SEE THE PRESSURE

Here you can see two proteins involved in DNA repair. Through modeling, we know that these proteins interact with each other physically, and through Nathan Clark’s ERC (or evolutionary rate covariation) scores, we can see how closely related they are in terms of their co-evolution in the mammals compared here. “These genes work together,” says Clark, noting the many similarities in the corresponding trees below each protein. The lengths of the branches represent the typical molecular divergence between the species. Bright yellow indicates the species that show rapid evolution in each gene, whereas blue indicates slower evolution. The evolutionary rates don’t match up perfectly for each gene, but they are remarkably similar. “Their rates of evolution are very correlated with each other,” he says. We don’t know exactly why the genes should be so similar, but we can deduce that whatever pressures are triggering evolution in one are likely to be the same pressures acting upon the other.
It all begins with a mutation.

A butterfly emerges from its chrysalis with a feature unlike that of any other butterfly, like perhaps a slightly lighter wing. If this new morphology helps the insect in some way—say, allowing the butterfly to gather more nectar or more effectively evade predators—then the chances of this lighter-wing gene getting passed on increase.

This is one of the simplest demonstrations of evolution at work: tiny, incremental changes caused by chance and passed down through generations at a nearly imperceptible pace.

But what if I told you we can see evolution at work in even smaller, more mysterious ways? Indeed, what if I told you scientists are learning how to track the path of evolution in our very cells, genes, and proteins?

The cabbage white butterfly (*Pieris rapae*) is considered a crop pest in the United States, but it may prove useful for scientists studying how organs evolve.

Understanding how genes co-evolve in animals could reveal links between human diseases.

**Feature**

**Co-evolution’s Witness**

*Page 28 Graphic: Courtesy Nathan Clark and Nolan Priedigkeit
Protein and Butterfly Illustrations: Michelle LeVeille*
“Proteins have their own traits,” says Nathan Clark, PhD assistant professor of computational and systems biology at the University of Pittsburgh School of Medicine.

“It’s not morphology we’re used to looking at, like the wing. But proteins have ways of sticking to each other, transporting things (like ions and amino acids) through their middles, and physically attacking each other.”

You could almost compare it to all the little critters in a drop of pond water, he says. Each cell is jam-packed with tiny actors, all of them trying to impose their evolutionary will on the others.

But while molecular biologists and structural biologists would look at what these components are doing presently, Clark is learning how proteins get their individual traits by looking into the past.

By analyzing present-day genomic sequences through their relations to one another—in 33 different mammals, from elephants to platypuses to humans—Clark can infer the historical picture and pinpoint areas where genes show signs of rapid evolution. The degree to which these hot spots match across the animal kingdom is measured using something called ERC, or the “evolutionary rate covariation.”

For instance, if genes related to a particular function, say, color vision, are evolving at a similar rate between two species, then their ERC score would be quite high. Likewise, a high ERC score across a collection of related animals implies that they are all responding to a similar evolutionary pressure.

It may seem counterintuitive that species that are seemingly so different from us might provide insight into human diseases, but it is easy to forget how similar we are on the molecular level. “The genes that form a retina in a mouse are the same as the ones that form a retina in a kangaroo,” reminds Clark. And by evaluating the
Waardenburg syndrome, Leigh syndrome, thyroid dyshormonogenesis—these are words you hope to never hear in a doctor’s office. These graphics include a veritable Who’s Who of diseases ranging from the unfortunate to the life-threatening. Even Clark admits he’d never heard of some of them before their ERC scores caused them to bubble up to the top of his list of correlations. (The ERC scores, you may recall, measure rates of evolutionary change at the genetic level.) On this page, each line drawn between items reflects a correlation between ERC scores. The big red spiderweb on the opposite page represents diseases, biological processes, and traits, many of which could be broadly classified as having something to do with our blood and skin. On the upper right of this page in light blue are diseases representing ciliopathies (in which cilia don’t work properly) and their association with mitochondrial disorders—a connection scientists had only guessed at before. In dark green, we see heterogeneous disorders with similar symptomologies and then a bevy of one-to-one links. Although Clark says this page’s graphic represents the strongest connections found out of the 48,205 disorder pairings he studied, he makes no bones about them being correlations—links suggested after rigorous data analysis. They are not reflective of shared genes, per se, or definitive associations.

