as seen in Table 5. With a single cross, little can be understood about the compatibility between two parents based on larvae ratios. Therefore, the other two batches, crosses, were analyzed.

 The colors in the parent colonies column correspond to genotype. For instance, in Batch one, the parent colonies in orange are all the same genotype; they are clones. Analysis of Batch 2 showed that the cross was between four parent colonies all of the same genotype. Thus, no implications about compatibility of the four colonies could be made. Batch 3 showed a diverse cross between three different parents. However, the larvae crashed within 24 hours and the only surviving larvae appeared to be self-fertilized.

 Despite the limited information about compatibility from the three crosses, it was discovered that clones of Montastraea faveolata can exist much farther from each other than expected. In future compatibility analysis, the parents will be genotyped before the crosses are made to ensure diverse crosses. Then the larvae ratios will be analyzed. Another possible approach to understanding coral reproduction could be research into find a compatibility factor such as S allele plant locus (3).

*References*

1. Bridge, Tom C. L., et. al., Call to protect all coral reefs. Nature Climate Change 2013; 3: 528-530
2. Trinh, Lan. Florida group rebuilds vital coral reefs. CNN U. S., Cable News Network, 1 Mar 2012. Web. 16 Dec 2013.
3. Schierup, M. H., et. al., Evolutionary Dynamics of Sporophytic Self-Incompatibility Alleles in Plants. Genetic Society of
 America 1997; 147: 835-846.
4. Severance, E. G., Szmant, A. M. and A. Karl, S. (2004), Microsatellite loci isolated from the Caribbean coral, Montastraea
 annularis. Molecular Ecology Notes, 4: 74–76. doi: 10.1046/j.1471-8286.2003.00573.x