Moriah Mitchell
American University

Differential Response to UV-C Radiation in Cave and Surface Populations of *Gammarus minus*

Physical isolation coupled with extreme, stable conditions make the cave environment an ideal setting in which to study evolution. Strong and constant selective pressure exerted by complete darkness, constant temperature, and low nutrient availability drives convergent evolution of both regressive and constructive traits in cave-dwelling, or troglomorphic, organisms[4]. Gradual reduction of traits that do not confer an evolutionary advantage in the cave environment leads to evolution of regressive traits including depigmentation, reduction of visual organs, and metabolic simplification in troglomorphic populations[5]. Conversely, neutral or beneficial mutations in cave populations can increase in frequency over time, leading to evolution of constructive traits including elaborated sensory structures[1]. Taken together, the selective pressure applied by extreme conditions in the cave environment and physical isolation make it possible to observe considerable evolutionary change over a relatively short period of time[4].

In order to study troglomorphic evolution, researchers often compare cave dwelling populations to closely related but morphologically different surface populations[2]. Though population pairs from closely related species within the same genus are often studied for this purpose, morphologically divergent cave and surface populations of the same species are of special interest to biologists[1]. Comparison of intraspecific differences between cave and surface populations is particularly useful because evolution can be observed on a shorter timescale and divergence within a species can lend insight to the processes and variables that precipitate speciation[1]. The freshwater amphipod *Gammarus minus* is a model organism for studying adaptive evolution because numerous physically proximate, morphologically divergent pairs of cave and surface populations exist in the eastern United States[1,2]. In light of evidence suggesting that each cave population was independently founded from an upstream spring population, it is not surprising that morphology varies between cave populations of *G. minus*[1,2]. However, in general, members of cave-dwelling populations have larger, less pigmented bodies with longer appendages and reduced or absent eyes in comparison to their respective upstream spring populations[2].

Although morphological divergence between sister populations of *G. minus* has been studied and documented extensively, the genetic basis for these differences had not been examined until recently. In order to better understand adaptive divergence of the species, Dr. David Carlini and Dr. Daniel Fong investigated the genetic differences between the morphologically divergent Organ Cave and Ward Spring populations in Greenbriar County, West Virginia. Transcriptome analysis by RNA-seq revealed that about two percent of transcripts were significantly differentially regulated between the two populations[1]. As expected, visually-related genes including opsin and arrestin were found to be downregulated in the Organ Cave population with noticeably smaller, less developed eyes; however, Carlini and Fong also identified a more unexpected genetic difference between the two populations. They observed an excess of premature termination codons in the Organ Cave population, including fixed premature termination of the photolyase gene at codon 264[1].

Ultraviolet radiation in the UV-B and UV-C spectra causes DNA damage through its action on pyrimidine bases[3,6]. Adsorption of ultraviolet light causes double bonds in

thymine and cytosine bases to open, allowing them to react with other neighboring bases[3]. When adsorption of ultraviolet light allows two new covalent bonds to form between neighboring pyrimidines, the product is known as a pyrimidine dimer[3]. If pyrimidine dimers are left unrepaired, proper DNA replication and transcription will be prevented[3]. In order to preserve normal cellular function after exposure to UV light, organisms exhibit a variety of pyrimidine dimer repair mechanisms including photoreactivation mediated by photolyase[3]. Photolyase is an enzyme that binds to pyrimidine dimers in a light-independent step, then converts them to pyrimidines by breaking the covalent bonds between bases upon exposure to 300-500 nm light[6]. This process of photoreactivation restores DNA to its natural and functional state. Because the Organ Cave population lives in complete darkness and is not exposed to ultraviolet radiation, it follows that mutations affecting the photolyase gene would be subject to relaxed selection[1]. Based on the discovery of this mutation, it was hypothesized that individuals from the Organ Cave population would be more susceptible to ultraviolet radiation than individuals from Ward Spring; however, this remained to be established[1].

