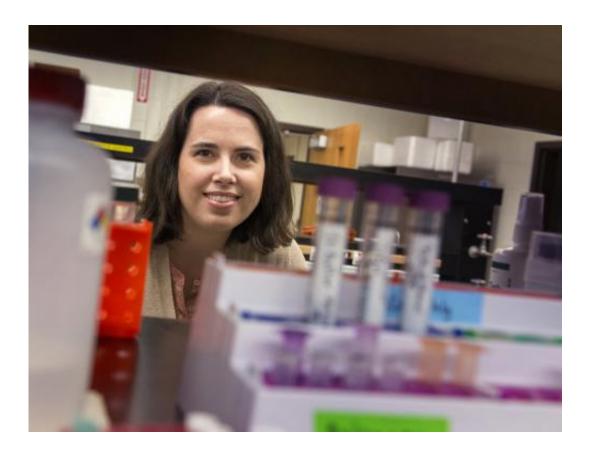


## **Colorful patterns of evolution mark butterflies and bumblebees**

May 15 2014, by David Pacchioli



Heather Hines is an assistant professor of biology and entomology in Penn State's Eberly College of Science. Credit: Patrick Mansell

(Phys.org) —As a graduate student, Heather Hines followed bumblebees all over the world. She was part of a successful effort to track the history of bumblebee evolution, painstakingly constructing from genetic and



geographic information a comprehensive family tree.

This was no small task. The genus Bombus encompasses some 250 species, its astonishing variety reflected in a diversity of color patterns: the interchangeable bands of orange-red, yellow, white, brown and black that cover bumblebee heads, thoraxes, and abdomens. From Mexico to the Pyrenees, and from California to western Burma, Hines, now an assistant professor of biology at Penn State, helped to document these patterns in all their many iterations.

While collecting specimens in Turkey, however, she observed something else. In bumblebees in the field, Hines kept seeing the same pattern, a white-banded body with a red tail. At first she thought she was looking at many members of a single species. Peering closer, however, she noted slight differences in coloring, and realized that what she was actually seeing was bees of several different species that had adapted themselves, albeit imperfectly, to look like one. "That," she says, "is what really got me interested in mimicry."

Henry Bates, the great Victorian naturalist, first noted mimicry in the Heliconius family of butterflies, whose brightly colored wing patterns he chased across the Brazilian Amazon for 11 years. Bates returned to England in 1859 and wrote a monograph that gave crucial early support to Darwin's theory of natural selection. By adapting the appearance of a species that was toxic to common predators, he posited, these copycat species shared the evolutionary advantage of protective coloring.

Heliconius has been scrutinized ever since. "It's been a textbook system for evolutionary genetics," Hines says. In particular, there's been ongoing effort to figure out the genetic basis for the wide variety of wing patterns the genus displays. "I and others have referred to these as 'canvases of evolution,' " she says. "You literally are seeing gene interactions on the wing, creating these complex patterns. So how did genes work together



to create them?"

In recent years, a large international consortium of evolutionary biologists has gathered to solve this challenge. Hines herself has been an active participant in this group. Her ultimate goal, however, has always been to carry what she learns from the study of butterflies back to her work with the system that first caught her attention: the bumblebees.

## **Evolution repeating itself**

Like Darwin's famous finches, butterflies and bumblebees are vivid examples of what geneticists call adaptive radiation, the rapid evolution of diversity from a single species in order to fill available ecological niches and ensure its survival.

What makes these two groups even more remarkable, however, is their use of mimicry. For as much as they fan out and diversify to fit into different habitats, in a given spot they choose the opposite strategy of making themselves alike. This convergence of species on a single color pattern provides researchers with a valuable tool: multiple copies of the same evolutionary process to analyze and compare.

"Basically, you have a trait that has evolved over and over again independently," Hines says. "Understanding the genetics involved allows us to answer questions like 'Does it involve the same genes each time? If it happened again, would it happen the same way? Are there certain types of genes that are especially prone to this kind of rapid change—what we call 'hot spots of evolution?' "





A bumblebee local to central Pennsylvania. Though of different species, this bee and the one in the following image appear similar because they represent a local mimetic pattern. Credit: Heather Hines

The first task, however, is to pinpoint the genes involved.

In the case of Heliconius, considerable progress has been made toward this goal. Genetic cross experiments conducted over the last 35 years have narrowed the possible locations of color pattern genes to three major regions on the butterfly genome. One of the three, it turns out, includes the controls for all red patterning. Two years ago, Hines went searching within that region for the specific gene—or genes—for red.

To do so, she compared two distantly related Heliconius species, H. melpomene and H. erato, that are considered co-mimics: they appear all but identical wherever they are found together. In a microarray



study—placing DNA samples on a tiny chip to compare expression of lots of genomic segments at once—she and her collaborators found that in both species, wherever red wing coloration occurred, there was higher expression of a gene called optix, and concluded that this was the gene she was looking for. So far, so good, but as Hines is quick to note, that's not the whole picture. Now they have to figure out how optix is regulated.

Gene regulation determines whether and how a gene is switched on, as opposed to lying dormant in the genome, its potential unused. If melpomene and erato each activated the optix gene by a different regulatory mechanism, Hines explains, that would be strong evidence in support of convergence—a case of evolution arriving twice at the same end from different starting points. If, however, both activate it by the same mechanism, that would suggest something different: that the two species share the exact same mutation, perhaps transferred from one to the other via some hybrid cross in the distant past.



