

1. What famous book did Darwin publish 150 years ago to introduce his ideas on evolution?
2. Where have biologists found “clues” into evolutionary biology?
3. When did one-celled organisms give rise to multicellular organisms?
4. What is interesting about proteins made in choanaflagellates?
5. Explain how the arthropods started out like a velvet worm.
6. What are the closest living relatives of vertebrates? Why do these organisms hold the origins to the vertebrate head?
7. Why is the eye ‘far from perfect’?
8. Did the eyes in flies and humans have common ancestor? What organism?
9. When and in what organism did the “first glimmers of a limb” appear?
10. What “jobs” do feathers have?
11. What other appendage do bird feathers share their origin with?
12. What is the closest living relative of flowering plants?
13. How many species of flowering plants exist today? Gymnosperms?
14. Explain this statement: “You see the baggage of history” in evolution.

"A Fin is a Limb is a Wing" Nov. 2006

By Carl Zimmer

Photographs by Rosamond Purcell

Scientists are tracing the steps through which evolution forged its successes. They're finding that the same genetic tool kit can build structures both simple and complex.

The father of evolution was a nervous parent. Few things worried Charles Darwin more than the challenge of explaining how nature's most complex structures, such as the eye, came to be. "The eye to this day gives me a cold shudder," he wrote to a friend in 1860.

Today biologists are beginning to understand the origins of life's complexity—the exquisite optical mechanism of the eye, the masterly engineering of the arm, the architecture of a flower or a feather, the choreography that allows trillions of cells to cooperate in a single organism.

The fundamental answer is clear: In one way or another, all these wonders evolved. "The basic idea of evolution is so elegant, so beautiful, so simple," says Howard Berg, a Harvard researcher who has spent much of the past 40 years studying one of the humbler examples of nature's complexity, the spinning tail of common bacteria. "The idea is simply that you fiddle around and you change something and then you ask, Does it improve my survival or not? And if it doesn't, then those individuals die and that idea goes away. And if it does, then those individuals succeed, and you keep fiddling around, improving. It's an enormously powerful technique."

But nearly 150 years after Darwin first brought this elegant idea to the world's attention when he published *The Origin of Species*, the evolution of complex structures can still be hard to accept. Most of us can envision natural selection tweaking a simple trait—making an animal furrer, for example, or its neck longer. Yet it's harder to picture evolution producing a new complex organ, complete with all its precisely interlocking parts. Creationists claim that life is so complex that it could not have evolved. They often cite the virtuoso engineering of the bacterial tail, which resembles a tiny electric motor spinning a shaft, to argue that such complexity must be the direct product of "intelligent design" by a superior being.

The vast majority of biologists do not share this belief. Studying how complex structures came to be is one of the most exciting frontiers in evolutionary biology, with clues coming at remarkable speed.

Some have emerged from spectacular fossils that reveal the precursors of complex organs such as limbs or feathers. Others come from laboratories, where scientists are studying the genes that turn featureless embryos into mature organisms. By comparing the genes that build bodies in different species, they've found evidence that structures as seemingly different as the eyes of a fly and a human being actually have a shared heritage.

Scientists still have a long way to go in understanding the evolution of complexity, which isn't surprising since many of life's devices evolved hundreds of millions of years ago. Nevertheless, new discoveries are revealing the steps by which complex structures developed from simple beginnings. Through it all, scientists keep rediscovering a few key rules. One is that a complex structure can evolve through a series of simpler intermediates. Another is that nature is thrifty, modifying old genes for new uses and even reusing the same genes in new ways, to build something more elaborate.

Sean Carroll, a biologist at the University of Wisconsin–Madison, likens the body-building genes to construction workers. "If you walked past a construction site at 6 p.m. every day, you'd say, Wow, it's a miracle—the building is building itself. But if you sat there all day and saw the workers and the tools, you'd understand how it was put together. We can now see the workers and the machinery. And the same machinery and workers can build any structure."

A limb, a feather, or a flower is a marvel, but not a miracle.

From One Cell to Trillions

In every human body roughly ten trillion cells—brainless units of life—come together to work as a unified whole. "It's a complex dance," says Nicole King, a biologist at the University of California, Berkeley, requiring organization and constant communication. And it began more than 600 million years ago when organisms containing just one cell gave rise to the first multicellular animals, the group that now includes creatures as diverse as sea sponges,

beetles, and us. It turns out that some of those single-celled ancestors were already equipped for social life.

