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Restoration of threatened *Acropora cervicornis* corals: intraspecific variation as a factor in mortality, growth, and self-attachment

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Abstract. The potential of farming *Acropora cervicornis* corals to generate material for restoring the species to Caribbean reefs was investigated. Coral colonies from both high and low energy areas were sampled, to determine the relative importance of intraspecific variability and source environment on mortality, growth, branching density, and self-attachment. Isolated *Acropora cervicornis* thickets, were sampled, half from the reef front and half from the back reef. Each thicket sampled was assumed to be of a single coral genotype. Replicate fragments were sampled from each thicket and transplanted to wire frames located in shallow back reef areas <2m deep, on five reefs in the La Parguera reef system, Puerto Rico. Results at one year indicate that coral genotype is a highly significant factor for all of the variables studied, with up to a six-fold difference in relative growth based on genotype alone. Back reef and reef front populations of *A. cervicornis* corals differed significantly in relative growth, branch diameter, and attachment ability even after growing together for one year, suggesting a genetic basis to morphology and adaptation to their original environment. Frame culture was an effective method to produce second generation stock, with annual growth rates often over ten fold.

Key words: *Acropora cervicornis*, coral restoration, coral farming, coral gardening.

Introduction

Acropora cervicornis is composed of low-diversity, predominantly clonal populations of corals maintained by asexual fragmentation with clones shown to span distances as great as 30-50 m (Bothwell 1981; Neigel and Avise 1983). The clonal nature of the species has major implications for the restoration of this threatened Caribbean coral (Bowden-Kerby 2001a; Bowden-Kerby et al. 2005).

While remnant *A. cervicornis* populations may be predominantly composed of a single genotype and thus unable to effectively reproduce sexually (Bowden-Kerby 2001a), the fact that the species easily fragments to create clonal populations creates the potential for developing low-tech restoration methods modeled after and accelerating these natural fragmentation processes (Bowden-Kerby 2001a, 2001b). Alternatively, the highly clonal nature of the species might also present unforeseen challenges, particularly for this "generalist" species which occurs over wide environmental gradients, as each genotype may be highly adapted to a narrow range of environmental tolerances. It can be assumed that a particularly abundant genotype would be well-adapted to the set of environmental conditions where it occurs and that long-term selective processes would eliminate maladapted coral strains over time while favoring particular genotypes. If the survival or growth of coral transplants is strongly related to the original environment of the corals, closely matching the source environment of the corals with that of the transplant site would be important to long-term success.

This study focused on providing information on the relative importance of intraspecific differences in the mortality, growth, morphology, and attachment of *Acropora cervicornis* and *A. prolifera,* the hybrid between *A. cervicornis* and *A. palmata*.

The major questions were:

1. Are there significant differences within a coral species in mortality, growth, branching or attachment?
2. Are differences related to the origin of the coral: low-energy back reef vs. high-energy reef front?
3. Are transplantation site differences more or less important than interspecific differences?
4. Is the culture of corals on wire frames an effective method for producing second generation coral fragments for use in restoration work?

Material and Methods

For the purposes of this study, each isolated coral thicket sampled from a distinct reef was assumed to be a unique genotype, even though somatic mutation

could potentially occur within a single coral colony, causing diverse responses within samples of a particular coral. Zooxanthellae strains were also assumed identical for all replicate fragments of a specific coral, but differences between fragments could potentially arise by incorporation of new algal strains. To help control for these unlikely factors, 30 fragments were used per genotype, six replicate fragments at each of five sites.

The hybrid coral *A. prolifera* was treated as a distinct species. For each of the two species, eight thickets were selected, four from back reef areas and four from reef fronts. Morphological differences were clearly observed between the two populations of each species, and so reef front and back reef populations of each species are referred to as morphotypes. The 16 genotypes were obtained from reefs within the La Parguera reef system. Samples spanned 6 km and with two exceptions each sample reef was separated from other reefs by wide channels 10-25 m deep. Fragments were sampled from within a single, large, distinct coral colony or from a tight group of adjacent colonies (thicket) widely isolated from other corals of the species.

