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Investigating Cryptic Coloration and Color Change in Peucetia viridans

Zulay Caridad Rodriguez

THE FLORIDA STATE UNIVERSITY

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INVESTIGATING CRYPTIC COLORATION IN PEUTICIA VIRIDANS

By

ZULAY CARIDAD RODRIGUEZ

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Dr. Thomas Miller

Thesis Director

Dr. Tanu Kohli

Outside Committee Member

Dr. Emily Duval

Committee Member

Introduction:

Understanding the development of distinct color patterns is ecologically important, as factors such as mimicry, disruptive, and cryptic coloration affect interactions within an environment, and can affect the process of natural selection. Coloration can vary both within and across species and serve different purposes. A species' coloration can evolve over generations or change across a lifetime or even a couple of minutes. Some organisms manipulate color patterns to disrupt the outline of an individual and confuse predators (Barbosa et al. 2007). Additionally, it has been documented that certain species manipulate their appearance so that it is similar to a more dangerous or distasteful species to avoid consumption (Uésugi 2010). This thesis is focused on cryptic coloration, commonly referred to as camouflage. This is a characteristic which affects predator-prey interactions and can change the appearance of a species over time.

The term cryptic coloration is used to refer to organisms that develop coloration which bears a resemblance to the habitat in which they reside (Merilaita & Lind 2005). Often this trait is used to evade predators or as a tactic for predators to deceive potential prey. This form of coloration is common in nature and has been observed across a huge spectrum of different animals. In many cases, individuals change color seasonally, such as when snowshoe hares turn from brown to white in the winter. However, other species have the capacity to actively change and reverse color changes to fit different backgrounds. Perhaps this is best known in organisms such as Old-World chameleons and cuttlefish, where the mechanisms of color change have been well investigated. However, this kind of color change has also been noted in once before in one species of spiders (Umbers et al. 2014, Oxford & Gillespie 1998), but much less is known about when and how it occurs. Peucetia viridans, ordinarily referred to as the green lynx spider has been reported in a single study to change color from green to a more reddish color when placed on a red flower (Neck 2009). However, virtually nothing else is known about how or when these spiders change color, nor its ecological importance.

Green lynx spiders are rather large (up to 2 cm length), non-web spinning, seasonal spiders (Kaston, 1972). The color of the dorsal surface of a spider's abdomen is generally green with a series of 1 to 4 dark or purple chevrons down the middle, but can vary in the intensity of colors and their arrangement (Figure 1). They are relatively successful predators capable of capturing multiple insects within a day (Arango et al., 2012). Adult females inhabit the tops of the inflorescences of a variety of species of plants and even a flower mimic, the tops of the tall pitcher plant Sarracenia flava, where they lie in wait to ambush insect prey. These host plants have colors that include purple, yellow, white, and green.

In this project I will be focusing on the relationship between the green lynx spider's host plant and the color values we can observe on their abdomens. There are two parts to my thesis project. First, I propose that this relationship is one of cryptic coloration within this species, which would result in the spider colors matching that of their host plants. In order to test this, I will analyze multiple photos of green lynx spiders taken from various plants in the field, using a novel quantitative method to identify standardized color intensities and patterns. A statistical method will then be used to ask which parts of a spider's abdomen differed most between individuals and if the observed pattern could be correlated with color of the host plant. Understanding this trait will lead to a better understanding of the ecology of this species and the role of coloration within an environment. Additionally, because green lynx spiders are ambush predators and lack webs, understanding this form of coloration could further explain their predatory behaviors.

For the second part of the project, I will investigate whether they are actually changing color to match their host plant. To address this question, new spiders will be collected and raised against backgrounds of different colors. These spiders will then be photographed, and their color patterns analyzed through time to see if they change to match their background and, if so, how long it takes for them to change.

Materials and Methods:

Experiment One: Establishing Color Variation

Figure 1. Image contrasting the differences in abdominal shape, size, and color between two individuals.

Before testing if spiders are changing colors or if they are displaying cryptic coloration, we need to establish that there is color variation and that these spiders are displaying different colors when compared to one another. For this portion of the study, we analyzed photos of 38 spiders that were collected in the Apalachicola National Forest in October of 2018 by graduate students in the Field Quantitative Methods. Green lynx adults were collected from purple flowers (Liatris spicata), white flowers (Eupatorium spp.), or green leaves (Sarracenia flava), and host plant color was recorded in the field. Individuals were transported to the laboratory and then photographed within 72 hours of capture. Each spider was lightly anaesthetized using carbon dioxide and photographed on a stage that included size and color standards under a "light tent" with a Canon EOS camera under standardized exposure.

