Science

Darwin 200 Collection

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Introduction
Darwin's Inspiration, Darwin's Legacy

The day has passed delightfully. Delight itself, however, is a weak term to express the feeling of a naturalist who, for the first time, has wandered by himself in a Brazilian forest. The elegance of the grasses, the novelty of the parasitical plants, the beauty of the flowers, the glossy green of the foliage, but above all the general luxuriance of the vegetation, filled me with admiration. A most paradoxical mixture of sound and silence pervades the shady parts of the wood. The noise from the insects is so loud, that it may be heard even in a vessel anchored several hundred yards from the shore; yet within the recesses of the forest a universal silence appears to reign. To a person fond of natural history, such a day as this brings with it a deeper pleasure than he can ever hope to experience again.

—Charles Darwin, *The Voyage of the Beagle,* Feb 29th [1832]

Like many other scientists raised in temperate latitudes, Charles Darwin was enthralled by his first glimpse of the tropical rain forest. His *Beagle* diary entry conveyed those immediate and thrilling first impressions, but the encounter with the Brazilian Atlantic Forest had an enduring influence on the development of his ideas over the following decades, with resounding echoes even today in 21st century evolutionary science.

The collection reprinted here is a sample of the articles published in 2009 by *Science* magazine in celebration of the Darwin bicentenary. We start with four of the essays from our "On the Origin of" series, prepared by *Science*'s news writers; further essays in this series are appearing monthly in *Science* throughout the year. A Perspective by Stephen Jackson then considers the legacy of Alexander von Humboldt, for whom, like Darwin, the South American tropics were a critical inspiration, and who died 150 years ago in the year of the publication of Darwin's *Origin.* (Humboldt's *Personal Narrative* of his tropical explorations was acknowledged by Darwin as 'far exceed[ing] in merit anything I have read' on the subject.) A book review by Lynn Nyhart explores two recent volumes on Ernst Haeckel's work, his interpretations of Darwin and his contributions to evolutionary thought.

In the first of four Review articles reprinted here, Peter Bowler analyzes the originality of Darwin's contribution to the understanding of the diversity and diversification of the living world. Michael Benton examines the extent to which biotic and abiotic factors have shaped species diversity in the fossil record. Dolph Schluter reviews how research on speciation has shifted in focus from morphological evolution to reproductive isolation, tracing the links between Darwin's ideas and current thinking. Christophe Fraser and colleagues discuss the contentious area of microbial species formation, an issue that would surely have vexed Darwin horribly had the bewildering diversity of microbes been known in his day.

Finally, with a focus on conservation, a Report by Ana Carnaval et al., who model evolutionary processes in endemic tree-frog species in the Brazilian Atlantic Forest, the very biodiversity hotspot that so inspired Darwin on his South American landfall, and that is now reduced to a collection of small fragments scattered along the coast. Darwin returned to the Brazilian coast on his final homeward leg, more than four years after his first landfall there. His enthusiasm for the tropical forested landscape was undiminished.

In my last walk I stopped again and again to gaze on these beauties, and endeavoured to fix in my mind for ever, an impression which at the time I knew sooner or later must fail ... they will leave, like a tale heard in childhood, a picture full of indistinct, but most beautiful figures.

—Charles Darwin, *The Voyage of the Beagle,* August 1836

Andrew Sugden, Deputy Editor

On the Origin of
Life on Earth

AN AMAZON OF WORDS FLOWED FROM Charles Darwin's pen. His books covered the gamut from barnacles to orchids, from geology to domestication. At the same time, he filled notebooks with his ruminations and scribbled thousands of letters packed with observations and speculations on nature. Yet Darwin dedicated only a few words of his great verbal flood to one of the biggest questions in all of biology: how life began.

The only words he published in a book appeared near the end of *On the Origin of Species*: "Probably all the organic beings which have ever lived on this earth have descended from some one primordial form, into which life was first breathed," Darwin wrote.

Darwin believed that life likely emerged spontaneously from the chemicals it is made of today, such as carbon, nitrogen, and phosphorus. But he did not publish these musings. The English naturalist had built his argument for evolution, in large part, on the processes he could observe around him. He did not think it would be possible to see life originating now because the life that's already here would prevent it from emerging.

In 1871, he outlined the problem in a letter to his friend, botanist Joseph Hooker: "But if (and Oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, etc., present, that a protein compound was chemically formed ready to undergo still more complex changes, at the present day such matter would be instantly devoured or absorbed, which would not have been the case before living creatures were formed."

Scientists today who study the origin of life do not share Darwin's pessimism about our ability to reconstruct those early moments. "Now is a good time to be doing this research, because the prospects for success are greater than they have ever been," says John Sutherland, a chemist at the University of Manchester in the United Kingdom. He and others are addressing each of the steps involved in the transition to life: where the raw materials came from, how complex organic molecules such as RNA formed, and how the first cells arose. In doing so, they are inching their way toward making life from scratch. "When I was in graduate school, people thought investigating the origin of life was something old scientists did at the end of their career, when they could sit in an armchair and speculate," says Henderson James Cleaves of the Carnegie Institution for Science in Washington, D.C. "Now making an artificial cell doesn't sound like science fiction any more. It's a reasonable pursuit."

Raw ingredients
Life—or at least life as we know it—appears to have emerged on Earth only once. Just about all organisms use double-stranded DNA to encode genetic information, for example. They copy their genes into RNA and then translate RNA into proteins. The genetic code they use to translate DNA into proteins is identical, whether they are emus or bread mold. The simplest explanation for this shared biology is that all living things inherited it from a common ancestor—namely, DNA-based microbes that lived more than 3.5 billion years ago. That common ancestor was already fairly complex, and many scientists have wondered how it might have evolved from a simpler predecessor. Some now argue that membrane-bound cells with only RNA inside predated both DNA

> The English naturalist had built his argument for evolution, in large part, on the processes he could observe around him.

and proteins. Later, RNA-based life may have evolved the ability to assemble amino acids into proteins. It's a small step, biochemically, for DNA to evolve from RNA.

In modern cells, RNA is remarkably versatile. It can sense the levels of various compounds inside a cell and switch genes on and off to adjust these concentrations, for example. It can also join together amino acids, the building blocks of proteins. Thus, the first cells might have tapped RNA for all the tasks on which life depends.

For 60 years, researchers have been honing theories about the sources of the amino acids and RNA's building blocks. Over time, they have had to refine their ideas to take into account an ever-clearer understanding of what early Earth was like.

In an iconic experiment in 1953, Stanley Miller, then at the University of Chicago, ignited a spark that zapped through a chamber filled with ammonia, methane, and other gases. The spark created a goo rich in amino acids, and, based on his results, Miller suggested that lightning on the early Earth could have created many compounds that would later be assembled into living things.

By the 1990s, however, the accumulated evidence indicated that the early Earth was dominated by carbon dioxide, with a pinch of nitrogen—two gases not found in Miller's flask. When scientists tried to replicate Miller's experiments with carbon dioxide in the mix, their sparks seemed to make almost no amino acids. The raw materials for life would have had to come from elsewhere, they concluded.

In 2008, however, lightning began to look promising once again. Cleaves and his colleagues suspected that the failed experiments were flawed because the sparks might have produced nitrogen compounds that destroyed any newly formed amino acids. When they added buffering chemicals that could take up these nitrogen compounds, the experiments generated hundreds of times more amino acids than scientists had previously found.

Cleaves suspects that lightning was only one of several ways in which organic compounds built up on Earth. Meteorites that fall to Earth contain amino acids and organic carbon molecules such as formaldehyde. Hydrothermal vents spew out other compounds that could have been incorporated into the first life forms. Raw materials were not an issue, he says: "The real hurdle is how you put together organic compounds into a living system."

Step 1: Make RNA
An RNA molecule is a chain of linked nucleotides. Each nucleotide in turn consists of three parts: a base (which functions as a

"letter" in a gene's recipe), a sugar molecule, and a cluster of phosphorus and oxygen atoms, which link one sugar to the next. For years, researchers have tried in vain to synthesize RNA by producing sugars and bases, joining them together, and then adding phosphates. "It just doesn't work," says Sutherland.

This failure has led scientists to consider two other hypotheses about how RNA came to be. Cleaves and others think RNA-based life may have evolved from organisms that used a different genetic material—one no longer found in nature. Chemists have been able to use other compounds to build backbones for nucleotides (*Science*, 17 November 2000, p. 1306). They're now investigating whether these humanmade genetic molecules, called PNA and TNA, could have emerged on their own on the early Earth more easily than RNA. According to this hypothesis, RNA evolved later and replaced the earlier molecule.

But it could also be that RNA wasn't put together the way scientists have thought. "If you want to get from Boston to New York, there is an obvious way to go. But if you can't get there that way, there are other ways you could go," says Sutherland. He and his colleagues have been trying to build RNA from simple organic compounds, such as formaldehyde, that existed on Earth before life began. They find they make better progress toward producing RNA if they combine the components of sugars and the components of bases together instead of separately making complete sugars and bases first.

Over the past few years, they have documented almost an entire route from prebiotic molecules to RNA and are preparing to publish even more details of their success. Discovering these new reactions makes Sutherland suspect it wouldn't have been that hard for RNA to emerge directly from an organic soup. "We've got the molecules in our sights," he says.

Sutherland can't say for sure where these reactions took place on the early Earth, but he notes that they work well at the temperatures and pH levels found in ponds. If those ponds dried up temporarily, they would concentrate the nucleotides, making conditions for life even more favorable.

Were these Darwin's warm little ponds? "It might just be that he wasn't too far off," says Sutherland.

Step 2: The cell

If life did start out with RNA alone, that RNA would need to make copies of itself without help from proteins. Online in *Science* this week (www.sciencemag.org/cgi/content/abstract/1167856), Tracey Lincoln and Gerald Joyce of the Scripps Research Institute in San Diego, California, have shown how that might have been possible. They designed a pair of RNA molecules that join together and assemble loose nucleotides to match their partner. Once the replication is complete, old and new RNA molecules separate and join with new partners to form new RNA. In 30 hours, Lincoln and Joyce found, a population of RNA molecules could grow 100 million times bigger.

Lincoln and Joyce kept their RNA molecules in beakers. On the early Earth, however, replicating RNA might have been packed in the first cells. Jack Szostak and his colleagues at Harvard Medical School in Boston have been investigating how fatty acids and other molecules on the early Earth might have trapped

> "Now making an artificial cell doesn't sound like science fiction any more. It's a reasonable pursuit."
>
> —HENDERSON JAMES CLEAVES, CARNEGIE INSTITUTION FOR SCIENCE

RNA, producing the first protocells. "The goal is to have something that can replicate by itself, using just chemistry," says Szostak.

After 2 decades, he and his colleagues have come up with RNA molecules that can build copies of other short RNA molecules. They have been able to mix RNA and fatty acids together in such a way that the RNA gets trapped in vesicles. The vesicles are able to add fatty acids to their membranes and grow. In July 2008, Szostak reported that he had figured out how protocells could "eat" and bring in nucleotides to build the RNA.

All living cells depend on complicated channels to draw nucleotides across their membranes, raising the question of how a primitive protocell membrane brought in these molecules. By experimenting with different recipes for membranes, Szostak and his colleagues have come up with protocells leaky enough to let nucleic acids slip inside, where they could be assembled into RNA, but not so porous that the large RNA could slip out.

Their experiments also show that these vesicles survive over a 100°C range. At high temperatures, protocells take in nucleotides quickly, and at lower temperatures, Szostak found, they build RNA molecules faster.

He speculates that regular temperature cycles could have helped simple protocells survive on the early Earth. They could draw in nucleotides when they were warm and then use them to build RNA when the temperature dropped. In Szostak's protocells, nucleotides are arranged along a template of RNA. Strands of RNA tend to stick together at low temperatures. When the protocell warmed up again, the heat might cause the two strands to pull apart, allowing the new RNA molecule to function.

Now Szostak is running experiments to bring his protocells closer to life. He is developing new forms of RNA that may be able to replicate longer molecules faster. For him, the true test of his experiments will be whether his protocells not only grow and reproduce, but evolve.

"To me, the origin of life and the origin of Darwinian evolution are essentially the same thing," says Szostak. And if Darwin was alive today, he might well be willing to write a lot more about how life began.

–CARL ZIMMER

Carl Zimmer is the author of Microcosm: E. coli *and the New Science of Life*.

Protocell. Researchers at Harvard are trying to make simple life forms, shown here in a computer image.

CREDIT: JANET IWASA

On the Origin of
Art and Symbolism

CREDITS (LEFT TO RIGHT): KATHERINE SUTLIFF/SCIENCE; THE BOXGROVE PROJECT

Since their discovery by French spelunkers in 1994, the magnificent lions, horses, and rhinos that seem to leap from the walls of Chauvet Cave in southern France have reigned as the world's oldest cave paintings. Expertly composed in red ochre and black charcoal, the vivid drawings demonstrate that the artistic gift stretches back more than 30,000 years. These paintings are almost sure to be mentioned in any article or paper about the earliest art. But what do they really tell us about the origins of artistic expression?

The prehistoric humans who decorated Chauvet's walls by torchlight arrived at the cave with their artistic genius already in full flower. And so, most researchers agree that the origins of art cannot simply be pegged to the latest discovery of ancient paintings or sculpture. Some of the earliest art likely perished over the ages; much remains to be found; and archaeologists don't always agree on how to interpret what is unearthed. As a result, instead of chasing after art's first appearance, many researchers seek to understand its symbolic roots. After all, art is an aesthetic expression of something more fundamental: the cognitive ability to construct symbols that

communicate meaning, whether they be the words that make up our languages, the musical sounds that convey emotion, or the dramatic paintings that, 30,000 years after their creation, caused the discoverers of the Chauvet Cave to break down in tears.

While sites like Chauvet might be vivid examples of what some researchers still consider a "creative explosion" that began when modern humans colonized Europe about 40,000 years ago, an increasing number of prehistorians are tracing our symbolic roots much further back in time—and in some cases, to species ancestral to *Homo sapiens*. Like modern humans themselves, symbolic behavior seems to have its origins in Africa. Recent excavations have turned up elaborate stone tools, beads, and ochre dating back 100,000 or more years ago, although researchers are still debating which of these finds are really demonstrate symbolic expression. But there's widespread agreement that the building blocks of symbolism preceded full-blown art. "When we talk about beads and art, we are actually talking about material technologies for symbolic expression that certainly postdate the origins of symbolic thought and communication, potentially by a very wide margin," says archaeologist Dietrich Stout of University College London.

The evolution of symbolism was once thought to have been as rapid as "flicking on a light switch," as archaeologist Clive Gamble of the Royal Holloway, University of London, put it some years ago. But given new evidence that symbolic behavior appears long before cave paintings, Gamble now says that his much-cited comment needs to be modified: "It's a dimmer switch now, a stuttering candle."