Frames for supporting coral fragments were constructed by bending 0.5 m x 1 m pieces of vinyl-coated 2.5 x 5 cm wire mesh into A-shaped frames, one meter long and standing about 25 cm high. Unbranched 8-12 cm apical coral fragments were attached to the frames with 10 cm plastic cable-ties, color-coded to denote replicate fragments of each genotype. Each fragment was secured at a mesh junction so that frame overgrowth could occur in all four directions along the wire. Separate frames were used for each species and for back reef and reef front corals, so there were four frames per site. All frames were located directly adjacent to one another on the sand substratum, randomly ordered and within 1-3 m of each other (split-plot design). The frames were weighted with short segments of heavy metal bars (Bowden-Kerby 2001a).

For the ANOVA analyses, mortality and relative growth means included data from all branches of each coral genotype. To minimize the hidden effects of breakage and mortality on branching, a branching density for each genotype was calculated based on half of the branches (3 out of 6), those with the highest relative growth per site. Branch density index was calculated as the length of the main branch in mm + 2 x length secondary branches + 3 x length tertiary branches ÷ total length of branches x 100. For overgrowth (attachment of the fragments to the frames), means were calculated based on the three maximum overgrowths per genotype per site, effectively eliminating branches or branch bases that had died.

For data presentation, each coral genotype was assigned a letter code: 'B' for back reef, 'F' for reef front, 'c' for *A. cervicornis*, 'p' for *A. prolifera.* Thus, Bc = back reef *A. cervicornis*, Fc = reef front *A. cervicornis*, Bp = back reef *A. prolifera*, Fp = reef front *A. prolifera*. Each genotype was also assigned a number from 1-4, with the number assigned based on the mean relative growth rates in descending order.

Results

Figure 1 shows mean mortality after one year for each coral genotype by site of origin. Comparing the genotypes in a randomized complete block design ANOVA, arcsine transformed data showed significant differences in mortality among reef front coral genotypes for both *A. cervicornis* ($p<0.0005$) and *A. prolifera* ($p<0.025$), however the ANOVA showed no significant differences in mortality among back reef genotypes of either *Acropora* species ($p<0.25$ in both cases).

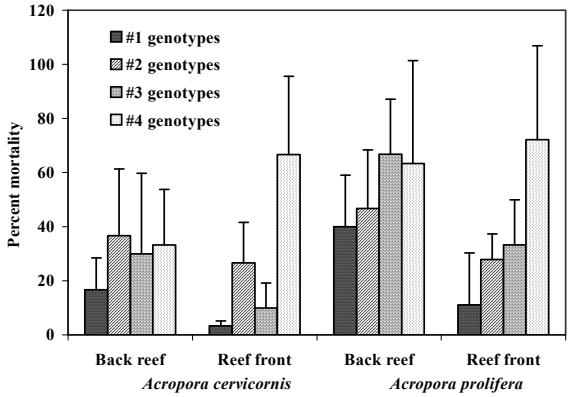

Figure 1: Mean percent mortality +SD of *Acropora* genotypes grown together for one year (n=30 per genotype, 5 sites x 6 branches; 3 sites for reef front *A. prolifera*. Genotypes are ordered based on relative growth: #1 with the highest, #4 the lowest.

Mean relative growth after one year is summarized in Figure 2. The results indicate the rapid growth of the various genotypes of each *Acropora* morphotype, ranging from a ten to twenty fold increase in branch lengths for *A. cervicornis* and from six to over thirty-fold increase over the year for *A. prolifera*. Two-way ANOVA comparisons indicate significant differences in relative growth among genotypes within coral morphotypes with transplant site as a factor (Bc: $p<0.025$, Fc: $p<0.025$, and Bp: $p<0.05$), except for genotypes of reef front *A. prolifera* (Fp: $p<0.50$). This lack of significance is likely due to smaller sample size, as only two sites included this morphotype due to a shortage of coral material.

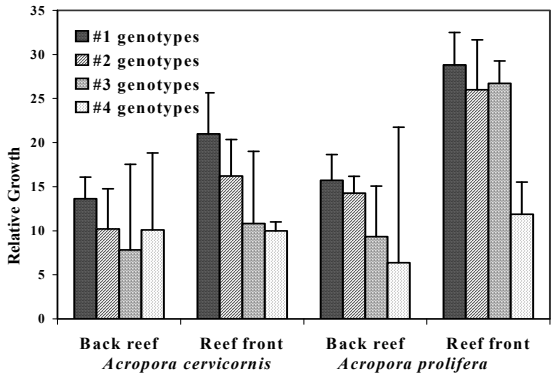

Figure 2: Mean relative growth based on maximum branch per site per coral genotype +SD (n = 4: 1 branch per coral genotype x 4 sites, 2 sites for reef front *A. prolifera*).