King studies some of our closest living single-celled relatives, known as choanoflagellates. Choanoflagellates are easy to find. Just scoop some water from a local creek or marsh, put a few drops under a microscope, and you may see the tadpole-shaped creatures flitting about. You can tell them apart from other protozoans by a distinctive collar at the base of their tail.

When King and her colleagues examined the proteins made by choanoflagellates, they found several that were thought to be unique to animals—molecules essential to maintaining a multicellular body. "It really blew our minds," says King. "What are these single-celled organisms doing with these proteins?"

Some of the proteins normally create what King calls "an armlock between cells," keeping animal cells from sticking together randomly. King and her colleagues are running experiments to figure out how choanoflagellates use these adhesive proteins—perhaps to snag bacteria for food. Others play a role in cell-to-cell communication. Choanoflagellates, which presumably have no need to talk to other cells, may use these proteins to sense changes in their environment.

The discoveries suggest that many of the tools necessary to build a multicellular body already existed in our single-celled ancestors. Evolution borrowed those tools for a new task: building bodies of increasing complexity.

Blueprints for Bodies

A developing fly larva looks as featureless as a grain of rice. But it already bears a map of the complex creature it will become. Across the larva, different combinations of genes are active, marking it off into invisible compartments. These genes turn on other genes that give each compartment its shape and function: Some sprout legs, others wings, others antennae. An invisible anatomy becomes visible.

Flies aren't the only animals that build their bodies this way. Scientists have found that the genes responsible for laying out the fly's body plan have nearly identical counterparts in many other animals, ranging from crabs to earthworms to lampreys to us. The discovery came as a surprise, since these animals have such different-looking bodies. But now scientists generally agree that the common ancestor of all these animals—a wormlike creature that lived an estimated 570 million years ago—already had a basic set of body-plan genes. Its descendants then used those genes to build new kinds of bodies.

To appreciate how this tool kit can generate complexity, consider the velvet worm. The velvet worm creeps along the floors of tropical forests on nearly identical pad-shaped legs. It is, frankly, a boring little creature. Yet it is also the closest living relative to the single most diverse group of animals, the arthropods. Among arthropods, you can find a dizzying range of complex bodies, from butterflies to tarantulas, horseshoe crabs, ticks, and lobsters.

Scientists studying body-plan genes think arthropods started out much like velvet worms, using the same basic set of body-building genes to lay out their anatomy. Over time, copies of those genes began to be borrowed for new jobs. The invisible map of the arthropod body plan became more complex, with more compartments and new body parts sprouting from them.

Some compartments, for example, developed organs for breathing; later, in insects, those breathing organs evolved into wings. Early insect fossils preserve wings sprouting from many segments. Over time, insects shut off the wing-building genes in all but a few segments—or used some of the same genes to build new structures. Flies, for example, have just one pair of wings; a second pair has turned into club-shaped structures called halteres, which help flies stay balanced in flight.

"The segments have all become different, the appendages have all become different, but the machinery for making appendages is the same," says Sean Carroll. "Evolution is a tinkerer, an improviser."

How We Got a Head

The human head is, inch for inch, the most complex part of our body. Not only does it contain our brain, but it also packs in most of our sense organs: eyes, ears, a nose, and a tongue. The intricate bones of the skull add to the head's complexity, from the cranium that keeps the brain safe to the jaws that allow us to eat. Thousands of variations on the theme exist—think of hammerhead sharks, of anteaters, of toucans.

All those heads become even more remarkable when you look at two simple sea creatures that are the closest living relatives of the vertebrates (animals with backbones). These humble organisms have no heads at all. But they have the makings of one in their genes.

The larvacean, a tiny gelatinous tadpole, lives in a floating house it builds with its own mucus. Its nervous system, such as it is, is organized around a simple nerve cord running along its back. Even stranger is its cousin, the sea squirt. It starts out as a swimming larva, with a rodlike stiffener in its tail. When it matures, it drives its front end into the ocean floor, eats most of its nervous system, and turns its body into a basket for filtering food particles.

