# ENABLING DISCOVERY THROUGH GENOMIC TOOLS PROGRAM

## 1st Annual Investigator Meeting

## National Science Foundation

April 28-29, 2019

The Embassy Suites, Alexandria, VA

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# Agenda

## National Science Foundation
## 1st Annual EDGE Investigator Meeting
## April 28-29, 2019
## Embassy Suites, Alexandria

**Sunday, April 28, 2019**

4:00 pm - 8:00 pm **Registration and Meeting Room Open**

6:00 pm - 8:00 pm **Welcome Meeting**

*Welcoming remarks and meeting goals from Dr. Donal Manahan, Division Director, Division of Integrative Organismal Systems*

**Monday, April 29, 2019**

7:30 am **Registration and Meeting Room Open**

8:30 am - 9:00 am **Welcome from the Directorate of Biological Sciences**

*Dr. Alan Tessier, Deputy Assistant Director, Directorate of Biological Sciences*

*Dr. Donal Manahan, Division Director, Division of Integrative Organismal Systems*

**Welcome and Introduction from EDGE**

*Ted Morgan, Program Director*

9:00 am - 9:45 am **Project Flash Talks**

*Chair: Cliff Weil, Program Director*

Jason Gallant, Michigan State University, *Enabling Genotype-Phenotype Studies in Weakly Electric Fish*

Andrea Sweigart, University of Georgia, *Enabling Functional Genomics in Monkeyflowers (Mimulus)*

Lauren O'Connell, Stanford University, *Developing Techniques for Linking Genotype to Phenotype in Amphibians*

Virginia Weis, Oregon State University, *Functional-Genomics Tools for Cnidarian-Dinoflagellate Symbiosis*

Georg Jander, Cornell University, *Development of Genetic and Genomic Resources for Milkweed, Asclepias syriaca and Asclepias curassavica.*

Scott Juntti, University of Maryland, *Expanding the Functional Genetics Toolkit to Link Genes to Phenotypes in Cichlid Fish*

Leonid Moroz, University of Florida, *Nanoscale Probles and Infrastructure for Real-Time and Single-Cell Genomics Across Metazoa*

Lillian Fritz-Laylin, University of Massachusetts, *Genetic Transformation of Chytrid Fungi*

Amy Toth, Iowa State University, *Functional Genomics in Polistes Wasps, A Model System for Integrative Organismal Biology*

Jonathan Henry, University of Illinois, *Generating Tools to Study Spiralian Development*

Gary Gorbsky, Oklahoma Medical Research Foundation, *Rapid and Efficient Gene Editing of Amphibians Through Nuclear Transfer from Engineered Cell Lines*

James Westwood, Virginia Tech, *Generating Transgenic Cuscuta as a Tool for Studying Plant Interactions*

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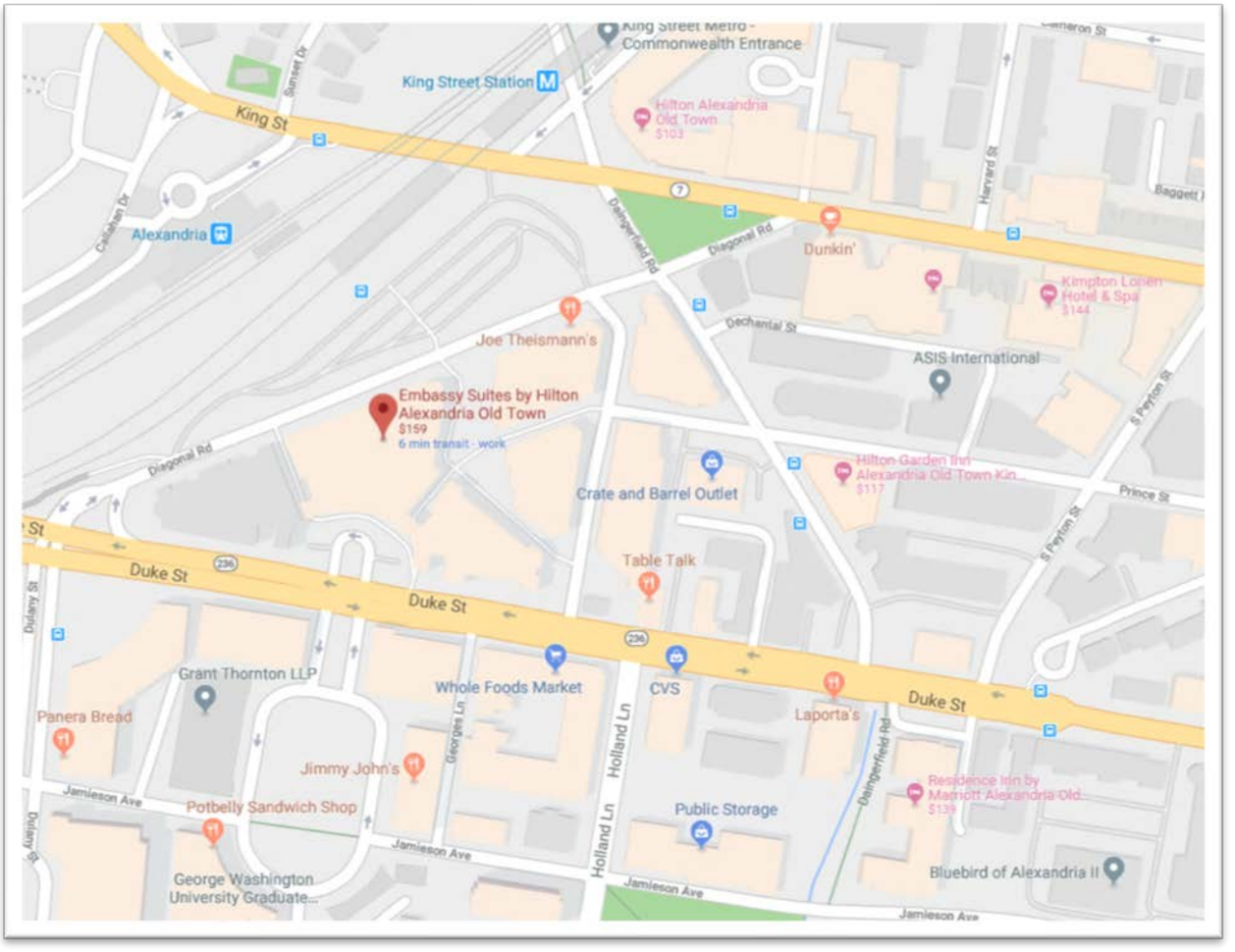
|---|---|
| | Joshua Rosenthal, The Marine Biological Laboratory, *Creation of a Genetically Tractable Cephalopod Model using the Hawaiian Bobtail Squid*<br>Zoe Donaldson, University of Colorado, *Tools for Studying Gene Function in Voles*<br>Doug Menke, University of Georgia, *Establishment of Genome-editing and Transgenic Tools in Anolis Lizards* |
| 9:45 am - 10:15 am | **Break** |
| 10:15 am - 11:45 pm | **Full Length Talks**<br>*Chair: Paul Krieg, Program Director*<br>Claudio Mello, Oregon Health & Science University<br>*Gene Manipulation Resources for Zebra Finches, a Vocal Learner Species*<br>Dan Bolnick, University of Connecticut<br>*Stickleback Evolutionary Genetics Embedded in Ecology*<br>Penny Chisholm, Massachusetts Institute of Technology<br>*Juggling Genes in Prochlorococcus: Challenges, Discoveries, and Opportunities*<br>Celina Juliano, University of California, Davis<br>*Establishing Tools in Hydra to Study Development and Regeneration*<br>Blake Meyers, Danforth Plant Science Center<br>*High Efficiency Identification of Products of Homologous Recombination in Plants as a Tool to Test Gene Function*<br>Jason Rasgon, Penn State<br>*General Tools for CRISPR Editing by ReMOT Control* |
| 11:45 am - 12:05 pm | **Dissemination Resources**<br>Joanne Kamens, Addgene<br>*Addgene: A Better Way to Share Science*<br>Nirav Merchant, CyVerse<br>*CyVerse: Cyberinfrastructure for All of Us* |
| 12:05 pm - 2:30 pm | **Working Lunch and Poster Session** |
| 2:30 pm - 2:40 pm | **Meeting Announcements** - *Ted Morgan* and *Dan Bolnick* |
| 2:40 pm - 2:55 pm | **Interagency Synergy with EDGE**<br>Jake Basson, National Institute of General Medical Sciences |
| 3:00 pm - 5:15 pm | **Meeting Discussion Groups**<br>Discussion topic #1 - *What do we need to meet the G to P grand challenge?*<br>*Introduced by Michelle Elekonich, Program Director*<br>Discussion topic #2 - *Bioethics and public outreach - starting the conversation*<br>*Introduced by Clare Palmer (Texas A & M) and Jason Delborne (NCSU)* |
| 5:15 pm - 5:30 pm | **Meeting wrap up and the next EDGE Awardee meeting** |

# Meeting Arrangements

The meeting will be held at the Embassy Suites in Alexandria, VA, starting at 4:00 PM on Sunday April 28, 2019 in the Virginia Ballroom. The Embassy Suites Hotel is located at 1900 Diagonal Road (see map below). *Light refreshments will be served during the meeting.*

