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Independent Project

Bioinformatics for Data Science (Biol 792)

**Background:**  Viviparity, or live birth, has evolved in squamate reptiles over 100 times. In comparison to oviparity (egg-laying), viviparity tends to occur in populations that occupy higher elevation habitats. The most widely supported explanation for this phenomenon is the Cold Climate Hypothesis, which posits that live birth confers a fitness advantage in cooler climates due to the ability of the mother to better control the temperature of an internally retained clutch, and thus ensure her offspring a better chance at survival. This hypothesis does not, however, account for the fact that closely related species with broadly overlapping elevational ranges often display contrasting modes of parity. In this project, we aim to assess variability in gestation between paired high and low elevation sites, as well as the fitness consequences of these differences in terms of offspring survival. We hope to use a combination of ecological, physiological and molecular data to understand the nature and mechanisms of such variation in the genus Sceloporus (spiny lizards). Spiny lizards are among the most abundant reptiles in the Western United states and viviparity has evolved several times in this group, whose elevational range spans from below sea level to above 10,000 feet Additionally, extensive museum records and genomic data are available for this genus, which will greatly facilitate future analyses through which we hope to shed light on the molecular underpinnings of observed phenotypic variation.

The sharp elevational gradients in the Nevada high desert provide an excellent landscape for exploring this topic, as they boast an exceptional diversity of both ecological conditions and reptiles, including oviparous and viviparous species of phrynosomatid lizards. Additionally, Nevada has recently evidenced the increasing thermal variability predicted to accompany global climate change, with record-breaking high and low temperatures recorded across the state over the past few years. Previous studies have shown differential energy efficiency in viviparous as opposed to oviparous females, as well as a reduced critical thermal maxima in gravid females. Consequently, the establishment of a long term squamate reptile study system in the Nevada Great Basin will shed light on the potential implications of parity mode on survival and reproductive success, and will inform conservation and management policies for species vulnerable to increasingly variable thermal conditions precipitated by global climate change.

**Methods:** At the start of mating season, we will begin performing surveys at one pair of high and low elevation sites per day for 5-6 days a week. Following capture, we will measure the temperatures of each lizard using a cloacal thermometer. We will follow a standard Capture-Mark-Recapture protocol using toe-clipping to mark individual lizards. Toe clips will be stored in RNAlater for later use in parentage analyses as well as transcriptomics studies. Using toe clips collected from gravid female lizards in addition to blood samples in later seasons, we will perform RNA-Seq to assess differences in gene expression among populations and across different stages of gestation. We will compare these transcriptomic results with phenotypic and developmental data from ultrasound scans, as well as environmental data from iButtons placed in 3D printed model lizards at field sites in order to continuously record ecologically relevant thermal data. We will use a small portion of each tissue sample to create a microsatellite dataset, which will be used to assign parentage and track reproductive success.

We will conduct ultrasound scans on all captured adult females for assessment of pregnancy and gestational progression. These scans, which will be performed using a miniature portable ultrasound scanner, are a nondestructive approach to examining gestational variation in individuals not only over the same season, but also over the course of multiple years. They will provide an ideal tool for assessing populations where there is substantial variability in the reproductive output of individuals from year to year, and combined with our other sources of data, this noninvasive approach will shed light on the environmental and molecular processes underpinning such variability.

Once eggs are laid, nest sites will be marked for regular revisitation, and time between oviposition and hatching will be recorded. We will continue the process of capturing and marking individuals from subsequent generations, as well as taking temperatures and performing ultrasound scans on gravid females to expand our dataset. This project will provide a much-needed integration of fitness data, phenotypic traits, and molecular variation to explore the spectrum of parity in the context of an abundant, wide-ranging genus with mixed parity and existing genomic reference data.

**Independent Class Project Proposal:** The first field season will primarily consist of preliminary data collection, including identifying and narrowing down candidate sites to focus on those with the highest abundance of the target species. Since we were not able to conduct surveys last summer and it is presently too cold for lizards to be out, I will be relying on locality data from databases to make these judgement calls. I will utilize records from Arctos and the UNR Herpetology collection in addition to observations from iNaturalist to select candidate sites that are likely to have the highest abundance of lizards at the desired elevational ranges.

Ideally, I hope to select 2 pairs of sites for the coming summer: 2 at a relatively low elevation (3500-4500 ft) and 2 at a high elevation (6500-7500 ft). I will export records from the databases as .csv files, and use pandas to concatenate, manipulate and sort these records according to species, elevation, date, sex and locality information. I will categorize potential sites within a 2 hour radius of UNR according to the desired high and low elevational ranges. Additionally, I will use ggplot/ qGIS to visualize this in figures and generate a map of potential sites.

This work will save us valuable time and resources that would otherwise be spent poring over spreadsheets with a variety of data formats and scouting out sites to assess whether they have a sufficient abundance of spiny lizards for a long term population study.

**Independent Project Approach:**

Instead of using data from separate databases, I used the database GBIF, which combines records from several of the museum databases mentioned above with iNaturalist. I used GBIF’s map tool to search for my focus species and select a region within the desired distance from Reno, then exported the data as a .csv file. I then used Open Refine to clean these data and create an extra column combining the latitude and longitude coordinates in order to get a sense for the number of specimens at each locality in addition to their respective elevations, year of collection / observation and coordinate uncertainty in meters. I used SQLite to select only the records from the past few decades with a reasonably low coordinate uncertainty (100 meters) and exported these to qGIS followed by the remaining records that did not meet the coordinate uncertainty criteria.

I generated a map with the candidate localities color coded by elevation (red for those in the high elevation bracket and blue for those in the low elevation bracket), with the remaining localities whose coordinate uncertainty was excessive colored white. I was able to use this map to select a set of sites, and subsequently visited these sites to verify the presence of my focus species. I did not end up using pandas or ggplot, as I felt the above-mentioned tools were sufficient to organize, filter and visualize my data. I am extremely happy with the outcome of this process, which saved me substantial time and energy and enabled me to successfully narrow down my potential study sites before validating them in person.

Map showing candidate high (red) and low (blue) elevation localities. Localities with excessive coordinate uncertainty shown in white


Figure Map showing candidate high (red) and low (blue) elevation localities. Localities with excessive coordinate uncertainty shown in white