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A Geometric Morphometric Analysis of Tail Morphology in the Colombia Spotted Frog, Rana lutieventris

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A Geometric Morphometric Analysis of Tail Morphology in the Colombia Spotted Frog, *Rana lutieventris*

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Keywords: *Rana lutieventris*, geometric morphometrics, phenotypic plasticity, dragonfly larvae, multivariate multiregression statistical analysis.
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ABSTRACT

Phenotypic plasticity is the result of environmental factors interacting with genes to influence the phenotype of individuals. Developing amphibian larvae may be influenced by the presence of tadpole predators. *Rana lutieventris* tadpoles were reared in the presence of a predator, presence of an injured conspecific, presence of both a mashed conspecific and predator, and a control of no predator or conspecific for a period of six weeks. Photographs were taken weekly to monitor tail growth. The photographs were digitized and the data was analyzed using geometric morphometries. Geometric morphometric software pooled the data, eliminated differences in size, position, and orientation to analyze the shape differences between the tadpoles in these four treatments. The data was statistically analyzed using multivariate analysis of covariance (MANCOVA).

All three experimental treatments were found to cause enlarged tails, showing that *R. lutieventris* has the ability to respond to chemical cues and exhibit phenotypic plasticity.
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INTRODUCTION

Phenotypic plasticity in nature is an important factor in maintaining the survival of individuals. The seed beetle will alter the size of their eggs according to the quality of their host. Fox and colleagues studied *S. limbatus* on two of its natural hosts: an acacia (a good host) and a palo verde (a poor host). It was found that female seed beetles will reduce the number of eggs laid in the palo verde to increase the size of the eggs. Increasing the size of the eggs, while reducing the clutch size, gives each individual egg a higher probability of surviving while growing in the poor host.

Phenotypic plasticity allows individuals the ability to phenotypically adapt to presence or absence of specific predators. Karban and Baldwin (1997) and Havel (1987) have shown that animals and plants both exhibit morphological change in response to predation. This is important due to the costly energy investment it takes to initiate and maintain these changes (McCullum and Leimberer, 1997).

In aquatic environments, Havel (1987) showed that chemical cues from predators can induce morphological changes. Being able to morphologically adapt according to chemical cues present in the environment is beneficial to the prey because it allows the prey to adapt to the environment without having direct interaction with predators (Dodson, 1989). These cues also allow prey to adapt only if necessary. If there is an absence of predators within a microenvironment (e.g. a pond), then there is no reason to use energy reserves to change morphology.
Recently researchers have found that certain amphibian species have the ability to alter their morphology, in their larval stage, when reared in environments where predators are present. Smith and Van Buskirk (1995) showed that tadpoles of the chorus frog (*Rana pseudacris*) had tail morphologies that differed between ponds that contained and didn’t contain predators. In a similar study, Relyea (2000) showed that when leopard frogs (*Rana pipiens*) and wood frogs (*Rana sylvatica*) were exposed to predation by dragonfly larvae, both species had reduced mouth and tail lengths accompanied by an increase in muscle depth of the tail. These adaptations to their environments give these amphibians an evolutionary advantage. Having the ability to change morphologically only when necessary allows these species to conserve energy for other purposes.

Simons (2002) studied phenotypic plasticity of the Columbia spotted frog (*Rana luteiventris*) within the Helena National Forest of Montana and found a correlation between predator populations and morphology. He was able to show that increased predator presence in natural ponds (e.g. more dragonfly larvae and tiger beetle larvae) resulted in longer tail lengths, increased depth of the tail muscle and narrower mouth widths in spotted frog tadpoles. This correlation suggests that phenotypic plasticity is very likely a contributing factor to the differences in tail length, muscle depth and mouth widths.

Bodley (2003) studied groups of spotted frog tadpoles in the laboratory that were exposed to one of four factors; injured conspecifics only, predator (dragonfly larvae) only, injured conspecifics with a predator, or none of these. He hypothesized that the group exposed to both factors (injured conspecific and
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predator) would produce the greatest difference in tail lengths and muscle depth, and narrower mouth widths. He found that the factors did not interact, although each had an individual effect on tail morphology. Therefore, although his original hypothesis had to be rejected, his results suggest that either the presence of a predator or an injured conspecific can result in changes in tadpole tail morphology.