*Location of Embassy Suites by Hilton Alexandria Old Town*

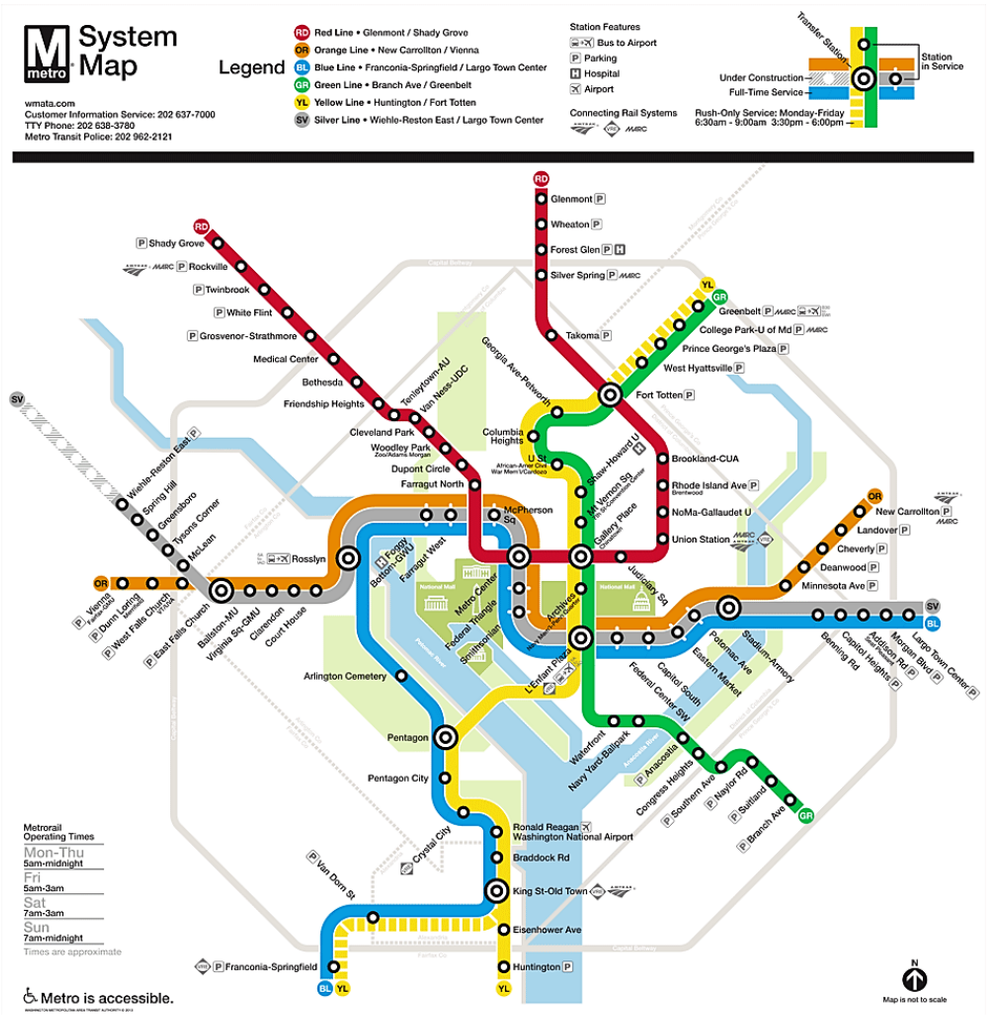


# Transportation to the Embassy Suites Hotel

From Ronald Reagan National Airport, you can take DC Metrorail, Uber, Lyft or taxi. For Metrorail, take the Yellow line in the direction of Huntington (left side of the platform as you go up the escalator) and then stay on the Yellow line until King St Metro station. The Embassy Suites Hotel is across the Metro parking lot and then head to your right, away from Joe Theismann's Restaurant. Fares are $2.40 (peak) to $2.15 (off-peak). SmarTrip cards can be purchased at the Metro station for $10, which has $8 stored fare value.

Alternatively, you can use Uber, Lyft or taxi. At Reagan National Airport, the taxi stands are located soon after you exit baggage claim are. For Uber or Lyft, you will need to be at specific rideshare stands (Terminal A: indicated by a Lyft sign, Terminal B/C: Door 6 & 7). Taxi rate is about $26.20-30.15 while Lyft varies from $16 to $26 and Uber varies from $15 to $35 depending on availability.

From Dulles International Airport – The best option is Lyft (Arrival Door 4), which will cost about $47, Uber will be about $52, and taxi will be about $72. Other lower cost options (~$10) are (1) taking the 5A bus to L'Enfant Plaza Metro Station and then taking the Yellow line down to King St Metro station (1 hr and 30 min) (2) taking the SL1 bus to Wiehle-Reston Metro station and then the Silver line to L'Enfant Plaza Station and then transfer to the yellow line to go to King St Metro station (1 hr and 43 min).



# Contact Information for Program Directors

**Enabling Discovery Through Genomic Tools Program**
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To reach all EDGE staff: bioiosedge@nsf.gov

# Posters in the Meeting Room

**Poster Session:** Mon. April 29, 12:30 pm – 2:30 pm. Posters will be mounted in the Virginia Ballroom displayed by number. The Poster session will be held on the Monday. ***Please identify your poster number in the first column***, stand by your poster during your assigned session.

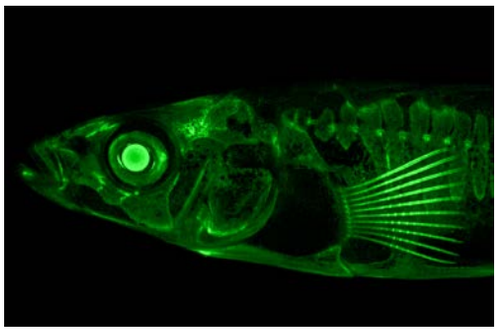
**Poster numbers are assigned alphabetically by project PI's last name:**

| Poster # | Award ID | Last Name |
|---|---|---|
| **1** | 1645170 | Bolnick |
| **2** | 1645061 | Chisholm |
| **3** | 1827990 | Donaldson |
| **4** | 1827257 | Fritz-Laylin |
| **5** | 1644965 | Gallant |
| **6** | 1645105 | Gorbsky |
| **7** | 1827533 | Henry |
| **8** | 1645256 | Jander |
| **9** | 1829158 | Juliano |
| **10** | 1825723 | Juntti |
| **11** | 1645199 | Mello |
| **12** | 1827647 | Menke |
| **13** | 1827761 | Meyers |
| **14** | 1645219 | Moroz |
| **15** | 1827333 | O'Connell |
| **16** | 1645331 | Rasgon |
| **17** | 1827509 | Rosenthal |
| **18** | 1827645 | Sweigart |
| **19** | 1827567 | Toth |
| **20** | 1645164 | Weis |
| **21** | 1645027 | Westwood |

# One-Page Project Descriptions and Updates

# Expanding Functional Genetics in Threespine Stickleback to Place Genomics Into Its Natural Context

Bolnick, Daniel · 1645170· University of Connecticut · daniel.bolnick@uconn.edu · (860) 486-3156



A stable transgenic adult threespine stickleback fish showing GFP expression in skeletal tissues.

## Project Objectives

Threespine stickleback present a massively-replicated natural evolutionary experiment. Marine stickleback repeatedly and independently colonized freshwater watersheds in postglacial environments. Their (partly) parallel adaptation to disparate freshwater habitats provides an opportunity to investigate the genetic basis of adaptive evolution in many disparate organismal traits: development, immunity, neurobiology, and more, set within a well-understood historical and ecological setting. Extensive genomic data provides a valuable foundation for this genetic research, but key tools remain underused or unavailable, hindering progress at functionally characterizing candidate genes. We seek to expand the functional genetics toolkit for stickleback, by optimizing methods for CRISPR-Cas9 editing of embryos by microinjection, adult brain tissue by viral transformation, and in vitro cell culture by nucleoporation. In support of these tools, we are also establishing a stickleback stock center where researchers globally can order fish eggs, adults, cryopreserved sperm, or cryopreserved cell cultures. Lastly, we are developing a gene expression atlas to improve gene function annotation needed to guide experimental design.

**Participants**

| Last Name | First Name | Role | Institution | Email |
|---|---|---|---|---|
| Bell | Alison | co-PI | University of Illinois | alisonmb@illinois.edu |
| Bolnick | Daniel | PI | UConn | daniel.bolnick@uconn.edu |
| Milligan-Myhre | Kathryn | co-PI | University of Alaska | kmillig1@alaska.edu |
| Miller | Craig | co-PI | University of California Berkeley | ctmiller@berkeley.edu |
| White | Mike | co-PI | University of Georgia | whitem@uga.edu |

**Keywords:** Threespine stickleback, *Gasterosteus aculeatus*, teleost, evolutionary developmental biology, evolutionary immunology, neurobiology, microbiome

**Project Website:** Stickleback wiki: http://sticklewiki.pbworks.com/w/page/124292094/FrontPage

## Progress to Date

We are developing several parallel lines of functional genetics tools for threespine stickleback:

Craig Miller's lab has established that **CRISPR-Cas9 homology-directed repair** is feasible in stickleback embryos with minimal genetic scarring. They have shown that mutations in *Hps5* result in albino larvae and fish, making a simple tool for scoring Cas9 editing efficiency, as well as better visualization of fluorescent transgenes. They have also been optimizing transgenesis in sticklebacks to study enhancer and gene function.

Dan Bolnick's lab generated fibroblast cell cultures from multiple source populations. They developed protocols to cryogenically preserve cultures, and measured population differences in cell function. They **are testing in vitro gene editing protocols**.

Kathryn Milligan-Myhre's lab has surveyed the stickleback research community to determine demand for stock center materials and **initiated a breeding program** at the University of Alaska.

Mike White's lab has improved the reference genome sequence and **annotation of coding and regulatory regions**. Long-read sequencing characterized full length transcripts and isoforms across the genome. ATAC-seq profiled regions of open chromatin across multiple tissues and developmental stages.

Alison Bell's lab has successfully **altered brain gene expression** in adult stickleback brains using the HSV1 derived virus with both a short-term (mCMV, peak expression by 4 days post-injection) and longer-term (hCMV) promoter.

## Highlights

We developed a community website (Sticklewiki) where researchers throughout the community can post protocols, collection locations, data, etc., http://sticklewiki.pbworks.com

Small travel grants will be distributed in May 2019 to encourage between-lab exchanges for collaboration and protocol dissemination.

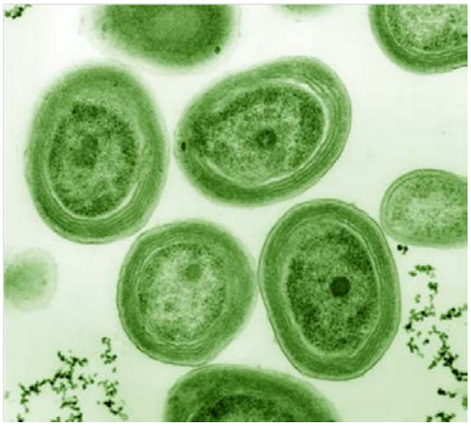
A summer course emphasizing gene editing experimental design, with hands-on practicals, is planned for summer 2020.

IOS-EDGE supported postdoc, Lauren Fuess, received a year-long American Association of Immunologists Intersect Fellowship to use single-cell RNAseq to identify immune cell types in stickleback,

IOS-EDGE supported postdoc Natalie Steinel published "Melanomacrophage centers as a histological indicator of immune function in fish and other poikilotherms" in *Frontiers in Immunology. 2017, 8:827.*

# Development of Genetic Tools for the Dominant Phototroph in the Oceans

Chisholm, Sallie · 1645061 · Massachusetts Institute of Technology · chisholm@mit.edu · (617) 253-1771



*TEM image of Prochlorococcus, the smallest and most abundant oxygenic phototroph on Earth*

## Project Objectives

The goal of this project is to develop an efficient transformation method and a panel of functional genomics tools for *Prochlorococcus*, the numerically dominant phototroph in the oceans.
The first aim was to develop an efficient way to deliver DNA inside *Prochlorococcus* cells without compromising the cell viability. To that end a collaboration was established with co-PI, Cullen Buie, who has designed electroporation microfluidic technology specifically for this purpose. The second aim has been to develop transposon genetic tools that will allow us to perform global mutagenesis experiments and provide a way to link genes to their phenotype in a genome-wide manner. The third aim is to develop targeted mutagenesis tools - using the CRISPR method - to allow specific gene knockout to answer more focused biological questions.
In terms of broader impacts, *Prochlorococcus* is an amazing vector to foster public interest on topics such as ocean ecology, climate change, biodiversity and genetics. It attracts the attention of science journalists, and serves as an 'ambassador' for microorganisms, drawing young scientists into the field.

