Toxicon 152 (2018) 137–149

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Female-biased population divergence in the venom of the Hentz striped scorpion (*Centruroides hentzi*)

Micaiah J. Ward[a], Schyler A. Ellsworth[a], Michael P. Hogan[a], Gunnar S. Nystrom[a], Paul Martinez[a], Amisha Budhdeo[a], Roxana Zelaya[a], Alexander Perez[a], Barclay Powell[a], Huan He[b], Darin R. Rokyta[a,*]

[a] Department of Biological Science, Florida State University, Tallahassee, FL, 32306, USA
[b] Institute of Molecular Biophysics and College of Medicine, Florida State University, Tallahassee, FL, 32306, USA

ARTICLE INFO

Keywords:
Scorpion
Venom
Sex-biased genes
Population variation

ABSTRACT

Sex-biased genes are expressed at higher levels in one sex and contribute to phenotypic differences between males and females, as well as overall phenotypic variation within and among populations. Venom has evolved primarily for predation and defense, making venom expression a highly variable phenotype as a result of local adaptation. Several scorpion species have shown both intraspecific and intersexual venom variation, and males have been observed using venom in courtship and mating, suggesting the existence of venom-specific, sex-biased genes that may contribute to population divergence. We used reversed-phase high-performance liquid chromatography (RP-HPLC), Agilent protein bioanalyzer chips, nano-liquid chromatography mass spectrometry (nLC/MS/MS), and median lethal dose (LD_{50}) assays in fruit flies (*Drosophila melanogaster*) and banded crickets (*Gryllodes sigillatus*) to investigate proteomic and functional venom variation within and among three Florida populations of the Hentz striped scorpion (*Centruroides hentzi*). We found significant venom variation among populations, with females, not males, being responsible for this divergence. We also found significant variation in venom expression within populations, with males contributing more to within population variation than females. Our results provide evidence that male and female scorpions experience different natural and sexual selective pressures that have led to the expression of sex-biased venom genes and that these genes may be consequential in population divergence.

1. Introduction

Among metazoans, males and females within a species often exhibit unmistakable phenotypic differences. These sexually dimorphic traits are usually the result of sex-biased gene expression and include not only sex-specific reproductive tissues, but also differences in size, coloring, and behavior (Ellegren and Parsch, 2007; Mank, 2008). Sex-biased gene expression is thought to be especially prevalent in species that experience differences in maternal (or paternal) care, as well as male-male mate competition, sperm competition, female mate choice, or differences in ecology (Shine, 1989; Ellegren and Parsch, 2007; Mank, 2008), as these traits are under the strong evolutionary forces of natural and sexual selection (Chenoweth et al., 2007). Sex-biased genes may be exclusively expressed in one of the two sexes (sex-specific genes), or be expressed at higher levels in one sex compared to the other (sex-enriched genes), and are not limited to genes located on sex chromosomes (Ellegren and Parsch, 2007). The most extensive studies of sex-biased genes have included *Drosophila* (Parisi et al., 2003; Ranz et al., 2003; Zhang et al., 2004; Connallon and Knowles, 2005; Pröschel et al., 2006), mice (Torgerson et al., 2002; Yang et al., 2006), and comparisons of chimps and humans (Khaitovich et al., 2005; Nielsen et al., 2005), all of which found that male-biased genes exhibit a greater amount of rapid, positive selection and are therefore more responsible for population and/or species divergence. Most of these rapidly evolving male-biased genes, however, are primarily expressed in reproductive tissues (Ellegren and Parsch, 2007), and the degree of male or female-biased gene expression may differ among tissue types (Yang et al., 2006) or species (Ranz et al., 2003; Mank and Ellegren, 2009).

