Introduction: Coevolutionary dynamics between predators and prey are an important focus of evolutionary theory. Deadly anti-predator defenses (toxins), and resistance to these defences has been poorly understood within the context of coevolutionary theory[1]. Many studies have examined this enigma through resistance to dermal toxins (newts/frogs) however the genetic basis of dermal toxins is not ideal for understanding this dynamic[1]. Snake venom, a good example of this phenomenon, is a complex secretion whose biological actions and genetic basis have been the focus of much research. Resistance to these complex cocktails has been reported in several mammals that are both snake prey and predators, though comparatively little research has been devoted to understanding the diversity of mechanisms of resistance in these mammals[2,3]. To date, most studies of resistance have described single resistance mechanisms expressed in single individuals. In contrast, my research will aim to characterize the variability of resistance across populations, examining the role that multiple mechanisms of resistance contribute to this complex phenotype. Uniquely, my research will pair venom toxicity with measures of mammalian resistance across regions representing predator/prey sympatry and allopatry, to attempt to quantify the degree to which these traits are engaging in an evolutionary arms-race.

The opossum *Didelphis marsupialis* (*DM*) has been shown to aggressively prey on and survive envenomation from several species of South American pit vipers (*Bothrops spp*)[4]. This species has both serum protein inhibitors, and a modified protein target (Von Willebrand Factor, or vWF) that likely contributes to venom resistance[5,3]. I will examine (1) how venom resistance varies in response to sympatry and allopatry with vipers on varying evolutionary time scales (2) if predatory mammals are a driving force in the evolution of venom toxicity (3) how serum proteins and molecular changes in venom targets provide the mechanistic basis of resistance.

Q1: Does venom and venom resistance show signs of reciprocal, "arms race" evolution? The islands of the Lesser Antilles represent an ideal natural experiment with pairwise associations of venomous snakes and venom resistant mammals. These islands have all undergone a recent Holocene invasion of mammals, including *DM* with the exception of Martinique (*Bothrops* have been present since the Pliocene)[8,6]. Trinidad is home to both *DM* and *B. atrox*, St Lucia has *DM* and *B. carribaeus*, Tobago and Grenada have populations of *DM* but no viper species, and Martinique is home to *B. lanceolatus* but harbors no *DM*. On the Venezuelan mainland, *B. asper/atrox* shares a distribution with many snake-eating opossums, resulting in non-overlapping sites with one, two, and three species of resistant opossum predators. Importantly, unlike other well studied island populations of divergent *Bothrops spp*, the Lesser Antilles *Bothrops spp.* did not undergo a significant change in diet from their mainland counterparts[6].

Hypotheses 1: a) If venom resistance via serum inhibitors from *DM* is a quickly evolving trait, it will have evolved venom specificity over the time scale of its island introduction (~700ad). **b)**Venom will be reciprocally modified in island vipers to be more toxic when in sympatry with *DM*; conversely, when *DM* is absent, venom will be less toxic due to relaxed selection pressure.

I will sample *DM* blood serum from the islands of Trinidad, Tobago, St, Lucia, and Grenada, as well as six mainland populations representing varying degrees of opossum sympatry with *B. asper/atrox*. I will use a flourometric proteolytic assay to measure both the proteolytic capability of venom to dissolve a substrate, as well as the relative ability for opossum serum to inhibit this activity[7]. This assay has been optimized for inexpensive and fast processing, lending itself to an experimental design with multiple pair-wise tests (with replicates). This makes it possible to sample many individuals within populations thereby including intraspecific variation in both venom and inhibition[7]. Venom will be obtained from collaboration with local researchers, who I have already been in contact with. For all sites, I predict that local *DM* will inhibit venom from

sympatric snakes, but will be best able to inhibit venom from populations of *B. lanceolatus* from Martinique (which has been released from mammalian predation pressure). I predict venom proteolytic capability to follow suit, with *B. lanceolatus* being least proteolytic, and mainland populations (in the presence of multiple mammalian predators) being most proteolytic. Since island vipers have only recently been re-exposed to mammalian predation pressure, their venom should be overall less toxic and better inhibited compared to mainland snakes. Interspecific and intraspecific variation in mammalian venom immunity has been surveyed in only one other system and was shown to vary significantly with both contemporary snake densities and historical sympatry[2]. However there has been no study to date that has isolated reciprocal evolution of snake venom in response to resistant mammalian predation. This study will provide the unique opportunity to measure the influence of resistant predators in venom evolution, as well as assess the time scale of evolutionary response (loss or gain) in mammalian predators.

Q2: How do mammals cope with thrombocytopenia induced by non-proteolytic snake venom C-type Lectin (SVCL) proteins on a spatial and evolutionary scale?

While the proteolytic components of venom are often regarded to be the most important in determining overall toxicity, they are not the only source of selection pressure. Many non-proteolytic SVCL molecules have been well characterized in venom from *Bothrops spp*. These molecules cause systemic thrombocytopenia by binding to intermediate agglutinating factors (vWF). While accelerated positive selection has been found in opossum vWF, the mechanism by which this modified target aids SVCL resistance has not been characterized[3].

Hypothesis 2: Molecular target modification of vWF provides protection from platelet agglutinating venom by decreasing binding affinity for SVCLs. This protection will be greatest for mainland species pairs who have been co-evolving longest, and least for island associations.

With the aid of Dr Tony Dean, I have already begun to test binding affinities of *DM* vWF sequences with Botrocetin (a SVCL from *B. jararaca*) *in silica*, and will soon begin *in vitro* binding assays via yeast-expressed vWF and chemically isolated Botrocetin, as well as platelet agglutination assays. These assays will provide a way to quantify the amount of protection that a modified vWF will provide against different SVCL venom components in *Bothrops spp*. I will collect tissue samples from each site and sequence vWF along with neutral markers to assess if this trait also varies in accordance with venom composition or population divergence. Together with proteolytic assays this study will represent the first attempt to include both target modification and serum inhibition in assessments of mammalian resistance, as well as the first to assess their capacity for variation on both long (mainland) and short (island) evolutionary time scales. Broadly, this study presents the unique opportunity to understand how coevolution works on a suite of rapidly evolving molecular traits that together produce a complex functioning phenotype on varying evolutionary time scales.

Broader Impacts: It is a personal goal of mine to encourage woman in science, both at UMN and during field work in developing nations. I will recruit undergraduates to participate in laboratory projects, as well as assemble a local field team in the Lesser Antilles. I am currently coordinating an extension of TeachSMART, an outreach program, to share my research and develop science curricula in an area of Minneapolis where the achievement gap for children of color is one of the worst in the country. I am deeply passionate about communicating this research across disciplines and to the public, and will continue to do so throughout my career.

SOURCES: **[1]** Williams *et al.* (2003) *Herpetologica* **59:** 155-163. **[2]** Biardi *et al.* (2006) *J. Chem Ecol* **32**:137–154. **[3]** Jansa and Voss (2011) *PLoSONE* **6**:e20997. **[4]** Pifano *et al.* (1993) *Roum Arch of Microb Immun* **52**:131-136. **[5]** Perales *et al.* (1994) *Toxicon* **32**:1237-1249. **[6]** Wuster *et al.* (2002) *Bull. nat. Hist. Mus. Lond.(Zool.)* **68**:101-106. **[7]** Biardi *et al.* (2011) *Toxicon* **57**: 342-347. **[8]** Giovas *et al.* (2012) *J. Biogeogr.* **39**: 476-487.