**Participants**

| Last Name | First Name | Role | Institution | Email |
|---|---|---|---|---|
| Chisholm | Sallie (Penny) | PI | MIT | chisholm@mit.edu |
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| Laurenceau | Raphael | Postdoc | MIT | rlauren@mit.edu |
| Capovilla | Giovanna | Postdoc | MIT | giocapo@mit.edu |
| Church | George | Collaborator | Harvard | gmc@harvard.edu |
| Kehoe | David | Collaborator | U. Indiana | dkehoe@indiana.edu |
| Johnston | Christopher | Collaborator | F. Hutch Institute | johnston@fredhutch.org |

**Keywords:** *Prochlorococcus*, cyanobacteria, bacterial transformation, phototrophy, marine ecology, phytoplankton

**Project Website:** https://chisholmlab.mit.edu/

## Progress to Date

We have developed an electroporation protocol for *Prochlorococcus*. We are now able to successfully deliver DNA inside cells while keeping cells viable.

We have designed and tested several transposon tools using various antibiotic resistance genes. We have attempted to transform these elements in several strains of *Prochlorococcus*, without success so far.

We have identified and characterized small mobile genetic elements within *Prochlorococcus* genomes which are promising targets to repurpose for genetic engineering. They are small, inducible, integrative and replicative elements that we are currently modifying to carry a selectable marker.

We have established new collaborations on the project that promise to advance our work significantly. With David Kehoe we are leveraging his success in developing a CRISPR system with *Synechococcus.* With Christopher Johnston we are drawing on his expertise to avoid bacterial defenses to exogenous DNA.

Finally, we have been working with colleagues at the U. Cordoba, Duke, and Roscoff Marine Station (CNRS) to organize a Symposium and Workshop focused on marine picocyanobacteria. The Workshop will be funded in part by this grant with supplemental funds from private foundations and will be focused on distributing tools developed under the auspices of this grant. The goal is to train the next generation of researchers on the nuances of working with recalcitrant microbes like *Prochlorococcus,* that have only recently been domesticated.

## Highlights

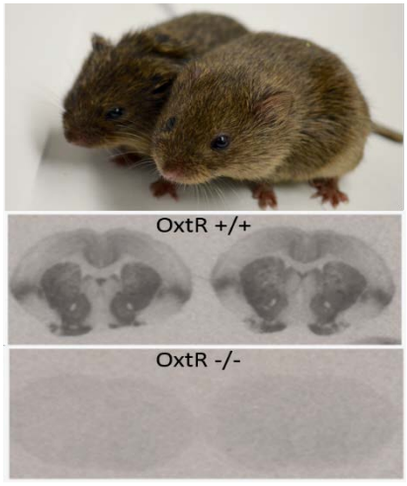
***DNA delivery via electroporation in the marine cyanobacterium Prochlorococcus***
Laurenceau R, Bliem C, Becker JW, Biller S, Chisholm SW.
(in preparation)

***Integrative elements as drivers of genome plasticity in the marine cyanobacterium Prochlorococcus***
Laurenceau R, Hackl T, Ankenbrand MJ, Bliem C, Cariani C, Thomas E, Chisholm SW.
(in preparation)

***ProSynFest 2020***
This conference on marine picocyanobacteria at the U. Cordoba in Spain will be preceded by a one-day workshop to disseminate the methods developed in the EDGE project.

# Engineering the Social Brain in *Microtus*

Donaldson, Zoe · 1827990 · University of Colorado Boulder · zoe.donaldson@colorado.edu · (303) 735-8879




Oxytocin receptor knockout prairie voles.

## Project Objectives

One goal of biological investigation is to understand the organizing principles of natural variation. To accomplish this goal requires causal manipulations of non-traditional model species. Voles of the genus *Microtus* stand out as popular study subjects. They vary in mating system, social attachment, and other behaviors that cannot be studied in traditional laboratory species. This project addresses a current bottleneck in integrative research with voles: the lack of technologies to directly interrogate neurogenetic function. To address this, the team of three PIs will: 1) Optimize tools for germline manipulation of *Microtus* embryos; 2) Develop an intravenously deliverable genetic vector; and 3) Test methods for post-mitotic gene manipulation in cells and regulatory circuits. The teams will disseminate these advances and train other researchers at all levels of their careers, spawning a cohort of scientists versed in these technologies and who will engage the broader public regarding the need for these technologies and the exciting discoveries that await their implementation.

**Participants**

| Last Name | First Name | Role | Institution | Email |
|---|---|---|---|---|
| Donaldson | Zoe | PI | CU Boulder | Zoe.donaldson@colorado.edu |
| Loth | Meredith | Senior Personnel | CU Boulder | Meredith.loth@colorado.edu |
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| Tripp | Joel | Senior Personnel | UT Austin | Joel.tripp@utexas.edu |
| Manoli | Devanand | Co-PI | UCSF | Devanand.manoli@ucsf.edu |
| Sharma | Ruchira | Senior Personnel | UCSF | Rsharma1810@gmail.com |

**Keywords:** Microtus, vole, oxytocin, vasopressin, CRISPR, AAV

**Project Website:** https://www.colorado.edu/project/volecrispr/

## Progress to Date

Initial proof-of-concept manipulations will focus on oxytocin and vasopressin systems, and the materials generated will be of immediate use to the vole research community. We are in the 6th month of this award.

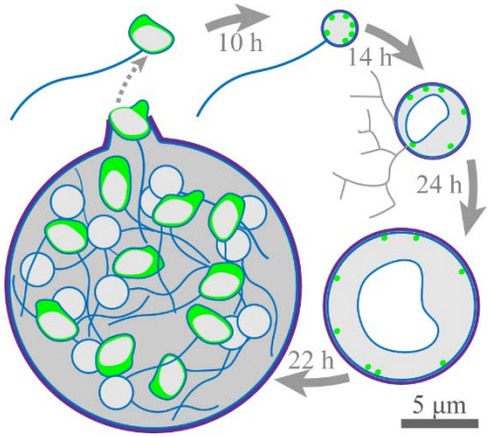
- Manoli Lab has generated knockout voles for a number of the neuromodulatory genes specified in the award. They are now optimizing conditions for CRISPR-mediated gene knock-in.
- Donaldson Lab has begun generating lentiviruses for CRISPRa and CRISPRi for brain region-specific gene manipulation in voles.
- Phelps lab has developed a novel Cre-recombinase independent strategy for screening for AAV capsids that infect neurons when injected intravenously and has begun generating capsid libraries.

## Highlights

- Vole meeting 2019 will be held in Austin, TX August 23 – 24, 2019. Opportunities for hands-on training are available in each of the collaborating labs.
- Additional information is available via the website below.
- Initial gene-editing results will be submitted for publication in early May.

# Genetic Transformation of Chytrid Fungi

Fritz-Laylin, Lillian · 1827257 · UMass Amherst · lfritzlaylin@umass.edu · (413) 545-0428




Life cycle of the chytrid *Bd* showing motile "zoospores" that develop into a cell walled "sporangium" that grows ~1000 fold and releases new zoospores.

## Project Objectives

Chytrids are a diverse polyphyletic group of >1000 species of fungi that play key roles in freshwater, marine, and soil ecosystems, including the infectious *Batrachochytrium dendrobatidis* (*Bd*) that is devastating frog populations. Despite their importance, no genetic tools have been developed for any species of chytrid fungus. This project seeks to overcome the main bottleneck to modern genetics in chytrids: molecular transformation of one species from each of the two main chytrid lineages: Batrachochytrium dendrobatidis (Bd), a member of Chytridiomycota, and *Allomyces macrogynus* (*Am*), a system used to study alternation of generations and a member of Blastocladiomycota.

**Objective 1**: develop panels of transformation vectors with drug resistance and fluorescent protein markers to facilitate screening for successful transformation.
**Objective 2**: optimize methods of intracellular delivery of said vectors.
**Objective 3**: use these tools developed to test gene function and relate genotype to phenotype. As a proof of principle, we will target components of cell motility, a key feature of chytrid biology with obvious phenotypes. We will disseminate the tools through rapid and open data and protocol sharing, and by directly training scientists from laboratories with distinct interests in chytrid molecular genetics.

**Participants**

| Last Name | First Name | Role | Institution | Email |
|---|---|---|---|---|
| Fritz-Laylin | Lillian | PI | UMass Amherst | lfritzlaylin@umass.edu |
| Buchler | Nicolas | Collaborator | NCSU | nebuchle@ncsu.edu |
| Medina | Edgar | Collaborator | NCSU | edgar.medina@duke.edu |

**Keywords:** Batrachochytrium dendrobatidis, Allomyces macrogynus, Spizellomyces punctatus, chytrids, fungi, transformation, gene expression

**Project Website:** chytrids.org

## Progress to Date

In the first 6 mos. of the award, we made progress on recruiting personnel and Objectives 1 and 2:

**Personnel:** We recruited technician Shane Hussey to aid in optimizing electroporation-based molecular delivery, cell culture, and project dissemination efforts. We have also recruited a postdoc with expertise in *Allomyces* (Andrew Swafford) who will start in August and work full time on developing genetic tools for *Am*, attempting both electroporation of plasmids and agrobacterium-mediated transformation (see "Highlights" below). Finally, we recruited graduate student Kristyn Robinson who will start in May and lead the *Bd* transformation effort.

**Objective 1:** We have conducted kill curves in liquid media to identify effective 5 selection drugs and developed transformation plasmid for each that is codon optimized to match codon pair usage in the Bd genome. We are now repeating kill curves on solid media to identify concentrations to use for colony selection.

**Objective 2:** We have optimized electroporation such that >80% of viable cells are loaded with fluorescent molecules and are beginning trials with plasmids.

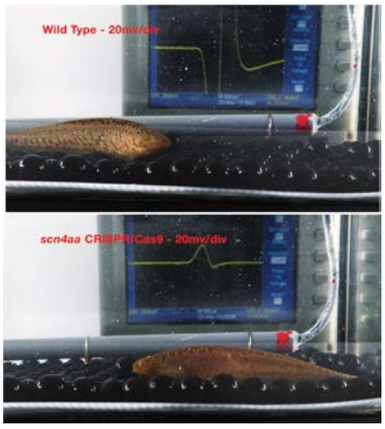
**Dissemination**: We are currently in the process of our first wave of data and material dissemination and expect to have submitted our plasmids to Addgene, our current transformation protocol to Protocols.IO, and our kill curve data to BioRXiv by the end of April.

## Highlights

We were contacted by Dr. Nicolas Buchler (NCSU) with the news that his postdoc Edgar Medina had achieved transformation of a different species of chytrid fungus: *Spizellomyces punctatus* (*Sp*). The Buchler lab asked us to help verify the transformation procedure and validate transformed lines. Dr. Medina visited the Fritz-Laylin lab in January and we recapitulated the transformation procedure and are currently vetting one of their transformants. We have agreed to provide assistance in developing dissemination materials, including detailed photographs and videos of key steps. We will also extend our attempts at transforming *Am* and *Bd* to include similar strategies. We also plan to begin Objective 3 using *Sp* within the year and are actively seeking a second postdoc to lead these experiments.

