Predator Diversion Adaptation in Juveniles of *Plestiodon Multivirgatus*, the Many-Lined Skink

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Abstract

Plestiodon multivirgatus, commonly known as the many-lined skink, possesses a blue tail as a juvenile that it loses as it matures. The trait is believed to serve as a predator diversion tactic but this hypothesis has not been tested in this species. We used a clay model predation experiment to examine the effects of the trait on avian predation. We found that the presence of a blue tail significantly increased the average distance of avian attack towards the tail of the model and altered the distribution of attacks on the body and tail. Blue-tailed models were also found to be attacked less than black-tailed ones. These results suggest that the blue tails do serve to aid in evading predation, and additionally suggest an aposematic function as well. The second conclusion contradicts previous experiments examining similar traits, opening new avenues for future research into this understudied species.

Introduction

Figure 1. (A) Adult *Plestiodon multivirgatus*; Credit: Diana-Terry Hibbits (B) Juvenile *Plestiodon multivirgatus*; Credit: Pierson Hill

In nature, it is commonly accepted that bright coloration in a species commonly indicates toxicity or the mimicry of a toxic species (Stevens & Ruxton, 2011). In some instances, however, vivid coloration occurs in species that are not toxic or mimicking a toxic organism. Vivid coloration without an aposematic function would seem to be disadvantageous, as the coloration would attract the attention of predators, but closer study and research might suggest differently: the coloration in some cases may actually make individuals more likely to survive predation attempts. Vibrant or conspicuous coloration, when reserved to the extremities of an animal, can serve to divert predator attacks away from vital parts of the body. The use of colored extremities has been attributed to greater rates of survival from predation attempts amongst multiple animal species, including the long-tailed weasel (Powell, 1982) and cricket frog (Caldwell, 1982). Lizards can make exceptional use of this predator diversion tactic, as they possess caudal autotomy: the ability to lose their tails. Losing a tail is energetically costly to lizards and results in reduced fitness for individuals who lose their tails (Bateman, 2008; Kuo et al., 2013). However, this trait also helps them survive predation attempts: when attacked, a lizard loses its tail, which distracts the predator and allows the lizard to escape (Arnold, 1984). In lizards that possess both colorful tails and caudal autotomy, the evolution of caudal autotomy precedes the evolution of colorful tails, suggesting that colorful displays augment the trait (Murali et al., 2018). Thus, tails with bright coloration serve to strengthen this function, further attracting the attention of attacking predators. Some lizard species are observed to have these colorful patterns exclusively in juvenile stages. For instance, the lacertid *Heliobolus lugubris* mimics the coloration of a noxious beetle (Huey & Pianka, 1977) and the lacertid *Acanthodactylus beershebensis* possesses a black body and blue tail (Hawlena et al., 2006). Both patterns only appear during these species' juvenile stages. These distinct colorations are theorized to help counteract the increased predation that is associated with juveniles' greater rates of foraging (Sousa et al., 2016; Hawlena et al., 2006). In both previous examples, unique juvenile color patterns thus help to increase the survivorship of individuals from predation during a vulnerable developmental stage.

One Wyoming species possesses similar traits. *Plestiodon multivirgatus* is a lizard species that resides throughout the western United States, ranging from Texas to South Dakota (Stebbins & McGinnis, 2018). This species undergoes an ontogenetic color change: while it is a juvenile, it is black along its body and its tail is bright blue, but it loses the blue tail coloration at maturity (Stebbins & McGinnis, 2018; Figure 1A; Figure 1B). No toxins have been identified in this species nor do any toxic species with similar coloration reside within its range. Based on this evidence, it seems the coloration of juvenile many-lined skinks might serve as a predator diversion strategy, deflecting attacks to non-vital parts of the body. In this study, we seek to quantify the effectiveness of this trait by measuring and comparing the number of attacks on the body and tail and the distance of attacks from the tip of the snout based on the presence of a blue tail. We accomplished this via a clay model predation experiment, which utilized methods similar to a study conducted by Charles M. Watson with similar goals. In this study, Watson and colleagues conducted a clay model predation experiment in a riparian environment in Texas to examine the function of blue coloration in various lizard species (Watson et al., 2012). This study did not examine the blue tail in *P. multivirgatus* specifically, with the size of models and target habitat misaligned with the species' characteristics, and had higher rates of avian predator attacks due to anthropogenic influence.

