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EDUCATION

2015 Ph.D. Evolution, Ecology, and Behavior. Dept. of Integrative Biology, University of Texas at Austin (UT)
Advisor: Dr. David Cannatella. First year graduate student.
2010 B.A. Biology with Distinction; specialization: Ecology and Conservation Biology. Boston University (BU)
Advisor: Dr. Karen Warkentin.

PUBLICATIONS

Tarvin, RD, P Peña, and SR Ron. Bd or habitat degradation? Evaluating the causes of population decline of *Atelopus spumarius* (Anura: Bufonidae) at Río Pucayacu, Ecuador. *Journal of Herpetology.* In review.

Muñoz, M, NG Crawford, **RD Tarvin**, JM Hopwood, E Mock, AL Schneider, and CJ Schneider. Divergence with gene flow during the adaptive evolution of colorful pigmentation in *Anolis marmoratus* from Guadeloupe. *Molecular Ecology*. Submitted.

Tarvin, RD, CS Bermúdez, and KM Warkentin. Extended postmetamorphic mass loss and slower growth of larger metamorphs in red-eyed treefrogs. *Oecologia*. In preparation.

Tarvin, RD, NG Crawford, MM Muñoz, N Messana, CJ Schneider. Seventy-five polymorphic microsatellite loci from the Guadalupean Anole, *Anolis marmoratus* (Polychrotidae:Squamata), a morphologically and ecologically variable Caribbean lizard. *Conservation Genetics.* In preparation.

RESEARCH EXPERIENCE

2011: Captive amphibian husbandry, La Balsa de los Sapos, Pontificia Universidad Católica del Ecuador (PUCE), Quito, Ecuador. Supervisor: Dr. Andrés Merino. Head caretaker of a few hundred frogs.

2010: Harlequin frog monitoring, PUCE, Quito, Ecuador. Supervisor: Dr. Santiago Ron. Demographic study of a population of the Amazon Harlequin frog (*Atelopus spumarius*) in the Amazonian highlands of Puyo, Ecuador.

2009-2010: Variation of post-metamorphic growth with foraging, activity, and size at metamorphosis in the red-eyed treefrog, BU. Supervisor: Dr. Karen Warkentin. Senior thesis project. Field research on froglet growth and foraging behavior during a summer internship at the Smithsonian Tropical Research Institute in Gamboa, Panama, followed by laboratory research in Boston.

2009: *Nematostella* research, BU. Supervisor: PhD Candidate Myra Hughley. Volunteered as a research assistant to design, develop, and perform research on the reproduction of the Cnidarian *Nematostella*.

2008: *Anolis* genome scan, BU. Supervisor: Dr. Chris Schneider. Lab research in Boston during a summer internship, followed by work as a lab volunteer. Performed a genomic scan of the lizard Anolis marmoratus in search of loci associated with color polymorphism.

BU Tropical Ecology Program, Universidad San Fransico de Quito, Cumbayá, Ecuador. Supervisor: Kelly Swing. Designed, carried out, and analyzed seven short-term field research projects in the tropics.

GRANTS AND HONORS AWARDED

2012: Ecology, Evolution, and Behavior Program graduate student start-up grant, UT. $2000
Grant for graduate student research in herpetology, Chicago Herpetological Society. $500
Grant in herpetology, Texas Herpetological Society. $500.
Honorable Mention, National Science Foundation Graduate Research Fellowship Program.

2010: Ada Draper Award for independent studies abroad following graduation, BU. $3000
Summa Cum Laude Latin Honors, BU. Awarded to the top 5% of the graduating class.
Induction into the Phi Beta Kappa Honors Society.

2009: Undergraduate research opportunities program (UROP) grant for red-eyed treefrog research, BU. $4000
UROP travel award for travel to research site in Panama, BU. $310

2008: UROP grant for genome scan of *Anolis*, BU. $4000

2006-2010: Half tuition merit scholarship for four years, BU. $68,000

PRESENTATIONS

Tarvin, RD, CS Bermúdez, and KM Warkentin. 2009. Post-metamorphic Growth of Red-Eyed Treefrogs. UROP symposium, Boston, MA.

