Advancing threespine stickleback fish as an outbred immunogenetics model by pinpointing the onset of adaptive immunity

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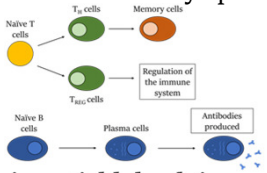
Abstract

- Genetic variation creates differences in immune function among individuals, particularly as the adaptive immune system first begins to develop.
- Understanding the genetics of developmental immunodeficiency is limited by the invasive nature of prenatal analysis in mammals.
- Threespine stickleback fish (*Gasterosteus aculeatus*) offer advantages as a developmentally accessible model for studying adaptive immunity in the context of genetic variation.
- The onset of adaptive immunity is currently unknown in stickleback, and is a necessary piece of baseline knowledge
- We will pinpoint the onset of adaptive immunity by analyzing early activators of adaptive immunity in genetically diverse stickleback.
- This work will further studies using stickleback as an immunogenetics disease model.

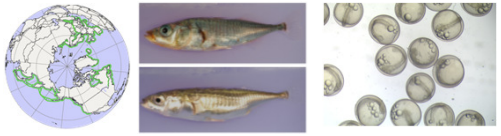
Introduction

1. Development of the adaptive immune system

- Adaptive immunity is fully functional after differentiation of T and B lymphocytes[3,4]



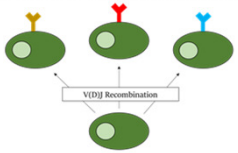
2. Threespine stickleback is an accessible model for studying adaptive immunity



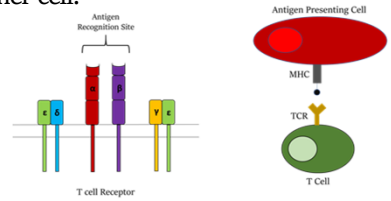
- Threespine stickleback live in a wide range of habitats and exhibit genetic variation within and between populations.
- External fertilization and transparent embryos facilitate developmental assays.

3. *rag1* and *tcr-β* are early genetic markers in the onset of a functional adaptive immune system

- Recombination Activation Gene *(rag1)* activates V(D)J recombination necessary for creating a diverse repertoire of antibodies and T cell receptors.[5]

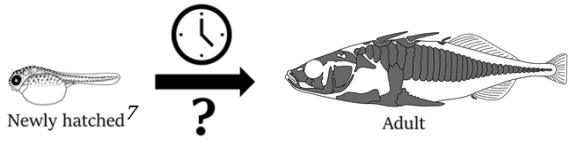


- A T cell becomes activated when receptors (TCR) on its membrane bind antigens presented by the major histocompatibility complex (MHC) on another cell.[6]



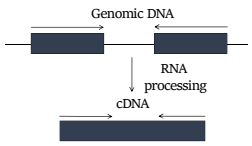
4. When does the adaptive immune system first develop in threespine stickleback?

- In other model fish, adaptive immunity is not activated until zero to three weeks after hatching.[1,2]
- We will harvest RNA from a developmental time series of stickleback, starting at hatching (6 days post fertilization).

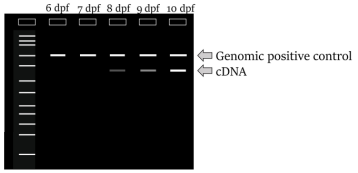


- rtPCR of early immune genes *rag1* and *tcr-β* will be used to detect the earliest onset of the adaptive immune system.

cDNA primer design

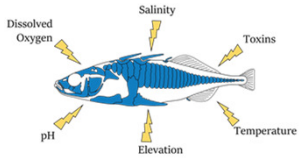


Expected Results



5. What environmental factors affect the robustness of the adaptive immune response?

- Many environmental factors have been shown to influence the adaptive immune system.



- We will collect wild adult stickleback from a variety of Oregon habitats and take environmental measures of temperature, pH, salinity, dissolved oxygen, elevation, and known toxins like perchlorate.
- We will measure the robustness of the adaptive immune response by measuring T cell abundance using flow cytometry.

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Citations

1. Moran, G, *et al. Vet Med* **56**(10). 2011
2. Nie, P, *et al. Immunol Immunopathol* **132**(2-4). 2009
3. Tedder, TF, *et al. Blood* **112**(5). 2008
4. Kondo, M, *et al. Semin Immunol* **20**(4). 2008
5. Yang, W, *et al. Mol Cell* **35**(2). 2009
6. Medzhitov, R, *et al. Science* **327**. 2010
7. Swarup, H. *J Embryol Exp Morphol* **6**(3). 1958

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