Because there was quite a bit of variability in abdomen size and the photos themselves (e.g., Figure 1), I used the "colormesh" method developed by Jenn Valvo and David Aponte (Valvo 2020) to standardize the photographs. The photos were first cropped to one standard size

(527 px. by 582 px). A color bar and size scale included in each of the photos was cropped and moved to a more useful position using Adobe Photoshop.

Figure 2. Image showing the final result of the cropping and rearrangement of the scale and color bar to prep photo for landmarking.

After all spiders were photographed, the photos were further processed for color pattern analysis. The photos were first given landmarks so that the same locations on different spiders could be compared. These landmarks were used to outline the general shape of the spider's abdomen. The landmarks included 4 major landmarks (red dots) based on discrete body locations (head attachment and spinnerets) with 20 equally spaced semi-landmarks (blue dots) in between (refer to Figure 3). All landmarks were then used to determine an "average" or consensus shape for the green lynx spiders to account for the wide variation in the sizes of each of the spider's abdomens. Morphing each photo into one shape eliminates allows similar body positions to be sampled for color on spiders that may differ significantly in shape or size. The landmarking was completing through the use of tpsDig2, a program developed by the Morphometrics group at SUNY Stony Brook (https://life.bio.sunysb.edu/morph/). The landmarks for all the spiders were then used to determine an average or "consensus" spider shape. Finally, the photograph for each spider was warped to fit the consensus shape. This leaves us with a suite of photographs of the spiders that have the same shape and landmark positions but retain the colors and relative color position.

Figure 3. Image depicting how the spiders were landmarked for creation of the consensus shape.

To obtain standardized body locations to sample color, lines were then drawn to connect the landmarks across from one another in a zig-zag fashion as part of the colormesh method (Valvo 2020). The centers of these triangles were used to create standardized points and where the program would then extract the colors. This triangulation method can then be repeated as many times a necessary using the triangle centers as the new landmarks, creating an exponentially increasing number of standardized sampling points (Figure 4). The red, green, and blue color intensity (RGB) can then be extracted from the pixels at each sampling point. We chose to use 4 rounds of triangulation for our analyses as it seemed to provide the best visual representation of the basic spider color patterns (T=4, Figure 4).

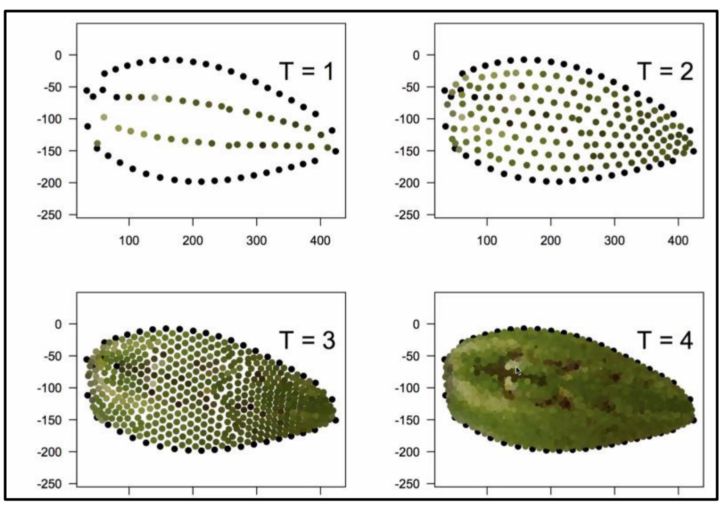


Figure 4. Photo depicting different levels of Delaunay triangulations. Lines were drawn between points to create triangles in which color values were sampled in the middle of each triangle.

