Focus on Everything

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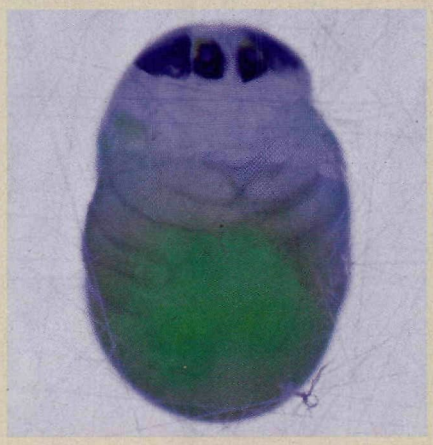
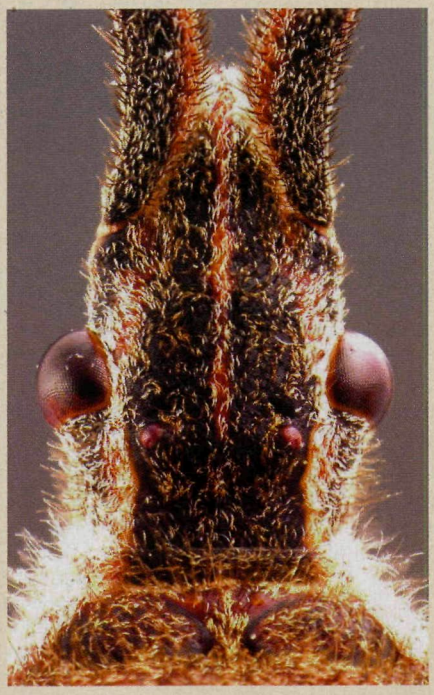
Biologist Cynthia Tedore uses a photo processing technique called focus stacking to obtain precise images of small organisms. Visible here, starting from the upper left and moving clockwise, are the tiger beetle *Cicindela sexguttata*, the fire ant *Solenopsis invicta*, the jumping spider *Lyssomanes viridis*, the katydid *Orchelimum pulchellum*, the common buckeye *Junonia coenia* (twice), the leaf-footed bug *Leptoglossus phyllopus*, hatching eggs of the jumping spider *L. viridis* (twice) and the large milkweed bug *Oncopeltus fasciatus*.





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Not long ago, it was difficult to produce photographs of tiny creatures with every part in focus. That's because the lenses that are excellent at magnifying tiny subjects produce a narrow depth of field. A photo processing technique called focus stacking has changed that. Developed as a tool to electronically combine the sharpest bits of multiple digital images, focus stacking is a boon to biologists seeking full focus on a micron scale. Cynthia Tedore, who recently finished her Ph.D. in biology at Duke University, is interested in the ways tiny animals filter complex sensory stimuli to make decisions important for their survival and reproduction. Focus stacking images made with a microscope camera lets her observe and measure color with new precision, she told American Scientist *contributing editor Catherine Clabby.*

Why is the effect of color as a signaling device among arthropods of interest?

Numerous scientists have explored the influence of color patch presence, size, brightness, and the patterning of its light and dark elements on another arthropod's behavior. However, little is known about the relation between natural variation in the dominant wavelengths and spectral purity of an animal's color patch and the behavior of another. There is evidence that color is important. A recent study of blue crabs, *Callinectes sapidus*, by Jamie Baldwin and Sönke Johnsen of Duke University, showed that males use both color and brightness of female claws to discriminate between potential mates and snappy immature females.

You used focus stacking image processing to observe the effects of color patches on a small jumping spider. Why didn't other techniques work?

I couldn't obtain repeatable measurements of tiny patches of color on the same individual using a handheld spectrometer, even when I worked under a microscope with the measurement probe and light source clamped into place.

When measuring color patches as small as 150 microns across, it's hard to be sure your specimen is positioned such that the measurement probe is pointing at the precise spot you're trying to measure. It's also difficult to ensure no glare distorts the measurement. Slight changes in the position of the spider relative to the measurement probe can produce divergent measurements of the same individual.

What did you set out to capture regarding color variation in the spider?

The average value of an entire color patch, rather than the value of a small spot within a color patch, is more representative of what tiny animals, with relatively coarse visual acuities, perceive. In male *Lyssomanes viridis*, this distinction is important because the pigmentation of the cuticle on the mouthparts bearing the fangs, called *chelicerae*, is not entirely uniform. The patch of colored forehead hairs can be sparsely or densely distributed, with or without the underlying cuticle showing through. Variation among individual hairs ranges from yellow to orange to red. Microscope photography made it easier to calculate the average values of entire color patches because I could choose the exact set of pixels I wanted to measure from each photograph.

By placing a grayscale calibration strip under the microscope next to the spider to generate a calibration curve unique to each photograph, I was able to convert the camera's charge-coupled device's values to brightness values in the camera's red, green and blue channels. I calculated the average brightness of the entire male forehead spot and the proximal half of the chelicerae in each of the red, green and blue channels, as well as overall brightness across channels. I also calculated the difference in average brightness between channels, normalized by overall brightness, as a proxy for color. This method gave me highly repeatable measurements of brightness and color across duplicated measurements of the same individual.

Once you had the measurements, what did they tell you?

In contests over females, males with less red (and more brown) chelicerae had an advantage, but only when the male with browner chelicerae was smaller than his opponent. This result was the opposite of what we expected, given that red looks more colorful and showy to people than brown does. However, jumping spiders do not see as far into the red portion of the spectrum as humans do. A complete lack of sensitivity in the red portion of the spectrum would mean that red chelicerae would be perceived as black (since they are not reflecting light that the spider can perceive). Brown chelicerae are more highly reflective in the green portion of the spectrum, a region that jumping spiders are highly sensitive to. Thus, to a jumping spider, brown chelicerae may appear more brightly colored than red. This finding challenges a lot of our assumptions about the selective pressures driving red coloration in animals with spectral sensitivities different from our own.

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