Tarvin, RD and CJ Schneider. 2008. Genome Scan Identifies Two Loci Associated with Color Polymorphism in *Anolis* Lizards. UROP symposium, Boston, MA.

TEACHING EXPERIENCE

2011–present: Graduate teaching assistant, UT. Lab instructor for introductory biology and comparative anatomy.

2010: Citizen Schools teacher, Edwards Middle School, Charlestown, MA. Designed and taught an after-school program focused on frog ecology to 16 six-graders, culminating in a poster which they presented to their peers.

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Phylogenetic origins and evolutionary consequences of alkaloid insensitivity in poison frogs.

Dart poison frogs (Anura: Dendrobatidae) are known for their bright colors and the use of their skin secretions for dart poison (1). Conspicuous coloration of chemically-defended poison frogs advertises their aversive taste, a defense strategy known as aposematism. Aposematism has arisen in parallel with diet specialization in Dendrobatidae at least three times, and aposematic diet specialists tend to have the highest levels of chemical defense (2-4). These trends present an opportunity to examine mechanisms underlying the evolution of aposematism and diet specialization. Defended species must be in some way resistant to their own chemicals; thus, alkaloid insensitivity is likely a characteristic driving the evolution of increased levels of defense in Dendrobatidae. The goal of this project is to elucidate the phylogenetic origin of alkaloid insensitivity in Dendrobatidae and determine what role insensitivity has played in the radiation of poison frogs.

Chemically-defended dendrobatids sequester alkaloids from arthropod prey, store them in cutaneous granular glands, and release them to the skin surface when threatened (5). Mere micrograms of certain alkaloids are lethal to humans because they attach to and disrupt the function of voltage-gated ion channels (VGICs), cell membrane channels responsible for neural communication and muscle contraction (6-8). For example, the alkaloids tetrodotoxin (TTX) and batrachotoxin bind to VGIC subunits that line ion channel pores, which disrupts the transport of sodium and/or potassium ions by directly blocking the pore or slowing channel deactivation (6, 9). However, the poison frogs themselves are not adversely affected by their own chemicals (1), which begs the question: what makes the poison frogs resistant? And has this resistance evolved once or multiple times?

In garter snakes (*Thamnophis*) that specialize in consuming newts defended by TTX, various VGIC amino acid (AA) substitutions that confer resistance to TTX have arisen multiple times (10). These mutations are present only in populations that preferentially consume toxic newts, suggesting that they arose in concert with multiple origins of diet specialization in garter snakes (10). Likewise, dendrobatids that consume many alkaloid-bearing organisms (*e.g.* ants) must be resistant to the adverse effects of these chemicals. Hence, VGICs of defended poison frogs should have AA substitutions that prevent alkaloids from interfering with VGIC function. In the most recent common ancestor (MRCA) of the clade Dendrobatidae, or of less inclusive groups within Dendrobatidae, newly-resistant VGICs likely diminished the negative effects of diet-derived alkaloid accumulation, simultaneously releasing dendrobatids from dietary constraint and permitting them to harness these chemicals for defense. Subsequently, resistance would have facilitated increased defense via more specialized diets and conspicuous coloration. Considering that most frogs are nocturnal and cryptic, and Dendrobatidae is the largest clade of diurnal, conspicuous anurans, it is likely that the evolution of alkaloid resistance and aposematism played an important role in the diversification of poison frogs. Surprisingly, the structure of dendrobatid VGICs has not been investigated. I aim to identify the molecular basis and phylogenetic origin(s) of alkaloid resistance in Dendrobatidae in order to understand how it influenced the evolution of these poison frogs.

Objectives

I hypothesize that functional VGIC mutations have arisen in concert with the multiple origins of aposematism in Dendrobatidae, facilitating the radiation of chemically-defended dendrobatid species. This hypothesis will be tested in three phases: a phylogenetic analysis will determine if nucleotide and AA mutations in VGICs are a synapomorphy of Dendrobatidae or of one or more subclades of Dendrobatidae (AIM 1); an experimental study will determine if AA changes in VGICs are the source of VGIC resistance to alkaloids (AIM 2); and an ecological analysis will determine if functional differences observed in VGICs are phylogenetically correlated with diet, coloration, and level of defense (AIM 3).