Methods

We conducted a clay model experiment to examine the potential for this trait to act as a predator diversion strategy. The study took place in southeast Wyoming, USA (Figure 2A). The study site consisted of grassland located on state land overlooking Spring Creek (41.244861, -105.123944). The study site contained areas of grassland with sandy soil, which is natural skink habitat, and fell within range estimates of the species (WYNDD, 2022a). In similar studies, clay predation model experiments focus on predation attempts made by avian predators (Watson et al., 2012; Paluh et al., 2015). Birds, especially raptors, are visual predators and possess some of the best color and contrast vision amongst the animal kingdom (Potier et al., 2018), making them the predators most likely to be able to discern between vivid coloration patterns. Accordingly, the study site falls within the ranges of many predatory birds, with suspected predators of *P. multivirgatus* such as the Swainson's Hawk, American Kestrel, and Loggerhead Shrike expected to reside within one mile of the study site (WYNDD, 2022b).

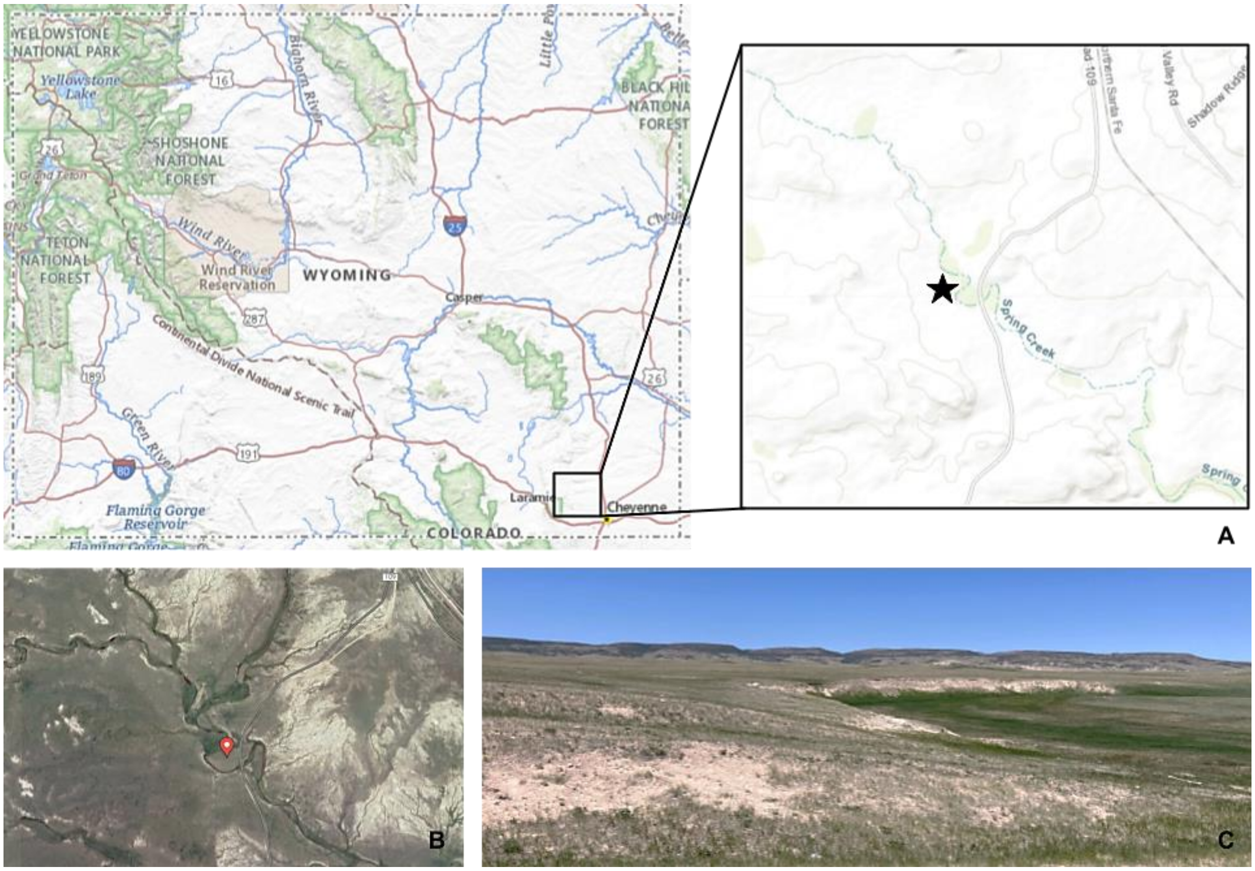

Figure 2. (A) Location of study site near Cheyenne, WY (41.244861, -105.123944); Credit: US Geological Survey (B) Satellite view of study site; Credit: Google Maps (C) Picture of the study site

We based the clay models on a ten-centimeter-long juvenile *P. multivirgatus* specimen from the University of Wyoming's Museum of Vertebrates. We created a 3-D digital model from the specimen via photogrammetry and used it to 3-D print plastic models at the Laramie Makerspace, which is located on the University of Wyoming campus. We cast silicon around these models, creating molds that clay could be pressed into. Using non-toxic, pre-colored Sculpey III clay, we pressed an initial layer of clay into the molds and inserted a strip of 22-gauge wire along the length of the body to provide stability. After