At first glance, these creatures seem unlikely to hold any clues to the origin of the vertebrate head. But a close look at the front tip of larvaceans and larval sea squirts reveals a small brainlike organ where a vertebrate would have a head. "There are 360 neural cells there. Compared with the vertebrate brain, that's nothing," says William Jeffery, a biologist at the University of Maryland. Yet scientists have seen a strikingly familiar pattern in how that tiny cluster of cells develops. Some of the same genes that build our own brains are at work there, and in roughly the same areas—front, middle, and rear.

Jeffery and his colleagues have also found that sea squirts have what appear to be primitive cousins of neural crest cells—the kind of cells that build much of the head in the developing embryos of vertebrates. Like our own neural crest cells, the sea squirt's emerge along the back of the developing embryo and migrate through the body. But instead of making a skull, neurons, and other parts of the head, they turn into pigment cells, adding brilliant colors to sea squirt bodies.

Over half a billion years ago our own headless ancestors may have resembled these modest creatures, already equipped with genes and cells that would later sculpt the faces and brains that make us human.

Catching the Light

Charles Darwin was well acquainted with the exquisite construction of the eye—the way the lens is perfectly positioned to focus light onto the retina, the way the iris adjusts the amount of light that enters the eye. An eye, it seemed, would be useless if it were anything less than perfect. In *The Origin of Species*, Darwin wrote that the idea of natural selection producing the eye "seems, I freely confess, absurd in the highest degree."

Yet the eye is actually far from perfect. The retina is so loosely attached to the back of the eye in humans that a sharp punch to the head may be enough to detach it. Its light-gathering cells point inward, toward the brain, not out toward the light. And the optic nerve starts out in front of the retina and then plunges through it to go to the brain. The place where the optic nerve burrows through the retina becomes the eye's blind spot. Evolution, with all its blunders, made the eye; Darwin himself had no doubt about that. But how?

A full answer has to account for not just our own eye, but all the eyes in the animal kingdom. Not long ago, the evidence suggested that the eyes in different kinds of animals—insects, cats, and octopuses, for example—must have evolved independently, much as wings evolved independently in birds and bats. After all, the differences between, say, a human eye and a fly's are profound. Unlike the human eye with its single lens and retina, the fly's is made up of thousands of tiny columns, each capturing a tiny fraction of the insect's field of vision. And while we vertebrates capture light with cells known as ciliary photoreceptors (for their hairlike projections, called cilia), insects and other invertebrates use rhabdomeric photoreceptors, cells with distinctive folds.

In recent years, however, these differences became less stark as scientists examined the genes that build photoreceptors. Insects and humans use the same genes to tell cells in their embryos to turn into photoreceptors. And both kinds of photoreceptors snag light with molecules known as opsins.

These links suggested that photoreceptors in flies, humans, and most other animals all evolved from a single type of cell that eventually split into two new cell types. If so, some animals might carry both types of photoreceptors. And in 2004, scientists showed that rag worms, aquatic relatives of earthworms, have rhabdomeric photoreceptors in their eyes and ciliary photoreceptors hidden in their tiny brain, where they appear to sense light to set the rag worm's internal clock.

With such discoveries, a new picture of eye evolution is emerging. The common ancestor of most animals had a basic tool kit of genes for building organs that could detect light. These

earliest eyes were probably much like those found today in little gelatinous sea creatures like salps: just pits lined with photoreceptor cells, adequate to sense light and tell its direction. Yet they were the handiwork of the same genes that build our own eyes, and they relied on the same light-sensing opsins.

Evolution then used those basic genes to fashion more sophisticated eyes, which eventually acquired a lens for turning light into an image. The lens too did not appear out of nothing. Lenses are made of transparent proteins called crystallins, which can bend light "like protein glass," as one scientist says. And crystallins, it turns out, existed well before evolution put them to work in the eye. They were just doing other jobs.

Scientists have discovered one crystallin, for example, in the central nervous system of sea squirts. Instead of making a lens, it is part of a gravity-sensing organ. A mutation may have caused cells in the early vertebrate eye to make the crystallin as well. There it turned out to do something new and extraordinarily useful: bring the world into focus.

From Fins to Limbs

Look at your arms holding this magazine. They are marvels of complexity, containing dozens of finely sculpted bones linked by tendons and muscles, supplied with blood by a mesh of arteries, controlled by an intricate network of neurons, and snugly wrapped in skin. Until about 380 million years ago, such limbs did not exist. Today they can be found not just on humans reading magazines, but also on bats flying out of Arizona caves, horses galloping across Mongolian steppes, moles burrowing through Connecticut gardens, and whales diving thousands of feet in the Pacific Ocean.

