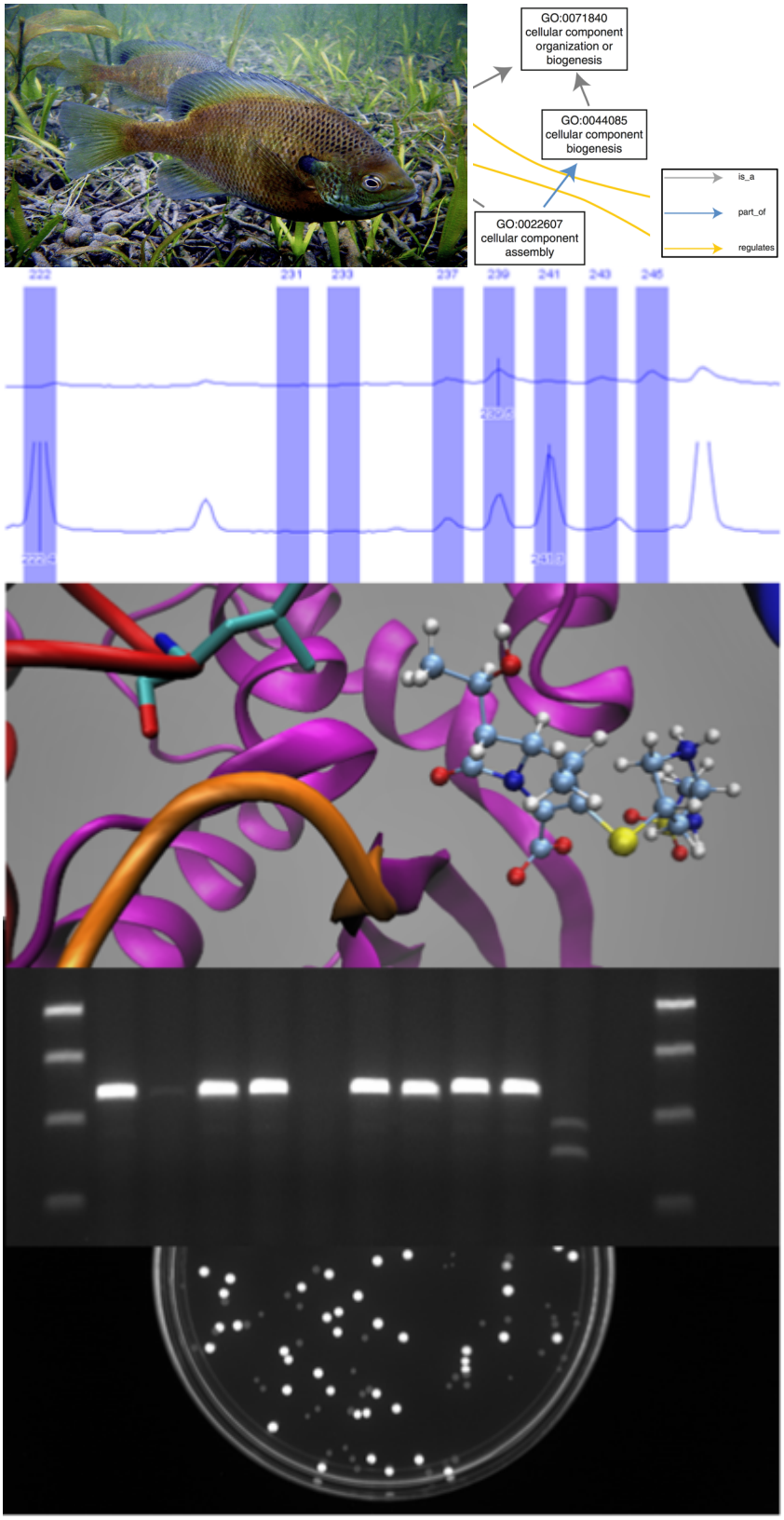
IV Cell & Molecular Biology Symposium



Isolating Hdc Gene Deactivation in *Drosophila Melanogaster* Through Transposon-Excision Mutagenesis