As they more precisely pinpoint when symbolic behavior began, scientists are hoping they might one day crack the toughest question of all: What was its evolutionary advantage to humans? Did symbols, as many researchers suspect, serve as a social glue that helped tribes of early humans to survive and reproduce?
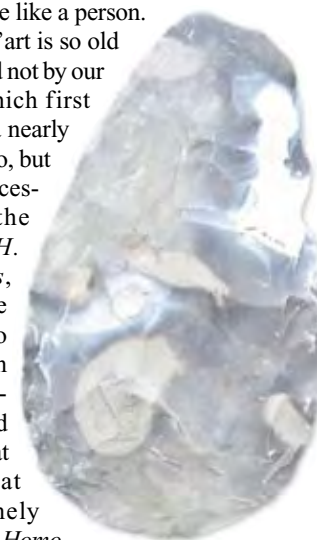
> Recent excavations have turned up elaborate stone tools, beads, and ochre dating back 100,000 or more years ago.
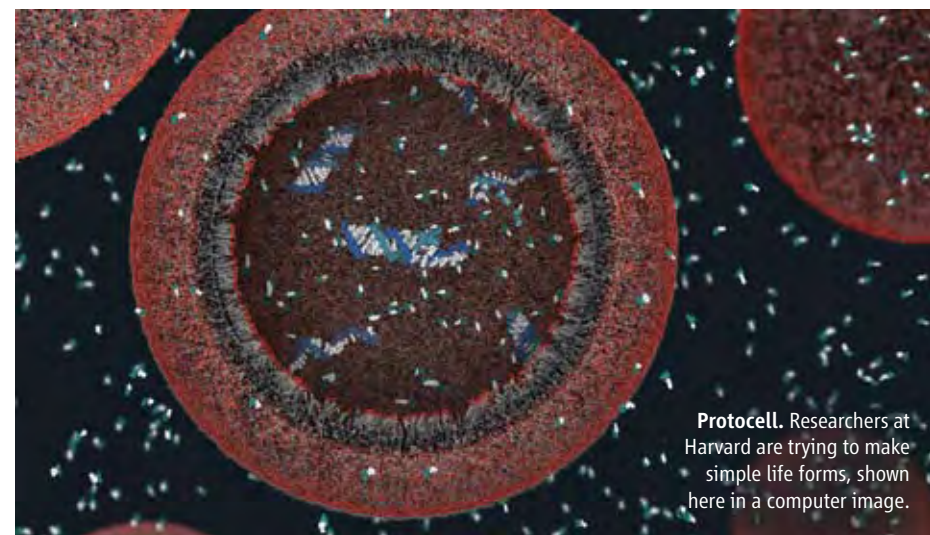
Venus, phallus, or pebble?

"I don't know much about Art, but I know what I like," quipped the humorist and art critic Gelett Burgess back in 1906. For archaeologists, distinguishing art from nonart is still quite a challenge. Take the 6-centimeter-long piece of quartzite known as the Venus of Tan-Tan. Found in Morocco in 1999 next to a rich trove of stone tools estimated to be between 300,000 and 500,000 years old, it resembles a human figure with stubby arms and legs. Robert Bednarik, an independent archaeologist based in Caulfield South, Australia, insists that an ancient human deliberately modified the stone to make it look more like a person. If so, this objet d'art is so old that it was created not by our own species, which first appears in Africa nearly 200,000 years ago, but by one of our ancestors, perhaps the large-brained *H. heidelbergensis*, thought by some anthropologists to be the common ancestor of modern humans and Neandertals. That would mean that art is an extremely ancient part of the *Homo* repertoire. "Ignoring the few specimens we have of very early paleoart, explaining them away, or rejecting them out of hand does not serve this discipline well," Bednarik wrote in a 2003 analysis of the Venus of Tan-Tan in *Current Anthropology*.

Symmetry in stone. Some stone tools require a mental image to create.

Yet many archaeologists are skeptical, arguing that the stone's resemblance to a human figure might be coincidence. Indeed, the debate over the Tan-Tan "figurine" is reminiscent of a similar controversy over a smaller stone discovered in 1981 at the site of Berekhat Ram in the Israeli-occupied Golan Heights. To some archaeologists, this 250,000-year-old object resembles a woman, but others argue that it was shaped by natural forces, and, in any case, looks more like a penguin or a phallus. Even after an exhaustive microscopic study concluded that the Berekhat Ram object had indeed been etched with a tool to emphasize what some consider its "head" and "arms," many researchers have rejected it as a work of art. For some, proof of symbolic behavior requires evidence that the symbols had a commonly understood mean-

A roaring start. Researchers agree that Chauvet Cave's magnificent paintings, including these lions, are full-blown art.

ing and were shared within groups of people. For example, the hundreds of bone and stone "Venus figurines" found at sites across Eurasia beginning about 30,000 years ago were skillfully carved and follow a common motif. They are widely regarded not only as symbolic expression, but full-fledged art.

Thus many researchers are reluctant to accept rare, one-off discoveries like the Tan-Tan or Berekhat Ram objects as signs of symbolic behavior. "You can imagine [an ancient human] recognizing a resemblance but [the object] still hav[ing] no symbolic meaning at all," says Philip Chase, an anthropologist at the University of Pennsylvania. Thomas Wynn, an anthropologist at the University of Colorado, Colorado Springs, agrees: "If it's a one-off, I don't think it counts. It's not sending a message to anyone."

Tools of the imagination

Given how difficult it is to detect the earliest symbolic messages in the archaeological record, some researchers look instead for proxy behaviors that might have required similar cognitive abilities, such as toolmaking. Charles Darwin himself saw an evolutionary parallel between tool-making and language, probably the most sophisticated form of symbolic behavior. "To chip a flint into the rudest tool," Darwin wrote in *The Descent of Man*, demands a "perfect hand" as well adapted to that task as the "vocal organs" are to speaking.

To many researchers, making sophisticated tools and using symbols both require the capac-

ity to hold an abstract concept in one's head—and, in the case of the tool, to "impose" a predetermined form on raw material based on an abstract mental template. That kind of ability was probably not needed to make the earliest known tools, say Wynn and other researchers. These implements, which date back 2.6 million years, consist mostly of rocks that have been split in two and then sharpened to make simple chopping and scraping implements.

Then, about 1.7 million years ago, large, teardrop-shaped tools called Acheulean hand axes appeared in Africa. Likely created by *H. erectus* and probably used to cut plants and butcher animals, these hand-held tools vary greatly in shape, and archaeologists have debated whether creating the earliest ones required an abstract mental template. But by about 500,000 years ago, ancient humans were creating more symmetrical Late Acheulean

> "If it's a one-off, I don't think it counts. It's not sending a message to anyone."
>
> —THOMAS WYNN, UNIVERSITY OF COLORADO, COLORADO SPRINGS

Symbolic start. Some scientists argue that this 77,000-year-old engraved ochre shows symbolic capacity.

tools, which Wynn and many others argue are clear examples of an imposed form based on a mental template. Some have even argued that these skillfully crafted hand axes had symbolic meanings, for example to display prestige or even attract members of the opposite sex.

The half-million-year mark also heralded the arrival of *H. heidelbergensis*, which had a much larger brain than *H. erectus*. Not long afterward, our African ancestors began to create a wide variety of finely crafted blades and projectile points, which allowed them to exploit their environment in more sophisticated ways, and so presumably enhance their survival and reproduction. Archaeologists refer to these tools as Middle Stone Age technology and agree that they did require mental templates. "The tools tell us that the hominid world was changing," says Wynn.

As one moves forward in time, humans appear able to imagine and create even more elaborate tools, sharpening their evolutionary edge in the battle for survival. By 260,000 years ago, for example, ancient humans at Twin Rivers in what is now Zambia could envision a complex finished tool and put it together in steps from different components. They left behind finely made blades and other tools that had been modified—usually by blunting or "backing" one edge—to be hafted onto handles, presumably made of wood. These so-called backed tools have been widely regarded as evidence of symbolic behavior when found at much younger sites. "This flexibility in stone tool manufacture [indicates] symbolic capabilities," says archaeologist Sarah Wurz of the Iziko Museums of Cape Town in South Africa.

Similar cognitive abilities were possibly required to make the famous 400,000-year-old wooden spears from Schöningen, Germany. One recent study concludes that these spears' creators—probably members of *H. heidelbergensis*—carried out at least eight preplanned steps spanning several days, including chopping tree branches with hand axes and shaping the spears with stone flakes.

The idea that sophisticated toolmaking and symbolic thought require similar cognitive skills also gets some support from a surprising quarter: brain-imaging studies. Stout's team ran positron emission tomography scans on three archaeologists—all skillful stone knappers—as they made pre-Acheulean and

Late Acheulean tools. Both methods turned on visual and motor areas of the brain. But only Late Acheulean knapping turned on circuits also linked to language, the team reported last year.

Color me red

At Twin Rivers, it's not just the tools that hint at incipient symbolic behavior. Early humans there also left behind at least 300 lumps of ochre and other pigments in a rainbow of colors: yellow, red, pink, brown, purple, and blue-black, some of which were gathered far from the site. Excavator Lawrence Barham of the University of Liverpool in the United Kingdom thinks they used the ochre to paint their bodies, though there's little hard evidence for this. Most archaeologists agree that the wearing of personal ornaments such as bead necklaces, was a key way that early humans symbolically communicated social identity such as membership in a particular group, much as people today declare social allegiances and individual personalities by their clothing and jewelry.

Yet while the Twin Rivers evidence is suggestive, it's hard to be sure how the ochre was actually used. There's little sign that it was ground into powder, as needed for decoration, says Ian Watts, an independent ochre expert in Athens. And even ground ochre could have had utilitarian uses, says archaeologist Lyn Wadley of the University of Witwatersrand in Johannesburg, South Africa. Modern-day experiments have shown that ground ochre can be used to tan animal hides, help stone tools adhere to bone or wooden handles, and even protect skin against mosquito bites.

"We simply don't know how ancient people used ochre 300,000 years ago," Wadley says. And since at that date the ochre users were not modern humans but our archaic ancestors, some experts are leery of assigning them symbolic savvy.

Yet many archaeologists are willing to grant that our species, *H. sapiens*, was creating and using certain kinds of symbols by 75,000 years ago and perhaps much earlier. At sites such as Blombos Cave on South Africa's southern Cape, people left sophisticated tools, including elaborately crafted bone points, as well as perforated beads made from snail shells and pieces of red ochre engraved with what appear to be abstract designs. At this single site, a number of what many archaeologists

Eye of the beholder. Archaeologists debate whether this modified stone was meant to represent a woman.

consider diagnostic elements of symbolic behavior came together. And in work now in press, the Blombos team reports finding engraved ochre in levels dating back to 100,000 years ago (*Science*, 30 January, p. 569).

There are other hints that the modern humans who ventured out of Africa around this time might also have engaged in symbolic behavior. At the Skhul rock shelter in Israel, humans left 100,000-year-old shell beads considered by some to be personal ornaments (*Science*, 23 June 2006, p. 1731). At the 92,000-year-old Qafzeh Cave site nearby, modern humans apparently strongly preferred the color red: Excavators have studied 71 pieces of bright red ochre associated with human burials. Some researchers argue that this represents an early case of "color symbolism," citing the universal importance of red in historical cultures worldwide and the apparently great lengths to which early humans went to gather red ochre. "There is very strong circumstantial evidence for the very great antiquity of the color red as a symbolic category," says anthropologist Sally McBrearty of the University of Connecticut, Storrs.

These finds of colorful ochre, fancy tools, and beads have convinced many researchers that the building blocks of symbolism had emerged by at least 100,000 years ago and possibly much earlier. But why? What selective advantages did using symbols confer on our ancestors?

To some scientists, the question is a no-brainer, especially when it is focused on the most sophisticated form of symbolic communication: language. The ability to communicate detailed, concrete information as well as abstract concepts allowed early humans to cooperate and plan for the future in ways unique to our species, thus enhancing their survival during rough times and boosting their reproductive success in good times. "What aspects of human social organization and adaptation wouldn't benefit from the evolution of language?" asked Terrence Deacon, a biological anthropologist at the University of California, Berkeley, in his influential book *The Symbolic Species: The Coevolution of Language and the Brain*. Deacon went on to list just some of the advantages: organizing hunts, sharing food, teaching toolmaking, sharing past experiences, and raising children. Indeed, many researchers have argued that symbolic communication is what held groups of early humans together as they explored new environments and endured climatic shifts.

As for art and other nonlinguistic forms of symbolic behavior, they may also have been key to cementing these bonds, by expressing meanings that are difficult or impossible to put into words. In that way, artistic expression, including music, may have helped ensure the survival of the fittest. This may also explain why great art has such emotional force, because the most effective symbols are those that convey their messages the most powerfully—something the artists at Chauvet Cave seem to have understood very well.
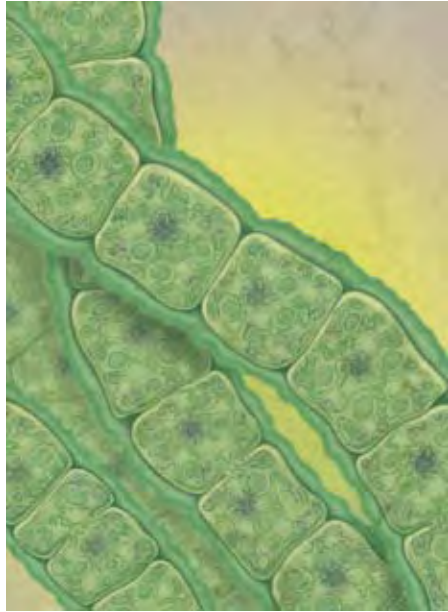
–MICHAEL BALTER

Online

sciencemag.org

Hear author Michael Balter discuss the roots of art at **www.sciencemag.org/darwin**.

On the Origin of
Photosynthesis

Try to picture the world without photosynthesis. Obviously, you'd have to strip away the greenery—not just the redwoods and sunflowers, but also the humble algae and the light-capturing bacteria that nourish many of the world's ecosystems. Gone, too, would be everything that depends on photosynthetic organisms, directly or indirectly, for sustenance—from leaf-munching beetles to meat-eating lions. Even corals, which play host to algal partners, would lose their main food source.

Photosynthesis makes Earth congenial for life in other ways, too. Early photosynthesizers pumped up atmospheric oxygen concentrations, making way for complex multicellular life, including us. And water-dwellers were able to colonize the land only because the oxygen helped create the ozone layer that shields against the sun's ultraviolet radiation. Oxygen-producing, or oxygenic, photosynthesis "was the last of the great inventions of microbial metabolism, and it changed the planetary environment forever," says geobiologist Paul Falkowski of Rutgers University in New Brunswick, New Jersey.

Given its importance in making and keeping Earth lush, photosynthesis ranks high on the top-10 list of evolutionary milestones. By delving into ancient rocks and poring over DNA sequences, researchers are now trying to piece together how and when organisms first began to harness light's energy. Although most modern photosynthesizers make oxygen from water, the earliest solar-powered bacteria relied on different ingredients, perhaps hydrogen sulfide. Over time, the photosynthetic machinery became more sophisticated, eventually leading to the green, well-oxygenated world that surrounds us today. In the lab, some biochemists are recapitulating the chemical steps that led to this increased complexity. Other researchers are locked in debates over just when this transition happened, 2.4 billion years ago or much earlier.