Fossils and embryos have provided a wealth of clues to the evolution of limbs. And they tell much the same story. "The limb was assembled over evolutionary time," says Neil Shubin, a paleontologist at the University of Chicago. "It didn't appear in one fell swoop."

About 400 million years ago, a new lineage of fish called lobe-fins emerged, bearing the first glimmers of a limb. From the outside, lobe-fins looked like any other fish, with fins for swimming. But the bones inside their fins were larger and more heavily muscled than in other fish.

Over tens of millions of years, new lineages of lobe-fins evolved, and true limbs took shape. *Eusthenopteron*, a 385-million-year-old fish found in Canada, had fins that contained one large rod-shaped bone linked to a pair of smaller bones—the same pattern of long bones now found in our arms and legs. *Tiktaalik roseae*, a 375-million-year-old lobe-fin that Shubin and his colleagues recently discovered in northern Canada, added wrist and ankle bones. The scientists think *Tiktaalik* used its fins not only to swim but also to crawl across coastal wetlands.

"It's pushing up and pushing forward," says Shubin. "Could it walk? Could it rotate its shoulder and the rest? No. It's doing half the function, but it's half the function that suits the animal fully well."

By 365 million years ago, lobe-fins had given rise to vertebrates with true limbs, known as tetrapods, meaning four feet. These tetrapods even had toes, although they were still adapted to the water, retaining the gill bones of their ancestors and finned tails for swimming. Land walkers evolved later. And later still, tetrapods took the basic plan of the limb and adapted it to new functions—digging, paddling, and flying.

Laboratories are uncovering the genes responsible for building limbs and finding that once again, evolution used the tools already at hand: versions of the same genes that lay out animals' body plans. Once these genes mark off our bodies from head to tail, they become active in the tiny buds that become our arms and legs. Evolution must have borrowed these genes in early fish and reused them to build fins. Later, subtle shifts in the patterns formed by these genes caused these append-ages to change shape into legs, arms, wings. Each transformation was profound. But, Shubin says, "you already had the machinery in place."

A Feather's Tale

As a feat of engineering, it's hard to beat the flight feather of a bird. From a central vane sprout hundreds of filaments called barbs. The barbs in turn sprout other, smaller filaments, some with grooves and some with hooks that zip the barbs together like Velcro. They create a lightweight plane that can lift a bird into the sky. When birds pull their feathers apart to clean them, the barbs simply zip back together by themselves.

Feathers do other jobs too. The club-winged manakin, a sparrow-size bird from the jungles

of Ecuador, can rattle its wing feathers so loudly they sing. Owl feathers are a kind of natural stealth technology, dampening sound so that the birds can surprise their prey. Fuzzy down feathers keep birds warm, while extravagantly curved feathers attract mates. Yet all these complex structures share their origins with prosaic reptile scales—a journey that Richard Prum, an ornithologist at Yale, is tracing.

The evolutionary link between feathers and scales is obvious on developing bird embryos. Disks of cells called placodes are scattered across the surface of the embryo. Some grow into scales, such as the ones that cover a chicken's legs. Others turn into feathers.

Prum's research indicates that feathers evolved in a series of steps, with old genes being borrowed each time for new uses. In reptile embryos specific genes mark off the front and back of each scale as it grows from a placode. In bird embryos, each feather begins as a tube growing from a placode, and the same front and back genes are at work in the tube. Some 150 million years ago, says Prum, those genes must have taken on this new role in dinosaurs, causing some to sprout the feathers and feather-like growths that recent fossil finds have revealed.

The appearance of branch-like barbs was the next step in feather evolution, Prum argues, and the development of a baby bird's downy feathers offers clues to how that happened. As a new feather tube grows, it divides into strips, which eventually peel away into barbs. And once again, only a little tinkering with genes may have been required to get the tube to split. Prum has shown that the same genes that mark the front and back of reptile scales and feather tubes also mark the points around the tube where it will split.

Later, birds evolved the ability to turn these fluffy feathers into feathers with vanes, and then to lock the barbs together to make flight feathers, all with slight genetic changes that Prum is tracing. And by tweaking the growth of different parts of the feather, birds evolved special plumage for hunting, swimming, courting, and other activities, Prum says. "All the kinds of stuff that the bird needs throughout its life, it can generate with the same basic information."