# Enabling Genotype-Phenotype Studies in Electric Fish

Gallant, Jason · 1644965 · Michigan State University · jgallant@msu.edu · (517) 884-7756




Sexually mature wild-type *B. gaduerio* (top) and *scn4aa* mutant F0. Mutant exhibits diminished electric discharges

## Project Objectives

This project is divided into two phases: the first phase of the project is rapid parallel protocol development, with the specific aims to: generate stable (1) mutant and (2) transgenic lines of electric fish using CRISPR/Cas9 technologies, (3) express transgenic material using a combination of viral-mediated and expression vector mediated techniques, and (4) knockdown specific endogenous gene activity using morpholinos.

The second phase of the project is dissemination of knowledge and community infrastructure improvement, with the specific aims to: (1) establish and distribute genomic resources and tools that enable the community to target genes of interest and (2) disseminate protocols developed in phase 1 by broadening participation in the field and training the next generation of electric fish biologists to harness these powerful new techniques.

## Progress to Date

Weakly electric fish evolved independently in Africa and South America: the goal of this project is to develop parallel functional tools in two representative species of these lineages to examine convergently evolved genotype-phenotype interactions. To date, we have completed draft genome assemblies for target species to enable development of sgRNAs. We have developed a protocol for obtaining high-throughput embryos in both lineages and have success at performing CRISPR/Cas9 editing using NEHJ in both species of electric fish. We have also developed a successful protocol for injecting bulk plasmids and Tol2-mediated transgenesis in adults and embryos. We are presently investigating the efficacy of electroporation and AAVs for use in the brain of weakly electric fish for transgenesis in the brain.

## Highlights

- April 2019 - BIOARXIV Preprint of Gene Editing Protocol (Constantinou et al.)
- March 2019 - Gene editing and bioinformatics workshop Montevideo, Uruguay
- July 2018 - Progress Update with Electric Fish Community Brisbane, Australia

**Participants**

| Last Name | First Name | Role | Institution | Email |
|---|---|---|---|---|
| Gallant | Jason | PI | Michigan State Univ. | jgallant@msu.edu |
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| Sawtell | Nathaniel | Co-PI | Columbia U. | ns2635@columbia.edu |

**Keywords:** Brachyhypopomus gauderio, Brienomyrus brachyistius, CRISPR, Morpholino, viral-mediated gene transfer, genomics, plasmids

**Project Website:** http://efishgenomics.integrativebiology.msu.edu

# Rapid and Efficient Gene Editing of Amphibians Through Nuclear Transfer from Engineered Cell Lines

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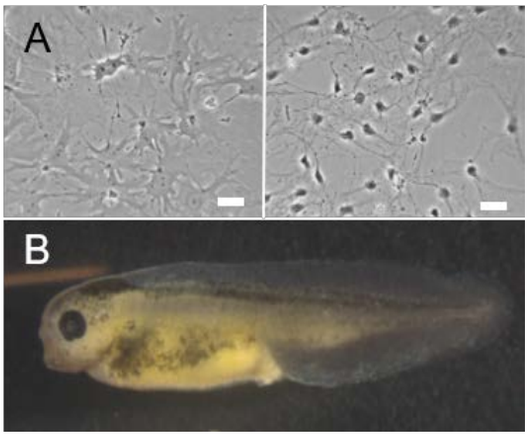


A. Two new *Xenopus laevis* J strain cell lines with distinct morphologies prepared from neurula stage embryos. (bars = 50 µm.) B. A *Xenopus laevis* tadpole developed from transfer of a cell line nucleus into an enucleated egg.

## Project Objectives

Producing homozygous mutant embryos in amphibians (Xenopus and Axolotl) is important for the analysis of gene function to better understand embryogenesis, regeneration and disease. The ideal test for gene function is to generate homozygous mutant animals. Although it is now possible to create mutations in amphibian genomic DNA, F0 embryos contain mosaic mutations, thus requiring additional breeding to generate homozygous null animals. A major objective of our EDGE project is producing homozygous null *Xenopus* embryos in the F0 generation by generating the mutations in cell lines and then generating F0 embryos through nuclear transfer into enucleated eggs. As we develop the technology we have been educating the community of scientists through presentations at conferences and we have hosted two genome editing workshops. We have also had broader outreach in presenting two high school courses teaching students about genome editing.

**Participants**

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**Keywords:** Amphibian cell lines, IPSC, *Xenopus laevis, Xenopus tropicalis, Ambystoma mexicanum*, Axolotl, nuclear transfer, reporgramming.

**Project Website:** n/a

## Progress to Date

We have made significant progress in the creation and manipulation of multiple cloned cell lines prepared from early embryos of inbred strains of *Xenopus laevis* and *Xenopus tropicalis*. We have successfully created derivative lines expressing fluorescent proteins and carried out CRISPR-Cas9 deletions of endogenous genes. As we anticipated a significant challenge has been getting normal development after nuclear transfer. The difficulty is the reprogramming of the donor nucleus to support normal development of an embryo from a cell line nucleus. We now have a wide variety of cell lines to test for better efficiency and to overcome the barrier of reprogramming of donor nucleus we are examining whether we can create induced pluripotent stem cells from these cell lines. We are also testing various treatments of the transferred nuclei and the host egg to increase efficiency. We are testing the use of various chemical inhibitors of methylation as well as activation of proteins involved in totipotency. To this end, we have tried several small molecules that alter epigenetic modifications. We have found that some of these have improved the survival of normal development to tadpole stages. Complementary, we have been testing other small molecule activators of reprogramming factors, such as Oct 4. The combination of these factors improves the efficiency of normal development after nuclear transfer.

## Highlights

**Progress:** We have focused on developing more cell lines, manipulating those lines, and improving the viability of embryos derived from nuclear transfer. We have made advances in all these areas. For the major challenge of increasing nuclear transfer efficiency, we have used inhibitor methyltransferase activity and inducers of Oct4 acitivity. Both improve reprogramming and increase the number of nuclear transfer embryos that survive to tadpole stages with normal morphology.

**Outreach:** This work has been presented in talks and posters at the 17th International *Xenopus* Conference and the 9th Aquatic Models of Human Disease conference. We have also hosted two advanced training workshops on genome editing in 2017 and 2018 which were attended by 30 total participants, including graduate students, postdocs, faculty and technicians. Last, we have begun outreach to high school teachers and students introducing them to genome editing in workshops hosted at the MBL.

# Generating Tools to Study Spiralian Development

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Ventral views of large female and small male black-footed slipper limpets, (*Crepidula atrasolea*). Rack aquarium system shown in the background.

## Project Objectives

**1) Design and construct an automated closed-system marine aquarium for rearing filter-feeding marine organisms.**
- design and modify aquarium system with Iwaki Aquatics
- design and develop automated feeding and monitoring systems

*This system is designed to be self-sufficient and run for days to weeks at a time without human intervention, reducing the challenges of rearing marine animals.*

**2) Raise *Crepidula atrasolea*, a model marine lophotrochozoan (spiralian), for biological study, including transgenic lines**.
- reduce generation time and increase reproductive output to produce sufficient numbers of animals for stable supplies of wildtype and transgenic animals
- develop SnailTrail App for tracking and reporting biological parameters
- use improved delivery methods (e.g., injection, electroporation) to generate transgenic animals (e.g., CRISPR/Cas9, plasmid-based integration, etc.)

**3) Broaden awareness of *Crepidula atrasolea* as a model marine system**:
- publication and online distribution of all plans, data, instructional videos, etc.
- solicitation of spiralian community input and feedback for future directions.

| Participants | | | | |
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**Keywords:** *Crepidula atrasolea*, aquaculture, automation, transgenic, spiralian lophotrochozoan, SnailTrail App

**Project Website:** http://www.life.illinois.edu/henry/tools.html

## Progress to Date

Completed conversion of a commercially available (Iwaki) freshwater rack aquarium system for use as a fully automated marine aquarium system, with delivery in April 2019. We have made excellent progress with the design and construction of accessory components, including the syringe-pump driven automated feeding system; real-time sensor to monitor feeding and adjust levels of phytoplankton, allowing for automated changes in response to consumption rates; and incorporation of an automated bypass loop to remove mechanical, biological and carbon filtration during feeding.

We have also designed the first version of the SnailTrailApp using FileMaker Pro. This interactive database system allows users to track the physical parameters of the aquarium system and uses relational databases to connect this information to biological outputs. We are measuring animal growth, sexual transitions, and reproductive output (brood frequency and brood size). Data will be available via a web-based portal. Incorporation of Bluetooth LE enabled devices such as a 2-D barcode scanner and digital calipers are being used to increase the efficiency of data collection from the system.

We have also developed electroporation protocols and had success in producing F0 transgenic animals via this method as well as by traditional micro-injection. We are currently using plasmid-based promoters to drive fluorescent marker expression and CRISPR/Cas9 to label specific cell types.

## Highlights

Design of automated feeding system for near-continuous feeding, including sensors to monitor and regulate food levels and consumption.

Marine-modified aquarium system delivered in April 2019. Specific modifications for marine aquatic system include: addition of protein skimmer, alteration of filtration systems for nitrate management (reducing need for frequent water changes), custom plugs and shields to limit evaporation and salt creep, additional filtration bypass loop for feeding.

Development of beta-version of SnailTrail App and database system for recording system parameters (automated and integrated into the aquarium system) and animal responses, tracking individual animals and transgenic lines, animal size, brood output, and sexual transitions.

Successful F0 integration of plasmid-based promoter-driven fluorescent markers. Successful F0 integration of CRISPR/Cas9 fluorescent markers.

# Development of Genetic and Genomic Resources for Milkweed

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Monarch larva on *Asclepias curassavica*

## Project Objectives

The further development of *Asclepias syriaca* (common milkweed) and *Asclepias curassavica* (tropical milkweed) as research model systems will require improved tools for studying the functions of individual genes. In this project, milkweed-specific genomic tools are being developed, including (i) stable transformation and transient expression systems, (ii) sequenced genomes, (iii) genetic mapping populations, (iv) transcriptomes, and (iv) metabolomic data. Transcriptomic and metabolomic analysis of A. syriaca and A. curassavica tissue types, with and without herbivory treatment, will provide a broad overview of the genetic and metabolic potential of these species. As part of outreach activities related to the project, school children raise monarch larvae on milkweed plants.

**Participants**

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**Keywords:** milkweed, Asclepias syriaca, Asclepias curassavica, monarch butterfly, Danaus plexippus

**Project Website:** https://www.milkweedbase.org/

## Progress to Date

- The genome of one *Asclepias syriaca* isolate was assembled using PacBio, Illumina, and Hi-C sequencing methods.
- A population of more than 200 genetically diverse *A.syriaca* plants was characterized by genotyping by sequencing, gene expression profiling with and without monarch caterpillar (*Danaus plexippus*) feeding, metabolite profiling, and measurement of latex exudation.
- Correlation analysis between gene expression and cardiac glycoside content in the *A. syriaca* mapping population has provided candidate genes for cardiac glycoside biosynthesis.
- Fifty *Asclepias curassavica* lines have been inbred for four to five generations in preparation for genome sequencing, genotyping, metabolite profiling, and insect bioassays.