Looking so far into the past is difficult. The geological record for that time is skimpy and tricky to interpret. Eons of evolution have blurred the molecular vestiges of the early events that remain in living organisms. But "it's a terribly important problem," says biochemist Carl Bauer of Indiana University, Bloomington, one well worth the travails.

To catch a photon

Over more than 200 years, researchers have ironed out most of the molecular details of how organisms turn carbon dioxide and water into food. Chlorophyll pigment and about 100 other proteins team up to put light to work. Plants, some protists, and cyanobacteria embed their chlorophyll in two large protein clusters, photosystem I and photosystem II. And they need both systems to use water as an electron source. Light jump-starts an electrical circuit in which electrons flow from the photosystems through protein chains that make the energy-rich molecules ATP and NADPH. These molecules then power the synthesis of the sugars that organisms depend on to grow and multiply. Photosystem II—the strongest naturally occurring oxidant—regains its lost electrons by swiping them from water, generating oxygen as a waste product.

However, some bacteria don't rely on water as an electron source, using hydrogen sulfide or other alternatives. These nonconformists, which today live in habitats such as scalding hot springs, don't generate oxygen. Their photosynthetic proteins huddle in relatively simple "reaction centers" that may have been the predecessors of the two photosystems.

Envisioning the steps that led to this complex biochemistry is mind-boggling. Similarities between proteins in photosynthetic and nonphotosynthetic bacteria suggest that early microbes co-opted some photosynthesis genes from other metabolic pathways. But protophotosynthesizers might also have helped each other piece these pathways together by swapping genes. Biochemist Robert Blankenship of Washington University in St. Louis, Missouri, and colleagues say they've uncovered traces of these lateral gene transfers by comparing complete bacterial genomes. For example, their 2002 study of more than 60 photosynthetic and nonphotosynthetic bacteria (*Science*, 22 November 2002, p. 1616) suggested that bugs had passed around several photosynthesis genes, including some involved in synthesizing the bacterial version of chlorophyll.

Gene-sharing might also explain the puzzling distribution of the photosystems, Blankenship says. A cell needs both photosystems to carry out oxygenic photosynthesis. Yet modern nonoxygenic bacteria have the presumptive predecessor either of photosystem I or of photosystem II, never both. To explain how the two protein complexes wound up together, Blankenship favors "a large-scale lateral [gene] transfer" or even a fusion of organisms carrying each photosystem. However, other researchers remain skeptical, arguing that one photosystem evolved from the other, possibly through the duplication of genes, creating an ancient cell with both. No one knows for sure.

The electron thief

Either way, it took some fancy fiddling to convert the primitive reaction centers to oxygen-generating photosystems. Oxygenic photosynthesis was a huge upgrade, leading to a land of plenty, says biochemist John Allen of Queen Mary, University of London. "Water is everywhere, so the organisms never ran out of electrons. They were unstoppable."

But water clings to its electrons. With its oxidizing power, photosystem II can wrench them away, but the reaction centers in nonoxygenic photosynthesizers cannot. Biochemists James Allen (no relation to John Allen) and JoAnn Williams of Arizona State University, Tempe, and colleagues are working out how a bacterial reaction center could have evolved photosystem II's appetite for electrons.

Taking a hands-on approach, they have been tinkering with the reaction center of the purple bacterium *Rhodobacter sphaeroides* to determine if they can make it more like photosystem II. First they targeted bacteriochlorophyll, the bacterial version of chlorophyll that's at the core of the reaction center, and altered the number of hydrogen bonds. Adding hydrogen bonds hiked the molecule's greed for electrons, they found.

The water-cleaving portion of photosystem II sports four manganese atoms that become oxidized, or lose electrons. So the team equipped the bacterial reaction center with one atom of the metal. In this modified version, the added manganese also underwent oxidation, the researchers reported in 2005. James Allen says that their creations aren't powerful enough to split water. But eventually, they hope to engineer a reaction center that can oxidize less possessive molecules, such as hydrogen peroxide, that would have been present on the early Earth. Even if the researchers never replicate photosystem II, "if we define the intermediate stages, we've accomplished a lot," he says.

Something in the air

How the photosystems got their start is crucial for understanding the origin of photosynthesis. But the question that's drawn the most attention—and provoked the most wrangling—is when photosynthesis began. Most researchers accept that nonoxygenic photosynthesis arose first, probably shortly after life originated more than 3.8 billion years ago. "Life needs an energy source, and the sun is the only ubiquitous and reliable energy source," says Blankenship.

The sharpest disputes revolve around when organisms shifted to oxygenic photosynthesis. At issue is how to interpret a watershed in the fossil record known as the great oxidation event (GOE). In rocks from

Catching rays. Long before plants got in on the act, photosynthetic cyanobacteria living in pools like this one in Yellowstone National Park were changing the composition of the atmosphere.

> Oxygenic photosynthesis "was the last of the great inventions of microbial metabolism, and it changed the planetary environment forever."
> —Paul Falkowski, Rutgers University

about 2.4 billion years ago, geologists see the first unmistakable signs of significant, sustained levels of atmospheric oxygen. These signs include red beds, or layers tinged by oxidized iron, i.e., rust. Further support that the GOE marks an atmospheric revolution comes from a technique that detects skewed abundances of sulfur isotopes that occur if the air lacks oxygen. These imbalances persisted until the GOE, when they vanished.

Hard-liners construe these data to mean that oxygenic photosynthesis could not have emerged until shortly before the GOE. But other scientists disagree. "We are finding more and more hints that oxygenic photosynthesis goes deeper into the fossil record,"

says astrobiologist Roger Buick of the University of Washington, Seattle. These hints could push the origin back 600 million years or more.

One line of evidence is oil biomarkers that researchers think are the remains of cyanobacteria. They've turned up in rocks that are up to 2.7 billion years old. And in western Australia, thick shale deposits that are 3.2 billion years old hold rich bacterial remains but no traces of sulfur or other possible electron sources, suggesting that the microbes were using water to make energy.

Geologist Euan Nisbet of Royal Holloway, University of London, and colleagues found additional support for an early origin when they went searching for traces of RuBisCO, a key photosynthetic enzyme. RuBisCO feeds carbon dioxide into the reactions that yield sugars. The enzyme version found in oxygenic photosynthesizers plays favorites: It prefers carbon dioxide that contains the carbon-12 isotope over the bulkier carbon-13. In 2007, Nisbet and his colleagues found disproportionately low carbon-13 values indicative of RuBisCO activity when they analyzed organic matter in rocks from three sites in Zimbabwe and Canada that are between 2.7 billion and 2.9 billion years old. Nisbet concludes that oxygen-making photosynthesis began at least 2.9 billion years ago.

The early-origin case isn't ironclad. For example, a 2008 paper that has some researchers fuming claims that the oil biomarkers are contaminants that seeped in from younger rocks. Advocates also have to explain why it took hundreds of millions of years for oxygen to build up in the air.

Although the last word on the origins of oxygen-making photosynthesis isn't in, researchers say they are making progress. One thing is for certain, however: Without this innovation, Earth would look a lot like Mars.

–MITCH LESLIE

> Given its importance in making and keeping earth lush, photosynthesis ranks high on the top-10 list of evolutionary milestones.

On the Origin of
Flowering Plants

IN 1879, CHARLES DARWIN PENNED A LETTER to British botanist Joseph Dalton Hooker, lamenting an "abominable mystery" that threw a wrench into his theory of evolution: How did flowering plants diversify and spread so rapidly across the globe? From rice paddies to orange groves, alpine meadows to formal gardens, prairies to oak-hickory forests, the 300,000 species of angiosperms alive today shape most terrestrial landscapes and much of human life and culture. Their blooms color and scent our world; their fruits, roots, and seeds feed us; and their biomass provides clothing, building materials, and fuel. And yet this takeover, which took place about 100 million years ago, apparently happened in a blink of geological time, just a few tens of millions of years.

The father of evolution couldn't quite fathom it. Darwin had an "abhorrence that evolution could be both rapid and potentially even saltational," writes William Friedman in the January *American Journal of Botany*, which is devoted to this "abominable mystery." Throughout his life, Darwin pestered botanists for their thoughts on the matter, but they couldn't give him much help.

Now, 130 years later, evolutionary biologists are still pestering botanists for clues about what has made this plant group so successful, as well as when, where, and how flowers got started—and from which ancestor. Today, researchers have analytical tools, fossils, genomic data, and insights that Darwin could never have imagined, all of which make these mysteries less abominable. Over the past 40 years, techniques for assessing the relationships between organisms have greatly improved, and gene sequences, as well as morphology, now help researchers sort out which angiosperms arose early and which arose late. New fossil finds and new ways to study them—with synchrotron radiation, for example—provide a clearer view of the detailed anatomy of ancient plants. And researchers from various fields are figuring out what genomic changes that might explain the amazing success of this fast-evolving group.

These approaches have given researchers a much better sense of what early flowers were like and the relationships among them. But one of Darwin's mysteries remains: the nature and identity of the angiosperm ancestor itself. When flowering plants show up in the fossil record, they appear with a bang, with no obvious series of intermediates, as Darwin noted. Researchers still don't know which seed- and pollen-bearing organs eventually evolved into the comparable flower parts. "We're a bit mystified," says botanist Michael Donoghue of Yale University. "It doesn't appear that we can locate a close relative of the flowering plants."

Seeking the first flower

One of two major living groups of seed plants, angiosperms have "covered" seeds that develop encased in a protective tissue called a carpel (picture a bean pod). That's in contrast to the nonflowering gymnosperms, such as conifers, which bear naked seeds on scales. An angiosperm's carpel sits at the center of the flower, typically surrounded by pollen-laden stamens. In most flowers, the carpel and stamens are surrounded by petals and an outer row of leaflike sepals. Seeds have a double coating as well as endosperm, tissue surrounding the embryo that serves as its food supply.

Darwin was perplexed by the diversity of flowering plants; they were too numerous and too varied, and there were too few fossils to sort out which were more primitive. Throughout much of the 20th century, magnolia relatives with relatively large flowers were leading candidates for the most primitive living flowers, although a few researchers looked to small herbs instead.

In the late 1990s, molecular systematics came to the rescue, with several reports presenting a fairly consistent picture of the lower branches of the angiosperm tree. An obscure shrub found only in New Caledonia emerged as a crucial window to the past. *Amborella trichopoda*, with its 6-millimeter greenish-yellow flowers, lives deep in the cloud forests there. In multiple gene-based assessments, including an analysis in 2007 of 81 genes from chloroplast genomes belonging to 64 species, *Amborella* sits at the base of the angiosperm family tree, the sister group of all the rest of the angiosperms.

Given that placement, *Amborella*'s tiny flowers may hint at what early blossoms were like. It's one of "the most similar living flower[s]" to the world's first flower, says James Doyle of the University of California, Davis. The petals and sepals of its single-sex flowers are indistinguishable and vary in number; so too do the numbers of seed-producing carpels on female flowers and pollen-generating stamens on male flowers. The organs are spirally arranged, and carpels, rather than being closed by fused tissue as in roses and almost all familiar flowers, are sealed by a secretion.

Most genetic analyses showed that water lilies were the next branch up the angiosperm tree, followed by a group represented by star anise, which also has a primitive look about it, says Doyle, "though each of these has deviations from the ancestral type."

Fossil records

Although some fossil pollen dates back 135 million years, no credible earlier fossil evidence exists. In Darwin's day, and for many decades afterward, paleobotanists primarily found leaves or pollen but almost no fossil flowers. They had the wrong search image, says Else Marie Friis of the Swedish Museum of Natural History in Stockholm. "When we started, the search profile was bigger, a magnolia [flower]," she recalls. But 30 years ago, she and others discovered tiny ancient flowers by sieving through sand and clay sediments. With this technique, they have now collected hundreds of millimeter-size flowers, some preserved in three dimensions, from Portugal and other locations with Cretaceous deposits 70 million to 120 million years old.

This fossil diversity shows that angiosperms were thriving, with several groups well-established, by 100 million years ago. In some, the flower parts are whorled like those of modern flowers; in others they are spiraled, considered by some researchers as the more primitive arrangement. Some flower fossils have prescribed numbers of petals, another modern feature, whereas in others the petal count varies.

In 1998, Chinese geologist Ge Sun of Jilin University in Changchun, China, came across what seemed to be a much older flower. The fossil, called *Archaefructus*, was an aquatic plant that looked to be 144 million years old. By 2002, Sun and David Dilcher of the Florida Museum of Natural History (FLMNH) in Gainesville had described an entire plant, from roots to flowers, entombed on a slab of rock unearthed in Liaoning in northeastern China.

In one sense, *Archaefructus* wasn't much to look at. "It's a flowering plant before there were flowers," Dilcher notes. It lacked petals and sepals, but it did have an enclosed carpel. When Kevin Nixon and colleagues at Cornell University compared its traits with those same traits in 173 living plants, *Archaefructus* came out as a sister to living angiosperms and closer to the common ancestor than even *Amborella*.

Archaefructus's distinction was short-lived, however. Within months, better dating of the sediments in which it was found yielded younger dates, putting this first flower squarely with other early fossil flower parts, about 125 million years old. Also, a 2009 phylogenetic analysis of 67 taxa by Doyle and Peter Endress of the University of Zurich, Switzerland, placed the fossil in with water lilies rather than at the base of the angiosperms, although this conclusion is contested.

> "We are realizing that this huge diversity is probably the result of one innovation piled on top of another innovation."
> —Peter Crane, University of Chicago

These fossils often spark debate because specimens tend to be imperfectly preserved and leave room for interpretation. To help remedy that, Friis and her colleagues have begun to examine flowers using synchrotron radiation to generate a 3D image of their inner structures, allowing the fossil to remain intact while Friis peers inside it from many angles (*Science*, 7 December 2007, p. 1546). "We can get fantastic resolution," says Friis. "It's really exciting." But so far, the flowers Friis finds are too diverse to trace back to a particular ancestor. "From these fossils, we cannot say what is the basic form," she says.

Before flowers

Although they have yet to find the oldest fossil flowers, researchers assume that the ancestral angiosperm evolved from one of the nonflowering seed plants or gymnosperms, whose heyday was 200 million years ago. Modern gymnosperms include conifers, ginkgoes, and the cycads, with their stout trunks and large fronds. Before angiosperms came along, these plants were much more diverse and included cycadlike species, such as the extinct Bennettitales, and many woody plants called Gnetales, of which a few representatives, including the joint firs, survive today (see family tree, p. 31). Also common in the Jurassic were seed ferns, a group now long gone; their most famous member is *Caytonia*, which seems to have precarpel-like structures. These groups' perceived relevance to flower evolution and their relationships to angiosperms have ping-ponged between camps, depending on how the evolutionary trees were constructed.