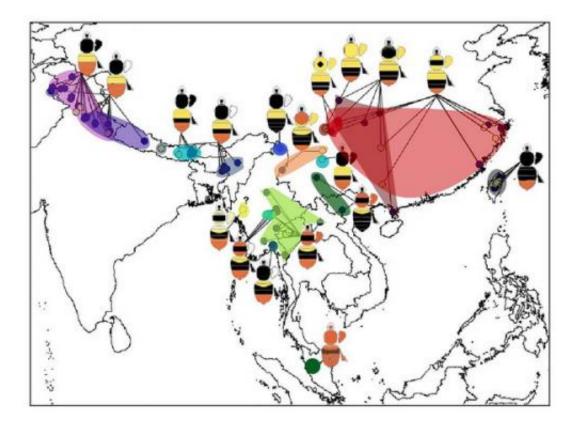


Chart depicting mimetic color forms and their ranges of the Southeast Asian bumblebee species Bombus trifasciatus. While the color pattern highlighted in red and reddish gray in central China is argued to be a "ground plan" color pattern for bumblebees and is not strongly mimetic, the rest of the color patterns are all part of different local mimicry complexes. Credit: Heather Hines

## **Evo-Devo**

"The real power of having an extremely diverse system," Hines continues, "is that we don't have to stick to just melpomene and erato—we can look across all Heliconius species. This comparative approach can help us understand how mutations flow across species and populations."

Bumblebees have even greater potential in this regard. With 250 species

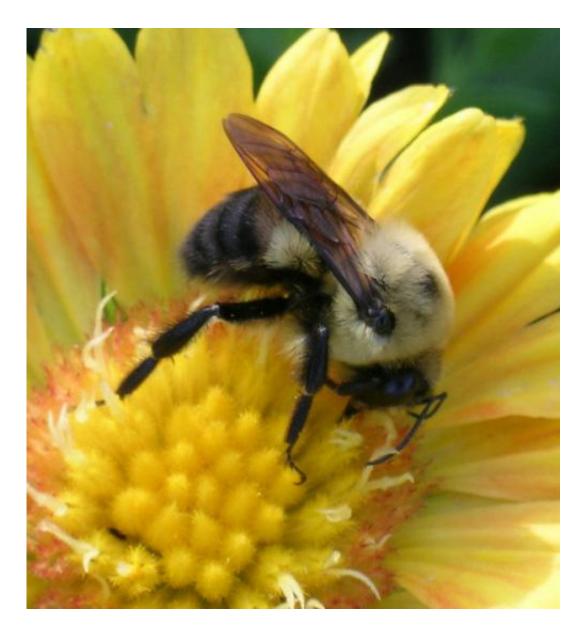


worldwide, and over 600 different color patterns—roughly three patterns per species—they triple the diversity of Heliconius. And unlike the butterflies, whose range is limited, bumblebees can be found almost anywhere on Earth. "They have a lot of specialization to different kinds of habitat," Hines notes.

In the U.S. alone, Bombus exhibits three basic patterns, in three distinct habitats: there's a Pacific coast pattern, a Rocky Mountain pattern, and an Eastern temperate pattern. All bumblebee species in each of these regions share a similar pattern. Species that overlap more than one region adopt the coloring of the habitat they're in. That means lots of opportunities for comparing one case of mimicry against another, and for better understanding how mimicry complexes form.

What's more, unlike the <u>wing patterns</u> of Heliconius butterflies, bumblebee color patterns occur along the elemental segments of the body. Presumably, then, they involve segmentation genes, the same ones that determine fundamental stages in an organism's development. Understanding the role these genes play in color variation could yield important insights into the role that development plays in evolutionary processes, a growing field of study known as evo-devo.





A bumblebee local to central Pennsylvania. Credit: Heather Hines

## A predictable genome

For all its promise, the bumblebee system has until now remained largely unused as a genetic model. Ironically, the bottleneck has been the same variety that gives the system such potential. Because the cost of sequencing and analyzing all those variant genomes has been prohibitive,



evolutionary geneticists have tended to stick to relatively simple model organisms, like the fruit fly, for answers to their questions.

With the emergence of next-generation sequencing technologies, however, genomics has exploded. "Genome sequencing can now be done for a reasonable cost," Hines says. The results are dramatic. Already the international Heliconius consortium has performed whole genome sequencing on more than 100 butterflies.

For the smaller cadre of researchers who are working on bumblebees, advance has been considerably slower. It's not so much the sequencing but the time needed for analyzing all the incoming data—and the lack of solid reference points—that pose the real challenges now.

"When we try to map back to a reference genome, only half of the new genome maps because there's so much variation," Hines explains. "So we have a lot of missing data, and we don't know what missing means. Does it mean there's just a chunk of DNA missing there, or that the genomes are so variable that they don't match up at all?"





Mimetic color form from east-central Burma. This color pattern is represented by four species in the region, and is restricted to an area less than 20km in radius. The bee in the picture is Bombus haemorrhoidalis. Credit: Heather Hines

These difficulties notwithstanding, the genetics behind Bombus are beginning to come into view. Hines reports. "We've sequenced four each of the two different color variants, red and black, of the West Coast species melanopygus, and we're currently sequencing four more. We are narrowing in on the genes behind the mimetic forms."

"What we're beginning to see is that there are trends in this—there are certain kinds of genes that are typically modified," she says. "These appear to be the hotspots that we talk about. Eventually, we should be able to predict them.



"Basically it comes down to the concept of a predictable genome. Once we can really understand these mechanisms, if we see a mutation in nature, we should be able to predict—from what it looks like—how it was generated."

Provided by Pennsylvania State University

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