## Highlights

- A draft genome of the common milkweed has been made publicly available on the project website.
- More than 200 genotyped milkweed isolates will be planted in a common garden experiment in 2019.
- A phylogenetic analysis showed geographic patterns in the relatedness of *A. syriaca* isolates from the eastern and central United States.

# Establishing Functional Genomics in *Hydra* to Study Stem Cells and Regeneration

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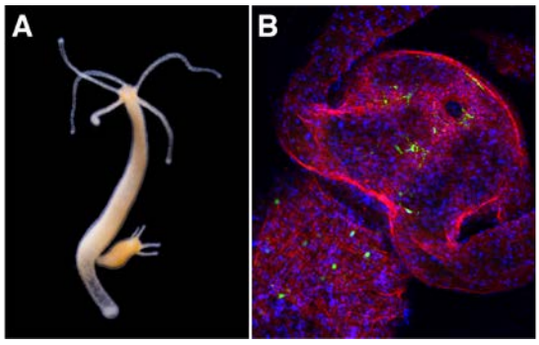


*A) Hydra vulgaris*, photo credit Stefan Siebert. B) *Hydra* transgenic line with GFP expression in a specific subtype of neurons located in the endodermal nerve net.

## Project Objectives

This proposal aims to establish fast and precise control of transgene expression and gene perturbations in *Hydra*, thus enabling broad studies of genotype-phenotype relationships during development and regeneration. This will be accomplished by: 1) Optimization of transgenic approaches to accelerate the time between microinjection and functional gene testing, 2) Leveraging our single cell transcriptomic resources to gain precise control over transgene expression, and 3) Obtaining temporal control over transgene expression and CRISPR-Cas9 gene editing by importing the hormone-inducible GeneSwitch system to *Hydra*. As a proof-of-principle we will use these tools to test gene function during neural circuit regeneration. We aim to automate *Hydra* care by leveraging existing liquid handling technologies to maintain large numbers of different transgenic lines in multi-well plates with minimal human effort. This will ultimately support the entire *Hydra* community by allowing us to maintain and share a large number of transgenic strains. To disseminate our new technologies and protocols we will: 1) build a website (openhydra.org) where researchers can access protocols and request reagents, including transgenic lines, and 2) hold annual *Hydra* workshops at the Marine Biological Laboratory. Finally, the broader impacts of our work include developing tools that can be applied to additional animal models, the organization of Cnidarian and neuroengineering scientific conferences, the mentoring of trainees, and community outreach.

**Participants**

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**Keywords:** *Hydra vulgaris*, Genetic Regulation of Development, Regeneration, Neuroscience

**Project Website:** openhydra.org (under development)

## Progress to Date

**Increase efficiency of creating transgenic lines:** We are implementing the AttB/AttP landing site approach to control the site of transgene integration. We obtained a transgenic line from Dr. Rob Steele which contains a single AttP site integrated into the genome and we have purified and tested phiC31 recombinase. We are now using these reagents to attempt to integrate a transgene with an AttB site; we are trying both embryo microinjection and electroporation.

**Spatial control of transgene expression:** We are leveraging our single cell RNA sequencing and ATAC-seq data sets to identify and test regulatory regions to drive expression in specific cell types. We have successfully generated reporter lines for 3 neuronal subtypes and for neuronal progenitors. We have also identified two putative pan-neuronal promoters that we are currently testing.

**Temporal control of transgene expression:** To import the inducible GeneSwitch system into *Hydra* we are attempting to create a transgenic line that carries the GeneSwitch hormone-inducible transcription factor in the germline. Several constructs are being tested.

***Hydra* vivarium:** We have developed a graphical user interface for operating a robotic system for maintaining transgenic *Hydra* lines that is compatible with open-source liquid handling robots from OpenTrons. In addition, we have developed a software environment to catalog and archive records of the *Hydra* maintenance based on QR-tagged multi-well plates.

## Highlights

***Hydra* EDGE Workshop at the Marine Biological Laboratory:** We held our first workshop at MBL on April 1-3, 2019; this will be an annual event. Twelve students attended the course and the class included graduate students, post docs, and PIs. We taught attendees *Hydra* techniques such as grafting and re-aggregation, behavior tracking, nervous system imaging, and embryo microinjection for creating transgenic lines. Based on the positive attendee feedback this was a very successful workshop and we hope to increase the class size to 20 next year.

**Supplying resources to the community:** We released our single cell RNA-seq data set to the public (before publication) on the Broad Single Cell Portal. This allows research to interrogate the expression of almost any gene in *Hydra*.

# Expanding the Functional Genetics Toolkit to Link Genes to Phenotypes in Cichlid Fish

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Cichlids in the Juntti Lab, University of Maryland
Photo credit: John Consoli

## Project Objectives

Cichlids are a family of >2000 species with a combination of phenotype diversity and rapid diversification rate that leads to an ideal system for genetically mapping traits. However, functional validation of genes predicted to control these traits is not yet feasible in the vast majority of these species. We seek to develop protocols that will enable us to collect fertilized embryos that can be manipulated through modern functional genetics including Tol2 transgenesis and CRISPR/Cas gene targeting. Furthermore, we will establish the first DNA sequence insertions at selected genetic sites (i.e. knock-ins) in cichlids. This will enable the use of gene replacement to test the function of evolved sequences. It will also allow conditional mutagenesis of genes in selected cell types. Ultimately these tools will result in powerful tools that can be employed by researchers in a wide variety of fields as they utilize the cichlid fish model. We will share the technologies we develop freely through workshops held at the University of Maryland.

| Participants | | | | |
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**Keywords:** cichlid, Astatotilapia burtoni, CRISPR, gene editing, hormones

**Project Website:** cichlidengineering.weebly.com

## Progress to Date

As success of genome editing technologies ultimately depends on the rate at which manipulable embryos can be obtained and injected, we have increased the number of embryos that a given fish produces through hormonal manipulations and have streamlined the injections process. Together we now achieve ~2-fold more mutant animals, in fewer scientist-hours. This has permitted us to generate more genetic lines carrying mutations for analysis. Regarding gene insertions, we have standardized the process by which we analyze potential gene knock-ins. Our preliminary results suggest we achieve knock-in at low rates; we are at work finding ways to increase this rate.

## Highlights

We have obtained 6 new CRISPR mutant lines with our streamlined protocols. We have also begun sharing our approaches with the wider cichlid community. Two additional labs are now working with animals generated through our protocols.

# Gene Manipulation Tools and Resources for a Vocal Learning Species

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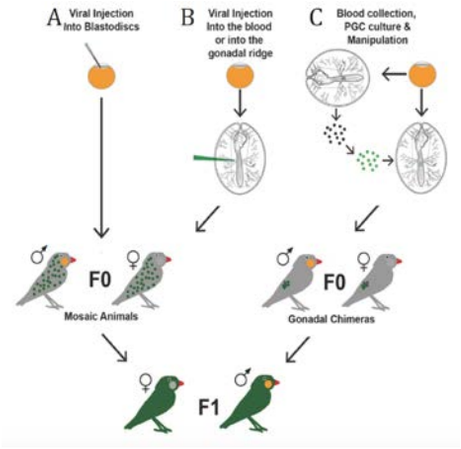


Strategies for avian transgenesis

## Project Objectives

Zebra finches are a key model organism to study the neural and genetic basis of vocal learning, a trait that subserves spoken language learning in humans. In spite of progress in understanding vocal learning and related circuitry, an in-depth knowledge of vocal learning mechanisms has been hindered by limited availability of gene manipulation tools. To address this issue, we proposed: Aim 1 - To generate a general purpose transgenic Cas9-CRISPR finch line. This line is expected to have broad applicability, including brain gene manipulations through local injections of viral vectors with guide RNA constructs. Aim 2 - To improve the technology for generating transgenic zebra finches using primordial germ cells (PGCs). Our goal is to isolate and culture PGCs that can be genetically modified *in vitro*, and become germ cells when transplanted into recipient animals, to enable germline genetic modifications. This strategy is expected to increase the efficiency and speed of generating transgenic zebra finches. Aim 3 - To improve the tools, resources and protocols for using viral vectors for genetic manipulation in finches. It is also our goal is to maintain and broadly disseminate these resources, through presentations, online postings and direct communications.

**Participants**

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**Keywords:** Zebra finches, vocal learning, birdsong, speech and language evolution, transgenesis, viral vectors, primordial germ cells

**Project Website:** finch related: http://songbirdscience.com , http://www.zebrafinchatlas.org

## Progress to Date

We have gained an in-depth understanding of key issues that limit our ability to generate transgenic finches and to manipulate gene expression using viral vector tools in this species. This includes confirming the pseudogenization of the receptor for VSVg-pseudotyped lentivirus (LV) and low conservation for the AAV receptor and obtaining detailed data on the dynamics of PGC formation and migration in finch embryos. Progress in addressing these issues includes: 1) We have generated mosaic zebra finches that express the genome-editing enzyme Cas9, using our proven method of injecting VSVg-pseudotyped LVs into freshly-laid fertilized eggs. We are currently screening the mosaics for germline transmission. 2) We have further developed protocols for isolating and culturing zebra finch PGCs. Our early stage cultured PGCs are transfectable and incorporate into the embryonic gonads of recipient embryos when injected in the blood stream. Manipulating these PGCs *in vitro* would allow for more targeted gene manipulations in transgenic lines such as knockins and knockouts. 3) We have established protocols for conducting screenings for AAV capsid variants that may result in more efficient transduction of finch brain, using targeted evolution and a bar-coded library of capsid variants. Library screenings are being pursued and candidate efficient variants being tested for brain infectivity. We have presented the ongoing efforts and progress at annual meetings (SfN, Bird Song and Animal Communication Meeting) and in songbird listserv postings.

## Highlights

Publications:
- Jung et al., 2019, Identification and characterization of primordial germ cells in a vocal learning Neoaves species, the zebra finch. *Submitted to FASEB J*.

Abstracts and presentations at meetings:

- Keyte et al., 2018, NSF EDGE consortium transgenic songbird project. Annual Bird Song and Animal Communication Meeting. Millbrook, NY.

- Velho et al., 2018, EDGE consortium transgenic songbird project: Developing gene manipulation tools and resources for a vocal learning species. Society for Neuroscience Abstracts, Vol. 43. Poster presentations at the Vocal Communication and Neuroethology session and at the Songbird Satellite

# Establishment of Genome-Editing and Transgenic Tools in Anolis Lizards

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Wild-type (bottom) and gene-edited albino (top) brown anole hatchlings from the University of Georgia.

## Project Objectives

The objective of this project is to establish CRISPR/cas genome editing and transgenic methods in the brown anole lizard, *Anolis sagrei*.

