The effect of elevation on the venom quality of Northern Pacific rattlesnakes and venom resistance of California ground squirrels

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**INTRODUCTION**

The selection pressures of inheritable functional traits of organisms have been shown to be influenced by the environment. Understanding these environmental pressures could help us better understand local adaptation and how it arises (Holding et al. 2018). Venom has evolved multiple times, but all together shares some major similarities. Organisms that produce venom generally produce large amounts, and the venom they produce is very complex chemically (Sachkova et al. 2020). Venom is considered a viable example of a functional trait that is genetically inherited. Organisms that use venom as a method of predation rely on the quality of their venom for survival, directly linking that organism’s fitness with their ability to produce quality venom. Likewise, the prey’s fitness is directly related to the resistance to the venom (Holding et al. 2018). Overall, diet is viewed as the prominent driver of selection pressures on the evolution of snake venom (Davies and Arbuckle 2019).

The selection pressures on organisms in different geographical locations are due to both biotic and abiotic factors. These selection pressures can influence the evolution of traits that increase an organism’s fitness in a particular area, while its fitness in a different area is unaffected (Smiley-Walters et al. 2017). Unfortunately, the patterns and occurrences of selection influenced local adaptations are quite understudied in predator and prey relationships of vertebrates. An organism’s local adaptations and our ability to measure them could prove to be insightful when trying to understand coevolutionary models (Holding et al. 2016) as well as local adaptations (Smiley-Walters et al. 2017).

Data for 10 Northern Pacific rattlesnakes (*Crotalus o. oreganus*) and 12 California ground squirrels (*Otospermophilus beecheyi*) containing 1440 data points displaying the quality of the venom and the resistance of the venom was used to evaluate the effect of elevation on venom strength of the snakes, and venom resistance of the squirrels (Holding et al. 2016). The purpose of this study was to provide further data to investigate and understand environmental selection pressures causing local adaptation. Holding et al. tested just the 10 snakes venom strength against elevation and 12 ground squirrels venom resistance against elevation directly, while this study tested all 1440 data points. This study also tested the possibility that random chance may have influenced Holding et al.’s results (Holding et al. 2018). There will be a strong positive relationship between elevation of venomous snakes, and baseline metalloproteinase activity (quality of venom) in their venom. Likewise, ground squirrel metalloproteinase serum activity (resistance to venom) will be strongly correlated with elevation.

**METHODS**

*Data Collection and Variable Calculations*

Data for this study was obtained from the Dryad data repository using the paper “Coevolution of venom function and venom resistance in a rattlesnake predator and its squirrel prey”. For the study conducted in the paper, venom from 10 Northern Pacific rattlesnakes (*Crotalus o. oreganus*) and blood serum from 12 California ground squirrels (*Otospermophilus beecheyi*) was collected. They then tested the quality of venom and resistance to the venom numerous times, resulting in 1440 total data points (Holding et al. 2016).

Data for metalloproteinase activity in snake venom, metalloproteinase serum activity, and the elevation where the animals were sampled from was extracted from the data set for the purposes of this study. Also, two random sets of elevation measurements were generated for further comparison: one for metalloproteinase activity in the snakes and another for metalloproteinase serum activity in the squirrels. This resulted in a total of 4 subsets of data: sample squirrel elevation and sample metalloproteinase serum activity, sample snake elevation and sample metalloproteinase activity, random squirrel elevation and sample metalloproteinase serum activity, and random snake elevation and sample metalloproteinase activity.

*X Y Scatterplot and Linear Regression Analysis*

Four different scatter plots were generated for using Rstudio statistical programming.

(Rstudio, 2015). For each of the four plots, the x-axis is represented by elevation. The y-axis is represented by either metalloproteinase activity for the snakes, or metalloproteinase serum activity for the squirrels. To, hopefully, observe any possible trends between elevation and venom strength or resistance, a general fit line and regression analysis model were fitted to the data for each plot.

*Linear Regression Analysis*

Using the lm() function in Rstudio statistical programming, a regression analysis was performed on each of the for subsets of data and was used to detect any correlation between elevation and venom quality in snakes and elevation and venom resistance in squirrels (Rstudio, 2015). The accepted p-value used was 0.05.

*Pearson’s Correlation*

The cor.test() function was used to conduct a Pearson’s Correlation test on the 4 sets of data using a 95% confidence interval and the scatterplot() function was used to create scatter plots. (Rstudio, 2015).

*Anova Analysis*

An ANOVA test was conducted using the aov() function in Rstudio statistical programming on each of the 4 subsets of data and p-value of 0.05 was used to access the data (Rstudio, 2015). This was done to add further comparison of the possible effects of elevation on venom quality or venom resistance.