Scorpions are often sexually dimorphic in appearance and exhibit many traits associated with sex-biased gene expression, including maternal care, mate competition, female mate choice, ecology, and even differences in venom composition and lethality (De Sousa et al., 2010; Rodríguez-Ravelo et al., 2015; Miller et al., 2016; Cid-Uribe et al., 2017), and such differences seem to be widespread among arachnids

* Corresponding author. Florida State University, Department of Biological Science, 319 Stadium Dr., Tallahassee, FL, 32306-4295, USA.
E-mail address: drokyta@bio.fsu.edu (D.R. Rokyta).

https://doi.org/10.1016/j.toxicon.2018.07.026
Received 4 May 2018; Received in revised form 27 June 2018; Accepted 31 July 2018
Available online 07 August 2018
0041-0101/

(Herzig et al., 2002, 2008; Zobel-Thropp et al., 2018). Venom consists of proteins and peptides that, in most venomous animals, have evolved primarily for the purposes of predation and defense (Biardi et al., 2005, 2011). Intraspecific venom variation, which has been established in multiple scorpion species (Abdel-Rahman et al., 2009; Ruiming et al., 2010; Rodríguez-Ravelo et al., 2013; Estrada-Gómez et al., 2014; Carcamo-Noriega et al., 2017; Schaffrath et al., 2018), is thought to be a result of local adaptation, especially when differences in prey availability or predator exposure exist among populations. Some scorpion species have also been observed using venom in hygiene (D'suze et al., 2015) and mating behavior (Polis and Sissom, 1990; Lourenço, 2000; Benton, 2001), where male-male mate competition (Benton, 1992) and female mate choice (Polis and Sissom, 1990; Tallarovic et al., 2000; Contreras-Garduño et al., 2006) have been documented. Male scorpions are often faster and more mobile in search for mates (Booncham et al., 2007; Carlson and Rowe, 2009; Kaltsas and Mylonas, 2010), and females are more sedentary because they are frequently gravid or carrying young on their backs, making long-distance travel more difficult (Shaffer and Formanowicz, 1996). The differences in ecological niches between males and females has resulted in females being more apt to sting defensively than males (Williams, 1987; Shaffer and Formanowicz, 1996; Carlson and Rowe, 2009; Carlson et al., 2014). Males are prone to greater predator exposure (Polis and Farley, 1979) and are likely to consume a broad range of prey types as they travel. The differences between male and female scorpions in morphology, behavior, environment, and venom composition suggest not only that sex-biased genes are present in scorpions, but that some sex-biased genes may be venom-specific and contribute to venom variation within and among populations.

To investigate intraspecific and intrapopulation venom variation as well as explore the potential contribution of venom-specific, sex-biased gene expression, we sampled male and female Hentz striped scorpions, *Centruroides hentzi*, from three Florida populations. *Centruroides hentzi* are sexually dimorphic scorpions found in the Southeastern United States. Males have smaller bodies with longer metasomal (tail) segments and females are larger with shorter, more rounded metasomal segments (Fig. 1). *Centruroides hentzi* are commonly found in tree bark of long leaf pines, such as those found in the Apalachicola National Forest (ANF), Osceola National Forest (ONF), and the Tosohatchee Wildlife Management Area (TSO) (Fig. 1). We used a combination of reversed-phase high-performance liquid chromatography (RP-HPLC), Agilent protein bioanalyzer chips, and nano-liquid chromatography mass spectrometry (nLC/MS/MS) to determine overall venom expression variation within and among populations, as well as between sexes within and among populations. To assess the correlation between molecular variation and venom function, we performed median lethal-dose (LD_{50}) assays in banded crickets (*Gryllodes sigillatus*) and fruit flies (*Drosophila melanogaster*).

2. Materials and methods

2.1. Animal and venom collection

Adult scorpions were collected from the ANF (n_{male} = 4; n_{female} = 8), ONF (n_{male} = 4; nf_{emale} = 7), and TSO (n_{male} = 5; n_{female} = 5). Scorpions were fed banded crickets 1–2 times per week, unless they were being prepared for venom collection. Venom was collected using methods previously described (Rokyta and Ward, 2017; Ward et al., 2018). Briefly, scorpions were starved for a minimum of seven days to ensure ample venom production and anesthetized with CO_2. Electrostimulation was performed using a transcutaneous electrical nerve stimulation (TENS) unit (9 V) on a medium setting and applying a positive and negative electrode to either side of the telson to induce a muscle contraction. Venom was collected on a sterile metal spatula and pipetted into tubes containing LC/MS water. Venom samples were quickly centrifuged (two minutes at 12,000 RPM), frozen, lyophilized, and stored at −80°C until later use. Each venom sample was quantified using the Qubit Protein Assay Kit (Thermo Fisher Scientific) prior to use in our analyses. The quantities of venom present in each sample used are provided in Table S1.

