Genetic isolation and plasticity in *Montipora dilatata* and Hawaiian congeners: Where are the species boundaries?

Previously funded National Marine Fisheries Service (NMFS) Species of Concern (SOC) work on *Montipora dilatata* resolved four distinct genetic groups of *Montipora* in Hawaii: I) *M. patula/M. verilli*, II) *M. incrassata,* III) *M. capitata,* and IV) *M. dilatata/M. turgescens/M. flabellata*, with mitochondrial and nuclear markers capable of resolving ~ 1 MY differences (Forsman *et al*. 2009). Further work confirmed that these groups are also congruent with fine-scale morphological features (Forsman et al. 2010). Species within these complexes are difficult to distinguish by molecular or morphological means; they are either newly forming species or within-species morphological variation. The goals of the 2010 project were: 1) to expand on the study of Hawaiian *Montipora* by including corals surrounding the Hawaiian Archipelago, and 2) to examine phenotypic plasticity of skeletal morphology in *Montipora* at the Hawaii Institute of Marine Biology (HIMB) by reciprocal transplant studies.

Brief descriptions of the current progress towards 2010 project goals are as follows:

1) Determine the degree of genetic similarity of *Montipora* in the Hawaiian Archipelago with congeners in surrounding waters:

Dr. Jim Maragos (U.S. Fish and Wildlife Service) has provided us with 98 *Montipora* samples from the Pacific Refuges surrounding Hawaii (Johnston Atoll, Baker Island, Howland Island, Jarvis Island, Palmyra Atoll, Kingman Reef), and 51 samples from the Papahānaumokuākea Marine National Monument located in the Northwestern Hawaiian Islands. Each sample is accompanied by an in-situ photographic voucher. We are currently processing these samples and investigating new molecular approaches (such as next-generation sequencing). We are interested in developing methods that will provide greater resolution within the Hawaiian species complexes than the PloS One study (Forsman et al. 2010). We have ordered next-generation sequencing primers that will allow deep coverage of nuclear ribosomal genes and spacers, and we will be testing these primers shortly. These markers will allow comparisons of recombination (which is a proxy for reproduction) between the morphologically defined species.

Previously developed microsatellite markers developed for *M. capitata* (Concepcion et al. 2009) were tested on samples from the *M. flabellata* clade. Ten individuals from the *M. flabellata* clade were tested for eight microsatellite loci. Four of the markers amplified and will be tested further for polymorphism. This preliminary trial indicates that it may be possible to apply some of the *M. capitata* makers on closely related species, which may provide a measure of gene flow within the Hawaiian species complexes.

2) Conduct a reciprocal transplantation experiment on *Montipora* at HIMB to examine plasticity of morphological traits:

The purpose of this experiment is several fold: 1) to determine nursery conditions for mass-propagating *Montipora capitata* as a test case for future work on *Montipora dilatata*, 2) to examine intraspecific morphological variation and plasticity in *Montipora*.

On June 17, 2010, a reciprocal transplant experiment was set up consisting of 1,080 small (~1cm) fragments of *Montipora capitata* divided into four mid-water platforms placed in four environments (lagoon full sun, lagoon shade, shallow forereef, deep forereef). Six colonies of five morphotypes were identified (thick branch, thin branch, smooth surface, plating, bushy morph; Figure 1) and fragmented onto nine meshes consisting of 30 fragments each (6 genotypes by 5 morphotypes). Each platform (Figure 2) has been equipped with a small data logger that records temperature and relative light levels, and each environment was further characterized with additional instrumentation to measure salinity and turbidity.

Figure 1. The five morphological extremes of *Montipora capitata* used for this study, from left to right: Thick Branch, Thin Branch, Smooth Surface, Plating, Bushy Morph.

Figure 2. Suspended mid-water platform or 'nursery' for the genotype by environment reciprocal transplant study.

This experiment is ongoing, but preliminary results indicate very high survivorship and growth in the lagoon environment, with less survival in shallow and deep reef treatments. Photographs show evidence of phenotypic plasticity of surface features, and preliminary microsatellite data show little correspondence between genotype and morphological form. In other words; the same genotype can grow to have a very different appearance in different environments. The preliminary data indicates that this experiment was successful, and will yield valuable information about culturing *Montipora*, as well as information about phenotypic plasticity.