In the mid-1980s, Peter Crane, now at the University of Chicago in Illinois, proposed a solution, the anthophyte hypothesis. Using several lines of evidence and noting that both Bennettitales and Gnetales organize their male and female organs together in what could be construed as a preflower, he considered them, along with angiosperms, as comprising a single angiosperm entity called anthophytes. For the next decade, most family trees based on morphology sup-

Out of the past. Tiny *Amborella* sits at the bottom of the angiosperm family tree.

Larger than life. Although merely 2.2 millimeters in diameter, this 3D fossil flower shows that grasses date back to 94 million years ago.

For more on flower origins, listen to a podcast by author Elizabeth Pennisi at **www.sciencemag.org/multimedia/podcast.**

Flowers, food, fuel. Darwin marveled at the diversity of angiosperms. Given that they represent nine in 10 land plants, it's no surprise that they serve as mainstays of both our welfare and sense of beauty. Clockwise from top left: aspens, orchids, grasses, sunflowers, tulips, apples, walnuts.

ported this idea. Crane and others carefully dissected and described fossils of these groups, looking for the precursors to carpels, the seed's double coat, and other distinctive angiosperm traits.

But they have run into problems. "We do not really know how to compare them because the structures are very different-looking; figuring out what's homologous is quite a difficult thing," says Crane. He and his colleagues argue, for example, that the seeds in the Bennettitales have two coverings, which may be a link to angiosperms. But in the January *American Journal of Botany*, Gar Rothwell of Ohio University, Athens, and two colleagues disagree, saying that what Crane calls the outer layer is the only layer, and find fault with the hypothesis in general.

To make matters worse for anthophyte proponents, gene-based evolutionary trees break up this grouping, pulling the Gnetales off any angiosperm branch and placing them among or next to the other gymnosperms. "The molecular work points in one direction; the paleobotanical
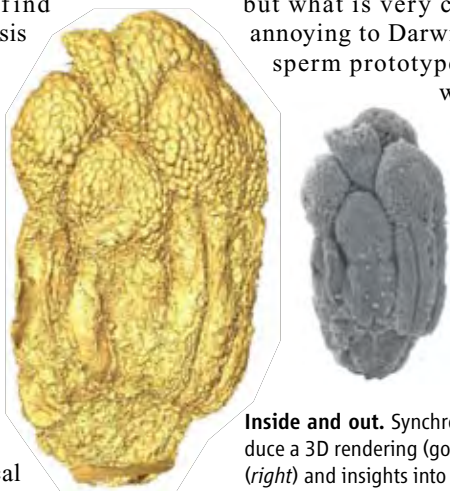
Inside and out. Synchrotron radiation helped produce a 3D rendering (gold) of this fossil male flower (*right*) and insights into its internal structure.

work points you in another direction," Crane says.

And if the molecular work is correct, then the field doesn't know in which direction to turn, because in most analyses the genetic data don't place any living plant close to angiosperms. The angiosperms group together, the living gymnosperms group together, and there's nothing in between. "The nonangiosperm ancestor just isn't there," says paleobotanist William Crepet of Cornell. "I'm starting to worry that we will never know, that it transformed without intermediates."

Seeds of success

The angiosperm's ancestor may be missing, but what is very clear—and was quite annoying to Darwin—is that the angiosperm prototype so readily proved a winner. Seed ferns and other gymnosperms arose about 370 million years ago and dominated the planet for 250 million years. Then in a few tens of millions of years, angiosperms edged them out. Today, almost nine in 10 land plants are angiosperms.

The exact timing of the angiosperms' explosion and expansion is under debate, as is the cause. At least one estimate based on the rate at which gene sequences change—that is, the ticking of the molecular clock—pushes angiosperm evolution back to 215 million years ago. "There appears to be a gap in the fossil record," says Donoghue, who also notes that molecular dating methods "are still in their infancy" and, thus, could be misleading. He and others think that flowering plants lingered in obscurity for tens of millions of years before radiating toward their current diversity.

Whatever the timing, there was something special about the angiosperm radiation. During the 1980s and again in 1997, Cornell's Karl Niklas compiled a database showing the first and last occurrences of fossil plants. When he and Crepet used that and more recent information to look at species' appearances and disappearances, they found that new angiosperms appeared in bursts through time, whereas other plants, such as gymnosperms, radiated rapidly only at first. Moreover, angiosperms proved less likely to disappear, somehow resisting extinction, says Crepet.

Once the angiosperms arrived, how did they diversify and spread so quickly? Darwin suspected that coevolution with insect pollinators helped drive diversification, though

such a causal relationship is not settled. Later, animals that ate fruit and dispersed seeds likely helped evolving species expand quickly into new territory. Some think the answer lies in genes: duplications that gave the angiosperm genome opportunities to try out new floral shapes, new chemical attractants, and so forth. This flexibility enabled angiosperms to exploit new niches and set them up for long-term evolutionary success. "My own view is that in the past, we have looked for one feature," says Crane. Now, "we are realizing that this huge diversity is probably the result of one innovation piled on top of another innovation."

The latest insights into diversification come from gene studies. From 2001 to 2006, Pamela Soltis of the FLMNH and Claude dePamphilis of Pennsylvania State University, University Park, participated in the Floral Genome Project, which searched for genes in 15 angiosperms. Now as a follow-up, the Ancestral Angiosperm Genome Project looks at gene activity in five early angiosperms and a cycad, a gymnosperm.

DePamphilis and his colleagues matched all the genes in each species against one another to determine the number of duplicates. They then looked at the number of differences in the sequences of each gene pair to get a sense of how long ago the duplication occurred. In most early angiosperms, including water lilies and magnolias, they saw many simultaneous duplications—but not in *Amborella*, they reported in the January 2009 *American Journal of Botany*, confirming earlier reports. The data suggest that a key genome duplication happened after the lineage leading to *Amborella* split off but before water lilies evolved. "We're beginning to get the idea that polyploidization may have been a driving force in creating many new genes that drive floral development," dePamphilis says.

Others have noted that a duplication occurred in the evolution of grasses, and the Floral Genome Project confirms that yet another duplication paved the way for eudicots, the group that includes apples, roses, beans, tomatoes, and sunflowers. "There are some real 'hot spots' in angiosperm evolutionary history," says dePamphilis, who is working to fully sequence the genome of *Amborella* with his colleagues.

SEED PLANT PHYLOGENY
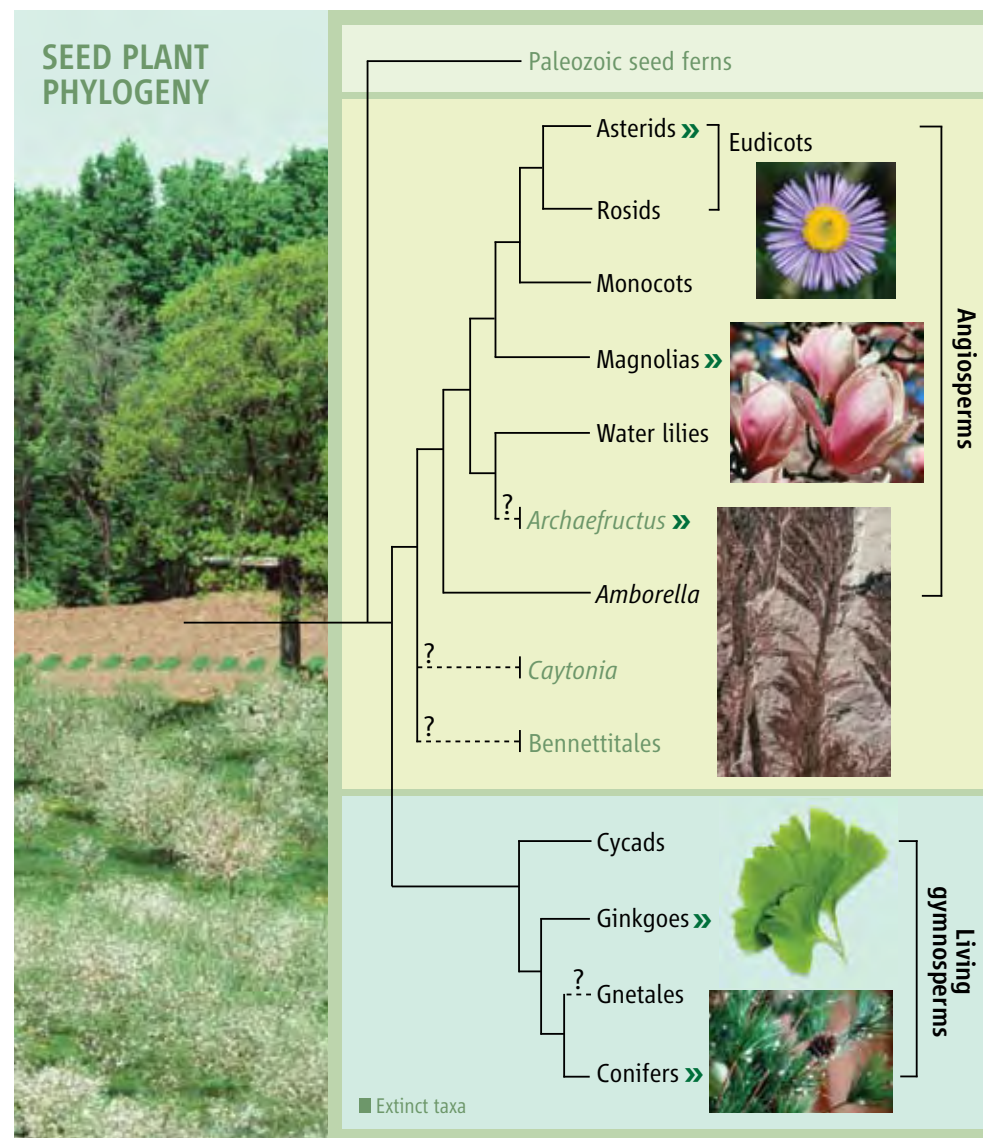
Shifting branches. As this simplified family tree shows, gene studies have helped clarify the relationships of many living angiosperms, but fitting in extinct species is still a challenge, and some nodes are hotly debated.

The Floral Genome Project also looked to see whether the genetic programs guiding flower development were consistent throughout the angiosperms. "We found that there are fundamental aspects that are conserved in the earliest lineages," says Soltis. "But there are differences in how the genes are deployed."

Take the avocado, a species on the lower branches of the angiosperm tree. In most angiosperms, the flower parts are arranged in concentric circles, or whorls, around the carpels, with stamens innermost, then petals, and finally sepals. Each tissue has its own distinct pattern of gene expression, but not in the avocado. Genes that in *Arabidopsis* are active only in, say, the developing petals spill over in avocado to the sepals. Thus in the more primitive plants, petals and sepals are not as well-defined as they are in *Arabidopsis*. This sloppiness may have made development flexible enough to undergo many small changes in expression patterns and functions that helped yield the great diversity in floral forms.

In his letter to Hooker, Darwin wrote that he would like "to see this whole problem solved." A decade ago, Crepet thought Darwin would have gotten his wish by now. That hasn't happened, but Crepet is optimistic that he and his colleagues are on the right track, as analyses of various kinds of data become more sophisticated. "We are less likely to go around in circles in the next 10 years," he says. "I believe a solution to the problem is within reach. … The mystery is solvable."

–ELIZABETH PENNISI

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Alexander von Humboldt and the General Physics of the Earth

Stephen T. Jackson
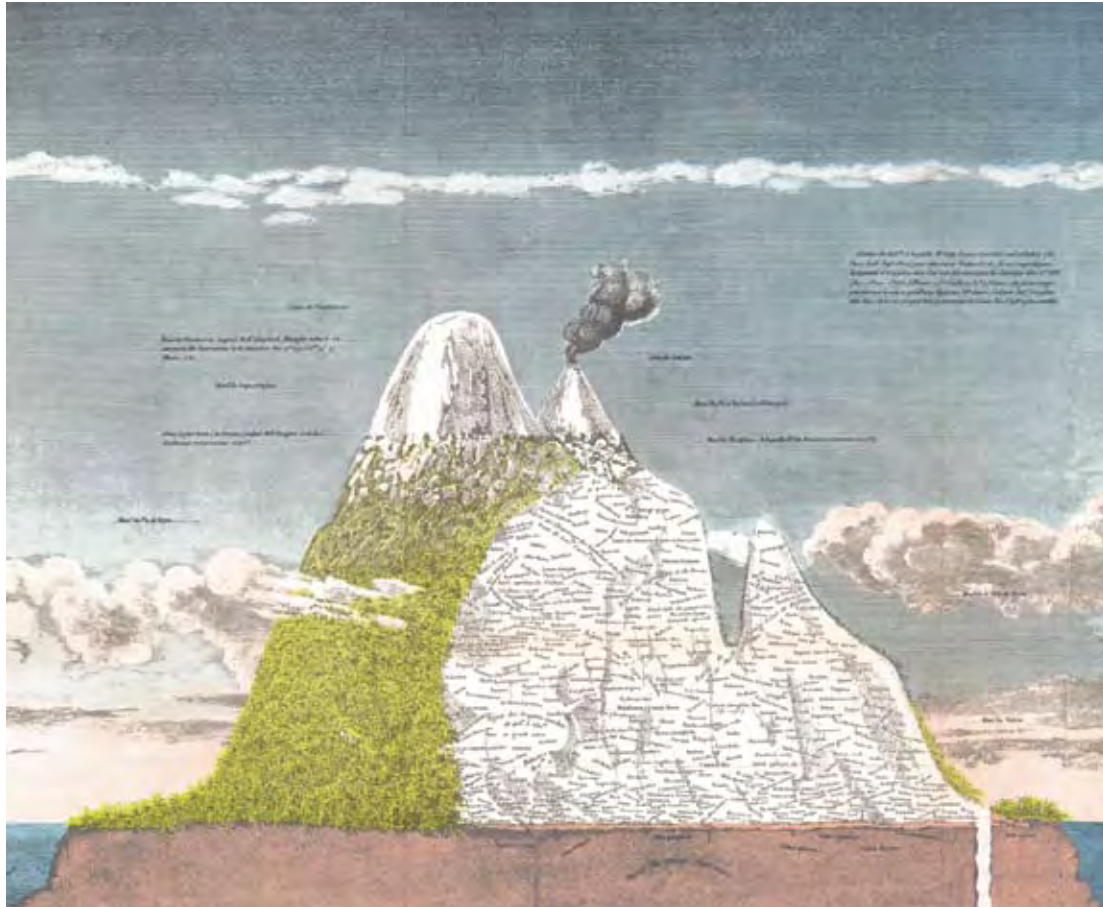
Intellectual riches. The central portion of Humboldt's *Physical Tableau of the Andes and Neighboring Countries*, published as part of (*2*, *3*), shows Chimborazo in profile, with vegetation zones, plant species, and snowline depicted at appropriate elevations. In the original, the profile is flanked on both sides by tables describing elevational patterns in temperature, humidity, light refraction and intensity, agriculture, fauna, and other physical, chemical, and biological features.