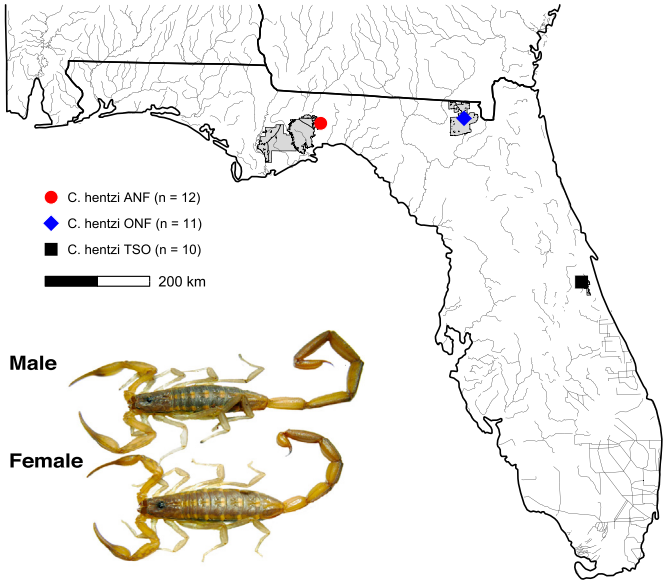

Fig. 1. Representative male and female *Centruroides hentzi* and Florida collection site map. Males have smaller bodies with longer metasomal segments and females have larger bodies with shorter, more rounded, metasomal segments. Gray areas indicate National Forest boundaries. The ANF and ONF forests are separated by roughly 257 km and the Suwannee river (SW), representing a substantial geographical boundary between the two populations. The ONF and TSO forests are separated by roughly 290 km and the ANF and TSO are separated by just over 482 km.

Banded crickets (*Gryllodes sigillatus*, size: 1.9 cm) were purchased from Ghann's Cricket Farm in Augusta, GA. Crickets were fed Ghann's Cricket Chow and housed at room temperature in a large aquarium cage with egg crates. Fruit flies (*Drosophila melanogaster*) used in this study were a single inbred line (line 11057) provided by the *Drosophila* Synthetic Population Resource (DSPR). Flies were kept on a light/dark cycle of 12 h held at a constant temperature of 25°C and fed standard cornmeal-agar media.

2.2. Reversed-phase high-performance liquid chromatography

Three venom samples for each *C. hentzi* individual were quantified using reversed-phase high-performance liquid chromatography (RP-HPLC). RP-HPLC was performed on a Waters 2695 Separations Module with a Waters 2487 Dual λ Absorbance Detector. Approximately 7 μg of protein was injected onto a Jupiter 5 μm C18 column (Phenomenex, Torrance, CA) using the standard solvent system of A = 0.1% trifluoroacetic acid (TFA) in water and B = 0.075% TFA in acetonitrile. A 125-min gradient from 10 to 75% solution B was performed at 0.2 mL/min, followed by 15 min at 10% solution B to wash the column. Data was analyzed using Empower Pro software version 5.00 (Waters Corporation, Milford, MA). Twenty-four RP-HPLC peaks were identified and quantified using methods previously described (Margres et al., 2015a; b). Briefly, the area under each peak was measured to determine the relative abundance of each peak to the total area of all protein peaks identified. According to the Lambert-Beer law, this relative abundance is related to the total percentage of peptide bonds in the sample (Gold et al., 1987) and has been shown to be representative of the relative amount of a specific protein by weight (Gibbs et al., 2009).