**RESULTS**

Results for the linear regression analysis for the sample squirrel data compared to the

Chart, scatter chart

Description automatically generatedsample metalloproteinase activity showed a slightly positive relationship. A slope of 0.19

Figure 1. *X-Y Scatter Plot comparing venom strength of snakes and elevation is displayed on the right with blue triangles (intercept=446.57779, slope=0.73127). On the left, an X-Y scatter plot comparing venom resistance of squirrels and elevation is displayed with the red circles (intercept=482.39619, slope=0.19451). Elevation measured in meters.*

was observed using the X-Y scatter plot (Figure 1). The sample snake data compared to sample

metalloproteinase activity showed a moderately positive relationship. An X-Y scatter plot showed a slope of 0.73 (Figure 1). The comparison of the randomly generated elevations and metalloproteinase activity did not display a significant correlation with a slight slope of 0.03 (Figure2). In the comparison of metalloproteinase serum activity to elevation, no significant correlation was observed with a slope of 0.08.

Chart

Description automatically generated The Pearson’s correlation test comparing sample snake elevation with sample metalloproteinase activity showed a strong correlation value of 0.48 (Figure 3). The same test comparing sample squirrel elevation and sample squirrel metalloproteinase serum activity yielded a weaker correlation value of 0.14 and can be observed easily in Figure 4. When sample metalloproteinase activity was compared with randomly generated elevations, no correlation was found with a correlation value of -0.02 being observed. The comparison of sample metalloproteinase serum activity and random squirrel elevation displayed a correlation value of 0.05, indicating almost no correlation.

Figure 2. *X-Y Scatter Plot comparing venom strength of snakes and randomly generated elevations is displayed on the right with blue triangles (intercept=* *812.556475, slope= -0.008427). On the left, an X-Y scatter plot comparing venom resistance of squirrels and randomly generated elevations is displayed with the red circles (intercept=* *607.90339, slope=* *-0.05880). Elevation measured in meters.*

ANOVA test results for the comparison of sample snake elevation with sample metalloproteinase activity and the comparison of sample squirrel elevation with sample squirrel Chart, line chart, scatter chart

Description automatically generatedmetalloproteinase serum activity displayed p-values <0.05 indicating a significant correlation (sample snake p-value = 2e-16, sample squirrel p-value = 1.86e-07). The random squirrel elevation comparison to sample metalloproteinase activity displayed no significant correlation. For the random snake elevation and sample metalloproteinase activity, no correlation was observed.

Figure 3. *Pearson’s correlation test of snake venom strength against snake elevation using a 95% confidence interval. P-value < 2.2e-6 and observed correlation value of 0.48. Elevation measured in meters.*

Chart

Description automatically generated**DISCUSSION**

The results of this study, although broad and lacking parameters, suggest that elevation should be considered when studying methods of local adaptations. It seems that elevation is a stronger driver of local adaptations in snakes. However, when compared to the randomly generated elevations, the metalloproteinase activity in snakes did not display any significant correlations. For the squirrels, elevation does not seem to have much of an effect considering only slight positive correlations were observed for all comparisons. The Pearson’s correlation scatter plot displays a visible significant correlation between snake metalloproteinase activity and elevation (Figure 3) but the strongest correlation of snake metalloproteinase activity with elevation was easily observed in the X-Y scatter plots (Figure 1). No correlation was found between the snake venom strength or the squirrel venom resistance when compared to the randomly generated elevations. This implies the correlation observed is indeed not due to random chance.

Figure 4. *Pearson’s correlation test of squirrel venom resistance against snake elevation using a 95% confidence interval. P-value < 1.9e-07 and observed correlation value of 0.14. Elevation measured in meters.*

The results of this study indicate that snakes, in terms of an evolutionary arms race, are a little better equipped for survival than their squirrel prey. It appears that snakes are more reliant on the strength of their venom, than squirrels are reliant on their resistance to venom. This is a little odd, considering the metabolic cost of making venom is quite high and snakes should be under selective pressures to reduce that cost (Sachkova et al. 2020). However, it can be presumed that strong selection should act on a snake’s venom, given the snakes reliance on the venom for survival (feeding/defense) (Strickland et al. 2018).

Previous studies have shown that venom quality/strength is inherently tied to prey subjugation. In saw scaled vipers, individuals who eat mostly arthropods possessed a venom that was specifically strong against scorpions. Specifically, the venom worked better on scorpions they would naturally encounter when compared to the effectiveness of the venom on lab raised species of scorpions. This implies that there are certain local ecological factors, like elevation in this study, that also influence the quality of venom in venomous organisms (Davies and Arbuckle 2019). The focus on venom research has been primarily on venomous snakes due to the global implications snake bite envenomation has caused (Cavigliasso et al. 2019). However, in recent years an effort has been made to understand the selection pressures on other venomous organisms such as parasitoid wasps (Cavigliasso et al. 2019) and sea anemones (Sachkova et al. 2020). Providing evidence of environmental factors influencing the selection of functional traits could prove to be a useful tool in better understanding how local adaption is generated through evolutionary mechanisms such as selection (Holding et al. 2018).

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