# Eye-Opening Gene

## How many times did eyes arise?

By JOHN TRAVIS

Several years ago, Walter J. Gehring of the University of Basel in Switzerland was working on a zoology textbook. When it came time to write a section that dealt with the evolution of eyes, Gehring unhesitatingly recited the traditional view that eyes had evolved independently dozens of times.

For the next edition, he'll pen a different scenario.

The discovery of a gene shared by fruit flies, mice, squid, and humans and the creation of unusual fruit flies that sprout eyelike structures in places such as wings, legs, and antennae have persuaded Gehring that all modern animals with eyes evolved from a common ancestor that possessed a primitive image-forming organ.

In essence, he contends that the eye probably evolved just once in life's evolutionary history—an assertion not everyone is willing to accept.

The eye has always been a thought-provoking organ in discussions of evolution. Creationists have regularly pointed to it as something so complex and specialized that it could not have developed on its own.

Charles Darwin also considered eyes a formidable challenge to his theory of natural selection. "To suppose that the eye, with all its inimitable contrivances for adjusting the focus to different distances, for admitting different amounts of light, and for the correction of spherical and chromatic aberration, could have been formed by natural selection, seems, I freely confess, absurd in the highest possible degree," he wrote in *On the Origin of Species.*

Yet Darwin quickly dismissed this concern, arguing that the complex eyes of modern animals could have evolved slowly from light-sensitive nerve cells and not much else.

In more recent years, evolutionary biologists have been asking a different question: How often can such an organ develop from scratch? While many creatures have an ability to sense light, a survey of the animal world shows that a minority of the major animal groups, or phyla, have true eyes.

"Only 6 out of 30 phyla have complex eyes, which are able to give you images, but because [such eyes] give them so many evolutionary advantages, these phyla dominate," says Stanislav I. Tomarev of the National Eye Institute (NEI) in Bethesda, Md. Researchers estimate that species with complex eyes comprise 95 percent of the animals on the planet, notes Tomarev.

While image-forming eyes are commonplace, no one design for eyes dominates. Scientists have described almost a dozen distinct blueprints, from the alien-seeming compound eyes of insects and many other species to the cameralike single eyes of vertebrates like us.

Gehring, P. Callaerts, and G. Halder

*Abnormal activity of the* eyeless *gene has generated an eye on the leg of a fly.*

The exotic appearance of the compound insect eye, with its hundreds of miniature eyes called ommatidia, helps explain why scientists have assumed that it evolved independently of the vertebrate eye.

Even superficially similar eyes provide evidence of independent evolution. At first glance, the eyes of cephalopods such as squid and octopuses closely resemble those of vertebrates. A closer examination reveals that the organs emerge from different embryonic tissues and differ considerably in the fine details of their construction. Consequently, the two groups of eyes have been thought a classic example of convergent evolution.

"They appeared independently and somehow evolved to form the very similar structures we observe now," says Tomarev.

Indeed, the majority of scientists studying eye evolution ultimately decided that the wide variety of eyes spread across the animal kingdom is evidence that the organ could not have developed just once. In 1977, L. Von Salvini-Plawen and Ernst Mayr, both of Harvard University, placed this conventional wisdom solidly on the record when they published a landmark paper concluding that eyes had arisen independently at least several dozen times.

That's where the story of eye evolution stood until 1993. That year, Gehring and Rebecca Quiring, also of the University of Basel, were studying fruit flies and looking for transcription factors—proteins that regulate the activity of genes.

Quiring finally identified a protein that binds to DNA, a common feature of transcription factors. Although it wasn't the kind of transcription factor Gehring was interested in, the researchers sent information on the discovery of this protein and its gene to a worldwide computer database to see if any similar genes, or homologues, had already been reported.

The database search highlighted two genes, one from mice that is called *Pax-6* (or *small eye*) and one from people that is called *Aniridia*. Both genes, which are nearly identical to the fruit fly gene, encode proteins crucial to eye development. If mutations exist in both copies of the mouse gene, embryos don't form eyes at all. In people, a mutation in one of *Aniridia*'s two copies usually produces defects in the eyes.

Gehring was surprised that the fly gene was so similar to the two vertebrate genes, but the real astonishment came when he realized that the insect gene also plays a role in eye development.

That finding, reported in the Aug. 5, 1994 SCIENCE, emerged after Gehring and his colleagues had mapped the location of the new gene. They found it at a chromosomal site harboring mutations in flies with developmental eye defects ranging from too-small compound eyes to a complete absence of the organs.

The new gene, named *eyeless*, turned out to be more than just a cog in the genetic machinery that makes a compound eye. When *eyeless* was turned on in parts of the developing fruit fly where it is normally inactive, it could sometimes initiate the development of additional eyes in odd places.

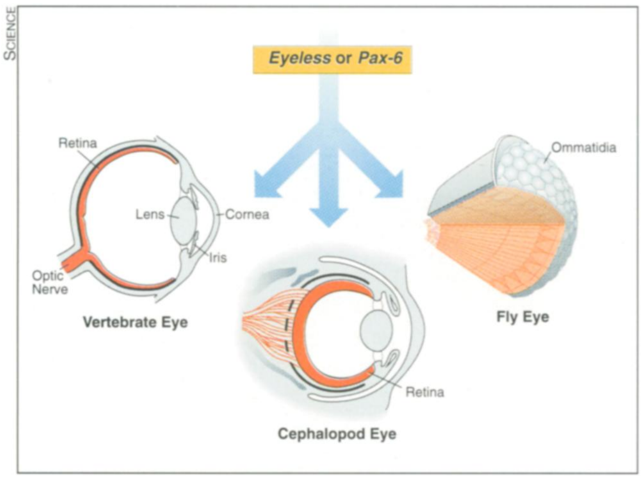
Even more remarkable, *Pax-6* and *Aniridia* did the same. Gehring's group added to fruit flies copies of the vertebrate genes that had been engineered to become active in imaginal disks, embryonic tissues that give rise to adult insect structures like wings and legs.