To quantify differences in color patterns among the spiders, we used Non-metric Multidimensional Scaling (NMDS), an ordination method which allows us to ask which parts of the spider body contributed the most to differences among spiders (pattern). We also used permutational multivariate analysis of variance (PERMANOVA) to determine if these color patterns differ among spiders found on different colored hosts. PERMANOVA is used to compare different groups to test the null hypothesis that the groups are not significantly different. The colormesh method and the statistical tests were conducted using R and the vegan package for R (R Core Team 2020). Finally, to ask which sample points on the spider body were most important for discriminating among different original color backgrounds, I used Discriminate Analysis of Principle Components (DAPC), a multivariate method designed to identify and describe groups of related individuals. In this case, I used the original natural color for each spider to delineate groups.

Experiment Two: Investigating Active Cryptic Coloration

I tested if spiders do change color in response to background by conducting a reciprocal background experiment, in which I selected spiders in the field from different background colors, then reared them in the greenhouse over a series of controlled colors. A preliminary project by T. Miller in 2018 suggested that the spider do change color, with spiders changing in response to both purple and white backgrounds (refer to Figure 5). However, these results are very tenuous, due to the lack of replication and use of older spiders in artificial lighting.

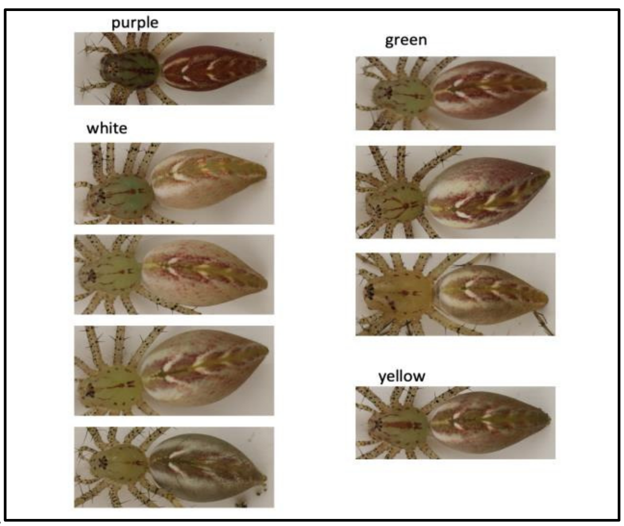


Figure 5. Image depicting Peucetia viridans color change in a preliminary experiment when placed onto either a green, purple, or white background (photos provided by T. Miller)

To initiate this experiment spiders were collected in the Apalachicola National Forest from along Highway 65 between Hosford and Sumatra. This was done over a series of weekends in October 2020 with the help of volunteer graduate students. We captured 9 spiders each from specific plant backgrounds (purple Liatris, white Eupatorium, or green Sarracenia flava) using 1-quart Tupperware cages, for a total of 27 spiders. All spiders were returned to lab and photographed within 30 hours of their capture. As before, spiders were knocked out with CO^2 in order to immobilize them long enough for us pose and to capture several photos of each individual to provide initial or 0-week photos.

The spiders were then placed into new cages with backgrounds of varying colors in a factorial design. Three spiders from each of the original plant backgrounds were placed on each of three different colors meant to mimic the field colors of purple, white, and green (Figure 6). The spiders were photographed at 0 weeks of captivity to capture their original color patterns. Each of the spiders as then rephotographed at two weeks and four weeks. This created a timeline for how fast color change is induced in green lynx spiders from different sources when placed on discrete color backgrounds.

Right now, we are in the process of data analysis but have already photographed all of our spiders across all replicates. Currently we are still standardizing and landmarking photos but for right now through this table we can see some visual differences across groups.

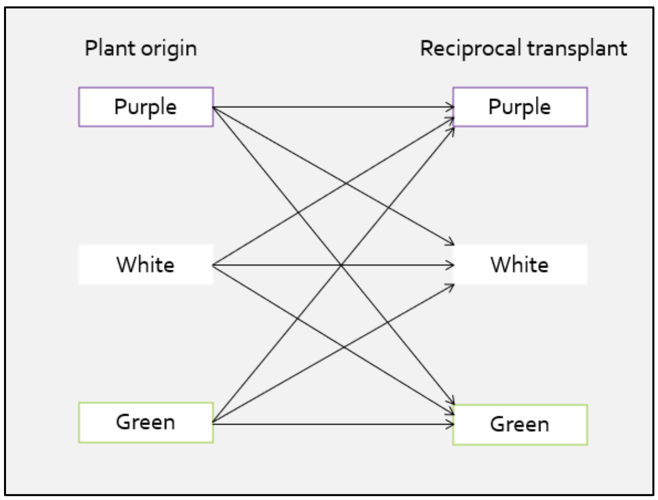


Figure 6. Diagram illustrating how the spiders found for each given color will be split and placed upon new colors within each replicate. The black lines represent what new background the spider will be placed on. Spiders will be collected from purple, white, and green color backgrounds in the field, then reared in the lab on purple, white, and green backgrounds in a reciprocal design.