Figure 3 exemplifies comparisons that included all six branches per genotype per site. ANOVA comparing genotype means gives highly significant results for all but the Fp morphotype. (Bc: $p < 0.00001$, Fc: $p < 0.0001$, Bp: $p < 0.035$, Fp: $p < 0.3$). An interesting genotype-specific ordering of growth was observed for all four coral morphotypes, with particular genotypes growing fastest or slowest nearly everywhere, regardless of transplantation location (Fig. 3). This figure also shows the poor growth at the Media Luna offshore site due to extensive breakage. As breakage was considered a nuisance variable to the experimental questions, this site was excluded from the analysis. Without exception, the mortality ranking (high to low) of each genotype was the same as their relative growth ranking. This was the case when analysis included dead, broken, and lost branches, as well as when analysis was based only on the maximum branch per genotype per site.

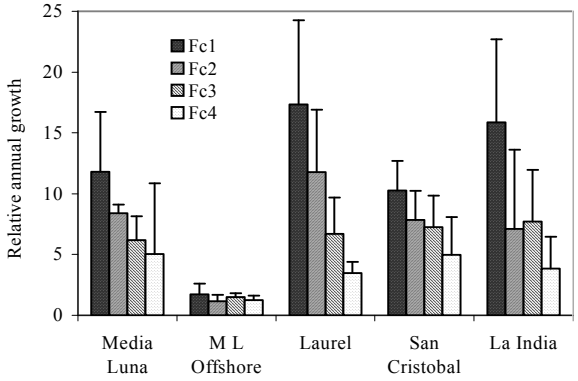

Figure 3: Site specific relative annual growth + SD of four genotypes of the reef front morphotype of *Acropora cervicornis* grown together on frames at five back reef locations. Each pattern represents a particular genotype (n = 6 branches per location).

Branch density data (Fig. 4) indicates that *Acropora prolifera* overall had a branch density index more than twice that of *A. cervicornis*. Comparisons between back reef and reef front morphotypes demonstrated no significant morphotype-specific differences in branching density within each species (paired *t*-tests: *A. cervicornis,* p=0.32; *A. prolifera*, p=0.39). Comparisons within each morphotype of the four genotypes indicated differences in branch density, confirmed to be statistically significant by randomized complete block ANOVA (Bc: $p<0.003$, Fc $p<0.005$, Bp: $p<0.025$, Fp: $p<0.05$). However, site was sometimes more significant than genotype in influencing branching (Bc: $p<0.005$, Fc $p<0.0005$, Bp: $p<0.0025$, Fp: $p<0.12$).

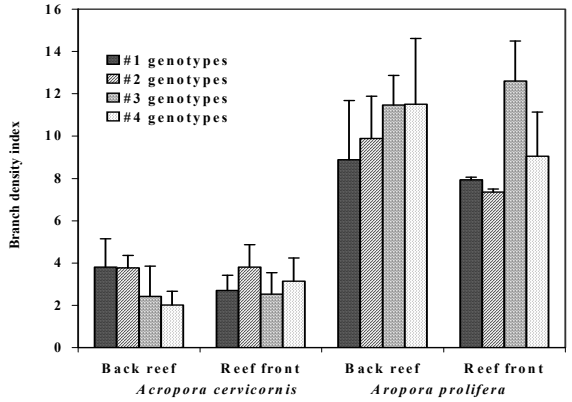

Figure 4: Mean relative branching +SD of *Acropora* genotypes grown together for one year in the back reef (n = 12: 3 branches x 4 sites; 2 sites for reef front *A. prolifera*).

Figure 5 shows mean overgrowth for each genotype of the four *Acropora* morphotypes, indicating a genetic basis for faster overgrowth of reef front corals, plus a generally positive relationship between relative growth and overgrowth. Randomized complete block design ANOVA of the data at one year gives statistically significant differences between genotypes for all morphotypes except for back reef *A. cervicornis* (Fa: $p<0.013$, Bp: $p<0.03$, Fp: $p<0.05$) (Bc: $p<0.38$.)

