

Yamashita: Investigation of a DNA fragment for scorpion molecular population genetics FRG-Fall 2007

B. Abstract

Research activities from this project will investigate the robustness of a molecular genetic marker for an ongoing study into the population differentiation and evolution of striped scorpion populations across its geographic range in the U.S. southwest. In this work I propose to conduct further studies to determine if a genetic marker initially employed in the scorpion study meets all the criteria necessary as a genetic marker to effectively separate genetic differences among scorpion populations. During the genetic data analysis in the initial study, it was observed that patterns produced from this marker may not adequately resolve genetic separation among populations. Conducting further analyses that determine if the genetic marker exists as single or multiple copy in the scorpion's DNA will help resolve how well the marker can separate among scorpion populations.

### C. Purpose/Objectives

In this research project I propose to investigate the appropriateness of a genetic marker for population separation in the striped scorpion *Centruroides vittatus*. This genetic marker was developed during a summer research experience at UA-F funded through the NIH (National Institute of Health) sponsored Arkansas BRIN program. When we first developed this genetic marker, it appeared to satisfy all the requirements needed to separate scorpion populations based upon DNA sequences. After many populations were surveyed with this genetic marker and after the DNA sequences associated with this genetic marker were analyzed, it became unclear if this was a robust marker that could effectively separate populations from each other on a genetic basis. I plan to review the data already collected, then conduct new analyses to determine if this genetic marker can adequately differentiate among scorpion populations.

### D. Significance/Need

The genetic marker considered in this project is a DNA fragment isolated from a screening of striped scorpion DNA to identify microsatellite markers. Microsatellite markers are a common genetic tool to separate populations in a wide variety of organisms (Estoup and Cornuet 1999). Although they are an important tool in population genetic research, they must be isolated for most species as they are commonly species specific. In our initial work, we were able to screen and identify several potential DNA regions; however, after subsequent analysis, we determined these DNA fragments did not exhibit the variability required for microsatellites. We performed further work and determined a one thousand nucleotide base DNA fragment numbered 1075 may show variability when sequenced. DNA sequencing is a technique in which all the nucleotides (DNA subunits) for a section of DNA are characterized. Upon sequencing several samples of the 1075 fragment from different populations, I discovered adequate genetic variability existed among the populations to incorporate this marker with another genetic marker in a large, regional study of striped scorpion population genetic variability and evolution.

The necessity of the 1075 fragment is that it represents a section of DNA found within the scorpion's nuclear DNA. Nuclear DNA represents the DNA commonly known to compose the genetic material in an organism's chromosomes. Nuclear DNA is more difficult to employ as a genetic marker as it undergoes crossing over and mixing during meiosis (Zhang and Hewitt 2003). Nuclear DNA and the genes found on the chromosomes are important as they represent the majority of the DNA housed in an organism. The other DNA region employed in the scorpion project is a marker located within the DNA of the mitochondria. Mitochondrial DNA (mtDNA) is genetic marker that has been commonly used in animal population studies (Ballard and Rand 2005). Mitochondrial DNA mutates at a faster rate than nuclear DNA and is maternally inherited, providing a cleaner signal than nuclear DNA. In the scorpion study, it has been a reliable and robust marker that has allowed discrimination of scorpion populations.

When compared to the mtDNA marker in our analysis, the 1075 fragment showed a more confusing pattern. I attributed the increased randomness of the pattern produced in the 1075 fragment as that often observed in nuclear DNA patterns when compared to mtDNA. When my co-investigator and I submitted a manuscript for publication, it became evident from the reviews that we would need to conduct further work on the 1075 fragment to be included in a publishable paper on the scorpion analysis.

E. Process of attainment of objectives/goals:

In order to determine the robustness of the 1075 fragment, I plan to conduct further DNA sequencing analysis and cloning of the fragment upon individuals from populations collected throughout the striped scorpion's geographic range in the 2007 summer. The expanded sequence work will allow a larger sample to better determine the variability in this marker and the extent of the variability among populations. In addition, I plan to isolate and clone samples of this marker from the same individuals to determine if the 1075 fragment exists as a single copy or if it exists as multiple copies within the scorpion's DNA. Genes and DNA fragments that exist as multiple copies can cause additional confusion beyond the problems encountered when utilizing nuclear DNA. If the 1075 fragment exists as a single copy region, it can be incorporated into the scorpion work with little additional concerns. If the 1075 fragment shows multiple copy within an individual, additional analyses will be required to determine the extent of these multiple copies among scorpion populations. Our previous analysis suggests the 1075 fragment does not exist in multiple copy as the initial cloning of this fragment was conducted when we first isolated it and showed no indication of this fragment as existing in multiple copy. In order to verify our previous work, the cloning of this fragment from several populations will allow us to determine if this fragment exists in multiple copy among individuals in separate populations across the scorpion's geographic range.

F. Report of Research activities:

In the 2007 summer, I was able to extract DNA from 77 new individuals and obtain 77 new sequences for this gene fragment. These DNA samples are currently undergoing analysis to determine their applicability to striped scorpion population genetics. In this analysis, I plan to review DNA sequence data files for accuracy and quantify the number of errors for each individual. In addition to these analyses, undergraduate students will amplify and clone questionable DNA sequences to determine if these sequences were the result of amplification errors or due to amplification of multiple DNA regions within each individual.