***Aim 1:*** ***Establish CRISPR/cas genome editing methods in Anolis sagrei.***

***Aim 2:*** ***Establish transposon-based transgenesis in Anolis sagrei.***

***Aim 3:*** ***Disseminate methods through workshops, videos, and manuals***

Our approach is to access maturing oocytes within adult *A. sagrei* females through a surgical incision and microinject either Cas9 RNPs or transgenes to accomplish targeted genome editing or transgenesis, respectively. We will develop training materials describing methods of genome manipulation appropriate for squamates, the surgical techniques required for oocyte microinjection methods, and screening methods for detecting stable changes in the *Anolis* genome. We will disseminate training materials that include a handbook available for download, video recordings, and intensive hands-on workshops hosted at the University of Georgia.

| Participants | | | | |
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**Keywords:** *Anolis sagrei*, lizards, CRISPR-Cas, transgenesis, oocyte, microinjection

**Project Website:** https://doi.org/10.1101/591446

## Progress to Date

We have successfully achieved one of our primary objectives – the production of live lizards that carry targeted mutations induced by CRISPR-Cas9. The first locus we targeted was the *tyr* gene. Injection into 146 oocytes yielded 9 offspring with targeted mutations, including 5 heterozygotes and 4 albino animals with bi-allelic mutations. The overall efficiency was 6%, though we determined that oocytes in the range of 1 – 5mm in diameter yielded higher efficiencies (~9%). These results have been released as a preprint, which includes a supplemental instructional video that demonstrates our approach. In a separate manuscript that is under review, we describe our survey of injectable anesthetic agents in anoles and identify the best drug combinations and injection sites for anesthetizing anoles for surgical procedures.

We have already begun targeting additional loci to further optimize gene editing in anoles and hope to try homology directed repair to create more precise gene edits. In addition, we will attempt transposon mediated transgenesis this summer.

## Highlights

***Preprint***

Rasys AM, Park S, Ball RE, Alcala AJ, Lauderdale JD, Menke DB. CRISPR-Cas9 gene editing in lizards through microinjection of unfertilized oocytes. https://doi.org/10.1101/591446

*Supplementary data includes an instructional video

***In Review***

Rasys AM, Divers SJ, Lauderdale JD, Menke DB. A systematic study of injectable anesthetic agents in the brown anole lizard (*Anolis sagrei*).

# High Efficiency Identification of Products of Homologous Recombination in Plants as a Tool to Test Gene Function

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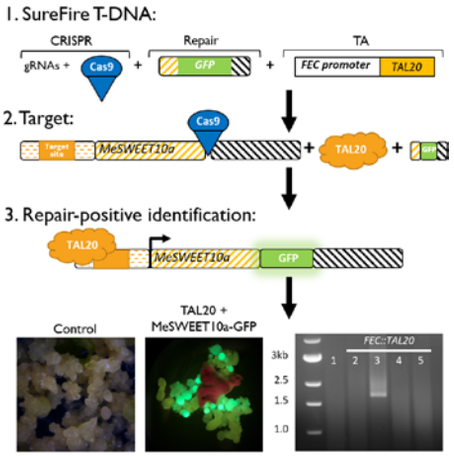


The process of "SureFire HR" Row 3: Left: FEC cells used for screening GFP expression. Right: PCR and sequencing confirmed SureFire integration.

## Project Objectives

The project will focus specifically on techniques to facilitate and accelerate the identification of successful products of HR by reporter-based methods using transcriptional activation. The approach combines insertion of a repair template (containing the desired genome edit and a selectable marker) with methods to rapidly find a desirable, rare HR event - the proverbial "needle in a haystack". Data from cassava demonstrates that this is an effective method for developing molecular and genomic tools for orphan crops and those lacking robust genomic resources. The work will be done in cassava, Arabidopsis, and Setaria as the primary species for analysis, because a demonstration in these species would support broad utility in eudicot and monocot species, including most crops. The questions to be addressed by this project include: 1) whether the efficiency of HR can be improved by focusing on selection of the products; 2) whether HR-based approaches be used to successfully tag non-coding RNAs such as microRNA and tasiRNA precursors; and, 3) whether it is possible to develop efficient processes for high-throughput, library-based knock-ins. The project will develop an approach for a key functional genomic tool (improved selection of products of HR) to enable direct tests of hypotheses about gene function in diverse organisms. Project members will be trained broadly in plant biology and genomics, gene function and functional genomics, and computational methods. All resources generated in this project will be made available for use. Specifically, constructs will be deposited in nonprofit global plasmid repositories and web-based tools will be designed to enable users unfamiliar with genome editing to successfully design custom components of SureFire for application to their organism(s) of study.

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**Keywords:** Cassava, *Setaria, Arabidopsis*

**Project Website:** Under development for release later in 2019.

## Progress to Date

While the project just started in the fall of 2018, the research team (Univ of Missouri graduate student Ryan Delpercio, and senior researcher Kira Veley) has been working to develop the constructs, make transgenic plants, and test & troubleshoot the first, entirely CRISPR-based SureFire HR approach, building off our preliminary data using TAL effectors. Current activities include the following:

- We developed CRISPR-based constructs, utilizing published versions of transcriptional activators (dCas9-TA) and *Cpf1.*
- We tested these constructs first in cassava, targeting the same gene that was successfully tagged previously using the TAL-based method (*MeSWEET10a*). We are testing the components of the system relative to the TAL-based method.
- We are using this system to test the requirements for successful HR in cassava, for example, varying repair template homology arm lengths.
- We developed GoldenGate-based constructs for rapid integration of guide RNAs, the promoter for the transcriptional activation ("TA") component, the programmable nuclease and TA enzymes, the homology arms, and the selectable marker/reporter.
- We developed gene-specific constructs with which to test these components in Arabidopsis, targeting a variety of genes including the largest gene in the genome (>25 kb) as well as other genes of 5 to 10 kb that would be difficult to tag using normal transgenic approaches.
- We are also testing multiple programmable (CRISPR-based) transcriptional activator systems that have been published. These tests are using transient expression systems including *Nicotiana benthamiana.*
- For the programmable nuclease, we are testing *Cpf1*, and growing plants at different temperatures to assess reports that the mutation rate is elevated at 28º C.
- We are also testing multiple promoters for callus- or seed-expression of the TA component of the constructs, for use in diverse species including *Setaria* and other grasses.

## Highlights

Publications, presentations, and mentoring highlights yet to come – the work is underway, in preparation for the 2020 PI meeting!

# Nanoscale Probes and Infrastructure for Real-Time and Single-Celled Genomics Across Metazoa

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Leonid L. Moroz,
Oceanic Research Expedition

## Project Objectives

The genetic material, or genome, first and foremost operates at the level of specific cells, and practically any animal tissue or embryo consists of thousands of highly diverse cells. How and why the same genome leads to such enormous diversity of cell types and functions are unanswered questions of modern biology. Yet, cell-specific approaches to link cause and effect are virtually absent for a majority of animal groups. This interdisciplinary project addresses these bottlenecks experimentally by developing novel genomic approaches and chemical labeling tools for genome-wide characterization of expression, classification, and mapping of thousands of individual cells in parallel. This information is used to (i) achieve a nearly complete census of cell types within a given organism, focusing on animal models critical to understanding mechanisms of learning and memory, such as Aplysia, and regeneration, such as Pleurobrachia, and (ii) generate nanoscale probes that selectively mark specific cells for genome editing, regardless of any advance knowledge about the cells' molecular diversity. Several communities are benefiting from the proposed research, including comparative neurobiology, development, biological oceanography, and the emerging field of synthetic biology. The project also affords cross-disciplinary training opportunities for trainees from the undergraduate to postdoctoral level and educational outreach activities in marine and comparative biology aimed at a diverse K-12 student body.

**Participants**

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**Keywords:** Aplysia, ctenophores, basal bilaterians, basal Metazoa

**Project Website:** n/a

## Progress to Date

There is more than one way to develop neuronal complexity, and animals frequently use different molecular toolkits to achieve similar functional outcomes (convergent evolution). Neurons are different not only because they have different functions, but also because neurons and circuits have different genealogies, and perhaps independent origins at the broadest scale from ctenophores and cnidarians to cephalopods and primates. By combining modern phylogenomics, single-neuron sequencing (scRNA-seq), machine learning, single-cell proteomics, and metabolomic across Metazoa, we began to reconstruct the evolutionary histories of neurons tracing them to ancestral secretory cells. Using novel Neuron-Selex chemical evolution, we also started to make novel nanoprobes to recognize living neurons in vivo.

Our data suggest that neurons, and perhaps synapses, evolved at least 2-3 times (in ctenophore, cnidarian and bilateral lineages) during ~600 million years of animal evolution. There were also several independent events of the nervous system centralization either from a common bilateral/cnidarian ancestor without the bona fide neurons or from the urbilaterian with diffuse, nerve-net type nervous system. From the evolutionary standpoint, (i) a neuron should be viewed as a functional rather than a genetic character, and (ii) any given neural system might be chimeric and composed of different cell lineages with distinct origins and evolutionary histories. The identification of distant neural homologies or examples of convergent evolution among phyla will not only allow the reconstruction of neural systems' evolution but together with single-cell "omic" approaches the proposed synthesis would lead to the "Periodic System of Neurons" with predictive power for neuronal phenotypes and plasticity. Such a phylogenetic classification framework of Neuronal Systematics (NeuroSystematics) might be a conceptual analog of the Periodic System of Chemical Elements. scRNA-seq profiling of all neurons in an entire brain or Brain-seq is now fully achievable in many nontraditional reference species across the entire animal kingdom. Arguably, marine animals are the most suitable for the proposed tasks because the world oceans represent the greatest taxonomic and body-plan diversity.

## Highlights

Whelan, Nathan V. and Kocot, Kevin M. and Moroz, Tatiana P. and Mukherjee, Krishanu and Williams, Peter and Paulay, Gustav and Moroz, Leonid L. and Halanych, Kenneth M. "Ctenophore relationships and their placement as the sister group to all other animals," ***Nature*** Ecology & Evolution, v.1, 2017.

Fu, Ting and Lyu, Yifan and Liu, Hui and Peng, Ruizi and Zhang, Xiaobing and Ye, Mao and Tan, Weihong. "DNA-Based Dynamic Reaction Networks," Trends in Biochemical Sciences, v.43, 2018.

Moroz, L.L. NeuroSystematics and Periodic System of Neurons: Model vs Reference Species at Single-Cell Resolution. ACS Chemical Neuroscience, v. 9:8, 1884-1903, 2018.

# Developing Techniques for Linking Genotype to Phenotype in Amphibians

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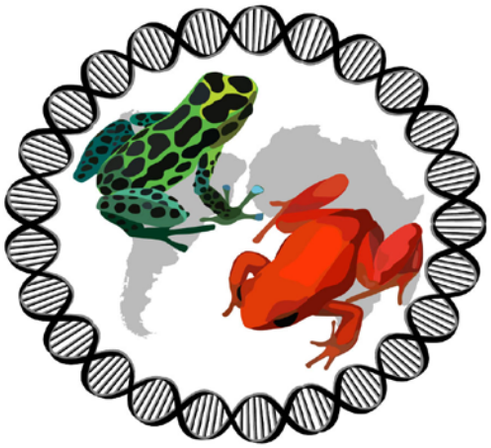

## Project Objectives

The goal of this project is to develop a robust genetic manipulation toolbox in poison frogs (Mantellidae and Dendrobatidae families) that can be readily expanded to other amphibian species. Our **specific aims** are:

1. Gene knockouts and knockdowns in the lab and field using CRISPR/Cas9 & morpholinos.
2. Expression of transgenes.
3. Spatial and temporal specificity of gene manipulation using electroporation of plasmids and morpholinos.