The data analysis will consist of NMDS of the color patterns at any given time, then using Permanova at each time to ask if the spiders in each group differ in color and if that color change is associated with either the original color of the spider source or the spider experimental background (in each case, purple, white, or green).

<u>Results:</u>

<u>Experiment One: Establishing Color Variation</u>

Principle components analyses (PCA) allows a visual representation of how similar or different spiders were from one another. The distance between points represents how similar they are in color values with ellipses being used to group spiders from different host plants. The ellipses encompass 65% of the variance and allow us to compare groups of spiders from different backgrounds to one another. I conducted separate PCA for each RGB color intensity. For blue, the spiders were quite variable from one another, with the first and second axes explaining 21% and 17% of the variance, respectively (Figure 7A). However, the PERMANOVA results showed no significance difference among the blue color patterns for spiders from different original backgrounds ($p = 0.84$). For red, again there was variation among the different spiders (1^{st} and 2^{nd} axes explaining 26% and 15% of the variance) and some indication that spiders from green and white backgrounds may have different patterns (Figure 7B). However, again the PERMANOVA showed no significant effect of original background color on the expression of red color in these spiders ($P = 0.168$). Finally, the green color patterns again seem to show that the green and white spiders have significantly different color patterns, with 1^{st} and 2^{nd} PCA axes explaining 27% and 15% of the variance (Figure 7C). In this case, the PERMANOVA shows a significant difference among spiders from different natural background colors ($P < 0.01$).

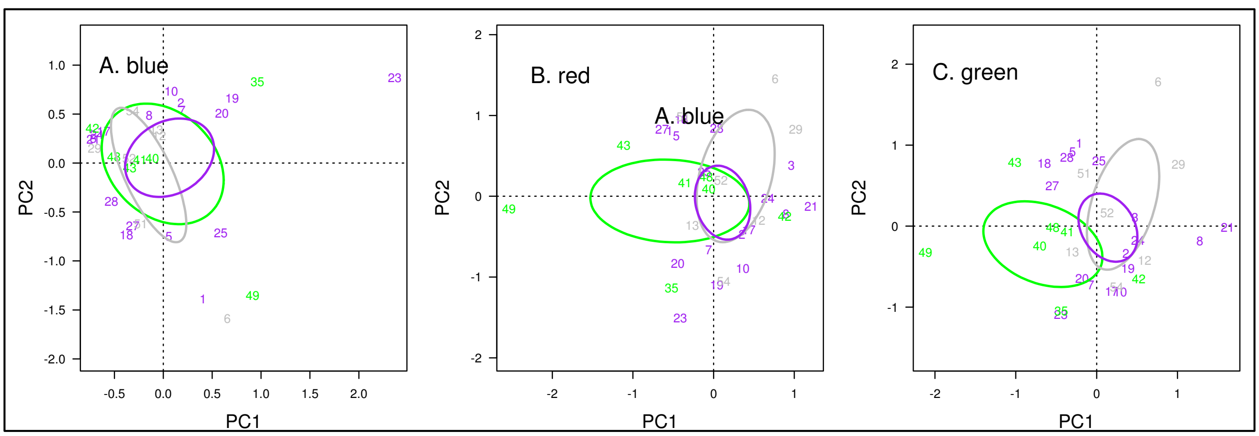


Figure 7. Graph depicting how similar or different individual spiders are for each of the colors based on Principle Component Analysis. Numbers represent individual spiders, numbers that are close together show spiders that have similar colors. The ellipses are visual guides to show each group of spiders from different backgrounds (purple= Liatris spp., green= Sarracenia flava, white=Eupatorium spp.).

