# The Evolution of Neotropical Electric Fish and Mechanisms of Electric Signal Production (Teleostei: Gymnotiformes)

Dawn D. Xiao, *Department of Cell and Systems Biology, University of Toronto*

## Introduction

Different taxa of fish have independently evolved the ability to produce electric signals. The Neotropical knifefishes (Teleostei: Gymnotiformes) are among the most diverse, with over 100 described species[1]. They are primarily nocturnal, relying on their electrosensory systems for navigation and species recognition. The commonly known electric eel can also produce strong electric discharges for predation and defence.

Electric signal discharge patterns of Gymnotiformes are species specific and well documented[2]. Some gymnotiform families produce pulses of electric discharge (Electrophoridae, Gymnotidae, Rhamphichyidae, and Hypopomidae), while others produce waves of electric discharge (Apteronotidae and Sternopygidae). Electric signals also vary in the frequency and number of phases in the waveform.

More derived lineages generally have more complex electric signal waveforms. This may be due to selective pressures from electroreceptive predators, then maintained by sexual selection[3,4]. However, it is unclear whether pulse-type or wave-type electric discharge evolved first in these fish. Systematic studies based on morphology hypothesized that the wave-type family Apteronotidae diverged first[5,6]. A phylogeny based on ribosomal mtDNA also found wave-type discharges to be plesiomorphic, but due to the early divergence of a subset of the Sternopygidae[7]. In contrast, phylogenetic analyses of electrosensory systems hypothesized that pulse-type electric discharges evolved first, in the families Electrophoridae and Gymnotidae[2]. A robust phylogeny consistent with the species phylogeny is essential to inferences on the evolution of novel traits.

Basic molecular mechanisms of electricity production are highly conserved in animals[8]. Electric potentials travelling down conducting pathways will lead to a series of electrochemical events which result in the opening of voltage sensing ion channels. The voltage gated sodium channels (VGSCs) are large pore-forming proteins, consisting of four homologous domains, each with six intramembrane domains[9]. Functional modulation occurs primarily on The N-terminus, C-terminus, and inter-domain regions. Gene duplication[10,11] and differential expression[9] of VGSC genes in different tissues has allowed for the evolution of gene-specific and tissue-specific electrical characteristics[12]. A paralog of the skeletal muscle VGSC gene is preferentially expressed in the electric organ. Previous research of this gene has shown amino acid sequence variation at a few functionally important sites using a limited sample of Neotropical electric fish species[13]. Electric fishes are a source of natural variation in electric signal waveforms, thus may be valuable in understanding the molecular mechanisms of electricity production.

## Objectives

The goals of my research are **1)** to clarify evolutionary relationships among the Neotropical knifefishes, **2)** understand the evolution of electric signal production in these species and **3)** use the natural variation in Neotropical knifefish electric signals to aid understanding of molecular mechanisms of electric signal production in vertebrates.

## Methods

### Objective 1: Construct a consensus species phylogeny for Gymnotiformes

In order to clarify evolutionary relationships among Gymnotiformes, I plan to sample species whose electric organ discharges have been quantified[2], with at least one species representing each gymnotiform genus. The outgroup will include Characiformes, Cypriniformes, and Siluriformes fishes[14,15].

Protein coding DNA sequences from single copy mitochondrial and nuclear genes will be obtained from genomic DNA. Cytochrome b (Cytb) is a housekeeping gene that accumulates mutations at a relatively constant

rate in vertebrates[16]. Recombination activating gene 2 (Rag2) is an immune system gene[17] successfully used for phylogenetic classification of fish[18,19]. Rag2 genes in fish contains no introns[20,21], which will simplify the sequence alignment on which the phylogeny will be based. Analyses based on housekeeping and single copy genes should prevent an inaccurate phylogeny due to differences in patterns of natural selection among species[22], and due to mistaken orthology[23,24].

Phylogenetic analyses will be performed using the 2000 bp of sequence from each of 75 ingroup and outgroup species. The model of molecular evolution that best fit the data will be selected using MrModeltest[25]. A Gymnotiformes phylogeny will be produced with the aid of maximum likelihood and Bayesian methods carried out in PAUP*[26] and MrBayes[27].

**Objective 2: Infer the evolution of electric signal patterns**

The phylogeny produced from Objective 1 will be used to test hypotheses of evolution in gymnotiform electric discharge pattern.

Characters important to the production of electric discharge will reviewed from the literature. Those known to vary with the electric discharge pattern will be mapped onto the species phylogeny. A program such as MacClade[28] may be used to test whether the increase in signal complexity resulted from evolution or common descent.

**Objective 3: Determine Functionally Important Sites on the Voltage Gated Sodium Channel**

Primers will be designed to amplify approximately 1000 bp of exon that covers sites of potential variation and modulation in the protein. The zebrafish homolog of the electric organ VGSC gene (GenBank Accession # NW_00150719) will be used to search the GenBank[29] database for orthologous sequences from fish to aid in primer design.

Electric organ VGSC gene sequences will be obtained from genomic DNA of the same species used to build the phylogeny in objective 1. Patterns of positive selection in Gymnotiformes will be tested using site and branch-site models in the PAML codeml program[30].