The **broader impacts** of the proposed work include:

1. Revitalize Amphibase as a web portal for amphibian genomics.
2. Host hands-on workshops at Stanford and UniAndes (Colombia).
3. Broaden the Frogger School Program to a nationwide resource for K-12 public schools. This program brings unique amphibian biology to biology education.

| Participants | | | | |
|---|---|---|---|---|
| Last Name | First Name | Role | Institution | Email |
| O'Connell | Lauren | PI | Stanford University | loconnell@stanford.edu |
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| Crawford | Andrew | Collaborator | UniAndes (Colombia) | crawfordaj@gmail.com |

**Keywords:** poison frogs, Mantellidae, Dendrobatidae, genome editing, CRISPR/Cas9, electroporation, transgenesis, amphibians

**Project Website:** under construction

## Progress to Date

- Established breeding colonies of three focal poison frog species.
- Standardized egg de-jelling.
- Reared injected larvae without egg jelly.
- Optimizing *in vitro* fertilization.
- Currently optimizing egg injections.
- Currently optimizing electroporation protocols.

## Highlights

- Currently revitalizing Amphibase.
- Preparing K-12 science curricula utilizing amphibians.
- Animal husbandry and embryo development protocols optimized.

# Accelerating Arthropod Genetic Manipulation Through ReMOT Control

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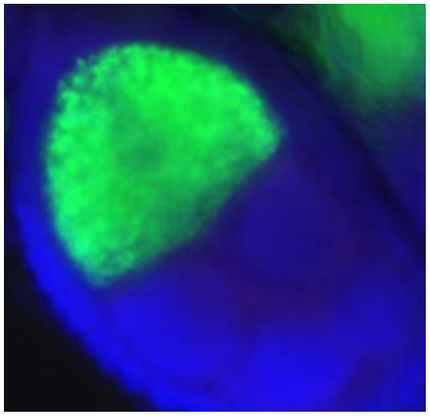

Cas9-EGFP transduced into developing mosquito ovary by receptor-mediated endocytosis

## Project Objectives

The overall goal of this project is to create a user-friendly and broadly applicable technology ("ReMOT Control") to apply the power of CRISPR/Cas9 gene editing to any arthropod species of interest by eliminating the requirement to microinject pre-blastoderm embryos. Our aims are to (1) identify small peptide ligands with specific tropism to the developing female germline that could be used to deliver Cas9 RNP by injection into vitellogenic females. We want to first identify species-specific ligands, then develop "universal" ligands with broad species activity. (2) Use the system to simultaneously deliver DNA constructs for gene insertion by homology-directed repair. (3) broadly disseminate the technology to research communities and end users. We have developed a series of ligand spanning a highly diverse group of arthropods (and even some vertebrates), as well as identifying a peptide sequence that functions broadly across insects and arachnids. We have been able to deliver DNA constructs to the germline. Broader impact activities include the creation of the ReMOT Control working group, dissemination of reagents to numerous labs across the globe, and multiple visiting scientists in the Rasgon Lab to learn the technology.

**Participants**

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|---|---|---|---|---|
| Rasgon | Jason | PI | Penn State | jlr54@psu.edu |
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**Keywords:** *Aedes aeypti, Aedes albopictus, Anopheles gambiae, Anopheles stephensi, Culex pipiens, Drosophila melanogaster, Ixodes scapularis, Bombus impatiens, Apis melifera, Acyrthosiphon pisum, Planococcus citri, Lutzomyia longipalpis, Tribolium castaneum, Dalotia coria, Gryllus bimaculatus, Spodoptera litura, Bombyx mori, Bemisa tabachi, Danio rerio,* MANY OTHERS! Receptor-mediated endocytosis, peptide ligand

**Project Website:** @ReMOTGeneEdits

## Progress to Date

We have identified a 41 amino acid peptide ("P2C") that mediates entry into arthropod ovaries by Receptor-mediated endocytosis. P2C functions in diverse arthropods including mosquitoes, ticks, moths, beetles, fruit flies, sandflies, mealybugs, crickets, whiteflies. We have also developed species-specific ligands for those taxa where P2C does not function (silkworm, zebrafish). When fused to Cas9, we and our collaborators have successfully used P2C to edit the genomes of Aedes aegypti, Aedes albopictus, and Ixodes scapularis. Editing experiments for other taxa are ongoing. We have also shown that we can use P2C to drag DNA into mosquito ovaries and are currently working on experiments to achieve HDR. We have formed the ReMOT Control working group to disseminate reagents, test species activity, and bootstrap protocols. Reagents have been disseminated to almost 20 laboratories. The Rasgon Lab has hosted numerous visitors to learn the technique.

## Highlights

Chaverra-Rodriguez D, Macias VM, Hughes GL, Pujhari S, Suzuki Y, Peterson DR, Kim D, McKeand S, Rasgon JL. 2018. Targeted delivery of CRISPR-associated endonuclease 9 (Cas9) into arthropod ovaries for heritable germline gene editing. Nature Communications. 9: 3008.

# Creation of a Genetically Tractable Cephalopod Model Using The Hawaiian Bobtail Squid

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Adult specimen of Euprymna scolopes

## Project Objectives

The coleoid cephalopods exhibit remarkable biological innovation. Their brain size and behavioral sophistication rival those of higher vertebrates. For example, the octopus's brain has ~ 4 orders of magnitude more neurons than non-cephalopod molluscs, and ~ 4 times the number of neurons as a rat. Besides their brains, they offer a wealth of other features that could stimulate broad areas of research. In spite of these opportunities, the cephalopod research community remains small. This is because it lacks a genetically tractable model. We propose to overcome a key bottleneck in cephalopod biology by creating the first transgenic cephalopod model using the *E. scolopes*. Cephalopods are difficult to culture in captivity, and this has been the primary impediment to developing a genetic model. We propose to create a life-cycle culture facility for *E. scolopes* and then use it as a resource to create transgenic lines using CRISPR-Cas9. The long-term goal is to create a resource center at the Marine Biological Laboratory that will provide animals, transgenic lines, and refined protocols to the research community. By creating the first transgenic cephalopod model, we will open new avenues to study these organisms on a mechanistic basis.

## Progress to Date

Our award started in September 2018. Since that time, we have made significant progress towards our two major aims: 1) establishing an *E. scolopes* culture facility and 2) creating transgenics using CRISPR-Cas9. Currently there are 490 animals in our facility, and this number has kept steady at close to ~500 animals over the past 6 months. Our facility has produced greater than 6000 embryos, and thousands of these have been used for microinjections. We have cultured animals for three generations within the facility. The initial brood stock was captured in Hawaii and we continue to capture wild animals to add to our facility. Currently, the greatest bottleneck for life-cycle culture has been low survivorship during the first 2-3 weeks after hatching. We have also established a culture of *E. berryii*, a highly similar species from Okinawa, Japan with many favorable characteristics for model development. For our gene knockout studies, we are focusing on Tryptophan Dioxygenase (TDO), an enzyme that catalyzes the first committed step in ommochrome pigment synthesis in the retina and chromatophores. Over the past 6 months we have been injecting embryos at the 1-8 cell stages with Cas9 and CRISPR guides to TDO. Needles, injection volumes and reagent concentrations have been optimized. Thus far, a total of 657 embryos have been injected. ~25% survive to hatching. Of these, 42% show clear lack of pigmentation in the eyes. All embryos that were injected at the 1-2 cell stages exhibit the eye phenotype. After hatching, animals were sacrificed for genotyping. Both a T7 nuclease assay and MiSeq sequencing on a small subset of animals have confirmed the presence of indels at the CRISPR gRNA directed sites.

## Highlights

We have established a culture facility that is capable of producing sufficient embryos for knockout studies. We are also performing life-cycle culture *E. scolopes*. We have also established the techniques to produce the first gene knockouts in a cephalopod. Although these animals are clearly mosaics in the F0 generation, they provide a good building block for establishing genetic lines.

**Participants**

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**Keywords:** Include species under investigation and keywords describing your project Euprymna scolopes, Hawaian Bobtail Squid, cephalopods,

**Project Website:** Not yet made.

# Enabling Functional Genomics in Monkeyflowers (*Mimulus*)

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Front page of the Mimubase site (http://mimubase.org/), a resource and information portal for *Mimulus* research

## Project Objectives

The wildflower genus *Mimulus* (monkeyflowers) combines extraordinary diversity in morphology, physiology, and life history with the ability to conduct genetic analyses and field experiments. Our research will enable *Mimulus* as a powerful model for ecological functional genomics and plant developmental genetics. Our objectives are to: 1) Optimize genetic transformation protocols in seven *Mimulus* species that are actively studied by many researchers and represent a wide range of phylogenetic diversity; 2) Generate large-scale chemical and transposon insertion mutant libraries for three of the most actively studied species. Together, these aims will enable a diverse community of *Mimulus* researchers to pursue causal links between genotypes and phenotypes that vary within and among natural populations and species and will position *Mimulus* as a powerful new model in plant developmental genetics.
Broader impact activities include three weeklong summer lab short courses, a comprehensive website that includes a searchable database and detailed instructions/videos of functional genomics protocols, and hands-on learning opportunities for undergraduate lab courses at two universities.

**Participants**

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**Keywords:** *Mimulus*, monkeyflowers, ecological functional genomics, developmental genetics, genetic transformation, mutant libraries

**Project Website:** http://mimubase.org/

## Progress to Date

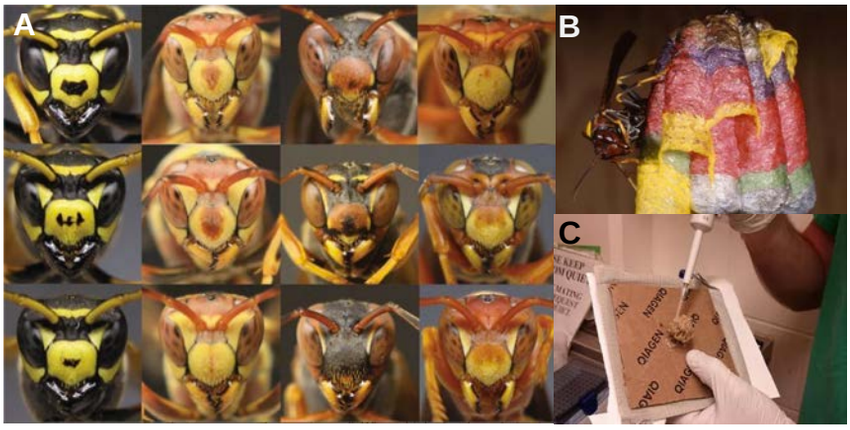
Aim 1: As a first step toward developing a reliable tissue culture-based transformation protocol, we have begun growing *M. nasutus* plants under sterile conditions. We have also successfully transformed this species and generated callus. Soon, we will scale up these efforts to additional *Mimulus* species.
Aim 2: We have begun our efforts to generate EMS-mutant libraries for *M. lewisii* and *M. verbenaceous*. So far, we have generated 500 M1 lines for each species**;** M1 flowers are being pollinated now to produce F2 seeds for the mutant screen.
We have also tested new transformation plasmids in *M. verbenaceus* for generating transposon insertion mutant libraries. The *TPase-ORF1* casette can now be expressed at high levels in transgenic plants, which is expected to result in high-frequency transposition once the *TPase-ORF* cassette and the *mPing-FMV* transgene cassette are crossed together into the same plant.
Education and dissemination: We have built the backbone of the Mimubase website, which will be a vital resource for our community. At this summer's *Mimulus* meeting (June 21, Providence, RI), we will provide instructions on how to use, maintain, and contribute to the site. We will also solicit feedback on how to improve the site. Additionally, we have announced our first short-course (June 16-20 at Univ. of Connecticut), which will cover methods in transformation by floral spray/infiltration.