A s scientists are celebrating the 200th anniversary of Charles Darwin's birth and the 150th anniversary of the publication of his *On the Origin of Species*, Darwin's ideas continue to shape and enrich the sciences (*1*). 6 May 2009 marks the 150th anniversary of the death of another 19th-century figure—Alexander von Humboldt—whose scientific legacy also flourishes in the 21st century. Humboldt helped create the intellectual world Darwin inhabited, and his writings inspired Darwin to embark on *H.M.S. Beagle*. More pertinent to our time, Humboldt established the foundation for the Earth system sciences: the integrated system of knowledge on which human society may depend in the face of global climate change.

Like Darwin, Humboldt undertook a major voyage that would shape his ideas and thinking. Humboldt spent 5 years (1799 to 1804) with botanist Aimé Bonpland exploring Venezuela, the northern Andes, and central Mexico, with visits to Tenerife, Cuba, and the United States. They collected botanical, zoological, geological, and ethnological specimens, made extensive atmospheric and geophysical measurements, and recorded the geographic location of their thousands of specimens and tens of thousands of measurements. Humboldt spent the next 22 years and most of his inherited fortune in Paris, preparing and publishing 45 volumes of a never-finished report on his travels.

Of these volumes, the first was a slim work entitled *Essay on the Geography of Plants* (*2*, *3*). The modest title belies the intellectual richness within. In the text and accompanying color plate (see the figure), Humboldt lays out a vision of a comprehensive "general physics of the Earth" aimed at nothing less than a synthesis of atmospheric, oceanic, geological, ecological, and cultural phenomena across the globe. Humboldt's obsession with geographically referenced measurements and collections was central to his vision. He recognized that spatial arrays of observations could be aggregated to reveal patterns that would in turn reveal underlying processes—such as the distribution of incident radiation, the transport of heat and materials in winds and ocean currents, the influence of temperature on plant form, and the effect of latitude and continentality on mountain snowline.

He expanded this vision in the succeeding years, establishing international cooperative networks of meteorological and geomagnetic measurement stations, inventing isotherms and other graphical devices to portray spatial patterns, and noting that plant form is often better predicted by local environment than by taxonomic affinity (a paradox resolved by Darwin). Humboldt's genius lay in his geographical vision, and in his intuition that Earth's land surface, oceans, atmosphere, and inhabitants form an integrated whole, with linkages among the various components (*4*, *5*). Humboldt's general physics of the Earth envisioned climate as a major control of Earth-surface phenomena, with vegetation serving as both an index of climate and a proximal control of microclimate, animal habitat, and cultural practices (*6*–*8*).

Humboldt's dream of systematic observational arrays across the globe took hold in the 19th century. Throughout the century, countless Humboldt-inspired explorations were launched, each involving systematic measurement and mapping of physical, biological, and often cultural features of landscapes and oceans (*8*–*10*). These surveys were relentlessly inductive, typically producing detailed descriptive reports with little integration within or among the component entities. However, for a few intellectually nimble participants—including Charles Darwin, T. H. Huxley, Matthew Maury, Asa Gray, C. Hart Merriam, and Peter Kropotkin—these explorations provided data and experience that spurred the development of biogeography, ecology, oceanography, and other environmental sciences (*11*).

Unfortunately, the conceptual unification among the sciences of the Earth that Humboldt sought never developed in the century following his death. Disciplinary specialization played a large role in eclipsing Humboldt's integration, as did 20th-century trends toward reductionism, experimentalism, and fine-scale processes in many disciplines.

A new incarnation of Humboldt's general physics of the Earth began to emerge with the plate tectonics revolution in the 1960s. Drawing on Humboldtian spatial arrays of observations, this theory provided a unified explanatory framework for disparate geophysical, geological, paleontological, and biogeographic phenomena.

Today, a second, even broader manifestation of Humboldt's vision aspires to understand the interactions and feedbacks among the components of the Earth system, encompassing the lithosphere, atmosphere, hydrosphere, cryosphere, and biosphere as well as human societies and economies. This effort is often referred to as Earth system science, but it could just as well be designated "general physics of the Earth," using the early-19th century definition of physics as the study of the material world and its phenomena (which we now call science).

Global environmental change may be the greatest challenge faced by human societies since the advent of agriculture. Humboldt advocated for science that spoke to human needs and concerns (*5*). It is fitting that on the 150th anniversary of his death, we recognize his role in fostering the sciences that speak to the most profound human concerns—sustainability of human societies and the ecosystems on which they depend.

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In the early 19th century, Alexander von Humboldt laid the foundations for today's Earth system sciences.

Making German Evolution: Translation and Tragedy

Lynn K. Nyhart

In this year of Darwin anniversaries (the 200th year of his birth and the 150th anniversary of *On the Origin of Species*), *The Tragic Sense of Life* and *H. G. Bronn, Ernst Haeckel, and the Origins of German Darwinism* remind us that the history of evolutionary thought in the 19th century extended well beyond Darwin himself. Darwin did not launch his theory onto an unprepared public and scientific community, nor was the evolutionism that developed after 1859 a mere extension of his views—it was not even one thing. How, then, should we think about the history of evolution in the 19th century? What sorts of accounts best help us understand the reception of Darwin's theory, its relations to earlier ideas about nature, the directions that evolutionary investigation subsequently took, and the relations of all of these to the broader social, cultural, and religious concerns scientists shared with their contemporaries?

These questions become especially pointed when one considers German Darwinism, and especially Germany's best-known follower of Darwin, Ernst Haeckel. Most often remembered by biologists as the author of the biogenetic law ("ontogeny recapitulates phylogeny"), Haeckel has also been accused of promoting European fascism via his monistic philosophy and of presenting a eugenic, biologically determinist vision of humanity that led to Hitler's "final solution." Can one scientist be responsible for so much? Most historians would say no, arguing that it takes a community, rather than an individual, to make a movement; that single-cause explanations are insufficient to account for something as broad as fascism; and that an individual cannot be held responsible for the ways in which others (such as Hitler) took up his ideas and molded them to new agendas after his death. But that still leaves open the questions of how to write responsibly about what Haeckel actually believed and how we should situate him in the history of evolutionary thought.

The historians under consideration here have chosen two radically different strategies to understanding Haeckel's place within German evolutionism, and both have pro-

duced important books. Robert J. Richards, the director of the University of Chicago's Fishbein Center for the History of Science and Medicine and a much-published author on Darwin and German Romantic biology, has written a biography of Haeckel. Sander Gliboff, a professor in Indiana University's Department of History and Philosophy of Science, places Haeckel at the end of a study that examines the larger process through which Darwin's words were translated, and his ideas modified, in the context of German biology. Both illuminate the twists and turns that evolutionary thought took in Germany, but they do so in dramatically different ways.

Richards's book, though over twice as long as Gliboff's, is the more entertaining read of the two. In his characteristically rich and rolling prose, Richards weaves a compelling story of a life marked by tragedy and of an intense, larger-than-life figure whose passions drove his scientific research and philosophy. In Richards's rendering, the scientific Haeckel cannot be understood separately from the man's personality and private circumstances. His love of nature was surpassed only by his love for his first wife, Anna Sethe, who died in abdominal agony on his 30th birthday. Over the next year, he wrote his way through the despair that enveloped him, producing his foundational work, *Generelle Morphologie* (*1*). Although he remarried, the union was not happy, and passionate love would elude him until his sixties, when he had a secret affair that ended tragically with the death of his lover. Science remained his salvation and refuge.

His professional life was also filled with drama, much of which centered on his philosophy of evolutionary monism—a science-centered faith that became one of the most successful alternatives to the Judeo-Christian religion among those searching for a secular spirituality. Haeckel could not turn down a fight: He battled the physician-statesman Rudolf Virchow over the role of evolution in the schools (Haeckel argued that it should replace religious education), sparred with religiously conservative scientists and with former students who challenged his ideas as they gained intellectual independence, and debated the pro-evolution (but anti-Haeckel) Jesuit priest and entomologist Erich Wasmann—the list could go on and on. These were not isolated episodes but rather moments in a lifelong campaign to advance his philosophy, which was accompanied by a bitter hostility to organized religion.

Richards does not neglect Haeckel's science proper, treating us to fascinating and original discussions of his pathbreaking systematic and phylogenetic work on radiolaria and other marine organisms, the importance of linguistic analysis to his phylogenetic trees of the races of humans, and his remarkable experimental work with siphonophores. These constitute important contributions to our understanding of the technical development of evolutionary biology.

The big picture here, however, is an argument about the power of personality—at least one personality—to shape the course of science. In Richards's presentation, German evolutionism was profoundly shaped by both Haeckel's charisma and his combativeness. Perhaps the late-19th-century opposition of evolutionary science to Christianity would not have been so fiery, he suggests, had Haeckel not continually fanned its flames. And although Richards absolves Haeckel of personal responsibility for fascism and Nazism, in part by situating him firmly in his time and place, he does show how the scientist's ardent temperament led him to the occasional intemperate statement that could be taken up by extreme thinkers. One cannot leave this book without a deep appreciation for Haeckel as a tragic figure and for the force of personality in shaping the direction science may take.

Gliboff's account is of a completely different order. His is not a story of personalities or private lives (although he mentions salient details), but of German academics seeking to live up to the highest (if changing) ideals of *Wissenschaft* and of the ways in which Darwin's theory was translated into this environment. He thus situates Haeckel at the end of a revised intellectual history of 19th-century German evolutionism. Central to his account is the idea of translation, which he uses both synchronically, especially in treat-

ing the translation of *Origin of Species* into German, and (more intriguingly) diachronically, as scientists reworked older words such as "perfection" and "type" to lend them new meanings. Gliboff's own clear, crisp prose is key to the success of this analysis, as he deftly leads his reader through dense philosophical and terminological thickets with nary a thorn scratch. This is some of the best close reading I have seen. It also represents a profound challenge to our standard picture of 19th-century German biology.

The old story, crudely put, is that Haeckel's version of evolution was a Darwinism in name only, best understood as an update on early-19th-century idealistic morphologists such as Carl F. Kielmeyer and J. F. Meckel that retained their teleology, their typological emphasis on form, and their linear recapitulationism. This story, emphasizing the long persistence of a German transcendental approach to nature, has been deeply entrenched in the history of biology.

Gliboff challenges this history right from the beginning. The ascription of simple linear recapitulationism to the views of Romantic embryologists, he notes, owes much to a caricature developed by Karl Ernst von Baer in a polemical context, then adopted uncritically by influential historians such as E. S. Russell and Stephen Jay Gould. Gliboff's fresh reading of the original sources interprets Kielmeyer and Meckel as far less rigidly typological in their orientation and much more attentive to nature's variability than has been seen before. Both for these early-19th-century naturalists and for their intellectual heirs, Gliboff argues, the critical issue was to understand nature's manifold variety while seeking out underlying strict natural laws to account for it.

This provides a new starting point for analyzing Darwin's first translator, the prominent paleontologist H. G. Bronn—a figure little attended to in the standard story but the lynchpin of Gliboff's. Intriguingly and plausibly, Gliboff argues that Bronn's use of terms like "vervollkommnet" (perfect) as translations for Darwin's "improved" or "favored" were not about dragging Darwin backward into a German teleological view of nature (as has been claimed by those who have paid attention to Bronn at all). Instead, Gliboff asserts, Bronn's

translations involved an attempt to recast existing German terms in a newer, more up-to-date mode that encompassed selection yet tamed Darwin's emphasis on unpredictability to meet the more rigorous requirements of a German academic scientist's understanding of a "law" of organic nature. Simultaneously, Bronn sought to translate Darwin's ideas about selection into a language without an exact equivalent for the term, and for an academic audience lacking the gentlemanly traditions of breeding pigeons and dogs so central to Darwin's exposition. The selection metaphor was further fraught with an anthropomorphism foreign to Germans, who were not brought up on British natural-theological assumptions about a personified God who had created a perfectly adapted nature. Bronn's translation, though it altered key ideas to make Darwin comprehensible to a German academic audience, was not a conservative throwback. It represented the dynamic engagement of a leading paleontologist who had also long been working on many of the questions Darwin claimed as his own—a critical yet generous equal, who saw himself as moving science forward through the modifications he made to Darwin's flawed theory. Bronn's death in 1862 afforded him little chance to steer the conversation further.

And so, finally, we come to Haeckel. Gliboff's key insight here is that Haeckel originally read Bronn's translation of Darwin, not Darwin in the original. Gliboff shows Haeckel as both echoing and responding to Bronn's concerns, rather than either reflecting directly on Darwin's original writing or reaching directly back to the Romantic embryologists. (Although Gliboff acknowledges the centrality of monism to Haeckel's thought, he focuses on the working evolutionary theorist, not the popular ideologue.) Like Bronn himself, Haeckel made further amendments both terminological and intellectual, and Gliboff rereads Haeckel's research program as one not dominated by a typological and linear-recapitulationist mindset but rather as continuing to wrestle with the need to account for variability and unpredictable change in terms of mechanistic laws of nature—among which Haeckel included, at the top of his list, natural selection. Haeckel's Darwinism thus shows continuity with early-19th-century concerns, mediated through Bronn. But those concerns were always more flexible than has been acknowledged, and their articulation changed over time. Of course Haeckel's Darwinism was not Darwin's own, but it was not an aberration or a distortion of some true theory, any more than any other post-Darwinian additions or adjustments were. It was science moving on.

Gliboff's overall picture of scientific advance, in contrast to Richards's emphasis on charisma and passion, is one of scientists building and innovating incrementally, working with what their predecessors have handed them and sculpting it into something new yet understandable to those around them. His sensitive reading allows us to see post-1859 German evolutionists as rational actors rather than irrationally stuck in some early-19th-century moment with unmodern commitments. By challenging the very foundations of the standard narrative of German morphology, this careful, compelling account does at least as much as Richards's to undermine the association of 19th-century German Darwinism with a dangerously exceptional view of nature. But the two books offer very different reads. Is scientific progress a matter of personal anguish and triumph, or of intellectual chugging along? Our concept of it should be capacious enough to include both.

A painter, too. Haeckel's oil landscape of highlands in Java, from *Wanderbilder* (1905).

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Darwin's Originality

Peter J. Bowler

Charles Darwin's theory of natural selection has been hailed as one of the most innovative contributions to modern science. When first proposed in 1859, however, it was widely rejected by his contemporaries, even by those who accepted the general idea of evolution. This article identifies those aspects of Darwin's work that led him to develop this revolutionary theory, including his studies of biogeography and animal breeding, and his recognition of the role played by the struggle for existence.

The publication of Charles Darwin's *On the Origin of Species* in 1859 is widely supposed to have initiated a revolution both in science and in Western culture. Yet there have been frequent claims that Darwinism was somehow "in the air" at the time, merely waiting for someone to put a few readily available points together in the right way [for instance (*1*)]. The fact that Alfred Russel Wallace (Fig. 1) independently formulated a theory of natural selection in 1858 is taken as evidence for this position. But Darwin had created the outlines of the theory 20 years earlier, and there were significant differences between the ways in which he and Wallace formulated their ideas. In this essay, I argue that Darwin was truly original in his thinking, and I support this claim by addressing the related issue of defining just why the theory was so disturbing to his contemporaries.