Methods

I will collect specimens of four *Epipedobates* species, a clade of poison frogs that are phylogenetically very closely related, yet ecologically very distinct, properties that make the group ideal for the comparative study of VGIC evolution and aposematism (14; Fig. 1). I have selected a number of localities in accordance with my collaborator (Santiago Ron, Pontificía Universidad Católica de Ecuador, PUCE) and I have the necessary collection permit (No. 001-11 IC-FAU-DNB/MA, Ecuador). Starting in June 2012, I will sample different populations in Ecuador, collecting skin secretions for alkaloid profile characterization, tissue samples for genetic analysis, and gut contents for diet analysis; voucher specimens and tissue samples will be deposited in the Museo de Zoología (QCAZ) at PUCE. Tissue

samples of other dendrobatid species will be obtained from Texas Natural Science Center and QCAZ. The following analyses will be performed.

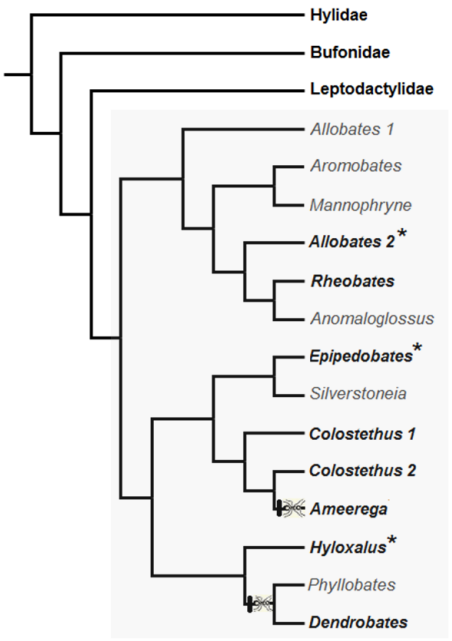

Figure 1. Diet specialization (indicated by ant) and aposematism (indicated by bar) have arisen multiple times in Dendrobatidae (indicated by gray box; *sensu* 3,14). In genera marked with asterisks, these characteristics are present in some, but not all species. Lineages that will be investigated in AIM 1 are in bold.

AIM 1: Determine if VGIC modification is phylogenetically correlated with the presence of chemical defense in Dendrobatidae.
Methods: Two genes encoding VGICs (Kcna3 and SCN4A) will be sequenced. Sequences will be aligned with MUSCLE, phylogenies will be inferred with RAxML, and origins and patterns of nucleotide and AA variation in VGICs, as well as phylogenetic correlations among these will be identified using Mesquite (11-13). The ingroup will be a selection of species (including populations within these) that span Dendrobatidae, including clades with and without chemical defense; outgroups will be species of Hylidae, Bufonidae, and Leptodactylidae (Fig. 1). We will focus our analysis on highly-conserved sites in channel pores to which alkaloids bind (5).
Expected results: Phylogenetic variation in VGIC genes will clarify if VGIC modification is present in the MRCA of Dendrobatidae or if it evolved in parallel with multiple origins of chemical defense. I expect that substitutions in alkaloid-binding sites are not present in outgroup species. **A)** If Mesquite ancestral state reconstructions indicate that VGIC modifications are a synapomorphy of Dendrobatidae and arose prior to the evolution of chemical defense, sequences in both defended and undefended species will contain very similar nonsynonymous substitutions. **B)** If VGIC modifications evolved multiple times, in concert with chemical defense, sequences in only the defended species will exhibit functionally convergent, but not necessarily identical, AA substitutions. As in garter snakes, multiple substitution patterns may produce similar resistance patterns (7).