In order to test this hypothesis, Dr. Carlini helped me to design an experiment to investigate the impact of this mutation on the ability of *G. minus* to repair ultraviolet-induced DNA damage, and I carried it out in his laboratory last summer. The project consisted of a light experiment and a dark experiment. Because existing research of this nature is very limited, I had to answer a number of preliminary questions before beginning the experiments. In order to identify an appropriate UV-C dose and perfect irradiating and housing *G. minus*, I performed a series of pilot studies with 2-3 organisms from each population. The goal was to identify a daily UV-C dose high enough to cause perceptible damage over time but low enough for variability in cumulative lethal dose between individuals to be observed. Results informed selection of the UV-C doses used in the experiments and established that *G. minus* can be maintained individually in refrigerated six-well culture plates filled with source water.

In the light experiment, ten individuals from each population were exposed to one of each of three daily UV-C doses: 1.0 J/m2, 0.25 J/m2, and 0.125 J/m^2. An additional ten animals from each population were placed in containers covered in foil to block UV-C exposure and irradiated in the same manner as controls. Following UV-C irradiation, all organisms were placed near a white light fixture in order to facilitate photoreactivation. Organisms were monitored daily for survival and cumulative UV-C dose at death was recorded for each organism.

The dark experiment was performed as an additional control. Ten organisms from each population were exposed to 0.25J/m^2 of UV-C radiation daily; however, organisms were deprived of white light required for photoreactivation. Determining how to isolate these organisms from white light was a challenge. I concluded that the best way was to cover the culture plates entirely in foil to block light, removing the foil only in a dark room lit with red light only to irradiate the organisms and check for survival.

In the light experiment, all control animals from both populations survived the duration of the experiment, suggesting that death of experimental individuals can be attributed to UV-C exposure rather than living in culture. As shown in Table 1, the mean lethal dose of UV-C radiation was higher in the Ward Spring population than in the Organ Cave population overall and within each daily dose regime. Results of two-way ANOVA show that differences in mean lethal UV-C dose were significantly different

between populations ($p<0.01$) and between daily dose regimes ($p<0.001$). These results suggest that *G. minus* in the Organ Cave population are more susceptible to UV-C radiation than those in the Ward Spring population as hypothesized.

Table 1: Mean and Standard Deviation of Lethal Doses for Light and Dark Experiments

	Light Experiment						*Dark Experiment*	
Daily UVC Dose(J/m^2)	1.0*		0.25**		0.125*		0.25	
Population	Cave	Spring	Cave	Spring	Cave	Spring	Cave	Spring
Mean Cumulative Lethal Dose	1.900	2.200	1.025	1.225	0.625	0.825	1.050	0.975
Standard Deviation	0.316	0.441	0.142	0.142	0.134	0.197	0.258	0.142

** Difference in mean lethal UV-C dose between populations was statistically significant at $\alpha=0.1$*
*** Difference in mean lethal UV-C dose between populations was statistically significant at $\alpha=0.01$*

In the dark experiment, the mean lethal UV-C dose was found to be slightly higher in the Organ Cave population than in the Ward Spring population (Table 1). One-way ANOVA results indicated that this difference was not significant ($p=0.431$). Because no significant difference in sensitivity to UV-C exposure was observed when photoreactivation was prevented by white light deprivation, there is reason to believe that the photolyase mutation rather than a lurking variable is responsible for the difference in sensitivity observed in the light experiment.

Although these results suggest that a significant difference in UV-C sensitivity between the two populations exists and can be explained by a fixed loss of function mutation to the photolyase gene in the Organ Cave population, there are still more questions to be answered. In order to investigate differences in biological activity of photolyase between the two populations, I started to work on modifying the transformation assay described by Sancar, Smith, & Sancar with Dr. Carlini's guidance, but ran out of time[6]. I will use this assay to determine the specific activity of photolyase in both populations in the coming semester.

Moreover, I plan to explore whether or not other Cave-Surface population pairs of *G. minus* follow the same pattern of UV-C sensitivity as the Organ Cave-Ward Spring populations. Specifically, I intend to repeat the light and dark experiments on the Fallen Rock Cave and Madison Spring population pair previously studied by Dr. Carlini in which photolyase is not differentially regulated. Due to the absence of a loss of function mutation in this cave population, one would not expect there to be a significant difference in UV-C sensitivity between the two populations.

References

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