completing the model with a top layer of clay, we inverted the molds to release the model. We covered any holes or exposed wires with small pieces of clay. This process ensured the rapid manufacturing of virtually identical models which closely approximated the size and shape of *P. multivirgatus* juveniles. The clay models created from these molds were nine centimeters long and consisted of a black body with either a black or blue tail (Figure 3A).

At the study site, we placed models 10 meters apart along a line following the ridge, alternating models based on tail color. The models were placed on or near objects such as rocks, logs, plant cover, and even cow pies to mimic natural basking behavior and expose the models to avian predators (Figure 3B; Figure 3C). We placed two trail cameras to observe a blue- and black-tailed model for animal activity (Figure 3D). After 48 hours, we collected the models and recorded the number of attacks and the distance of the attacks from the snout. We retrieved footage from trail cameras and off-site carefully examined it to identify common predators. We rotated the order of models between trials and locations of trail cameras weekly.

Figure 3. (A) Clay models (B) Trail camera placed to observe black-tailed model (C) Black-tailed model placed near cow pie (D) Blue-tailed model placed on top of a rock

Bird attacks were identified as distinct holes or scratches on the top of the models (Figure 4C; Figure 4D; Figure 4E; Figure 4F). Other markings, such as incisor marks and gnawing patterns, were attributed to the foraging behavior of rodents and not considered for data analysis (Figure 4A; Figure 4B). Both bird and rodent markings were similar to markings observed in other clay model experiments (Watson et al., 2012; Brodie, 1993). Multiple markings in close proximity to one another were defined as a single attack. We measured the distance of an attack from the tip of the snout from