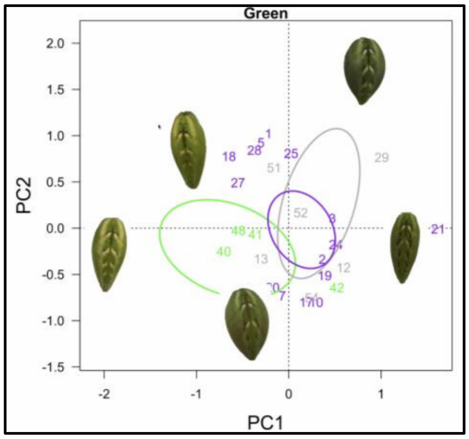


Figure 8. Graph of green values between different groups of spiders with the abdomens for reference. The ellipses are visual guides to show each group of spiders from different backgrounds (purple= *Liatris* spp., green= *Sarracenia flava*, white=*Eupatorium* spp.).

In order to visualize how the spiders from different plants vary in color, I have replaced some of the points in Figure 7C with the original photographs for these spiders, using the PCA for green intensity (Figure 8). If we look at the spiders from different plants, the ones on light green pitcher plants have light green bodies but those on purple plants have a darker green and are more purple. You can also see a difference in the number of these chevrons between these groups.

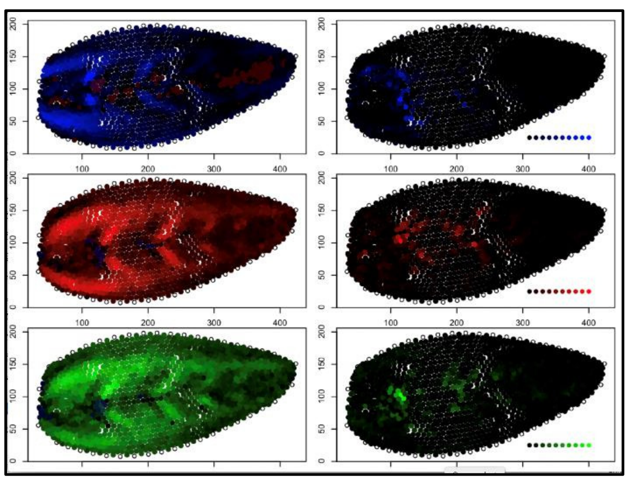


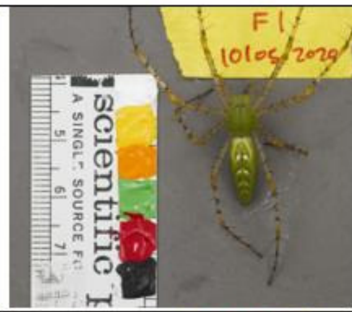
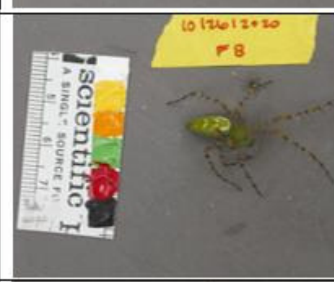
Figure 9. On the left is a visual representation of where spiders are most variable for different color values on their abdomen (based on PCA loadings). On the right shows which points on a spider's abdomen contributed the most to differences between spiders from different hosts (based on DAPC contributions).

I then used the PCA loadings for the sample points on each spider to ask where there was the most variation among the 38 spiders in the expression of blue, red, and green intensity (Figure 9). The graphs on the left depict the degree to which each point on the body is contributing to the principal component analysis. The brightness of each point just shows how much this point is contributing to differences across spiders (i.e. points on the body that contribute more to differences among the spiders are going to be brighter). Notably we can see bright patches on the sides and all along the chevrons, suggesting that these areas vary most among different spiders.

However, these figures do not show if this variation is due to different natural color background or is just variation among spider due to other causes. For this, I used the contributions of each sample point to the Discrimate Analysis of Principle Components. The DAPC for each color is shown in Figure 9 on the right, indicating which parts of the spider's abdomen from spiders of different backgrounds (aka different plant hosts) contributed the most to color differences. In other words, the figures on the right depict what parts of the spider's abdomen vary the most between spiders of different plant hosts. Most of these points on the graph are dark and did not contribute much to any of the changes we see but there are these notable bright spots by the upper portion of the abdomen where the first chevron occurs especially for green color values.