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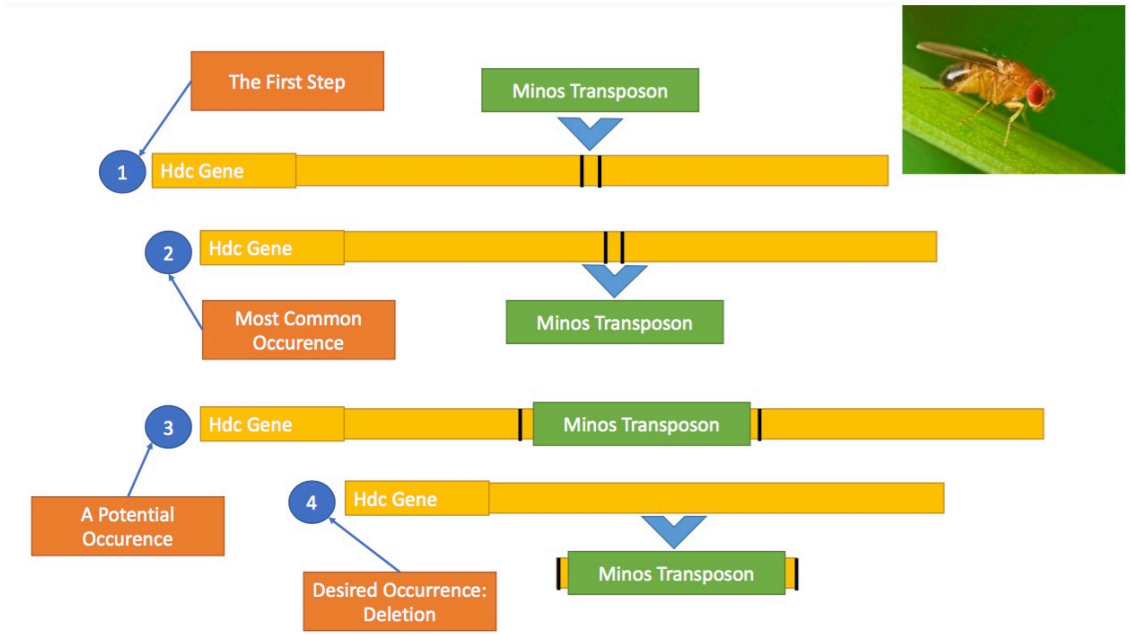

Gene silencing is a powerful tool that allows researchers to study the function of a particular genetic sequence and can be accomplished throughs several techniques. One method of gene silencing is to use transposase to excise an inserted transposon, which rarely causes an imprecise excision event, removing part of the gene sequence along with the transposon, effectively silencing the gene. There is a current lack of *Drosophila Melanogaster* null mutant lines that can used for further research on the genomic region and restricted expressivity involving Hdc. The primary focus of this study was to isolate multiple *Drosophila* lines that possess imprecise excision events within the Hdc gene in order to create null mutants that can be used for further research. The Minos transposon was inserted into the Hdc gene of a *Drosophila* line, which was then bred with another *Drosophila* line containing the transposase, producing up to 150 different lines isolated for observation. To determine which lines had an imprecise deletion of Minos we designed primers to anneal to the Hdc sequence outside of Minos. If the necessary sequence was present, an amplicon will be created, whereas if there was an imprecise excision event, the necessary sequence would have been excised, causing the amplicon to not be produced. The role of this project could create new opportunities for research regarding the effect of histamine and Hdc gene in relation to various other genomic alterations.