Darwin was certainly not the first to suggest the idea of evolution as an alternative to the creation of species by God. J. B. Lamarck's theory, published in 1809, had been widely discussed, although generally rejected (*2–4*). Robert Chambers's *Vestiges of the Natural History of Creation* of 1844 sparked a debate over the possibility that new species were produced from pre-existing ones in a progressive sequence leading up to humans (*5*). But if the general idea of evolution was not entirely new, Darwin's vision of how the process worked certainly was. Although the theory was eventually paralleled by Wallace, Darwin had conceived its basic outline in the late 1830s, after his return from the voyage of H.M.S. *Beagle*. He worked on it in relative isolation over the next 20 years, until the arrival of Wallace's paper in 1858 precipitated the flurry of activity leading to the publication of the *Origin*.

Historians have quarried Darwin's notebooks and letters to establish the complex process by which he developed his theory (*6–9*). Darwin was a highly creative thinker who synthesized a number of key insights, some derived from his scientific work and others from currents circulating in his cultural environment. Few would now accept the claim that natural selection was in the air. Darwin approached the subject in a way that was significantly different from any of the other efforts being made to explain the history of life on earth. He had a unique combination of scientific interests that alerted him to topics ignored by other naturalists. He certainly drew on ideas widely discussed at the time, but was forced by his scientific interests to use those sources of inspiration in a highly original way.

To some extent, Darwin may have been merely "ahead of his time," anticipating developments that would push other naturalists toward an evolutionary vision during the years he worked in isolation. By the late 1850s, the idea of progressive evolution was widely recognized, and the positive role of individual competition was being articulated by thinkers such as Herbert Spencer (Fig. 1). But key aspects of the Darwinian vision were truly original and would not have occurred to any other naturalist at the time. Here, Wallace provides a good comparison: He too moved toward the idea of branching evolution driven by local adaptation, but even he did not share Darwin's insight that the work of the animal breeders throws light on the process of natural selection.

The theory was both original and disturbing. It was not just that the idea of natural selection challenged the belief that the world was designed by a wise and benevolent God. There was a wider element of teleology or goal-directedness almost universally accepted at the time. Most thinkers—including Jean-Baptiste Lamarck and Chambers—took it for granted that the development of life on earth represents the unfolding of a coherent plan aimed at a predetermined goal. (This assumption is still preserved in the very term "evolution"; the Latin *evolutio* refers to the unrolling of a scroll.) The explanatory framework centered on the theory of natural selection challenged this vision of nature as an orderly pattern of relations.

Darwin's world view was profoundly different because he argued that the adaptation of populations to their local environment was the sole cause of transmutation. Many people found it hard to see natural selection as the agent of either divine benevolence or of a rationally structured cosmic teleology. Selection adapted species to an ever-changing environment, and it did so by killing off useless variations in a ruthless "struggle for existence." This did not seem the kind of process that would be instituted by a benevolent God, especially because its essentially "selfish" nature meant that a parasitic way of life was a perfectly natural adaptive response in some circumstances.

More seriously for the idea of cosmic teleology, Darwin's supposition that the production of the individual variants in a population was essentially undirected ruled out any possibility that evolution could be shaped by a predetermined developmental trend. There was no obvious goal toward which it was aimed, and it did not produce an orderly pattern of relations between species. The accusation that the theory depended on "random" variation indicated the concerns of his opponents on this score. As Darwin himself made clear, variation was certainly caused by something (later identified as genetic mutations), but it was not aimed in any one direction and, thus, left adaptive evolution essentially open-ended. He allowed a limited role for variation shaped by the organisms' own activities (the so-called Lamarckian effect), but this too permitted multiple vectors of change. Evolution had to be depicted as a branching tree in which each act of branching was the result of a more or less unpredictable migration of organisms to a new location. At the same time, Darwin's theory undermined the old idea that species were idealized types, fixed elements in a clearly defined natural order. Species had to be treated as populations of varying individuals, with no fixed limit on the range of possible variation.

The Tree of Life

One innovation at the heart of Darwin's theory seems so obvious today that it is hard for us to appreciate just how new and how radical it was at the time. Lamarck had proposed that there might be natural processes adapting species to changes in their environment. But Darwin was perhaps the first to realize that if adaptation to the local environment was the only mechanism of evolution, there would be major implications for the whole system by which species are classified into groups. Darwin's mentor in geology, Charles Lyell, had shown how his uniformitarian theory would allow the biogeographer to reconstruct the migrations of species on an ever-changing earth. Populations could sometimes become divided by geographical barriers, so that what was once a single species could split into multiple branches adapting to separate environments (*10*). Evolution would become a divergent process, with some branches splitting over and over again, whereas others came to a dead end through extinction.

The image of the tree of life had appeared in Darwin's notebooks of the late 1830s (Fig. 2) and was proposed independently by Wallace in a paper published in 1855. Both realized that it explained why naturalists were able to arrange species into groups within groups, using descent from a common ancestor to explain the underlying similarities. Closely related species have diverged recently from a common ancestor, whereas the ancestry of more distantly related forms must be traced further back down the family tree to find the common point of origin.
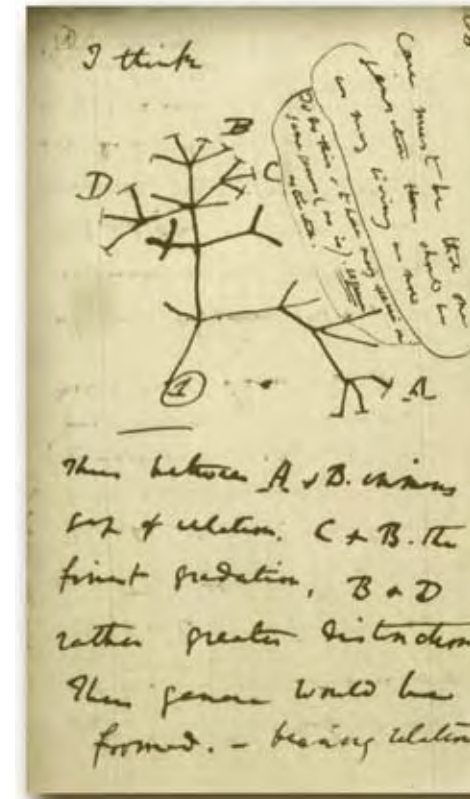

Fig. 2. Tree of Life, from Darwin's notebooks (*22*).

The idea of common descent now seems so obvious that we might wonder what alternative models could have been proposed to account for the relations among species. Several proposals available in the 1830s deflected attention away from the model of the branching tree (*11*). William Sharp Macleay's quinary or circular system of classification supposed that every genus contained five species that could be arranged in a circle; each family five genera, and so on through the taxonomic hierarchy. Chambers's *Vestiges of the Natural History of Creation* depicted evolution in terms of parallel lines advancing through a predetermined sequence of stages within each family, driven by force derived from individual development.

These rigidly structured models of taxonomic relations and evolution made good sense to anyone embedded in a vision of nature as a predictable, orderly system governed by a divine plan. Such a world view made it difficult to accept that the history of life on earth might be essentially irregular and unpredictable, dependant on the hazards of migration, isolation, and local adaptation. Darwin was led toward his alternative model in part because he was more interested in adaptation than cosmic teleology, thanks to the influence of William Paley's natural theology. Natural selection replaced divine benevolence as an explanation of adaptation. Unlike Macleay and Chambers, Darwin did not expect his theory to predict an orderly pattern of relations.

It has been argued that Darwin's move to a more historical viewpoint was inspired by German romanticism [e.g. (*12*)], but a more practical incentive was provided by the biogeographical insights gained on the *Beagle* voyage (1831–36). The Galapagos species provided the most obvious example of how the relations within a group can be explained by supposing that an original population became divided up, in this case by independent acts of migration to oceanic islands. Here, Darwin followed Lyell in seeing that biogeography must become a historical science, explaining present distributions in terms of past migrations, extinctions and (for Darwin but not for Lyell) evolutionary adaptations. Populations divided by geographical barriers will develop independently as each adapts to its new environment in its own way, and the possibility that barriers can be crossed occasionally allows for the branching process of evolution that Darwin conceived in the late 1830s. It was by approaching the problem of the origin of new species through a study of biogeography that Darwin was led to construct his model of open-ended, divergent evolution. Wallace developed a similar model and tested it during his explorations in South America and the Malay Archipelago (modern Indonesia).

Adrian Desmond and James Moore have recently proposed that Darwin's hatred of slavery prompted his move toward evolutionism (*13*). Because many slaveholders argued that the black race was separately created from the white, Darwin wanted to show that all races share a common ancestry, and he realized that this claim could be defended by extending the idea throughout the animal kingdom. As a basis for his thinking, this thesis is sure to generate much controversy, but if accepted it would emphasize the crucial role played by his move toward a model of branching evolution based on geographical diversity.

This model was so radical that many late 19th-century evolutionists were unable to accept it in full. Ernst Mayr argued that the theory of common descent was one of Darwin's greatest achievements, in addition to natural selection itself (*14*). So it was, but I think Mayr overestimated the rapidity with which other naturalists were converted to the theory. Many of the non-Darwinian theories of evolution proposed during the "eclipse of Darwinism" in the late 19th century were introduced with the aim of subverting the implications of the principle of common descent (*15*). The American neo-Lamarckians Edward Drinker Cope and Alpheus Hyatt proposed that the evolution of each group should be seen as a series of parallel lines moved through the same hierarchy of developmental stages, an updated version of the idea suggested in Chambers's
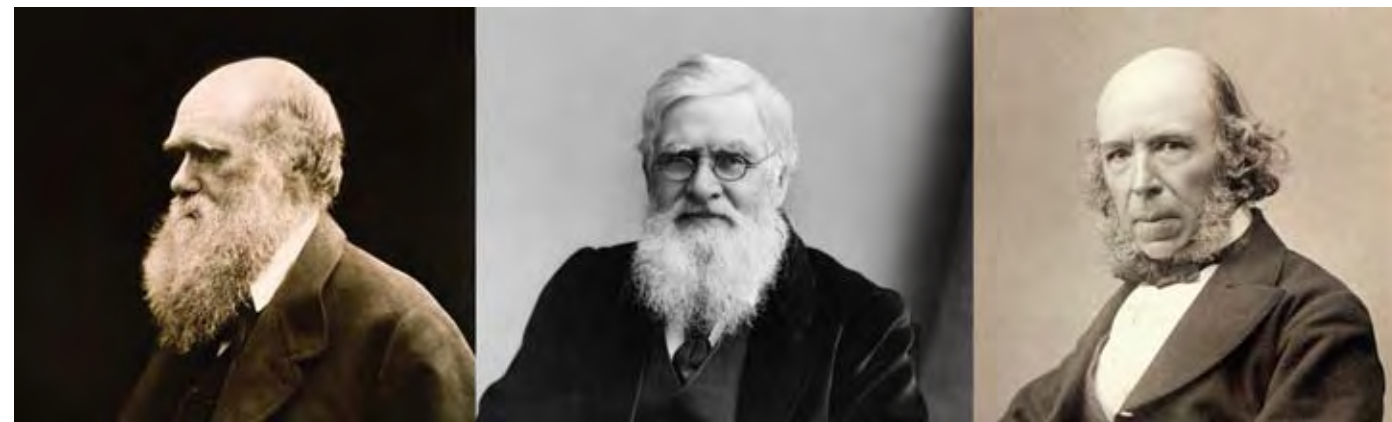

Fig. 1. Charles Darwin, Alfred Russel Wallace, and Herbert Spencer.

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Vestiges. The similarities linking the species in a genus were due not to a recent common ancestry, but to parallel trends independently reaching the same stage of development. Like Chambers, they endorsed the recapitulation theory (ontogeny recapitulates phylogeny, in the terminology introduced by Ernst Haeckel) and saw evolution as the addition of preordained stages to ontogeny. Adaptation was not crucial once the basic character of the group was established, and the linear, orthogenetic evolution of the group might eventually generate bizarre nonadaptive characters as a prelude to extinction—the theory of "racial senility." Darwin could make no sense of the theory proposed by Cope and Hyatt, because he could not imagine an evolutionary process driven by predetermined trends. But the fact that such theories flourished in the late 19th century demonstrates just how radical the theory of open-ended, divergent evolution was to the naturalists of the time.

Artificial Selection

These non-Darwinian models were ultimately marginalized by the synthesis of the selection theory and genetics in the early 20th century. Genetic mutations seemed to be essentially pluralistic and undirected, providing just the source of "random" variation that Darwin's mechanism required as its raw material. This later development highlights the importance of another insight gained by Darwin in the late 1830s, his decision to investigate the work of the animal breeders (Fig. 3) and his recognition that their method of artificial selection offered a useful way of understanding how the equivalent natural process operated. The exact role played by Darwin's study of breeding in the formulation of his theory is much debated by historians (*16–17*), but there can be little doubt of how important the analogy between artificial and natural selection became in his later thinking. In this case, Darwin was truly unique, because even Wallace did not take this step and dissociated himself from the link with artificial selection expressed in Darwin's later writings.

Darwin turned to the breeders in search of a clue as to how a population could be changed—here at least was a situation where modifications were actually being produced on a human time scale and that could be investigated directly. There was a well-developed network of breeders by this time, and although their ideas about heredity and variation were distinctly pregenetical (like Darwin's own), they had a very clear appreciation of how they produced changes in their artificially small populations. The insight that they worked by selection may have been important (this is the point of contention among experts studying Darwin's notebooks), but the breeders certainly taught him one thing. He realized that in a domesticated population there is always a fund of apparently purposeless and un-

directed variation among individual organisms. Although convinced that the degree of variability was artificially enhanced under domestication, Darwin, nevertheless, accepted that there must be some equivalent variability in every wild population. The analogy with artificial selection then allowed him to depict natural selection as a parallel process in which a few variant individuals, in this case with characters useful to the species rather than the human breeder, survive and reproduce. Those with harmful characters are eliminated by the struggle for existence, just as the breeder will not permit any animal to reproduce if it does not have the character he wants. It was the breeders who taught Darwin that variation is not directed toward some preordained goal, allowing him to build on his existing conviction that adaptive evolution must be an open-ended, branching process.

At the same time, the breeders' attitude toward variation pushed Darwin toward the view that the species is just a population of interbreeding individuals. Traditionally, species were treated as idealized types with a fixed essence, any variation from the norm being trivial and impermanent. The breeders knew that they could produce huge changes in structure by accumulating normal variations over a number of generations. When Darwin linked this information with his conviction that species could change indefinitely over time, he was driven toward a new form of species concept in which the population becomes paramount. The natural range of variability becomes part of the species' character, not the result of accidental deviations from a fixed norm. This is what Mayr called the transition from typological thinking to population thinking, and although he may have exaggerated the extent to which Darwin himself made the conceptual transition, the subsequent development of the selection theory brought this implication out more clearly.

