

Variation in the Major Histocompatibility Gene is Maintained By Selection Imposed By Infectious Disease.

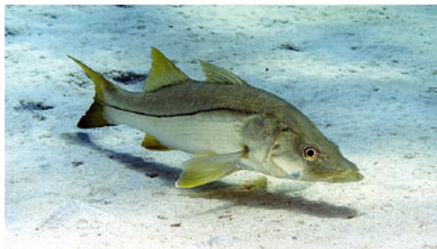
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**Why is polymorphism of the MH gene of great importance for adaptive immunity?
Why is exon 2 the focus of peptide-antigen binding for the MH gene?**

Introduction

- The Major Histocompatibility (MH) genes play critical roles in mounting adaptive immune responses to infectious diseases and parasites. There are two classes of MH genes: Class I and II; Class II can be further divided into Class II A and Class II B genes; this study focuses on Class II B. Class II B genes are exceptionally variable in sequence. Leading hypotheses propose that this genetic variation is maintained by selection for resistance to pathogens. Since pathogens are constantly evolving to avoid susceptibility to MH proteins, and since new pathogens regularly enter populations, individuals must protect themselves from a variety of infectious disease organisms. Individuals bearing a variety of MH Class II B are better protected at any one time, and populations harboring a variety are better protected over the long term.
- This study examines MH Class II B in *Centropomus undecimalis*, the common Snook. Genes will be sequenced, and their variability quantified as a step toward quantifying the importance of the MH genes in the reproductive success of this fish in Florida.

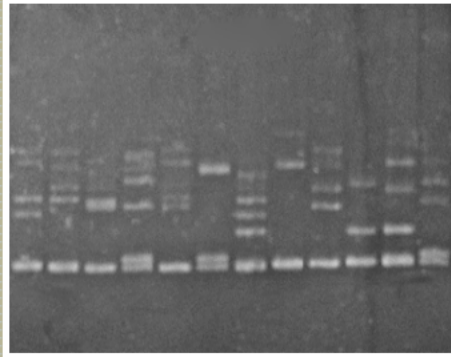


Methods

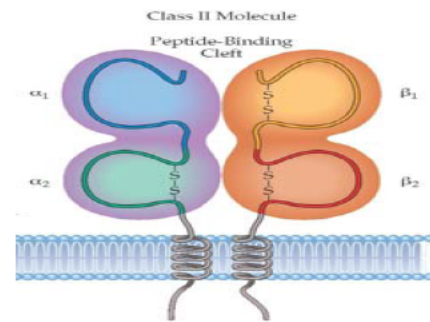
- Our approach utilizes database searches (NCBI), cloning, PCR, and sequencing. After obtaining sequences, contigs are then assembled using Sequencher.
- Comparison of sequences from single individuals will reveal how many copies of the MH Class II B genes are present. Comparison of sequences among individuals will provide insight on how gene variability is maintained by immune responses to infectious pathogens.

MHC Variation

Image Below: Gel Electrophoresis of sampled clones; the lowest (smallest) band has been shown to lack sequence variation in Exon 2. This project focuses on the upper bands.

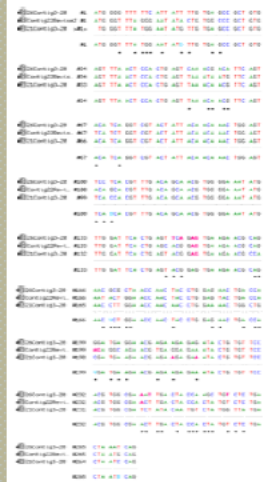


MHC Class II B Gene

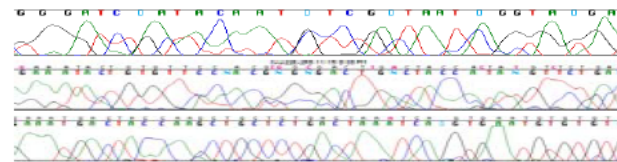


Above Image: Both classes of MH proteins present peptides to T cells, stimulating the T cells to initiate an immune response. The $\beta 1$ domain of the Class II protein is encoded by Exon 2 of the Class II B; this is where most of the sequence variability is found. Different sequences bind different peptides into the cleft.

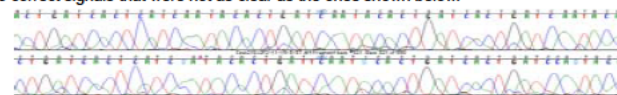
Preliminary Data



By sequencing three clones, I have shown that there is variation in exon 2 of the MH Class II B gene of the common Snook, *Centropomus undecimalis*. Each black marker beneath the individual bases, left, signifies polymorphism at that site in the gene. By obtaining 3 different alleles for 3 individual clones, this expresses the likelihood of many varying alleles in a given population. These individual clones are therefore unlike clones that have been sequenced prior to now.



Aligned above are the chromatograms extracted from bases 239-273. This is a more visual comparison of the variation in the 3 clones. Extensive editing was carried out in order to correct signals that were not as clear as the ones shown below.



Discussion

Though widely variable, MH genes are found in all vertebrates. MHC genes are the most polymorphic genes known, and for this reason, antigen recognition is affected. The peptide binding region of the MH gene is thus influenced. The evolutionary selection for polymorphism found within the MH gene provide insight that the this gene plays a vital role in immune response.

This research is currently still in progress, as more clones are in the process of being sequenced and their variability quantified. Once sequenced, progressions can be made in order to determine the number of loci found on the MH Class II B gene of the common Snook. The issue of flanking sequences (un-transcribed regions; Intron 1 and Intron 2) of Exon 2 can then be resolved in order to develop PCR primers capable of amplifying a single locus at a time. With the availability of primers that can amplify these un-transcribed regions, we can assuredly determine the degree of polymorphism of each locus. It is then in which the genotype of an individual species of *Centropomus undecimalis* can be compared to the genotype of a parasite burdened individual.

My presumptions are that there will be complete variability observed among the two individuals and each loci especially since this gene is maintained via pathogen-driven selection. What one individual may have immunity to, the other may possess an allele of the MH gene rendering it susceptible. Genetic diversity and adaptive immunity is thus important at the level of the Major Histocompatibility Class II B gene.

References

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