The molecular basis of fertilisation in coral *Acropora* and its role in speciation

Thesis submitted by

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Abstract

To shed light on diversification of the genus *Acropora* (Scleractinia, Cnidaria), one of the most widespread, abundant, and species-rich genera of hard corals (113–180 species), I searched for fertilisation-related genes in a model coral, *Acropora millepora*, and examined variations in these genes among *Acropora* species. First, by focusing on the ADAM–integrin interaction, which is involved in sperm–egg binding and membrane fusion in mammals, 15 ADAM–integrin interactions related to candidate genes obtained from an expressed sequence tag (EST) database of *A. millepora* were screened using hierarchical strategies (gene structures, gene expression patterns, fertilisation inhibition experiments). However, no evidence was found that these genes, other than integrin betacn1, are involved in *Acropora* fertilisation. To identify fast-evolving genes from *Acropora* species as fertilisation candidates, I then performed direct comparative sequence analysis with EST datasets from two acroporid species: *A. millepora* and *A. palmata* from the Caribbean Sea. Comparison of selected 849 independent genes from the *A. palmata* EST database (4,017 ESTs) to 10,232 ESTs from *A. millepora* resulted in the identification of 513 putative homologues. Within 163 homologous pairs in which dN and dS were examined, 93 homologous pairs had dN/dS ratios significantly <1, which suggests that these genes are under selection pressure associated with functional constraints. Six independent genes showed dN/dS ratios >1, and two of these had a significant deviation from one, suggesting that they are fast-evolving genes. It was unclear whether these fast-evolving genes are involved in fertilisation. Finally,

variations of integrin betacn1, which is involved in coral fertilisation, were compared among some *Acropora* species. Interestingly, comparison of integrin betacn1 sequences demonstrated that there are some mutations around the DxSxS motif, and two combinations of eight different clones showed significant possibilities of positive selection. However, it is unclear whether these variations are related to *Acropora* speciation. The next step is to characterise proteins making molecular complexes with integrin betacn1 or fast-evolving genes, and to compare these amino acid sequences among *Acropora* species.

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