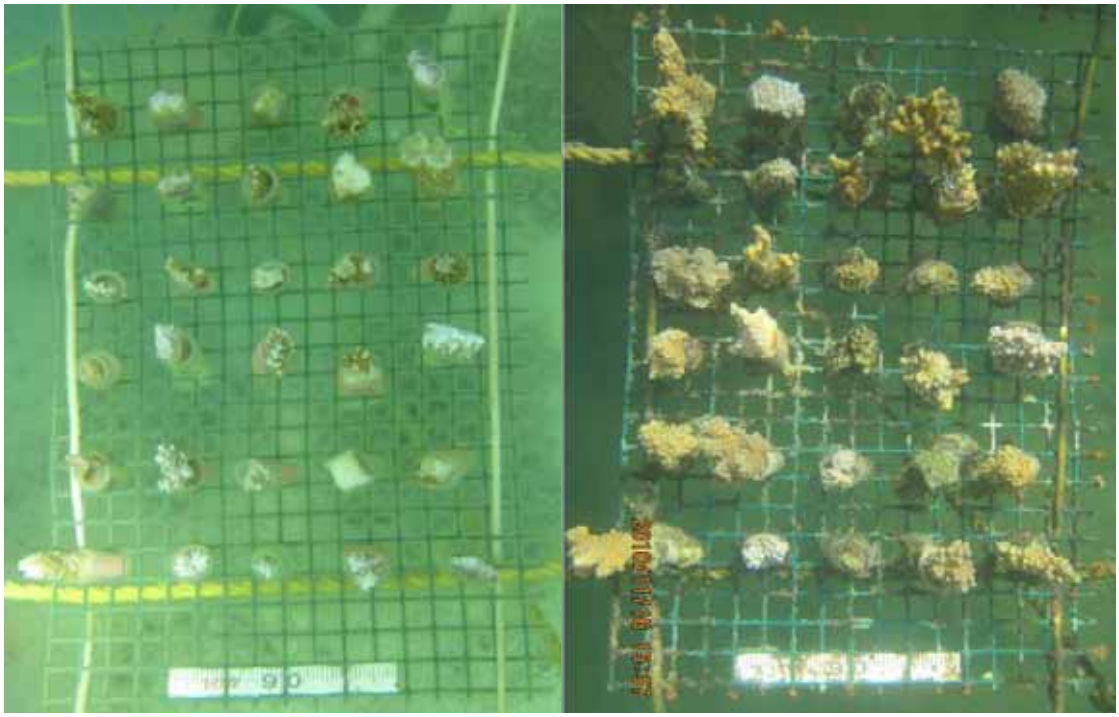

Figure 3. One of nine replicates of the lagoon full sun treatment (left: July 15, 2010; right: the same corals on November 16, 2010). Note that growth is primarily vertical.

Figure 4. Close up of corals in the lagoon shade treatment (left: July 15, 2010; right: the same corals on November 16, 2010). Note primarily horizontal growth. The morphology of the parental colony is listed on the image on the left. The smooth morph usually lacks verrucae (rice grain-sized bumps) associated with *M. capitata*, and is often confused for *M. dilatata*, or thought to be a hybrid between *M. capitata* and *M. dilatata*. The formation of verrucae on the image on the right demonstrates that this morphology is due to remarkable phenotypic plasticity.

Additional activities:

- Our manuscript (Forsman et al. 2010) was published in the open access journal PLoS One, and received considerable press attention including: interviews with Hawaii Public Radio and the Associated Press; press coverage by Science Daily, escience news, underwatertimes, and Coral Magazine; and an article in the Honolulu Advertiser.
- Findings were presented at the Evolution 2010 meetings in Portland, Oregon.
- Findings were presented to the NOAA Biological Review Team currently evaluating the status of the 83 coral species petitioned to be listed under the US Endangered Species Act.
- Six students were mentored over the summer and participated in the research; each student formally presented their research results:
    - Pauleen Fredrick (Pohnpei Micronesia Community College student)
    - Carrie Colbert (Punahou High School student)
    - Noelle Victoria, Kekainani Lucero, Anuhea Lym, and Jaslynne Chang (High School students enrolled in Windward Community College Paces program)

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