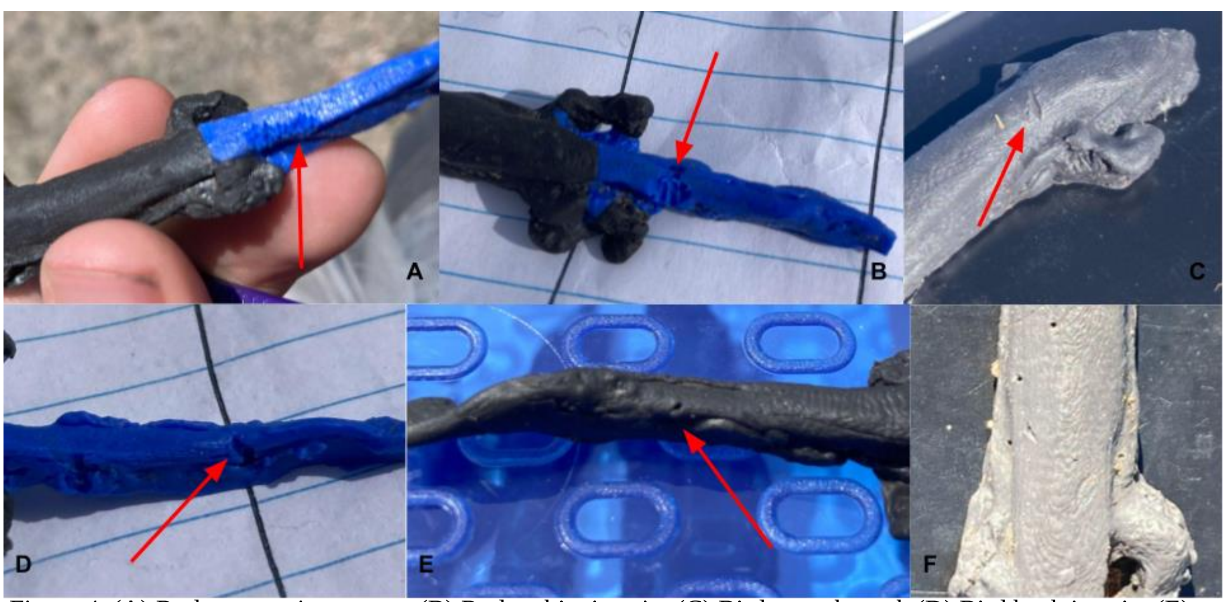

Figure 4. (A) Rodent gnawing pattern (B) Rodent bite imprint (C) Bird scratch mark (D) Bird beak imprint (E) Bird beak imprint (F) Bird foot imprint

the instance of the marking closest to the snout. Attacks on the tip of the snout, which usually completely removed it, were measured as beginning 0.1 centimeters from the snout.

	Body	Tail	Total
Blue-Tailed	29	13	42
Black-Tailed	44	3	47
			89

Figure 5. Raw data for frequency of attacks on the model types.

We completed all statistical analyses in R. We used a standard T-Test to test for differences between the number of attacks on the body and tail between model types. We used a Chi-Squared Independence test to test for differences between the distributions of body and tail attacks between model types. Finally, we used a Linear Model test to test for differences between distances of attacks from the snout between each model type.

Results

In total, 200 models (100 blue-tailed and 100 black-tailed) were placed at the study site over a period of three weeks from June 17th to July 1st. Eleven models (3 blue-tailed and 8 black-tailed) were not recovered, resulting in a recovery rate of 94.5%. The models that were not recovered were either missed or potentially taken by predators and squirrels. A total of 89 attacks were attributed to birds, with 42 on blue-tailed models and 47 on black-tailed models (Figure 5). Black-tailed models were attacked more frequently than blue-tailed models (T-test; p=0.03573). There were no significant differences between the number of attacks on the body (T-