Early Blooming

Like many other Victorian gentlemen, Charles Darwin was fond of plants. He packed his hothouses with sundews, cowslips, and Venus flytraps. He had exotic orchids shipped from the tropics. And yet, as he wrote to a friend in 1879, flowers were for him "an abominable mystery."

Darwin was referring to the sudden, unheralded emergence of flowers in the fossil record. Making the mystery all the more abominable was the exquisite complexity of flowers. Typical flowers have whorls of petals and petal-shaped sepals surrounding the plant's male and female sex organs. Many also produce brilliant pigments and sweet nectars to lure insects, which ferry pollen from flower to flower.

Today the mystery of flowers is less abominable, although big questions still remain. The first flowers must have evolved after the ancestors of flowering plants split from their closest living relatives, the gymnosperms—including pines and other conifers, cycads, and ginkgoes—which produce seeds but not flowers.

Some of the most important clues to this transition come from the genes active each time a plant blossoms. It turns out that before a flower takes shape, sets of genes mark out an invisible map at the tip of the stem—the same kind of map found on animal embryos.

The genes divide the tip into concentric rings. "It's like a stack of doughnuts on top of the stem," says Vivian Irish of Yale. Guided by the genes, cells in each ring develop into different flower parts—sepals in the outer ring, for example, and sex organs in the innermost rings.

As is so often the case with complexity, the genes that build flowers are older than the flowers themselves. Gymnosperms turn out to carry flower-building genes even though they don't make flowers. Scientists have yet to determine what those genes do in gymnosperms, but their presence indicates that these genes probably existed in the common ancestor of gymnosperms and flowering plants.

In the flowering plant lineage those genes were borrowed to map out the structure of the flower. The first flowers were simple. But over time, the genes were duplicated accidentally, freeing one copy to take on a new role in flower development. Flowers grew more complex, and some of their parts gained new functions, such as luring insects with bright colors and fragrance.

This flexibility may help explain the success of flowering plants. Some 250,000 known species of flowering plants exist today. Gymnosperms, their flowerless relatives, are stuck at just over 800.

Complexity in Miniature

Some of life's most marvelous structures are its smallest: the minute clockwork of molecules that make cells tick. *E. coli*, a bacterium found in the gut, swims with a tiny spinning tail made up of several dozen different proteins, all working together. Doubters of evolution are fond of pointing out that the flagellum, as this tail is called, needs every one of its parts to function. They argue that it could not have evolved bit by bit; it must have been created in its present form.

But by comparing the flagellar proteins to those in other bacterial structures, Mark Pallen of the University of Birmingham in England and his colleagues have found clues to how this intricate mechanism was assembled from simpler parts. For example, *E. coli* builds its flagellum with a kind of pump that squirts out proteins. The pump is nearly identical, protein for protein, to another pump found on many disease-causing bacteria, which use it not for building a tail but for priming a molecular syringe that injects toxins into host cells. The similarity is, in Pallen's words, "an echo of history, because they have a common ancestor."

Scientists have discovered enough of these echoes to envision how *E. coli*'s flagellum could have evolved. Pallen proposes that its pieces—all of which have counterparts in today's microbes—came together step-by-step over millions of years. It all started with a pump-and-syringe assembly like those found on pathogens. In time, the syringe acquired a long needle, then a flexible hook at its base. Eventually it was linked to a power source: another kind of pump found in the cell membranes of many bacteria. Once the structure had a motor that could make it spin, the needle turned into a propeller, and microbes had new mobility.

Whether or not that's the full story, there is plenty of other evidence that natural selection has been at work on the flagellum. Biologists have identified scores of different kinds of flagella in various strains of bacteria. Some are thick and some are thin; some are mounted on the end of the cell and some on the side; some are powered by sodium ions and some by hydrogen ions. It's just the kind of variation that natural selection is expected to produce as it tailors a structure to the needs of different organisms.

Darwin also argued that complex features can decay over time. Ostriches are descended from flying birds, for example, but their wings became useless as they evolved into full-time runners. It turns out that microbial tails can become vestigial as well. Although *E. coli* is believed to make only one kind of tail, it also carries the remnants of genes for a second type. "You expect to see the baggage of history," says Pallen.

Evolution, ruthless and practical, is equally capable of building the most wonderful structures and tossing them aside when they're no longer needed.