SEPARATED AT BIRTH?
Perhaps it isn’t so surprising that Clark’s research should find what appear to be patterns of evolutionary connections between the genes responsible for different blood disorders. What is surprising, however, is the connection his data suggested between other disease pairs, such as Hirschsprung’s disease and melanoma. In case you’re not familiar with it, Hirschsprung’s is a congenital condition in which a baby is born without the proper assortment of nerve cells that govern the colon. Melanoma, on the other hand, is a deadly serious skin cancer. There’s nothing in the medical literature that would indicate an overlap between these two afflictions—except for one single observational note published by a doctor who noticed that families with Hirschsprung’s disease also seemed to suffer from melanoma. Clark thought this was interesting, so he went digging for more information. “It turns out that melanocytes, which form our pigment and are of course important in melanoma, come from the exact same progenitor tissue as nerves.” This means that while diseases of the colonic nerves and skin may appear disconnected, when you look at the way they manifest in a fully formed human, they would have once been intimately related in the much smaller world of the developing embryo. Realizing this connection may not lead to the cure of either disease any time soon, says Clark, but it could at least encourage families with Hirschsprung’s disease to get vigilant with the sunblock.
co-evolutionary signatures of genes related to these areas, we might be able to zero in on the individual genes or groups of genes that contribute to, say, retinal disease. But it gets even more interesting.

In a paper published in the February issue of *PLOS Genetics*, Clark and MD/PhD student Nolan Priedigkeit used these signatures to show that there are some surprising evolutionary connections between what we think of as separate diseases.

“When you look at diseases affecting someone’s retina,” he says, “it actually turns out that similar genes are causing problems in people’s kidneys.”

The link? Something called ciliopathy, or a malfunction of cilia (a cellular structure found in both retinas and kidneys). Scientists had suspected a link between these disparate diseases, but Clark’s technique of finding correlations between ERC scores revealed the specific genes that are functionally related between the two diseases.

“If we can discover unforeseen connections between diseases, then that might lead to innovations in how we treat those diseases,” says Clark. “For instance, if a treatment works at the molecular level of one disease, it might work for the other disease, as well.” He adds that another way this approach could have therapeutic potential is drug repurposing—that is, taking a drug that’s already approved for the treatment of one disease and discovering its usefulness in treating another.

Systems-biology approaches to health, like this sleuthing out of molecular-level relationships between seemingly unrelated diseases or conditions, are revealing all sorts of interesting avenues for scientists like Clark to investigate. However, he warns that it’s no silver bullet for discovering the origins of disease. Instead, he envisions this method being just one layer in a larger, integrated network of approaches biologists can tap into.

In the meantime, Clark keeps at it, getting glimpses of the past to pull out all sorts of secrets about life.

It all starts with a mutation.
THE NEW ORGAN ON THE BLOCK

Clark says that four areas of the genome always tend to stick out when he’s looking for rapidly and adaptively evolving genes: those related to olfaction, immune system function, detoxification, and sexual reproduction. Recently, Clark teamed up with Nathan Morehouse, an assistant professor in the Department of Biological Sciences, for a project investigating the evolutionary origins of a reproductive organ found in butterflies.

“In the same way that the genes involved in diseases affect the evolution of other genes, male and female butterflies have a very similar kind of relationship,” Clark says. For instance, genes from viruses and the hosts they infiltrate are perpetually upping their armaments in attempts to best each other. In butterflies, this push-pull relationship can be seen during mating. Males give the females a spermatophore, or a packet full of sperm and nutrients. But the gift isn’t free—it comes covered in a hard shell that takes three days to digest, during which time the female cannot mate again. In response, the females of the species *Pieris rapae*, or the cabbage white butterfly, have developed an organ known as a bursa copulatrix to digest the spermatophore more efficiently, allowing the females the freedom to mate again.

“Most organs were formed hundreds of millions of years ago, so we don’t have a chance to see how they must have come about. The stomach has been there since we were worms,” says Clark. “But here’s this new organ that pops up.” (Quick note: “New” is a relative term when you’re talking about evolution. The bursa is likely millions of years old.) The hope is that if they can sequence enough species of butterfly, then Clark can use the same techniques he did with his disease study to pull back the curtain of history on the co-evolution of cabbage butterflies. “We have [the sequences of] most species in hand and will produce the others within the year.”