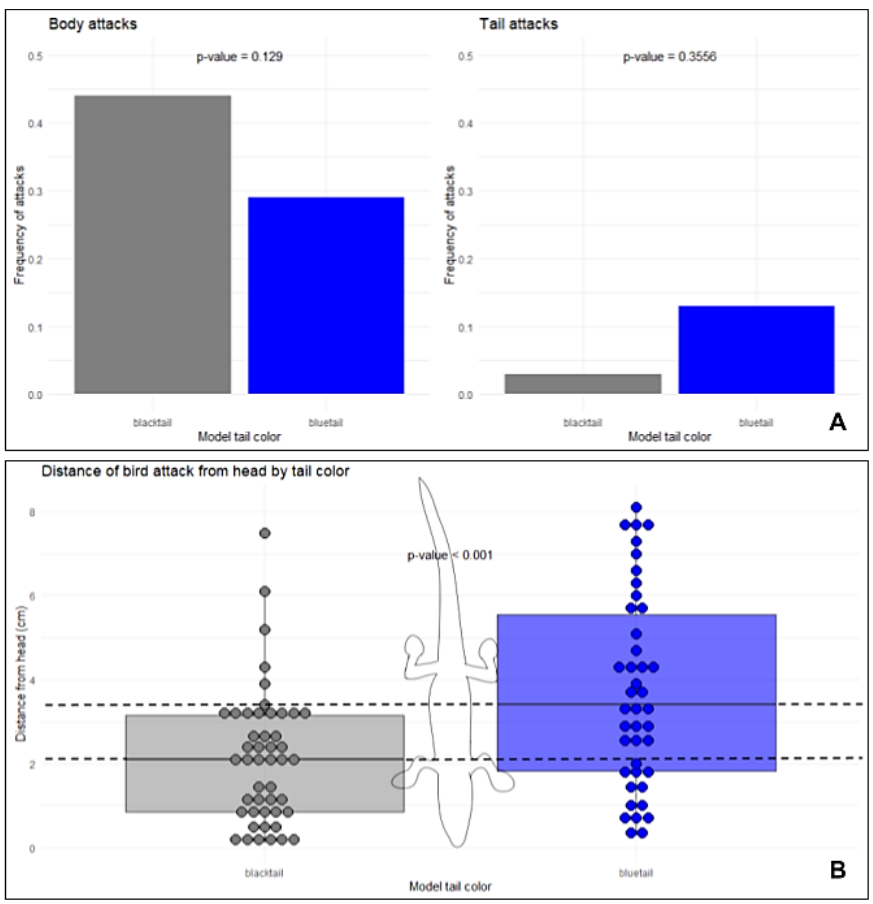

Figure 6. (A) Number of attacks on the body or tail based on model tail color (B) Distance of attacks from the tip of the model's snout based on model tail color

test; p=0.129) and the tail (T-test; p=0.3556) between model types. However, the distribution of attacks between the two model types was significant (χ^2=7.49027; p<0.01). The average distance of attack from the snout was 2.1383 cm on black-tailed models and 3.7024 cm on blue-tailed models (Figure 6B). The presence of a blue tail increased the average distance of attack from the snout by 1.5641 cm (Linear Model; p<0.01), or 17.38% of the total model length. The increase shifted the placement of attack from the forelimbs to the midsection of the model between model types (Figure 6B).

We identified a variety of avian and rodent species that reside at the site by direct observation and analysis of trail camera footage. Multiple killdeer (*Charadrius vociferus*), red-winged blackbirds (*Agelaius phoeniceus*), and thick-billed longspurs (*Rhynchophanes mccownii*) were directly observed, as well as an individual ferruginous hawk (*Buteo regalis*) and Swainson's hawk (*Buteo swainsoni*). Thick-billed longspurs, killdeer, horned larks (*Eremophila alpestris*), and vesper sparrows (*Pooecetes gramineus*) were observed foraging and moving near the models on trail cameras. Wyoming ground squirrels (*Urocitellus elegans*) were directly observed at the site and were also observed foraging around and even on clay models on trail camera footage (Figure 7A). The trail cameras also captured footage of North American deer mice (*Peromyscus maniculatus*) and thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*).

Figure 7. Species captured on camera near clay models (A) Wyoming Ground Squirrel (*Uroticellus elegans*) (B) Thirteen-Lined Ground Squirrel (*Ictidomys tridecemlineatus*) (C) North American Deer Mouse (*Peromyscus maniculatus*) (D) Thick-Billed Longspur (*Rhynchopanes mccownii*) (E) Vesper Sparrow (*Pooecetes gramineus*) (F) Horned Lark (*Eremophila alpestris*) (G) Killdeer (*Charadrius vociferus*)

Discussion

Based on these results, blue tails appear to serve as a predator diversion strategy. The finding that blue-tailed models were attacked less frequently was surprising. This finding differs from the study conducted by Watson, which found that models were equally conspicuous (Watson, et al. 2012), and contradicts the original hypothesis of this study. The initial reasoning was that blue-tailed models would be attacked more frequently, as their bright coloration would make the models more conspicuous to avian predators. The avoidance of blue tails might suggest the bright coloration serves some aposematic function or is the result of Batesian mimicry, though the species has not been identified to be poisonous in any measure and does not reside within the range of a toxic species with similar coloring. However, it could also be that blue-tailed models were more cryptic than black-tailed models. In most cases of aposematism, colors like red, yellow, and orange are generally used in contrast with black, as their wavelengths are less easily scattered, and are thus easier to see at distance (Stevens & Ruxton, 2011). In this way, the blue tails of *P. multivirgatus* may be less conspicuous than other colors, helping individuals achieve crypsis while residing in the shadows of plant or rock cover, but still diverting predators if they are spotted and attacked. Based on the species present at the site and the size of the recorded attacks, it is likely the main birds attacking these models are Passeriformes, smaller-sized birds known as “perching birds”. These bird species may be important predators during the juvenile stage of life for *P. multivirgatus*. If so, then this trait likely evolved to foil the attempts of these predators. Markings made by rodents were not considered for data analysis, as this experiment was not designed to consider predation by terrestrial species. Furthermore, though some of the rodent species are known to be opportunistic carnivores and scavengers (WYNDD, 2022c), rodent subjects in trail camera footage did not seem to exhibit predatory behaviors toward the models.