## Highlights

In collaboration with the Genetic Science Learning Center at the University of Utah, we are finalizing several online education modules on flowering plants and their pollinators (https://learn.genetics.utah.edu/content/flowers/).

# *Polistes* Wasps: A Model System for Integrative Biology

Toth, Amy · 1827567 · Iowa State University · amytoth@iastate.edu · (515) 294-3121




**(A)** Examples of diversity of color marking in paper wasp. Pictured from left to right are *P. dominula*, *P. exclamans, P. fuscatus* and *P. bellicosus.* **(B)** Paper wasp can be reared in the lab; nest progress can be monitored by providing colored paper. **(C)** Nest cells are accessible to manipulation, opening the possibility of RNAi treatments in the wild.

## Project Objectives

Paper wasps have traditionally been an important model for research on social behavior, kin recognition and the evolution of social organization because of the ease with which they can be observed in the field and lab. More recently, they have also been a key model for studies of visual signaling and cognition. There are currently 2 published Polistes paper wasp genomes and at least 5 more unpublished genomes soon to be released providing the opportunity for paper wasps to be an exciting system for exploring the functional genomics of traits such as caste development, social organization, color patterning, cognition and more. Our project has 3 aims:

**AIM1. RNAi in the field and lab** – leverage the unique open nest structure of paper wasps to treat individual larvae or pupae in the field to examine effects of gene manipulation in the wild.

**AIM2. Improve rearing** – wasps have been reared in captivity for many years, but systematic studies are needed to improve lab-based rearing outcomes.

**AIM3. Develop transgenic protocols** – combined with improved rearing, the use of transgenic tools such as CRISPR/Cas9 has the potential to provide important resources for knock-out, knock-ins, reporter lines, calcium imaging and more.

**Participants**

| Last Name | First Name | Role | Institution | Email |
|---|---|---|---|---|
| Toth | Amy | PI | Iowa State | amytoth@iastate.edu |
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**Keywords:** *Polistes* paper wasps with particular focus on *P. fuscatus, P. dominula, P. metricus,* and *P. exclamans*; social evolution; caste-determination; gene function in the wild; individual recognition; color patterning; cognition

**Project Website:** in progress

## Progress to Date

The project started in August 2018, at the end of the natural wasp colony cycle. Since August we have focused on improving rearing of wasps, specifically the ability to mate and overwinter wasps in the lab.

**Mating:** We discovered that application of juvenile hormone to unmated female wasps greatly increased receptivity. Female wasps tend to mate singly in the wild and appear to be rather choosy. This study provides an avenue for increasing success of planned crosses in the lab.

**Nest initiation post overwintering:** A key bottleneck in the establishment of functional genomics in paper wasps is the need to have wasps overwinter and then initiate nests in the lab. We have discovered that for wasps from upstate NY, rates of nest initiation increase precipitously after a few months of diapause, as opposed to nearly no nests initiated after only a few weeks of diapause. These 'longer' diapause individuals, however, still experienced shorter diapause (and very high survival) compared to natural populations indicating a means for more rapid production of new nests and speeding up the natural colony cycle.

**Functional genomic correlates of nest initiation:** We have sampled wasps that either did or did not initiate nests to look at differences in brain gene expression. This on-going work may reveal a molecular switch that regulates nesting and the start of maternal care.

**Egg injections:** With eggs laid by early nests we have been refining injection techniques for injecting eggs in the nest. We have learned from prior experience that eggs are well-attached to the nest and difficult to remove and replace.

## Highlights

Walton et al (in prep). Hormonal modulation of reproduction in *Polistes fuscatus* social wasps: dual functions in both ovary development and sexual receptivity

In conjunction with the Sciencenter (Ithaca, NY) we hosted 20+ middle school students to learn about paper wasps and our research.

# Functional Genomics Tools for Cnidarian-Dinoflagellate Symbiosis

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Aposymbiotic and symbiotic *Exaiptasia pallida* (Aiptasia)

## Project Objectives

Overall goal is to develop effective genetic methods in the sea anemone Aiptasia and its algal symbiont *Breviolum minutum* (Family Symbiodiniacea), a rapidly developing model system for the study of coral-algal symbiosis.

Aim 1: Genetic manipulation of *Breviolum minitum.*

Aims 2 and 3: Development of methods for gene knockdown, knockout, tagging, and overexpression in Aiptasia.

Aim 4: Achieving settlement and metamorphosis of Aiptasia larvae.

Broader impacts: Develop online communication platforms for rapid dissemination of tools and techniques developed for Aiptasia. Also develop a web site that will be a central repository for a variety of resources useful to the Aiptasia and coral symbiosis communities.

**Participants**

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| DeGennaro | Matt | Co-PI | Florida Int U | mdegenna@fiu.edu |

**Keywords:** *Exaiptasia pallida*, Aiptasia, Symbiodiniaceae, corals, zooxanthellae, symbiosis, cnidarian, host-microbe interactions

**Project Website:** https://www.protocols.io/groups/aiptasiasymbiodinium-model-system

## Progress to Date

Aiptasia:

- Regular spawning year-round.
- Plasmid-based expression of fluorescent proteins driven by Aiptaisa promoters delivered by microinjection and electroporation of zygotes.
- Successful expression of capped mRNA delivered via microinjection of zygotes.
- Testing different settlement cues is ongoing with no consistent metamorphosis or settlement observed.
- Initial attempts to electroporate pedal lacerates and are currently optimizing this using capped mRNA and recombinant fluorescent proteins.

Symbiodiniaceae:

- Attempted delivery of both Cas9 RNPs and CRISPR/Cas9 expression constructs via electroporation, biolistics, glass bead abrasion, lithium acetate transformation, freeze-thaw, and nanostraw methods for mutagenesis of *URA3*, *DHC1*, and *nitrate reductase* genes.
- No mutants have been obtained, but low-level editing activity was observed in *URA3* using Cas9 RNPs delivered through electroporation.
- We are optimizing the isolation of nuclei from *Breviolum minutum* for ATAC-seq. We expect the information on genome-wide chromatin accessibility will be useful in future editing attempts.

## Highlights

- Hosted an international workshop in 2018 that brought together 54 Aiptasia/Symbioiniaceae researchers and educators from 15 institutions and 4 nations to discuss Aiptasia as a model system for coral symbiosis.
- Launched a public Protocols.io site for posting techniques and for community interaction through discussions and resource sharing. Our site has 25 published protocols.
    - Of the 63 members on the public Aiptasia Protocols.io site, a) ~67% are non-EDGE and b) ~15% use Aiptasia strictly for undergrad research and education.
- Website for the Aiptasia model system is in development. Slated to roll out in summer 2019. It will strengthen and grow the Aiptasia community and promote interaction with the broader community.
    - Showcases research goals and directs visitors to: Protocols.io page, genomic resources and education tools.

# Generating Transgenic *Cuscuta* as a Tool for Studying Plant Interactions

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The parasitic plant *Cuscuta campestris* (field dodder; the leafless yellow vine) grows on the stem of a tobacco host.

## Project Objectives

Background:
*Cuscuta* spp. (dodders) are parasitic plants that are often significant weeds of agricultural and ornamental crops. *Cuscuta* spp. are also very interesting plants because of their abilities to locate and attach to host plants and establish open connections that permit exchange of macromolecules including RNAs. Study of these species has been hampered by a lack of methods for genetic transformation.

Project objectives are to:

1. Develop a protocol for generating transgenic *Cuscuta* plants.
2. Promote research on *Cuscuta* such that this plant can be used by a wide range of plant scientists.
   - Disseminate information on how to grow and manipulate *Cuscuta* for research purposes.
   - Train students in *Cuscuta* research.

| Participants | | | | |
|---|---|---|---|---|
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**Keywords:** *Cuscuta campestris*, parasitic plant, transformation, plant-plant genetic material exchange.

**Project Website:** https://www.ppws.vt.edu/research/westwood-lab/westwood-lab-nsf-plant-research/westwood-lab-cuscuta-research-resources.html

## Progress to Date

***Cuscuta* transformation:**
We have made encouraging progress in developing a methodology for transformation of *Cuscuta campestris* (field dodder), but have not yet achieved our goal.

We can regenerate *Cuscuta*. We have tested different types of tissues for callus production and transformation, including seedlings, shoots, meristems, flowers, immature embryos (*Cuscuta* does not have roots or leaves). We have been able to obtain callus-like tissues under tissue culture conditions, regenerate shoots, and successfully transfer these cultured shoots to hosts. This step was viewed as a major hurdle because of the parasite's obligate need for a host, but *Cuscuta* seems to regenerate easily.

We can transform *Cuscuta* tissues. We have tested several transformation methodologies, both *Agro-bacterium*-dependent and independent, and have obtained transient transformation of *Cuscuta* tissues. However, we have not succeeded in regenerating shoots from transformed sectors.

Alternative approaches. One novel approach is to use the host-parasite exchange of RNAs and protein to modify parasite genes in a trans-species manner. We are testing CRISPR/Cas9 components to see if they can be moved from host to parasite to achieve gene editing in the parasite.

**Methods for *Cuscuta* research:**
It seems that everyone who works with *Cuscuta* has their own protocols for growth and assays. We are creating a set of defined laboratory conditions (host, light quality, propagule type, etc.) for research on *C. campestris* in order to bring some standardization to the field and facilitate success by novice growers.

## Highlights

Provided research opportunities with parasitic plants:

- Training 1 Ph.D. Student
- Training 1 Postdoctoral Associate
- Training 6 Undergraduate Students
- Training 2 High School Students

Developed a website to serve as a resource for scientists interested in working with *Cuscuta* (https://www.ppws.vt.edu/research/westwood-lab/westwood-lab-nsf-plant-research/westwood-lab-cuscuta-research-resources.html)

We have consulted with several other researchers who are interested in working with Cuscuta.

# Meeting Attendee List

| Meeting Attendees List | | | | |
|---|---|---|---|---|
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