Experiment Two: Investigating Active Cryptic Coloration

Experiment two focused on answering the question whether the green lynx spider is performing active or passive crypsis and how long does any color change take. It involved capturing spiders from the field, noting their host plant's original color, and then placing them on different color backgrounds. Currently we are in the process of analyzing these photos and performing the same series statistical analysis as done for experiment one. However, as a visual guide to any possible color change I have provided the table below.

		Time exposed to new background	
Original Color	Experimental Color	2 weeks	4 Weeks
Green	Green		
Green	Purple		
Green	White		
Purple	Green		

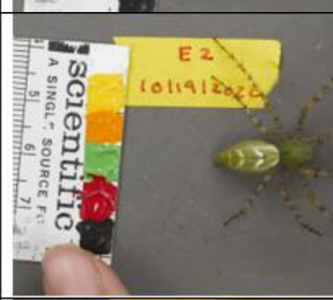
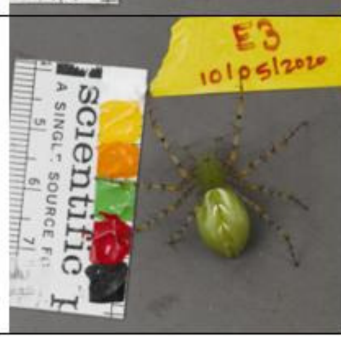
Purple	Purple		
Purple	White		
White	Green		
White	Purple		
White	White		

Figure 10. Table of photos following one spider over time as exposed to a color background. Spiders were either placed on green, purple, or white backgrounds meant to simulate the colors they often encounter the most in the wild via a plant host. Then when spiders were photographed every two weeks to capture and quantify any color change.

Discussion:

With this project I aimed to investigate the plasticity of cryptic coloration in the green lynx spider. I set out to answer the question if there is color variation between spiders of different plants hosts and if this variation is due to active cryptic coloration When comparisons where drawn in experiment one across all groups of spiders from various plant hosts we saw significant difference in green values (p-value: 0.017) and (while not significant) notable differences in red values (p-value: 0.14). This means that the spiders from different plant hosts varied the most in the green colors they displayed. Additionally, when investigating where on a spider's abdomen contributed the most to color differences it was found that the chevrons and lines along the chevron were most responsible for these visual differences between individuals of different color morphs. Again, as previously stated the analysis for experiment two is on hold but the data does show some promising results about color change and color change progression in the green lynx spider.

If the spiders are displaying color differences based on their host plant and have the capacity to display active cryptic coloration, what are the impacts of this? As stated within the introduction cryptic coloration can serve both as a means of deceiving prey and evading predators. Therefore, could this trait be aiding the green lynx spider in capturing more prey, or better eluding predators, or possibly even both. This trait also poses a new series of questions about spiders and their various hunting techniques. As previously stated, this trait has been recorded in various arachnids but only once before in spiders, in the white-banded crab spider. Between the white-banded crab spider and green lynx spider both do not spin webs and ambush their prey. Could cryptic coloration be a trait used to circumvent the lack of a web and ensure they can successfully capture prey?

In general, cryptic coloration is a trait that has evolved several times across multiple taxa. It is widely successful and appears in various forms and manners across different species. It is also well suited to variety of different environments from rocky sea bottoms, to tree bark, and even the tops of flowers. The green lynx spider is one out of several species (e.g. orchid mantis, tussock caterpillar, etc.) attempting to blend in or even appear as a part of an inflorescence. On the tops of flowers is where the green lynx spider hides and waits to ambush pollinators much like other predators who display crypsis. Since green lynx spiders consume mostly pollinators how could this trait possibly be affecting the rest of the community. First it poses the question if this trait could lead to fitness consequences for pollinators. Then if it could lead to ecological consequences for flowers since their pollinators are being consumed potentially leading to a decrease in fitness.

Cryptic coloration is a trait in the green lynx spider that leads to series of new, exciting questions about its implications on the evolution and ecology of the species it interacts with. The green lynx spider serves as a fantastic model organism to test even more questions about the bigger implications of this trait. Specifically, because the green lynx spider exists both as a predator and potential prey, we could investigate how both aspects of the spider's fitness is impact by this trait. Additionally, because of the spiders wide, wide range we could even investigate if this trait varies by region as different populations of spiders might rely on different plant hosts that display other colors. Moving forward and continuing to investigate cryptic coloration in the green lynx spider can help us comprehend how this trait evolves and affects local communities.

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