In the debates that followed the publication of *On the Origin of Species*, the analogy with artificial selection continued to play a key role by forcing even Darwin's critics to think about the problems of heredity and variation in a new way (*18*). Opponents such as Fleeming Jenkin, who saw selection working on large variations or "sports of nature," were, nevertheless, still working within the framework defined by this analogy. For supporters such as Francis Galton, artificial selection helped to clarify the nature of both heredity and selection, paving the way for the revolutionary impact of Mendelian genetics. The notion of "hard" heredity was introduced in opposition to the "soft" form of inheritance implied by the Lamarckian process. The undirected nature of variation was clarified both through the study of large populations by Galton and through the breeding studies of the geneticists. Although it took some time for the geneticists to accept the situation, their studies of mutation ultimately endorsed Darwin's claim that the only way the environment could affect the population was by selection. Modern evolutionary developmental biology has reopened the question of whether variation and evolution can be quite as open-ended as Darwin and his followers believed. But the non-Darwinian vision of evolution unfolding to an orderly, predictable plan has been essentially marginalized by acceptance of the key insights on which Darwin based his theory of natural selection.

Fig. 3. Pigeons (*23*).

The Struggle for Existence

One of the most disturbing aspects of Darwin's theory was its appeal to the struggle for existence as the natural process that equates with the breeder's activity as a selecting agent. This very harsh vision of nature certainly threatened the traditional belief in a benevolent Creator. The term "struggle for existence" occurs in Thomas Robert Malthus's *An Essay on the Principle of Population*, although used in the context of tribal groups competing for limited resources. Darwin saw that population pressure would lead to competition between individuals and was perhaps the first to realize that it might represent a means by which the population could change through time (*19*, *20*). The process worked by eliminating the least fit variants within the population and allowing the better adapted to survive and breed. This was what the philosopher Herbert Spencer would later refer to as the "survival of the fittest." Strictly speaking, natural selection requires only differential reproduction among variants, but Darwin thought that the pressure of competition was necessary to make it effective. It seems that without the input from Malthus, he would not have come up with the theory.

The idea of struggle was pervasive in the literature of the period, but could be exploited in many different ways. In the 1850s, Spencer had already seen how competition could be turned into a very different, and in some respects less disturbing, mechanism of progress (*21*). For Spencer, the interaction between individuals stimulated their efforts to adapt to the changing social and physical environment. He then invoked Lamarck's concept of the "inheritance of acquired characteristics" to explain how these self-improvements accumulated over many generations, leading to biological evolution and social progress. Spencer's self-improvement model of progress became immensely popular in the later 19th century, and because it too seemed to rely on struggle as the motor of change, it was often confused with the Darwinian mechanism. In fact, Spencer thought that all humans will eventually acquire the faculties needed to interact harmoniously with one another. But his occasional use of highly individualistic language allowed him to be perceived as the apostle of free enterprise. Much of what later became known as "social Darwinism" was, in fact, Spencerian social Lamarckism expressed in the terminology of struggle popularized by Darwin.

This point is important in the context of the charge raised by modern opponents of Darwinism that the theory is responsible for the appearance of a whole range of unpleasant social policies based on struggle. Darwin exploited the idea of the struggle for existence in a way that was unique until paralleled by Wallace nearly 20 years later. Their theory certainly fed into the movements that led toward various kinds of social Darwinism, but it was not the only vehicle

for that transition in the late 19th century. It did, however, highlight the harsher aspects of the consequences of struggle. The potential implications were drawn out even more clearly when Galton argued that it would be necessary to apply artificial selection to the human race in order to prevent "unfit" individuals from reproducing and undermining the biological health of the population. This was the eugenics program, and in its most extreme manifestation at the hands of the Nazis, it led not just to the sterilization but also to the actual elimination of those unfortunates deemed unfit by the state. Did Darwin's emphasis on the natural elimination of maladaptive variants help to create a climate of opinion in which such atrocities became possible?

It has to be admitted that, by making death itself a creative force in nature, Darwin introduced a new and profoundly disturbing insight into the world, an insight that seems to have resonated with the thinking of many who did not understand or accept the details of his theory. Darwinism was not "responsible" for social Darwinism or eugenics in any simple way. After all, some early geneticists endorsed eugenics by analogy with animal breeding even while dismissing natural selection as the mechanism of evolution. And the Nazis wanted to purify a fixed racial type, which they certainly did not want to admit had evolved gradually from an ape ancestry. But by proposing that evolution worked primarily through the elimination of useless variants, Darwin created an image that could all too easily be exploited by those who wanted the human race to conform to their own pre-existing ideals. In the same way, his popularization of the struggle metaphor focused attention onto the individualistic aspects of Spencer's philosophy.

Modern science recognizes the importance of Darwin's key insights when used as a way of explaining countless otherwise mysterious aspects of the natural world. But some of those insights came from sources with profoundly disturbing implications, and many historians now recognize that the theory, in turn, played into the way those implications were developed by later generations. This is not a simple matter of science being "misused" by social commentators, because Darwin's theorizing would almost certainly have been different had he not drawn inspiration from social, as well as scientific, influences. We may well feel uncomfortable with those aspects of his theory today, especially in light of their subsequent applications to human affairs. But if we accept science's power to upset the traditional foundations of how we think about the world, we should also accept its potential to interact with moral values.

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The Red Queen and the Court Jester: Species Diversity and the Role of Biotic and Abiotic Factors Through Time

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Evolution may be dominated by biotic factors, as in the Red Queen model, or abiotic factors, as in the Court Jester model, or a mixture of both. The two models appear to operate predominantly over different geographic and temporal scales: Competition, predation, and other biotic factors shape ecosystems locally and over short time spans, but extrinsic factors such as climate and oceanographic and tectonic events shape larger-scale patterns regionally and globally, and through thousands and millions of years. Paleobiological studies suggest that species diversity is driven largely by abiotic factors such as climate, landscape, or food supply, and comparative phylogenetic approaches offer new insights into clade dynamics.
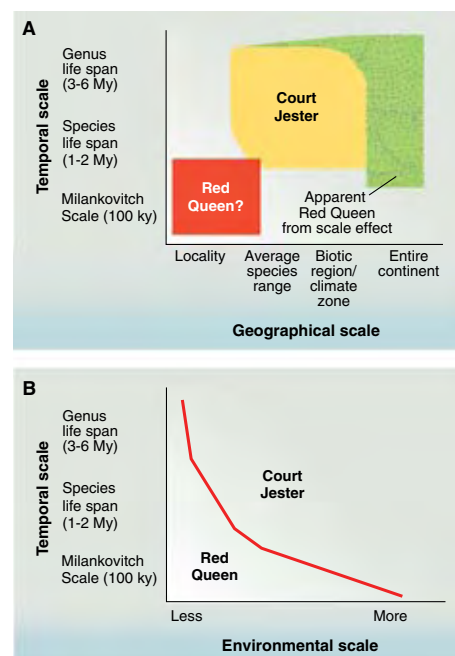
There are two ways of viewing evolution, through the spectacles of either the Red Queen or the Court Jester. The Red Queen model (*1*) stems from Darwin, who viewed evolution as primarily a balance of biotic pressures, most notably competition, and it was characterized by the Red Queen's statement to Alice in *Through the Looking-Glass* that "it takes all the running you can do, to keep in the same place." The Court Jester model (*2*) is that evolution, speciation, and extinction rarely happen except in response to unpredictable changes in the physical environment, recalling the capricious behavior of the licensed fool of Medieval times. Neither model was proposed as exclusive, and both Darwin and Van Valen (*1*) allowed for extrinsic influences on evolution in their primarily biotic, Red Queen views.

Species diversity in a Red Queen world depends primarily on intrinsic factors, such as body size, breadth of physiological tolerance, or adaptability to hard times. In a Court Jester world, species diversity depends on fluctuations in climate, landscape, and food supply. In reality, of course, both aspects might prevail in different ways and at different times, what could perhaps be called the multilevel mixed model. Traditionally, biologists have tended to think in a Red Queen, Darwinian, intrinsic, biotic factors way, and geologists in a Court Jester, extrinsic, physical factors way.

Much of the divergence between the Red Queen and Court Jester world views may depend on scale (*2*) (Fig. 1): Biotic interactions drive much of the local-scale success or failure of individuals, populations, and species (Red Queen), but perhaps these processes are overwhelmed by substantial tectonic and climatic processes at time scales above 10^5 years (Court Jester). It is important not to export organism-level processes to regional or global scales, and it is likely that evolution operates in a pluralistic way (*3*).

There are two broad methodologies for studies of species diversity through time, taxic and phylogenetic (*4*). The taxic approach involves treating species, genera, or families as independent entities and counting their occurrences against time and other factors. The phylogenetic approach uses cladograms or molecular trees to disentangle key aspects of clade histories. Clades are monophyletic, including all descendants of an ancestor, whereas taxa may be monophyletic or paraphyletic, excluding some descendants of the ancestor. Comparative macroecological studies add rigor to analyses showing that sister clades may vary in rate of evolution, timing of increases in species richness and morphospace occupation, and distributions of evolutionary novelties across lineages and subclades. Here, I will explore the largest-scale global, taxic in-

Fig. 1. Operation of Red Queen (biotic causation) and Court Jester (abiotic causation) models at different geographic and temporal scales (**A**). The Red Queen may prevail at organismic and species level on short time scales, whereas the Court Jester holds his own on larger scales. The stippled green shape shows an area where Red Queen effects might be identified erroneously, but these are likely the result of spatial averaging of regional responses to climate change and other complex physical perturbations that may be in opposite directions, and so cancel each other, suggesting no controlling effect of the physical environment on evolution. Physical-environmental disruptions may elicit biotic responses along the red line separating Red Queen and Court Jester outcomes (**B**). The usage here is the microevolutionary Red Queen, as opposed to the macroevolutionary Red Queen that posits constant extinction risk, a view that has been largely rejected (*31*). Illustration based on (*2*).

vestigations, provide an outline of how these and other studies correspond to the predictions of the Red Queen, Court Jester, and multilevel mixed models (Table 1), and outline some phylogenetic studies of the macroevolution of species diversity.

The Global Pattern of Diversification Through Time

A key question about the origin of modern biodiversity is how today's 10 million species arose from a single ultimate species of microbial life 3500 million years ago (Ma) (Fig. 1). Two models for global diversification are termed the saturation/equilibrium model (*5–7*) and the expansion model (*8–11*). The equilibrium model has prevailed, among marine paleobiologists at least, for a long time, and represents a classic Red Queen viewpoint because it implies primarily biotic controls (density dependence) on global diversity.

There are two versions of the equilibrium model, differing in the time when the global marine ecosystem became saturated. Sepkoski's coupled logistic model (*5*) identified three equilibria, in the Cambrian, most of the Paleozoic, and perhaps a third, beginning in the Pliocene and continuing to the present (Fig. 2A). These three equilibrium levels correspond to three sets of phyla, the Cambrian, Paleozoic, and Modern, that interacted and successively replaced each other through the Cambrian-Ordovician and Permo-Triassic intervals, reaching higher equilibrium levels after each long-term replacement event. The second model (*6, 7*) identifies a single equilibrium level from the early Paleozoic, perhaps 400 Ma, to the present (Fig. 2B). In both models, the equilibria correspond to biodiversity saturation in which

new taxa could become established only by driving others to extinction. Key evidence is that both origination and extinction rates appear to have been density-dependent (*5–7*), limiting rises in diversity and promoting rapid recovery after extinction events.

Alternative models for global diversification are expansionist, allowing global species diversity to rise, with damping, but without a predictable limit (*8–11*). Density dependence of origination and extinction rates does not preclude expansionist models because they may be dampened by limiting factors such as shortage of food or space, or active predation, as well as by climate and other physical factors. Further, it seems that the coupled logistic model may be partly an artifact of taxonomic scale (Fig. 2, red curve); it was worked out at ordinal and familial levels but does not work convincingly at generic or specific levels (*10, 11*), there are problems with key numerical assumptions (*11, 12*), and the background assumption of a global carrying capacity is doubtful (*8, 10, 11*). Further, it has proved hard to export the logistic model to the much more speciose terrestrial realm, whether one considers plants, insects, or vertebrates, because these groups seem to have radiated explosively, without diversity plateaus, particularly in the past 100 million years (My) (*10*).

Resolution between the equilibrium models, and between these and expansionist models, might seem straightforward, but the solution depends on adequate assessment of the quality of the fossil record. The long-term saturation model for global diversification (Fig. 2, blue
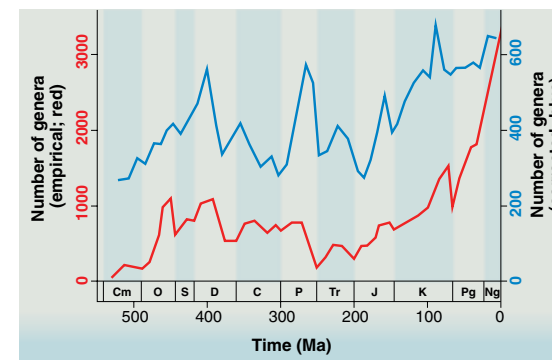

Fig. 2. Patterns of marine animal genus diversification through the past 530 My, the Phanerozoic. The two lines compare current estimates from the empirical (uncorrected) Sepkoski database (red line) and sampling-standardized (corrected) analysis of the Paleobiology Database (blue line). The empirical curve (red line) suggests that global marine diversity reached a possible plateau through the Paleozoic (450 to 250 Ma) and has risen, apparently exponentially, ever since. The sampling-standardized curve (blue line) suggests that global marine diversity reached near-modern levels some 400 Ma and there has been only modest increase since then. Cm, Cambrian; C, Carboniferous; D, Devonian; J, Jurassic; K, Cretaceous; Ng, Neogene; O, Ordovician; P, Permian; Pg, Paleogene; S, Silurian; Tr, Triassic. Based on (*6*).

curve) arises from extensive attempts to correct data sets for sampling error (*6, 7*), whereas the multiple-equilibria and expansion models originally used raw data, without correction (*5, 8–11*), although more recent analyses return a somewhat dampened but congruent signal when corrections are imposed. Correction for sampling is clearly essential (*6, 7*), and future investigation must determine appropriate independent proxies for preservation and human error; some current proxies (such as number of fossiliferous localities) are themselves dependent

on diversity, and other correction regimes may be so complex as to produce data in which geologic and biologic signals are not obviously separated.

Life on land today may be as much as 25 times as diverse as life in the sea, so it may be wrong to generalize from marine paleontological studies to all life. Perhaps land and sea show similar patterns of exponential increase in species numbers (*8, 9, 11*), or perhaps they differ in their key rules (*13, 14*), with the sea acting as a giant Gaussian petri dish, where species diversity is equilibrial and density-dependent, and the land witnessing continuing (dampened) exponential rise in diversity as ever new sectors of ecospace are conquered (*9, 14*). Any model for global diversification must encompass the independent evidence for increasing complexity of organisms, increases in the occupation of novel ecospace, explosive evolution within particular clades, and addition of novel clades without the loss of precursors (*9, 11, 15*), all of which have happened many times in the past 500 My.