Other hypotheses have sought to explain the function of the blue tail coloration as something other than predator diversion. One such hypothesis states that the coloration serves as intraspecies recognition, helping juveniles to avoid the aggression of adult males protecting their territory from rivals (Clark & Hall, 1970). However, this hypothesis does not account for the placement of the coloration being on the tail, when other coloration patterns reside further up on the body, such as the red-jaw coloration adorning males during the mating season (Stebbins & McGinnis, 2018; Figure 1A). Additionally, a trait used for predator diversion may additionally function in intraspecific signaling. As such, it seems that the blue tail may primarily serve as a predator diversion adaptation but may also be utilized in intraspecific communication.

There are some caveats to the results and conclusions reported here. For one, the number of attacks made by birds may have been limited by the activities of rodents. Much of the footage recovered from the trail cameras were of rodent species. If the footage is representative of other models, then extensive squirrel activity around the models might have deterred avian predation. Furthermore, there were multiple times where models were recovered outside the entrance to burrows, far away from their original placements. Over 150 instances of rodents interacting with the models were recorded. Some models were even gnawed beyond recognition, removing the model’s head, tail, and other limbs. The biting and gnawing may have destroyed the markings of previous bird attacks. Furthermore, we did not record any direct trail cam footage of bird attacks. While this could mean that markings recorded as bird attacks were misidentified rodent markings, this is unlikely, as markings made by birds were very distinct, and in multiple instances, bird attacks were present on models that did not possess markings made by rodents.

Although the study site was identified as skink habitat based on models from the Wyoming Natural Diversity database and knowledge of habitat preferences, no skinks were observed at the study site, leaving the potential that skinks do not reside there. Therefore, it is unclear whether the individual predators at this site have direct experience with this species. Furthermore, while many-lined skinks are active from April to August (Stebbins & McGinnis, 2018), there is the potential that there are at certain times where they are more active. If the experiment was misaligned spatially or temporally, meaning predators at the site were not familiar with predating upon skinks, this may have reduced the number of attacks observed on the models.

The findings of this study open quite a few avenues for potential future research. The potential for the blue tail to serve either as aposematic coloring or Batesian mimicry cannot be overlooked. Further research could investigate whether this species is poisonous or if it resides within the range of a toxic species with similar coloring. Previous studies of species with similar traits have suggested that the ontogenetic change in coloration could be to account for a decrease in foraging levels between juveniles and adults. Rates of tail loss increase with exposure to predators (Sousa et al., 2016), so the strategy is favored in early developmental stages since juveniles forage more boldly than adults (Hawlena et al., 2006). Since this change in coloration is also observed in *P. multivirgatus*, it could be reasoned that they have similar differences in foraging strategy between adults and juveniles. Further research could investigate this.

While passerine birds seem to be important predators of juvenile skinks, it is unclear whether they prey on adults. If the two life stages have different dominant predators, then the change in coloration might be ascribed to that. Rodent species were frequently observed interacting with models, but it was unclear whether the squirrels recognized the models as skinks. Further research could investigate how these species interact with novel objects in their environment and could be used to show if they viewed the models as prey or scavenging opportunities. These findings could help to establish whether clay model predation experiments could be used to identify predation by terrestrial predators.

While not exhibiting predatory behaviors, one of the rodent species present at this site provides another perspective on this trait. The Wyoming ground squirrel possesses a black tip on its tail, which contrasts vividly from its tan body (Figure 7A). Very few terrestrial predators reside near the study site (WYNDD, 2022b), so it is likely that this species is mainly predated upon by avian species. It has already been established that ground squirrels use their tails to avoid rattlesnake predation using infrared signaling (Rundus et al., 2007). As such, the black tip on their tail may work in a similar fashion to the skink's blue tail, diverting avian predator attacks. If this proves true, then these two species may have undergone convergent evolution and provide another avenue of potential research.

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