Podcast Interview: Robert Reed

PNAS: I'm your host Prashant Nair and welcome again to Science Sessions.

It is blue-butterfly day here in spring,
And with these sky-flakes down in flurry on flurry
There is more unmixed color on the wing
Than flowers will show for days unless they hurry.

That was from *Blue Butterfly Day* by the renowned American poet Robert Frost, who described butterflies as "flowers that fly." Butterflies have inspired poets and scientists for centuries. Yet much of the biology behind their beauty and variety remains a mystery. The genes that control the splendid colors that butterflies sport on their wings have long been known to science. But just how these genes produce such varied yet precise patterns has been hidden from view. Evolutionary biologist Robert Reed and his colleagues at Cornell University raised the curtain on the underlying mechanism in a PNAS article published last year. They discovered how a gene with the evocative name of *optix* goes about the painterly process of patterning butterfly wings. Color on the wings of butterflies comes in two main forms: pigmentary and structural. Pigmentary color, as the name suggests, stems from pigments that reflect light of certain wavelengths that we perceive as colors. Structural color, like shimmer, is actually an optical effect arising from the arrangement of physical structures on wings. Reed and his team found that *optix* controls both forms of color on butterfly wings. The aesthetic elegance and biological significance of Reed's experiments were not lost on his peers, and the article earned the 2017 PNAS Cozzarelli Prize in biological sciences. Reed explains the context for the work.

Reed: Wing pattern genetics, really in my mind started in the 1970s and 80s when people started doing crosses between butterflies with different wing patterns that come from different regions of Africa and South America primarily. In particular, there was this literature on these passion-vine butterflies from Central and South America where within one species you would get 20 or more wing patterns. So, people started taking these butterflies from different populations and doing crosses between them. And you could see that these color patterns would segregate and you would get this fairly straightforward pattern of inheritance suggesting that there was, maybe, a simple genetic basis to wing pattern variation. For many years there were many labs that had been working to try to find the genes that control the color pattern variation in these passion-vine butterflies, they did crosses, genetic mapping, and this was in the era before we had whole genome sequences, so it was really hard work. And, I was involved in a collaboration with Owen McMillan who's now at the Smithsonian, and some other labs, to try to identify a couple of these major genes. So, one controls black color patterns, and we've since discovered that gene is called *WntA* and another controls red color patterns, and we found that that one is *optix*. And for years we didn't really know exactly what this color pattern gene did. So we knew it had something to do with red color patterns, we knew it was under selection, but we didn't really have the mechanism.

PNAS: Which is where CRISPR, a tool that has revolutionized molecular biology, came into play. Reed and his team used CRISPR to tinker with the *optix* gene in four different species of butterflies. The tinkering revealed that *optix* is a major control switch for butterfly color patterns.

Reed: It acts, essentially like a paintbrush to paint red or orange colors on the wing, so wherever you express *optix*, you get red. I think a paintbrush is a perfect analogy for this gene. You just express the gene wherever you want on the wing and you get a little dash of red, right? And if we break the gene using CRISPR, not only does the red go away but all the color on the wing completely goes away in all the butterfly species we've looked at. CRISPR genome editing, yeah, it's a miracle for us. And so when I heard about CRIPSR, we jumped on it immediately. We were pretty early adopters of it, and it worked perfectly the first time, and with that first experiment, everything changed for us. What became the most frustrating challenge of my career, which was getting functional validation of these color pattern genes on butterflies, it turned into an undergraduate side project.

PNAS: Reed's CRISPR experiments also showed that in some butterfly species *optix* controls not just pigmentary colors but also a form of structural color called iridescence, which is scientific lingo for shimmer. Think of the brilliant blue wings of the famous Morpho butterflies.

Reed: If you look at a butterfly wing under a microscope, the color pattern's composed of thousands and thousands of little scale cells, so it's like a micro-mosaic, and each scale cell is a different color. If you zoom in even further with the microscope and look at these scale cells, they have these very fine structures on them of little cross-veins and ribs. And just the way that light reflects off of these small micro-structures will produce these colors. Not through pigments, but through the way the waves reflecting off of these structures will interact with each other. What we found, and this was a huge surprise, we weren't expecting this at all, was that if we knock out the *optix* gene in buckeye butterflies, so these are little brown and orange butterflies that occur in North America, if you knock out *optix* you lose the red and orange coloration, like you would expect, right, because this is a paintbrush gene for red pigmentary colors. Not only do you lose those colors, but you get this blazing blue iridescence. So these little buckeye butterflies actually are like little miniature Morphos. It was incredible. So they turn dark and iridescent blue. Okay, so genetically this is kind of amazing, right? Just to think that you have this one master regulatory gene that could switch between multiple pigment pathways, but also control structural coloration at the same time. It really is a master regulator of color. That was just amazing, genetically.

PNAS: What was equally amazing was that buckeye butterflies in which *optix* was knocked out looked like distantly related species in which iridescence had evolved independently. And that led Reed to hypothesize that *optix* might be part of a genetic command center in butterflies that allows color patterns to reemerge over evolutionary time. More broadly, he says, the findings could lead to clues to the origin of color itself in butterflies.

Reed: Just the idea that there is a single gene that can regulate all of colors in butterflies makes you ask, does this gene have something to do with the origin of color? And if so, how did that work? So, how does *optix* figure in to the origin of the gene network that makes colors, and how did that gene network get tapped to be expressed in wings, that's kind of what we want to know. This gene provides a really nice entryway into that question.

PNAS: Reed says the Cozzarelli Prize is not just an acknowledgment of his team's hard work, but a nod to evolutionary biology's role in unraveling the wonders of nature.

Reed: I would never have predicted that working on butterflies would lead to a prize. I'm very happy that, I think, people outside of the butterfly world are looking at our work and paying attention to it and think it's interesting and realize that it has implications for genetics and evolutionary biology more widely. Even beyond my own, maybe, ego and delight I think it's very satisfying to see the community seeing the value of research on butterflies, just the value of basic research in evolutionary biology.

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Paul Gabrielsen contributed editing and transcription to this podcast.