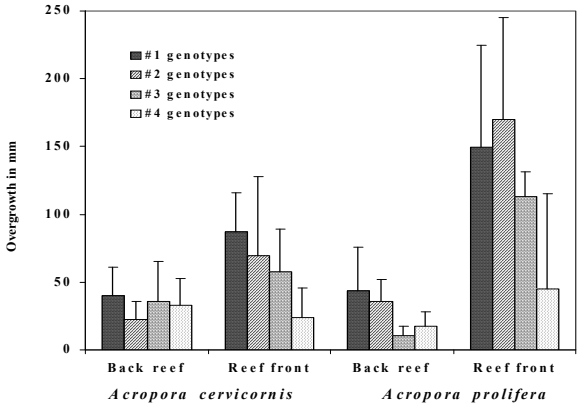

Figure 5: Mean overgrowth of *Acropora* genotypes in mm +SD onto wire mesh frames at one year (n= 15: 3 branches per genotype x 5 sites, 3 sites for reef front *A. prolifera*).

The experiment started with 10-cm fragments with little difference in thickness between apical fragments from the back reef and reef front. However, *A. cervicornis* from the reef front developed a distinctly more robust morphology than the back reef morphotype in the calm growth conditions of the back reef over the year. Statistically significant differences between the mean diameters of the two *A. cervicornis* morphotypes from the apical ($p=0.002$), middle ($p<0.00005$), and basal ($p<0.000005$) colony regions were revealed (Fig. 6) with *t*-tests. In addition to branch thickness, branch tips of the reef front morphotype were also noticeably softer and easily crushed, indicating a more porous, less heavily calcified skeleton than the lagoonal morphotype. Color differences were often apparent between the two morphotypes as well, the back reef type being more uniformly golden brown, while the robust reef front morph often had a darker brown or gray to purplish hue.

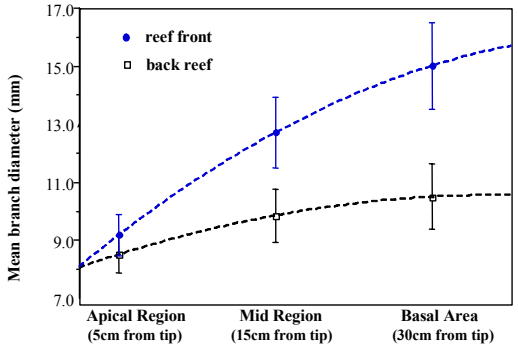

Figure 6: Comparison between reef front and back reef morphotypes of *Acropora cervicornis* grown together for one year, showing mean branch diameters ± SD for three colony regions.

Discussion

Based on this study, coral genotype appears to be more important than transplantation location, with 75% of all tests being significant for genotypic effects, as compared to only 44% of experimental outcomes having significant location effects. A G-test comparison of the observed frequencies confirms this ($p<0.005$, $G=8.3$, 1d.f.). However, genotype and location were equally as important for back reef morphotypes transplanted to the back reef, but for reef front morphotypes genotype was significantly more important than site in predicting experimental outcomes ($p<0.00001$, $G= 36.7$, 1d.f.). A potential explanation for the greater number of statistically significant differences among reef front corals transplanted to the back reef is that only certain genotypes from the reef front can adapt well to back reef environments, while genotypes from the back reef are in general well-adapted to the environment and therefore more even in their responses.

These results confirm that there is a biological basis for obtaining transplants from areas as similar as possible to the planned restoration site. *A. cervicornis* appears to be a highly diverse generalist species, not because each genotype has a high environmental tolerance, but because the species is composed of a diversity of genotypes, each adapted to a specific range of tolerances. Sourcing corals from environments similar to a restoration site and incorporating a high level of genetic diversity into transplantation projects would help ensure successful results for at least some of the transplants.