AIM 2: Determine if AA substitutions in VGIC genes are responsible for VGIC insensitivity to alkaloids.
Methods: Alkaloids will be extracted from four *Epipedobates* species (Fig. 2) with the Transcutaneous Amphibian Stimulator (15). Extracts will be analyzed by gas chromatography-mass spectrometry to determine the concentration and types of alkaloids present. To analyze VGIC sensitivity, channels will be expressed in *Xenopus* oocytes and analyzed using common methods: by measuring the extent to which channels are blocked by standardized mixtures of alkaloids from each species (16). I chose this clade because it varies in levels of defense (Fig. 2), so variation in alkaloid sensitivity may also exist. As a positive control, VGICs of the outgroups used in AIM 1 will also be tested.
Expected results: This experiment will assign VGIC phenotypes to the VGIC phylogeny of *Epipedobates* from AIM 1. I expect that outgroup VGICs are sensitive to all alkaloid extracts. **A)** If VGIC AA substitutions decrease VGIC sensitivity, I expect variation in VGIC function to positively correlate phylogenetically with VGIC AA variation. **B)** If AA substitutions are not the cause of VGIC insensitivity, I expect no relationship between AA variation and VGIC function.

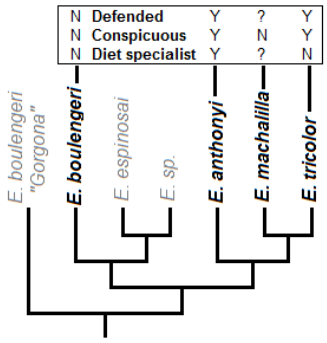

Figure 2. *Epipedobates sensu* (14). Species to be analyzed in AIMs 2 and 3 are in bold; presence (Y) and absence (N) of characteristics in focus species are described above tree. Characteristics that could not be assigned were marked as unknown (?).

AIM 3: Determine if physiological variation in VGIC sensitivity is associated with diet type, level of defense, and conspicuousness.
Methods: For *E.machalilla*, alkaloids and stomach contents will be analyzed and diet will be classified following standard methods (4). Then, using the VGIC genotype and phenotype phylogenies from AIMs 1 and 2, and published ecological descriptions for other species of *Epipedobates*, VGIC function will be compared with diet, defense, and conspicuousness.
Expected results: I expect *E. machalilla* to be defended, but its diet may be either generalized or specialized (both are equally parsimonious). With ecological information for four ecologically-distinct *Epipedobates* species, I will be able to parse out the order in which VGIC insensitivity, defense, conspicuousness, and diet specialization evolved in this clade. **A)** If decreased VGIC sensitivity evolved in concert with aposematism and diet specialization (hence, increased defense: 2-3), VGICs of the aposematic diet specialist will be least

sensitive to alkaloids while VGICs of cryptic generalists (less defended) will be most sensitive. **B)** If VGIC sensitivity did not change (*e.g.* insensitivity was already present) with origins of aposematism and diet specialization, sensitivity will not correlate with diet type, defense, or coloration.

Significance and future directions

Within Dendrobatidae, aposematism and diet specialization have evolved multiple times (3). In the clades of *Ameerega, Phyllobates,* and *Dendrobates*, all individuals are conspicuous, diet specialized, and defended, and in other clades, some but not all of these characteristics are present. However, four closely-related species of *Epipedobates* are characterized by different combinations of these ecological traits. By determining the phylogenetic origin of alkaloid insensitivity within this clade, I aim to identify the order in which insensitivity, conspicuousness, defense, and diet specialization have evolved. The origin of these traits is of interest because it will help to clarify under what types of ecological pressures traits such as aposematism can arise. In the future, I hope to examine VGICs of polymorphic dendrobatids that have intraspecific variation in diet to understand the role of VGICs in fine-scale ecological evolution. Furthermore, I will examine VGICs in mantellids (poison frogs of Madagascar), and attempt to clarify if there are costs associated with VGIC insensitivity in either dendrobatids or mantellids. This project will synthesize theory from systematics, physiology, genetics, and ecology, providing a novel multidisciplinary approach to understanding the ecology and radiation of Dendrobatidae as well as the evolution of aposematism and diet in other systems such as butterflies, nudibranchs, and toxic birds (pitohuis).