Impact of sampling length on loop dynamics of several clinical mutants of OXA-66 and parameterization of doripenem for use in MD simulations

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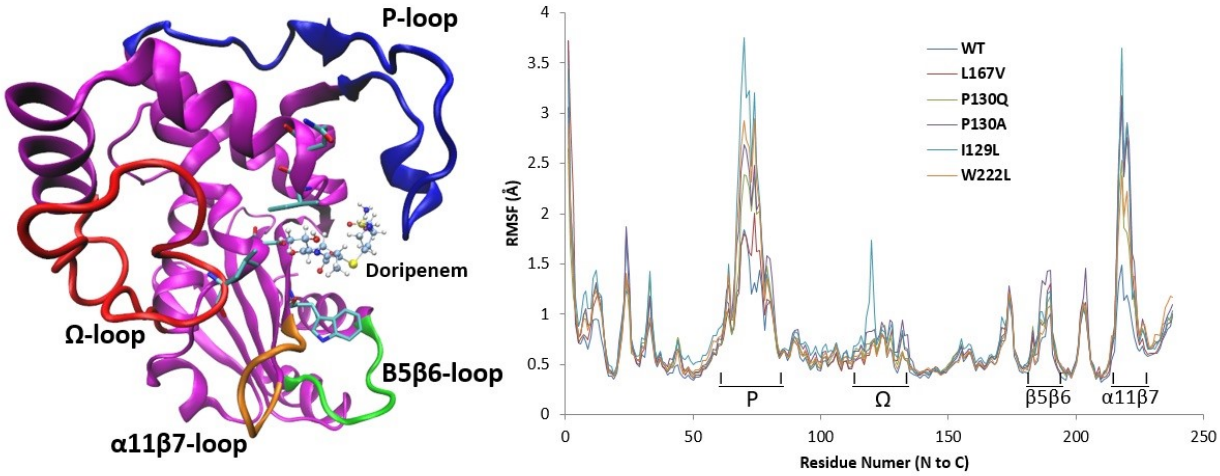

Antibiotic resistant bacteria are a leading cause of nosocomial infections. The primary mechanism of resistance against clinically significant β-lactam antibiotics is through the production of β-lactamases. Mutants of OXA-66 β-lactamase isolated from drug-resistant *A. baumannii* strains display alarming potential for carbapenemase activity. Previous simulations of OXA-66 mutants (e.g. P130Q, I129L) showed increased rotational flexibility of residues I129 and W222 that is consistent with improved carbapenem binding. The data also indicated a modulation of loop dynamics in the active site. However, it is not clear if the timescale of our simulations allows sufficient sampling of the loop conformations. Additionally, the simulations of apoenzymes provide limited insight into how the loop dynamics contribute to carbapenemase activity. Here we present data from medium and long timescale molecular dynamics simulations of OXA66 P130A, I129L/L167V, and W222L mutants. We compare all-Cα RMSD, Cα-RMSD for the loops surrounding the active site, Cα-RMSF, and the distinct loop conformations, from 250 nanosecond simulations to those calculated from microsecond-long trajectories. Our data indicate that longer timescales are necessary to sample the conformations of the 24 amino acid long P-loop. We also present preliminary parameter sets for doripenem for use in future MM/MD simulations of the protein-antibiotic complex. Our data will allow us to better determine the simulation endpoint and sample the conformations of the extended loops and residues interacting with the ligand.

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Investigating Social Evolution Using *Salmonella enterica serovar typhimurium*'s Production of Siderophores

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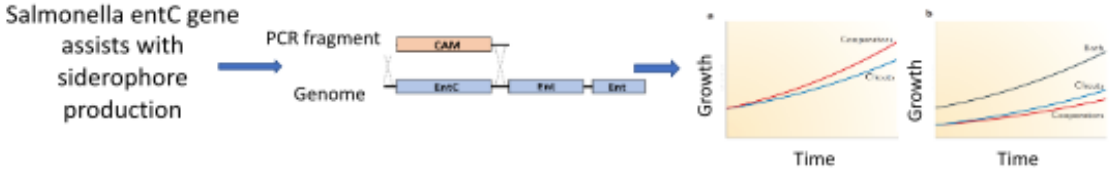

