

**Interspecific Hybridization in *Acropora*
(Cnidaria: Scleractinia): Mechanisms and Evolutionary
Consequences**

Thesis submitted by
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Abstract

This thesis addresses several aspects of the genetics and reproductive biology of cross-fertile, mass-spawning scleractinian corals, specifically in the genus *Acropora*, and the results presented contribute to our understanding of the evolutionary consequences of hybridization in this animal group.

The rDNA ITS1-5.8S-ITS2 region has been used for phylogenetic analysis of cross-fertile coral species in the genus *Acropora*, and has shown patterns of variation consistent with reticulate evolution. However, results from a number of analyses in this thesis, including the occurrence of deamination-like substitutions at methylation sites; differences in evolutionary rates among clades of a 5.8S phylogeny; and occurrence of non-compensatory mutations that may affect the rRNA secondary structure, suggest that at least part of rDNA diversity in *Acropora* is due to pseudogenes.

Natural hybridization in coral genera may cause taxa to merge through homogenization of gene pools or may create new hybrid species. Here I demonstrate that high cross-fertilization *in vitro* does not guarantee the merging of species. Data from eight polymorphic allozyme loci indicate small but significant differentiation between sympatric populations of *A. cytherea* and *A. hyacinthus*, a pair of acroporid corals with very high interspecific fertilization rates *in vitro*. The biological significance of differences between the species in sympatry is highlighted by the absence of genetic differentiation between widely allopatric populations within each species. Moreover, a Nested Clade Analysis using sequence data from a nuclear intron indicates that these two species constitute distinct evolutionary lineages. I conclude that *A. cytherea* and *A. hyacinthus* are neither merging nor constitute morphs within a single species, but rather conform distinct cohesion species.

Cross-fertilization trials may overestimate the rate of hybridization that occurs under natural conditions, because they are non-competitive, involving the exclusive combination of sperm from one species with eggs from another. I designed breeding trials using acroporid corals to test whether the mixture of conspecific and heterospecific sperm inhibits interspecific fertilization, promoting conspecific sperm precedence. However, spawning failure and low cross-

fertilization rate between the study species did not allow evaluating this hypothesis properly.

Integrins are proteins involved in cell adhesion that play major roles in gamete binding and fusion in mammals. A cDNA sequence encoding for a $\beta 1$ -class integrin has been identified in the scleractinian coral *Acropora millepora*. Given that the integrin mRNA is present in unfertilized eggs, the corresponding protein may have a potential role in coral fertilization. As a first attempt to elucidate the molecular basis of gamete specificity in corals, I studied the role of the *Acropora millepora* β_{Cn1} integrin in fertilization. I examined the effect of polyclonal antiserum raised against a substantial part of the β_{Cn1} integrin on fertilization rates of *A. millepora* eggs. The results indicate that *Acropora* β_{Cn1} integrin is involved in sperm-egg binding but does not confer reproductive specificity. The implication of a disintegrin-integrin binding in the fertilization process in *Acropora* suggests that some functions of these molecules may have been conserved in corals and humans.

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I had the fortune of having not one supervisor, but three excellent ones. I am very happy to admit that during my Ph. D., I remained silent when other graduate students ranted about their supervisors. My lack of complaining was not due to my thesis being "too easy". On the contrary, as any other, it was plagued by difficulties. But precisely in those hard moments was when I received good advice and full support from them. I especially appreciate the open and friendly discussions with Dave Miller, which surprised more than one overseas student used to strict hierarchical relationships between supervisors and students. I am very grateful of Madeleine van Oppen's kind recommendations on my everyday work at the lab, or during my struggle with the analyses, particularly her "that's weird, it never happened to me", that always made us laugh. I will never forget Bette Willis' strength, determination and stamina, which is an inspiration for all of her students, as well as her welcoming disposition to share her amazing knowledge of coral biology.

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