Based on genotypic differences alone, mean relative growth rates within *A. cervicornis* morphotypes growing together in the back reef varied by more than a factor of three, while mean relative growth rates within *A. prolifera* varied by a factor of six. These findings are in agreement with earlier studies which suggested that coral genotype strongly affected the ability of individual corals to thrive in a particular environment (Potts 1984; Edmunds 1994; Takabayashi and Hoegh-Guldberg 1995; Hoegh-Guldberg et al. 1997). A next step in advancing future research on the issue of environmental adaptation of coral genotypes might involve taking replicate fragments of specific genotypes and transplanting them into highly variable conditions of water flow, depth/light, and temperature.

A possible cause for the significantly slower growth and higher mortality of the reef front *A. cervicornis* genotype Fc4 could be that this genotype contains a maladapted zooxanthellae strain, perhaps even the zooxanthellae species identified by Baker et al. (1997), adapted to light conditions at greater than 9-12 m deep.

A possible non-genetic or non-zooxanthellae driven basis for differences in coral growth among genotypes could be the presence or absence of sublethal levels of pathogens within a corals sourced from the same thicket. In this study, there was an indication that disease may have suppressed the growth of genotype Fc4, which had significantly higher mortality ($p<0.0005$) than other genotypes. Of the 30 replicate Fc4 fragments, 66.7% died during the year, and much of this was visibly due to disease. This contrasts strikingly with the fastest growing genotype of this morphotype (Fc1), with only 3.3% mortality. Colonies of Fc4 appeared to be healthy during the first six months of the study, although displaying a noticeably slower growth rate. “White band” disease was later observed killing this genotype at several locations, while very few corals of the other genotypes had the disease. It appears that genotype Fc4 is either more susceptible to this disease, and the disease organisms were widespread throughout the reef system, or perhaps the disease was present in the

tissues of the source colony. The slower growth could have been the outcome of fighting a disease over several months, until the fragments eventually succumbed.

Bottjer (1980) studied the morphology of *A. cervicornis* from both reef front and back reef populations and surmised that the distinctly stockier morphology of the fore reef corals was environmentally induced. However this study suggests a genetic basis to this morphology. The reef front morphotype also had 14.6% less partially dead area in the lower portions of colonies than the back reef type (p=0.005, paired *t*-test), indicating that this morphotype of *A. cervicornis* invests more energy in maintaining healthy tissues in lower colony portions. The greater ability of reef front genotypes to attach themselves firmly to the substratum appears to be a genetically determined adaptation for survival and persistence in rocky reef front conditions, enabling coral fragments to attain a rapid and strong foothold. Baker et al. (1997) suggest the possibility of a cryptic species occurring within *A. cervicornis*, based on the occurrence of two distinctly different zooxanthellae taxa that were never found to co-occur in an individual coral. While the cryptic species proposed by them were segregated by depth, the *A. cervicornis* morphotypes of this study are segregated according to energy regime.

This study showed that morphological variation between back reef and reef front populations of *A. cervicornis* was relatively fixed. On the other hand, *A. prolifera* from the reef front changed growth form completely when grown in the back reef, transforming to become indistinguishable from the back reef genotypes. The thick-branched, open form described in Vaughan (1901), transformed into that of the undescribed slender-branched, bushy back reef type, indicating a plastic morphology based primarily on environmental influence. The back reef and reef front growth forms of *A. prolifera* thus conform to the definition of "ecomorphs", while the two forms of *A. cervicornis*, being independent of environmental causation are more accurately referred to as morphotypes.

If environmental conditions in the Caribbean have become unfavorable to the long-term survival of *A. cervicornis* as a species, coral transplantation might be used as part of a mitigation and recovery strategy. Artificially increasing the genetic diversity within monoclonal populations would potentially enhance natural fertilization rates, increasing sexual recruitment and genetic recombination and thus accelerating the recovery and adaptive potential of this ecologically important coral species. Another measure might include rescuing genetic material from declining *A. cervicornis* populations, transplanting and culturing coral fragments on frames as described in this study, as long as these transplantation efforts did not cause additional mortality. Reestablishing a more natural balance of herbivores on reefs where other factors such as siltation are not overpowering would potentially both increase the recruitment of coral larvae and the survival of remaining *A. cervicornis* populations. Effective interventions based on transplantation to conserve and restore *A. cervicornis* and *A. prolifera* will require further clarification of the population genetics of these species. Of course any recovery strategy will prove futile if the factors leading to coral reef decline in the Caribbean region are not reversed.

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