Using geometric morphometric analysis, I recreated the experiments of Bodley (2003) to investigate the morphological plasticity of spotted frog tadpoles in a laboratory setting. I wanted to further investigate the results of Simons (2002) and Reylea (2000) in a setting where the predator effects could be controlled. I hypothesized that the group of tadpoles exposed to predators and injured conspecifics would be most affected morphologically compared to those exposed to only predators or only injured conspecifics.

METHODS AND MATERIALS

In the spring of 2003 four R. lutieventris egg clutches were collected from a pond south of Park Lake in the Helena National Forest. An important predator on tadpoles, dragonfly larvae (Odonate spp.) were collected from a pond north of Park Lake at a later date. These locations were chosen because of the availability of the needed organisms. The egg clutches were found near the edge of the southwest corner of the pond. They were obtained by slowly scooping them into large plastic containers. The dragonfly larvae were captured by using dip-nets in
muddy shallows along the entire shore of the pond. Both the dragonfly larvae and egg clutches were transported back to the lab in coolers filled with ice.

Once at the laboratory, the egg clutches were equally divided between two 38-liter aquaria filled with well water. The eggs were allowed to develop and began hatching within a week and a half of their capture. At hatching, tadpoles were placed into four different treatments; each replicated 5 times, with ten tadpoles per replicate. The treatments, consisting of the presence of predators, injured-conspecifics, both injured-conspecific and predators, and no treatment (the control), were randomly placed along a laboratory bench. The twenty plastic rectangular aquaria (3.8 liter) each held a small 0.95 L plastic container with a 5.08 cm x 5.08 cm square cut out of both sides. A mesh screen glued to the side with a non-toxic silicon aquarium gel covered the holes. The containers physically separated experimental tadpoles from the treatments (predators and injured conspecifics) but allowed access to chemical cues.

The experiment ran from June 9th to July 17th. Tadpoles were fed a mix of three to five rabbit chow pellets ground with flake fish food every two days. The well water in each replicate was changed weekly, as were the injured-conspecifics. Injured conspecifics were obtained by rupturing the skin of the tadpole, usually by smashing it, and placing it into the treatment containers. Predators were rotated on a schedule of a week on followed by a week off. Dragonfly larvae in the predator treatments were not fed so the treatment did not mimic the predator, injured conspecific treatment. Predators that died during the
week were immediately removed and replaced. Tadpoles that died during the experiment were immediately removed from the aquaria.

At the end of each week, four tadpoles in each treatment were randomly collected and placed in individual glass containers. The glass containers were constructed by gluing large and small microscope slides together to make a trough with graph paper taped to the back of the structure (Fig. 1). The troughs were used to photograph the tadpoles so that their growth could be monitored. Once a photograph was taken the tadpole was returned to its replicate tank.

The photographs taken over six successive weeks were digitized into JPEG files. The files were analyzed using three geometrical morphometrics programs; tpsUTIL, tpsDIGIT, and tpsREGRES, all created by Adams (2003). The tpsUTIL software was used first to create the initial tps files needed to conduct the analysis. Next, using the tpsDIGIT software, seven landmarks on the tadpole tail were placed on each individual photograph for comparison within and between treatments. These landmarks were placed at the leg bud, dorsal anterior end of the tail, posterior tip of the tail, the medial dorsal and ventral areas of the
tail, and the medial dorsal and ventral spots in the tail muscle. Once all the files had been landmarked the tpsUTIL software was used to pool the landmark data from all groups. Next, the tpsREGR software was used to superimpose landmarks of all specimens and to calculate centroid size for all specimens. Once superimposition was accomplished the tpsREGR program was used again to calculate shape variables and perform multivariate analysis of covariance (MANCOVA) to determine overall effect of body size, predators, injured conspecifics, the interaction between predators and injured conspecifics, and all four factors together.

RESULTS

The MANCOVA test demonstrated that all of the factors had a significant effect on the morphology of the tadpoles (Table 1, Figure 2). When analyzed alone the presence of predator and conspecific both appeared to induce increases in tail size and muscle depth of tadpoles. Analysis of the presence of predator and conspecifics together also showed an increase in tail size and muscle depth.