In situations where members of a microbial population 'cooperate' to communally produce metabolites that facilitate the utilization of some resource (e.g. a nutrient), natural selection will favor the emergence of 'cheats': individuals that do not invest the metabolic energy required to produce the metabolite, but which enjoy its benefits (i.e. the ability to utilize the resource) thanks to its production by cooperator (wild type) cells. In such situations, cheater cells will gain a competitive advantage over wild type cells. *Salmonella enterica serovar typhimurium*, an enteric bacterium, produces siderophores, a ferric ion chelating agent, that are essential in obtaining extracellular iron for metabolic use. Our goal was to determine the growth relationship between *Salmonella* wild-type and a Salmonella 'cheater' strain. The wild type strain synthesizes and secretes siderophores while the 'cheats', which do not invest the energy to produce siderophores, are still able to utilize iron owing to the siderophore production by wild type cells. The cheater strain was developed by inactivating the gene pathway for producing enterobactin, a secreted compound for chelating iron. This was done by knocking out the entC gene, which controls the entire operon. Without a functional entC gene, Salmonella was unable to produce enterobactin. The 'cheats', who do not expend as much energy as the wild type, due to the lack of the EntC gene and disruption of the downstream pathway used to create the siderophores, will have a growth advantage in the presence of iron. We plan to analyze the ratio of growth between the wild-type and mutant *Salmonella* through competition assays.

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Differential Expression of Endocrine Genes in Females and Male Alternative Reproductive Tactics in Bluegill Sunfish, *Leopomis macrochirus*

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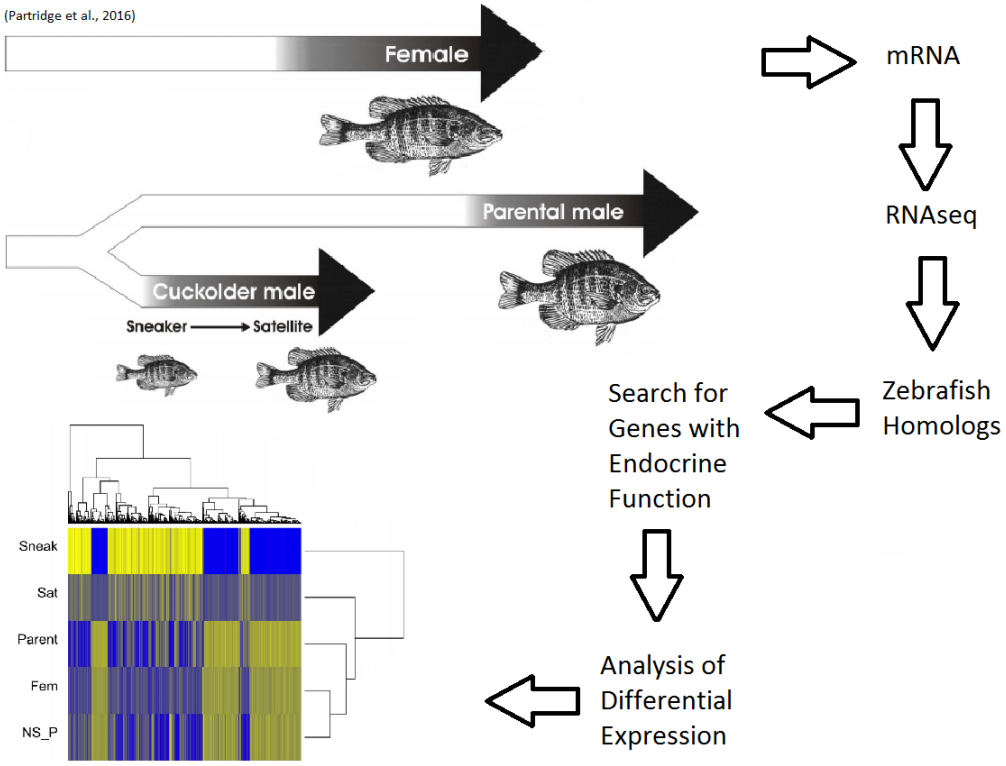

