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

Thesis submitted by
Luis Miguel Márquez, Licentiate (USB) MSc (IVIC)
in February 2002

for the Degree of **Doctor of Philosophy**
in the Department of Biochemistry and Molecular Biology at
James Cook University of North Queensland

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 ocurrence 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 precendence. 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 β1-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.

STATEMENT OF ACCESS

I, the undersigned, the author of this thesis, understand that James Cook University will make it available for use within the University Library and, by microfilm or other means, allow access to users in other approved libraries.

All users consulting this thesis will have to sign the following statement:

> In consulting this thesis, I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author; and to make proper public written acknowledgement for any assistance which I have obtained from it.

Beyond this, I do not wish to place any restriction on access to this thesis.

Signature

Date

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Signature

Date

Acknowledgments

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.

Many people were very influential on my work, but especially my lab mates who shared their knowledge friendship with me. Jason Mackenzie who, after starting his Ph. D. at the same time as I did, became my twin soul, sharing all the enjoyment and misery that the field and lab work had prepared for us. John Reece-Hoyes thought me all his very useful "dirty-quickie" lab techniques, while Julian Catmull made enormous efforts trying to erase them from my memory, teaching me the proper way of doing things. Julian also developed the antibodies against integrin that I used afterwards. Nikki Hislop, my office friend, was my compass through the lab and my faithful assistant during coral spawning. More recently, Carlos Sanchez, Danielle de Jong and Hiroki Go also lent me a hand when I needed it. The honours and small-project students that went through the lab were all very helpful and friendly: Kate Hardie (thanks to her fantastic work expressing coral Integrin I had a sixth chapter), Teun, Ingo, Robert, Brenda, Jill, "little" John, Hanneke, Anouk, Friso, Chris (my Oz and Japanese language teacher), Melanie, Karin U, Karin O, Dalma, Alejandro (who shared his Pax and mtDNA sequences with me), Lucija and Elisse. Members from other labs within Biochemistry at JCU were of great help at several

occasions: Prof. Jim Burnell, Prof. David Yellowless, Dr. Subash Vasudevan, Ross, Moira, Ellessa, Bill, Andrew and Paula.

I am very grateful to all the people that shared a piece of their expertise with me. Beth Ballment, Sven Uthicke and David Ayre gave me technical advice with the allozymes. Eldon Ball provided me some very good ideas in relation to my integrin work. Steve Croft helped me with the pictures. David Posada, Lars Jermiin, Lynn Atkinson and Line Bay advised me with NCA and phylogenetic analyses. Carden Wallace and Jackie Wolstenholme guided my incursion into *Acropora* identification.

During my fieldwork I received help from many people, particularly during coral spawning, many thanks to all of them. I want to acknowledge some of them in particular: Cathy, J. J., Brita, Melissa, Brian, Oliver, Xavier, Ben, Ameer, Rick, Andrew Baird, and Adam. Maggie and Michael Fogg were also helpful during my visits to OIRS. I thank Katharina Fabricius for allowing me to collect samples during her field trips on the "The Harry Messel", whose crew was amazingly helpful. Luke Smith and Andrew Heyward assisted me during the fieldwork in Dampier.

I thank the Australian Institute of Marine Science, for allowing me to use its facilities at Dampier and Cape Ferguson for the allozymes work. This project was funded by the Australian Research Council and James Cook University. I also acknowledge a scholarship from the Venezuelan Council for Scientific and Technologic Research (CONICIT), and an educational credit from Fundayacucho.

Last but not least, I want to acknowledge all the people closer to my heart. My wife and best friend, Carolina Bastidas helped me throughout each step of my Ph. D., since we looked for funds to come to Australia, during my fieldwork, lab work, and writing. I am the happiest man on earth sharing my life with such a wonderful person. My parents Miguel and Amparo, my sister, Lina, and my mother in law, Hilda, also gave me all the emotional support that I needed, even when the distances made it difficult. Maggie, Roberto, R. Andrés, Ana Corina, Angel, Aurora, Victor and Cañemo also contributed their fair share. Many friends were always eager to share their ideas and listen and to my rambling: Pedro, Mauricio "Teque", Yamil "Pocho", Viviana, Claudia, Guillermo "G", J.J., Aniuska, Bernardo, Mark, Irene, and Debbie.

Contents

List of Figures

List of Tables