<table>
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DISCUSSION

These results provide a missing link between the results of Simons (2002) and those of Bodley (2003). In 2002, Simons conducted a field study that showed a correlation between the tail length and musculature of *R. lutieventris* tadpoles and the presence of predators. He found that in ponds that contained large predator populations there was an increase in depth of tail muscles and tail length. In laboratory experiments, Bodley (2003) observed induced growth in tail length and muscle depth of tadpoles among treatments that contained only predators and only injured conspecifics. However, due to extreme variability from a small sample size he was not able to show that predators and injured conspecifics, when exposed together, induced a morphological effect on tadpoles (Bodley 2003).

In my study, the three treatments (predator, injured conspecific, and predator with injured conspecific) were shown to affect morphology of tadpole
tails. These results show that *R. lutieventris* is able to morphologically respond to chemical cues released from injured tadpoles and predators. This supports the hypothesis that larval *R. lutieventris* are affected by chemical cues released from injured conspecifics and predators when presented alone and in concert. Additionally, my study is the first to use geometric morphometric analysis to test for the effects of predator cues on tadpole morphology.

My results correspond with other studies using different amphibian species. Relyea (2000) found a reduced mouth width and tail length, and an increased muscle depth when wood frog and leopard frog tadpoles were reared in the presence of larval dragonflies. However, tadpoles still showed a morphological response to the presence of predators. Van Buskirk and McCollum (1999) also found that tadpoles of the eastern gray tree frog, *Hyla versicolor*, reared with predators developed larger tail muscle depths than those reared without the presence of predators. Van Buskirk and Relyea (1998) discovered that when wood frogs, *Rana sylvatica*, were reared in environments which contained predators, the tadpoles developed deeper tail fins, and longer tails.

My results and the results of other studies show the important role that phenotypic plasticity plays in the development of amphibian species. It is important for the tadpoles to be able to morphologically adapt because the tail of the tadpole makes up such a large proportion of the organisms biomass and performs an important role in locomotion. Different explanations on why the tadpole increases its tail size have been hypothesized. The most obvious, that a larger tail allows for a faster and quicker tadpole has been discussed by many
researchers (Caldwell et al. 1980; Lawler 1989; Van Buskirk and McCollum 2000). It has been shown that an increase in swimming speed helps tadpoles escape predators (Caldwell et al. 1980; Lawler 1989). These results coincide with the idea that an increase in muscle depth and surface area of the tail would help in creating “burst swimming” and an increase in ability to avoid predators (Van Buskirk and McCollum 2000).

However, Van Buskirk and McCollum (2000) showed that the increase in tail muscle depth and length does not improve swimming abilities. They suggest that the reasons for the morphological changes are due to tail autonomy, the ability to distract predators with tail parts long enough for the individual to escape. Many lizard species are known to exhibit this phenomenon. They found that the swimming ability of a tadpole was not seriously affected until 30 percent of the tail was removed (Van Buskirk and McCollum 2000). They suggest that if there is more tail there initially, there is more tail to distract predators with.

Tail autonomy is supported from the research of Blair and Wassersug (2000) who found that the most commonly damaged area of the tadpole is the tip of the tail. Since this area tears easily, the tadpole has the ability to escape and regenerate the damaged portion (Hoff and Wassersug, 2000). These data suggest that having a larger tail creates a bigger target, while at the same time creating a less lethal target.

Phenotypic plasticity plays an important role in increasing individual fitness. My results and those of others show that tadpoles adapt to an
environment through phenotypic plasticity to increase their fitness by responding to chemical cues released from predators and injured conspecifics.
ACKNOWLEDGMENTS

Firstly, I would like to thank Dr. Grant Hokit for all of his help and patience that he has shown me throughout the process of putting this project together. Without his help none of this research could have been accomplished. I would also like to thank Dr. Gerald Shields for his advice and contagious passion for the scientific process. I am also grateful to the James Manion Scholarship Fund for giving me the financing to carry out this research. Carroll’s Natural Science department cannot be forgotten, without their guidance and compassion for teaching I would not of been able reach the heights that I have reached in the past four years. Kate Menninger deserves special thanks as well for all of the hours that she helped me with in this process. I can’t forget my friends and family for helping me stay focused on my goals, thank you.
LITERATURE CITED


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