Bluegill sunfish *(Lepomis macrochirus)* are a classic model for the study of alternative reproductive tactics (ARTs). Males have two life histories: parental and cuckholder, and three reproductive tactics: parental, satellite, and sneaker. It is unclear how the endocrine system is involved in producing the distinct behavioral phenotypes observed in male ARTs. In a previous study, RNAseq was used to identify differentially expressed genes from the brains of females, spawning and non-spawning parental males, sneaker males and satellite males. In the current analysis, we focus on genes specifically related to endocrine function in order to better understand the role of the endocrine system in driving these behaviors. From the previously constructed bluegill transcriptome, we identified homologous genes in zebrafish *(Danio rerio)*, then identified the subset of those genes related to endocrine function - using text searches of the Gene Ontology database – and, finally, analyzed these genes for differential expression. Four genes were differentially expressed in females relative to parental males, 63 in females relative to sneakers, 111 in parental males relative to sneakers, 5 in spawning parental males relative to satellite males, 5 in satellite males relative to sneaker males, and 6 in spawning parental males relative to non-spawning parental males. A differentially expressed gene, which was not found in the previous study, Shisa family member 7, an AMPA receptor, related to contextual memory, was downregulated in females and parental males relative to sneaker males. Understanding which genes related to endocrine function are differentially expressed among ARTs will contribute to our understanding of the evolution and maintenance of alternative reproductive tactics in Centrarchid fishes.

Patterns of Population Structure in Gray Bats, *Myotis grisescens*

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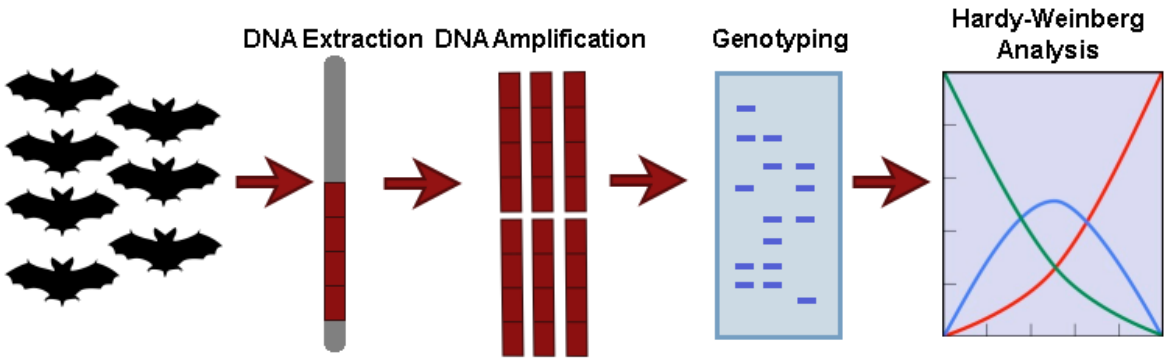

Bat populations of the United States have suffered major declines in the past 12 years due to the emergence of the fungal pathogen *Pseudogymnoascus destructans* (*Pd*). The gray bat (*Myotis grisescens*) has been listed as endangered since 1975 by the U.S. Fish and Wildlife Service due to decreasing habitat from commercialization and the flooding of caves from artificial reservoirs; it has also recently been documented as a host of *Pd*. Gray bats have previously been studied for population structure and diversity, but the microsatellite primers used were for another species of bat. By examining allelic frequencies of non-coding loci in a sample population with specifically designed primers, a species specific neutral determination of genetic diversity can be established. Fifteen microsatellite loci were genotyped in a sample population of 45 *M. grisescens* individuals. We will test these data for Hardy-Weinberg equilibrium, and evaluate patterns of population structure in the sample. The analysis will provide insight into the population health of gray bats in the United States and allow focused efforts towards their conservation.

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