Schedule

AIM 1	Collect samples	June – August	2012
	Extract DNA and sequence SCN4A and Kcna3	September – October	2012
	Create VGIC phylogenies	November – December	2012
AIM 2	Analysis of alkaloid extracts	January – May	2013
	Channel cloning and VGIC testing	June – August	2013
AIM 3	Analyze gut contents	September – October	2013
	Analyze correlation between ecology & VGIC function	November – December	2013

References

1. Albuquerque, EX, JE Warnick, FM Sansone, and J Daly. 1973. The pharmacology of batrachotoxin. V. A comparative study of membrane properties and the effect of batrachotoxin on sartorius muscles of the frogs *Phyllobates aurotaenia* and *Rana pipiens. J Pharmacol Exp Ther* 184: 315-29.
2. Summers, K, and ME Clough. 2001. The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). *Proc Nat Acad Sci USA* 98: 6227-6232.
3. Santos, JC, LA Coloma, and DC Cannatella. 2003. Multiple, recurring origins of aposematism and diet specialization in poison frogs. *Proc Nat Acad Sci USA* 100: 12792-12797.
4. Darst, CR, PA Menéndez-Guerrero, LA Coloma, and DC Cannatella. 2005. Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): a comparative analysis. *Am Nat* 165: 56-69.
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6. Daly, JW, TF Spande, and HM Garraffo. 2005. Alkaloids from amphibian skin: a tabulation of over eight-hundred compounds. *J Nat Prod* 68: 1556-1575.
7. Daly, JW, CW Meyers, JE Warnick, and EX Albuquerque. 1980. Levels of batrachotoxin and lack of sensitivity to its action in poison-dart frogs (*Phyllobates*). *Science* 208: 1383-1385.
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12. Maddison, WP, and DR Maddison. 2006. Mesquite: a modular system for evolutionary analysis. Version 2.75 http://mesquiteproject.org
13. Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
14. Santos, JC, LA Coloma, K Summers, J Caldwell, R Ree, and DC Cannatella. 2009. Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages. *PLoS Biology* 7: e1000056.
15. Grant, JB, and B Land. 2002. Transcutaneous amphibian stimulator (TAS): a device for the collection of amphibian skin secretions. *Herpetological Review* 33: 38-41.
16. Geffney, SL, E Fujimoto, ED Brodie III, ED Brodie Jr, and PC Ruben. 2005. Evolutionary diversification of TTX-resistant sodium channels in a predator-prey interaction. *Nature* 434: 759-763.

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Budget

Category	Details	Amount	Total
Travel	Plane ticket to and from Ecuador	$1000	x1 = $1000
	Transportation in Ecuador	$1000	x1 = $1000
	Visa	$280	x1 = $280
Fieldwork	Accommodation and food while in field	$25/day/person	x40 = $1500
	Transcutaneous Amphibian Stimulator	$325	x1 = $325
	Accommodation and food in Quito	$25/day/person	x10 = $250
Lab work	Primer design and DNA sequencing at University of Texas Core Facility	$6/sample	x50 = $300
	DNA extraction kit (Qiagen)	$140	x1 = $140
	RNA extraction kit (Qiagen)	$160	x1 = $160
	Channel cloning	$3/sample	x30 = $90
Total			**$5045**
Grant support obtained to date (from UT, THS, and CHS grants; see CV)			$3000
Amount still needed			$2045
Amount requested			**$2000**

Budget Justification

Travel: $2280 will cover my round-trip plane ticket from Texas to Ecuador and (approx. $1000), and a car rental for one month, including gas (approx. $1000). It will also pay for my visa ($280).

Fieldwork: $2075 will cover the cost of obtaining a Transcutaneous Amphibian Stimulator ($325), lodging and food for me and my field assistant for one month of travel [($25/person/day) x (2 people) x (30 days) = $1500)]. It will also cover 10 days of lodging in Quito for me, while I organize and deposit specimens at PUCE [($25/person/day) x (1 person) x (10 days) = $250].

Labwork: $690 will cover lab expenses, including DNA extraction kits ($140), RNA extraction kits (required to analyze sodium channel gene; $160), DNA sequencing ($6/sample x 50 samples = $300), and channel cloning ($3/sample x 30 samples = $90).

Reference Letters

(1) Dr. David Cannatella. Current Advisor, UT. 2011 – present.

(2) Dr. Karen Warkentin. Undergraduate Advisor, BU. 2009 – 2010.