**Significance**

Systematic relationships can be used to inform many disciplines in biology. The proposed research would demonstrate the versatility of systematics by using a phylogeny to help answer questions relevant to ecology and evolution, as well as cell biology.

South America has one of the most diverse populations of freshwater fish, including the order of electrogenic fish Gymnotiformes[1]. Their wide distribution, species diversity, and diversity of electric signal patterns make them an ideal model for patterns of biogeograpy, evolution, and neuroscience. The proposed research will provide an evolutionary roadmap to help explain the origins of diversity in Neotropical electric fish species, and origins of diversity their intrinsic electric signals.

Natural variation and evolution of electric signal production in these fish can also be used to inform medical science. Mutations in voltage gated sodium channel genes can cause several different neuromuscular diseases[31]. Analysis of genes involved the production of electric signals in gymnotiform fish can contribute to a greater understanding of the evolution of channel protein function, and mechanisms of neuromuscular diseases.

**Schedule**

This project would represent the core of my Masters dissertation. Most of the specimens and genomic DNA have come from my supervisor Dr. Nathan Lovejoy's tissue collection. The rest will be sought from museums and collaborators.

Half of the DNA sequences needed for the project have been obtained during this first year of my graduate studies. Over the next few months, I plan to obtain the rest of the sequences and perform the analyses. Compilation and publication should occur in 2009 or early 2010.

## Literature Cited


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**Budget**

| | |
|---|---|
| DNA Extraction (75 samples x $2 each) | $ 150 |
| PCR and gel electrophoresis (150 samples x 3 loci x $1.50 each) | $ 675 |
| PCR cleanup (150 samples x 3 loci x $1.50 each) | $ 675 |
| Sequencing (150 samples x 3 loci x 2 reactions x $5 each) | $ 4500 |
| Other consumables (tubes, tips, gloves, etc) | $ 50 |
| | |
| Total | $ 6050 |
| **Total requested** | **$2000** |

**Budget Justification**

This project will require genomic DNA samples from 150 study species and outgroups. Half of the DNA samples were already available in the lab. Each sequencing reaction will produce approximately 600 bp, so each gene will require 2 reactions.

Lab equipment and software are available in Nathan Lovejoy's lab at the University of Toronto. Funds for work already done have come from a Natural Sciences and Engineering Research Council grant awarded to my supervisor and a Sigma Xi grant awarded to me in 2008.

# DAWN D. XIAO

702 Brimorton Drive • Toronto, ON, Canada • M1G 2R9 • (647) 838 9416 • D.Xiao@UToronto.ca

## EDUCATION

**University of Toronto**

Candidate Masters of Science — September 2008 - Present
Honours Bachelor of Science — April 2008

## LAB EXPERIENCE

**University of Toronto**

***TEACHING ASSISSTANT, CELL AND MOLECULAR BIOLOGY LAB*** 2009-PRESENT

- Facilitates undergraduates' understanding of practical aspects of lab techniques

***MASTERS STUDENT, LOVEJOY LAB*** 2007-PRESENT
***PREVIOUSLY RESEARCH PROJECT STUDENT, AND NSERC SUMMER STUDENT***

- Analyzes the molecular phylogenetics and evolution of electric fish
- Performs bioinformatic analysis on cDNA and genomic DNA sequences
- Executes molecular lab work for genetic analysis: design primers, extract DNA, amplify gene sequences by PCR

***RESEARCH PROJECT STUDENT, BROWN LAB*** 2006-2008
***PREVIOUSLY VOLUNTEER LAB ASSISTANT***

- Investigated the time course expression of heat shock proteins after heat shock and Celastrol in a human neuronal cell line
- Executed experiments in an efficient manner: treated and harvested tissue culture, produced precise protein assays, performed quantitative western blots

***VOLUNTEER LAB ASSISTANT, MASON LAB*** 2005-2006

- Organized and sustained multiple species of crickets and jumping spiders, population totaling over 4000

**Toronto Western Hospital**

***RESEARCH PROJECT STUDENT, MILLS LAB*** 2004

- Helped run a team experiment on the effects of beta amyloid on PC-12 cells
- Fed tissue culture cells, acquired confocal images, and measured cell processes

## PUBLICATIONS, CONFERENCES, AND AWARDS

- Awarded a Grant in Aid of Research from Sigma Xi, the Scientific Research Society (2008)
- Acknowledged in Chow, A. M. and Brown, I.R. (2008). Interaction of heat shock protein 70 (Hsp70) family members in differentiated human neurons. Program No. 652.20. Presentation in the 2008 Neuroscience Meeting, Washington, DC.
- Awarded a Natural Science and Engineering Research Council (NSERC) Undergraduate Summer Student Scholarship (2008)
- Acknowledged in Elias, D. O., Lee, N., Hebets, E. A. and Mason, A. C. (2006) *Seismic signal production in a wolf spider: parallel* versus *serial multi-component signals*. The Journal of Experimental Biology. **209**, 1074-1084