This unusual experiment, described in the March 24, 1995 SCIENCE, generated flies that had extra eyes growing out of their legs, wings, and other body parts. While the eyes were not wired to the brain, they were light-sensitive and looked superficially much like normal compound insect eyes.

Even before the startling pictures of these mutant insects hit the newspapers, Gehring and other researchers set off to find *eyeless* counterparts in more species, especially ones that might offer further insight into eye evolution.

The group began to collaborate with Tomarev and Joram Piatigorsky, also of NEI, to study the squid *Loligo opalescens*, for example. This animal indeed has its own version of *eyeless*, the researchers reported in the March 18 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

Moreover, the squid gene, like the vertebrate genes, initiated formation of extra eyes when activated in developing fruit flies.



*The insect gene* eyeless *and its counterparts in vertebrates and cephalopods appear to control the formation of distinctly different eyes.*

Although the ability to create eyes where none should exist is an impressive feat, researchers are still struggling to understand what *eyeless* and its noninsect relatives do during development.

Gehring likes to call *eyeless* a "master control gene" for eye development, one that sits at the top of the network of genes, estimated at more than 2,000, used to form eyes. "It's like the main electrical switch in a building. You turn on the main switch and all the lights can go on," explains Gehring.

This interpretation of *eyeless*' role rubs some researchers the wrong way.

"It greatly oversimplifies the way you build organs," argues Graeme Mardon of Baylor College of Medicine in Houston. "The idea that one gene can do the whole job is wrong."

Mardon emphasizes that there are many genes before and after *eyeless* in the genetic hierarchy driving eye development, making it impossible to pick *eyeless* or any other single gene as a master control gene. For example, the fruit fly gene *dachshund*, which he studies, can also trigger the formation of extra eyes, though Mardon acknowledges that it may work by turning on *eyeless*. (In addition to eye defects, flies with mutant *dachshund* genes have short legs.)

Mardon further points out that *eyeless* clearly needs the proper environment to make an eye. "There are many cells in the developing [fruit fly] larva that will not respond to *eyeless*. You turn on *eyeless* and they go 'Ha, we're not making an eye here,'" he says.

Adding to the confusion about *eyeless* are observations indicating that the gene does much more than guide eye development. The fruit fly gene is naturally active in embryonic regions other than those destined to give rise to eyes, as are the gene's counterparts in other animals.

In mice, *Pax-6* plays a role in the formation of the nose. If both copies of *Pax-6* have mutations, a mouse embryo will die because it has no nose and cannot breathe properly. The squid version of *Pax-6* is active in the animal's tentacle development, adding to the idea that the gene plays a role in the formation of the many organs that process sensory information.

Even more controversial than Gehring's calling *eyeless* the master control gene for eye development is his belief that its discovery in several disparate species shatters the dogma that eyes evolved independently on many occasions.

"We now think that this event happened only once," asserts Gehring.

Mayr, however, is emphatic that the new research does not conflict with his proposal of 2 decades ago. He notes that species of worms that do not have eyes also employ *eyeless*-like genes during development.

"What we claimed, and it's as correct as ever, is that the eyes themselves, the photoreceptive organs, developed independently at least 40 times. This is not in the slightest touched by finding that there are genes used in making eyes that existed long before eyes," says Mayr. "You should go to the species that have no eyes but have this gene and find out what it's doing."

Mayr and other researchers suspect that *eyeless* was originally part of a group of genes shaping the developing nervous system. As eyes evolved in various organisms, this genetic cascade was adapted to the specific task of eye development.

"These genes may have been involved in some sort of primitive genetic network that then got co-opted into eye development, either once or multiple times," says Nancy Bonini of the University of Pennsylvania in Philadelphia. "I'm not sure how easy it will be to resolve those two choices."

Even Mardon, who says that he favors Gehring's hypothesis that eyes evolved just once, agrees that the jury is still out. "It's not a foregone conclusion that because *eyeless* and *Aniridia* are both involved in eye development, it must be true that there was a common eye precursor in a species prior to the divergence of insects and vertebrates," he says.

The challenge facing Gehring and other scientists is to identify many more genes involved in the early stages of eye formation, understand how those genes function as a group, and then determine whether all animals share this developmental genetic network.

There has already been significant progress in that respect. Scientists have identified other fruit fly genes—*eyes absent* and *sine oculis*, as well as *dachshund*—that play some role in the early stages of insect eye formation and have vertebrate counterparts.

Ultimately, they hope to chart how these and other genes are linked to *eyeless* and together form the compound insect eye. If large parts of this genetic network are in fact shared by widely divergent animals, then it may become more and more difficult for Mayr and other evolutionary biologists to argue that eyes evolved independently dozens of times.

"When you have a pathway that's conserved, the argument that [eyes] evolved independently is much more tenuous," says Mardon.

As for Gehring, he's already confident enough in his interpretation that eyes probably developed just once that he has begun to plan how he should revise his textbook's section on eye evolution.

"It's nice to disprove your own text, as long as it doesn't happen too many times," he laughs. □