Large-Scale Controls on Species Diversity

Taxic paleobiological studies have provided a great deal of evidence about controls, mainly abiotic, on species diversity. Biotic factors, such as body size, diet, colonizing ability or ecological specialization, appear to have little effect on the diversity of modern organisms, although abundance and r-selected life-history characteristics (short gestation period, large litter size, and short interbirth intervals) sometimes correlate with high species richness (*16*).

Table 1. Macroevolutionary phenomena and their support for either the Red Queen (biotic, intrinsic) or Court Jester (physical, extrinsic) models. Many could fit either worldview, and so are noted as "multilevel mixed."

| Red Queen	Court Jester	Multilevel mixed
Interspecific competition	Waxing and waning of clades in association with tectonic and oceanographic events (*2, 17*)	Vicariance and dispersal in major phylogenetic splits (*17*)
Character displacement	Mass extinctions and smaller extinction events triggered by extrinsic causes such as eruptions, climate change, anoxia, impact (*10, 11*)	Latitudinal diversity gradient (*22–24*)
Evolutionary arms races (*1*)	Coordinated turnovers, originations, and extinctions in response to physical perturbations— termed "coordinated stasis" or "turnover pulse" hypothesis (*2, 29, 30*)	Occupation of new ecospace (*25*)
Constancy of ecological guilds through time (*25*)	Nonconstant probability of extinction (*3, 11*)	Subdivision of niches/specialization (*10, 25*)
Incumbency advantage (*3, 24*)	Lack of evidence for a global carrying capacity and equilibrium levels (*8, 10*)	Declining global extinction rates through time (*1, 5*)
	Lack of cohesiveness of the great "evolutionary faunas" (*12*)	Onshore-offshore patterns and disturbance (*3*)
	Species richness–energy relationship (*18, 19*)	Resource use: stenotopes are more speciose than eurytopes (*29, 30*)
	Inverse relationship between global temperature and biodiversity (*21*)	
	Lack of clear correlation of species richness with body size or other biotic factors (*16*)	
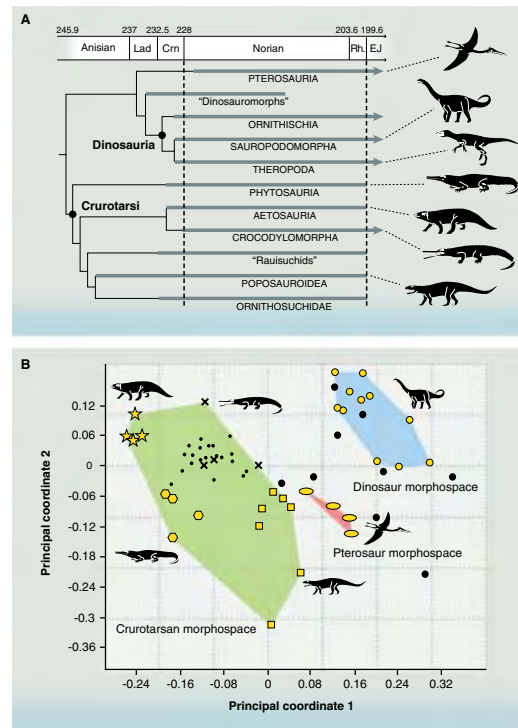
Fig. 3. Phylogenetic relationships and morphospace occupation for Triassic archosaurs. (**A**) Framework phylogeny for Triassic crurotarsans scaled to the Triassic time scale. Numbers at top refer to millions of years before the present; gray bars represent the observed durations of major lineages; vertical dashed lines denote two extinction events, at the Carnian-Norian and Triassic-Jurassic boundaries; arrowheads indicate lineages that survived the latter event. Lad, Ladinian; Crn, Carnian; Rh, Rhaetian; EJ, Early Jurassic. (**B**) Empirical morphospace for Late Triassic archosaurs, based on the first two principal coordinates. Large circles, dinosaurs; ovals, pterosaurs; squares, poposauroids; hexagons, phytosaurs; stars, aetosaurs; crosses, crocodylomorphs; smaller black dots, "rauisuchids"; larger black dots, nondinosaurian dinosauromorphs, *Scleromochlus*. Based on (*28*).

Geographic and tectonic history has generated patterns of species diversity through time. The slow dance of the continents as Pangaea broke up during the past 200 My has affected modern distribution patterns. Unique terrestrial faunas and floras, notably those of Australia and South America, arose because those continents were islands for much of the past 100 My. Further, major geologic events such as the formation of the Isthmus of Panama have permitted the dispersal of terrestrial organisms and have split the distributions of marine organisms. A classic example of vicariance is the fundamental division of placental mammals into three clades, Edentata in South America, Afrotheria in Africa, and Boreoeutheria in the northern hemisphere, presumably triggered by the split of those continents 100 Ma (*17*). Other splits in species trees may relate to dispersal events, or there may be no geographic component at all.

Species richness through time may correlate with energy. The species richness–energy relationship (*18*) posits correlations with evapotranspiration, temperature, or productivity, and studies of terrestrial and marine ecosystems have shown that these factors may explain as much as 90% of current diversity, although relationships between species diversity and productivity change with spatial scale (*19*). Over long time spans, there are strong correlations between plankton morphology and diversity and water temperature: Cooling sea temperatures through the past 70 My, and consequent increasing ocean stratification, drove a major radiation of Foraminifera, associated with increasing body size (*20*). More widely, there is close tracking be-

tween temperature and biodiversity on the global scale for both marine and terrestrial organisms (*21*), where generic and familial richness were relatively low during warm "greenhouse" phases of Earth history, coinciding with relatively high origination and extinction rates.

A much-studied manifestation of energy and temperature gradients is the latitudinal diversity gradient (LDG), namely the greater diversity of life in the tropics than in temperate or polar regions, both on land and in the sea. There are two explanations (*22*): (i) the time and area hypothesis, that the tropical belt is older and larger than temperate and polar zones, and so tropical clades have had longer to speciate, or (ii) the diversification rate hypothesis, that there are higher rates of speciation and lower rates of extinction in the tropics than elsewhere. There is geological and paleontological evidence for a mixture of both hypotheses (*23*, *24*).

Species diversity may increase by the occupation of new ecospace. The number of occupied guilds, that is, broad ecological groupings of organisms with shared habits, has increased in several steps through time, from 20 in the early Paleozoic to 62 in post-Paleozoic marine faunas (*25*). Further, marine animals have shown several step increases in tiering, the ability to occupy and exploit different levels in the habitat: At times, burrowers have burrowed deeper, and reef-builders have built taller and more complex reefs. Analogous, if even more dramatic, expansions of ecospace have occurred on land, with numerous stepwise additions of new habitats, from the water-margin plants and arthropods of the early Paleozoic to the forests

and upland habitats of the later Paleozoic when land animals first burrowed, climbed, and flew, through the introduction of herbivory, giant size, endothermy, and intelligence among vertebrates, and the great blossoming of flowering plants (with associated vast expansions in diversity of plant-eating and social insects and modern vertebrates) during the Cretaceous Terrestrial Revolution 100 Ma (*26*).

The other mode of species increase globally or regionally is by niche subdivision, or increasing specialization. This is hard to document because of the number of other factors that vary between ecosystems through time. However, mean species number in communities (alpha diversity) has increased through time in both marine (*15*, *25*) and terrestrial (*10*) systems, even though niche subdivision may be less important than occupation of new ecospace in increasing biodiversity. Further, morphological complexity may be quantified, and a comparative study of crustaceans shows, for example, that complexity has increased many times in parallel in separate lineages (*27*).

Phylogenetic Studies of Clade Histories
Species are not randomly distributed; they have an evolutionary history, and so occur as twigs on a great phylogenetic tree. Studying species as members of clades is a fruitful approach to understanding the drivers and controls on speciation. Key questions include (i) Do species diversify early in a clade's history? (ii) How do diversity and disparity (variance in characters or morphology) covary? (iii) Do major lineages within a clade follow similar, or different, patterns, and if different, why? (iv) Do evolutionary radiations follow the acquisition of new characters or emptying of ecospace? (v) How do major clades of apparent competitors interact over long spans of geologic time? and (vi) How do sister clades vary in species diversity and why?

For such analyses, the ideal is a complete species tree, a phylogenetic tree that contains all species living and extinct, plotted accurately against geologic time (*4*). Simple to say; hard to achieve. More commonly, incomplete trees have been used, with the risk of error in calculations of evolutionary rates or comparisons of subclades. In paleontology, it has proven much easier to work with higher taxa such as genera or families because species fossil records are less complete than those of higher taxa, and yet it is not clear how higher-level patterns relate to those at species level. Many key questions can be tackled by comparing a real tree to a hypothetical tree that follows an equal-rate Markov (ERM) model, equivalent to tree growth after a random walk, where equal chances of speciation and of extinction are shared by all species (*4*).

Major biotic replacements, where one clade replaces another, have been a focus of debate about the roles of competition and progress in

macroevolution, and dinosaurs provide a classic example. The standard view was that dinosaurs originated in the Late Triassic, some 230 Ma, by a process of competition in which they prevailed over their precursors, the crocodile-like crurotarsans and others, because of superior adaptations. A comparative phylogenetic study (*28*) shows, however (Fig. 3), that the Dinosauria expanded in two steps, one after an extinction event 225 Ma that removed dominant herbivores, and the second following the end-Triassic extinction 200 Ma that removed most of the crurotarsans. Dinosauria remained at moderate diversity and low disparity, and at lower disparity than the crurotarsans they supposedly out competed, during the 25 My between the events, suggesting that there was no insistent competition driving other groups to extinction but rather that the dinosaurs occupied new ecospace opportunistically, after it had been vacated.

A further study on Dinosauria explored the subsequent evolution of the clade (*26*). Classic views that the dinosaurs arose with a flourish, and then finally gave way in the Cretaceous to the superior mammals, or that they dwindled to extinction because of "racial senility," had long been abandoned. The dinosaurs seemed to be radiating actively in the Cretaceous, with many new clades appearing through their last 55 My, and especially in their final 15 My. The new study (*26*) shows that most diversification shifts (departures from ERM assumptions) fall in the first one-third of the history of the clade and that their continuing diversification in the Late Jurassic and Cretaceous was mainly indistinguishable from a random walk. In particular, dinosaurs did not participate in the Cretaceous Terrestrial Revolution, some 130 to 100 Ma, when flowering plants, leaf-eating insects, social insects, squamates, and many other modern groups radiated substantially. There is no geometric reason that diversification shifts should mainly occur low in a clade's history: Clade shapes vary from bottom-heavy to top-heavy, and diversification shifts may be concentrated low (dinosaurs and bats) or high (insects and ants) in a clade (*26*).

In the future, the identification of diversification shifts across numerous taxa may provide evidence for the relative importance of the Red Queen and Court Jester worldviews. If the majority of diversification shifts are coordinated, and associated with particular climatic, tectonic, and geographic drivers, then the Court Jester model of macroevolution would prevail. This would link most increases in species diversity to particular large-scale radiation events, such as the Cretaceous Terrestrial Revolution (*26*), or recoveries after mass extinctions. If, on the other hand, the majority of diversification shifts are unique to particular clades, and not coordinated temporally with others, then the Red Queen worldview might be considered.

Comparing Sister Taxa
A powerful element of the comparative phylogenetic approach to species diversity through time is the opportunity to compare sister taxa. Sisters arose from a single ancestor, and so their trajectories occupy the same amount of time, and they started with the same genotype and phenotype. Any similarities in their subsequent evolution probably reflect this phylogenetic signal of a common origin, but differences reflect independent aspects of their separate histories.

Comparisons of sister taxa have allowed tests of the resource-use hypothesis (*29*), that generalists are less speciose and have longer species durations than specialists. Specialists divide the physical environment into small patches, each occupied by a species, and each probably more subject to environmental crises than their generalist relatives. Classic examples in support of the resource-use hypothesis come from studies of Neogene mammals (*29*). For example, two antelope subgroups, the tribes Alcelaphini and Aepycerotini, diverged 6 to 8 Ma. The former is now highly speciose, with some 7 living and 25 extinct species, and the latter is represented by two species, only one, the impala *Aepyceros*, surviving. The slowly evolving Aepycerotini consists of few species at any time, and each of those is long lived, whereas the speciose Alcelaphini consists of many short-lived species. The ecological habits of both clades differ: The impala has a broad, generalist diet, whereas the speciose alcelaphines show more dietary specialization. In wider studies of many clades of Neogene African and South American mammals (*30*), the resource-use hypothesis was supported, and some subsidiary predictions confirmed: Specialists are more common than generalists, carnivores include more generalists than herbivores, and there are more specialists in habitats that underwent recent environmental change (tropical rain forests and deserts). The resource-use model then stresses the role of climate and tectonic movements in determining species diversity rather than biological controls such as competition and predation.

Outlook
Paleontologists and evolutionary ecologists have debated species diversity largely independently. The realization that the Red Queen and Court Jester models may be scale-dependent, and that evolution may be pluralistic (*3*), opens opportunities for dialog. Taxic studies in paleontology continue to have great value in highlighting correlations between species richness and other factors, but comparative phylogenetic methods will illuminate questions about clade dynamics, species richness, and the origin of novelties. Further, methods are shared by paleontologists and neontologists, and this allows direct communication on the patterns and processes of

macroevolution. Viewed close up, evolution is all about biotic interactions in ecosystems (Red Queen model), but from further away, the large patterns of biodiversity are driven by the physical environment (Court Jester model).

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Evidence for Ecological Speciation and Its Alternative

Dolph Schluter

Natural selection commonly drives the origin of species, as Darwin initially claimed. Mechanisms of speciation by selection fall into two broad categories: ecological and mutation-order. Under ecological speciation, divergence is driven by divergent natural selection between environments, whereas under mutation-order speciation, divergence occurs when different mutations arise and are fixed in separate populations adapting to similar selection pressures. Tests of parallel evolution of reproductive isolation, trait-based assortative mating, and reproductive isolation by active selection have demonstrated that ecological speciation is a common means by which new species arise. Evidence for mutation-order speciation by natural selection is more limited and has been best documented by instances of reproductive isolation resulting from intragenomic conflict. However, we still have not identified all aspects of selection, and identifying the underlying genes for reproductive isolation remains challenging.

It took evolutionary biologists nearly 150 years, but at last we can agree with Darwin that the origin of species, "that mystery of mysteries" (*1*), really does occur by means of natural selection (*2*–*5*). Not all species appear to evolve by selection, but the evidence suggests that most of them do. The effort leading up to this conclusion involved many experimental and conceptual advances, including a revision of the notion of speciation itself, 80 years after publication of *On the Origin of the Species*, to a definition based on reproductive isolation instead of morphological differences (*6*, *7*).

The main question today is how does selection lead to speciation? What are the mechanisms of natural selection, what genes are affected, and how do changes at these genes yield the habitat, behavioral, mechanical, chemical, physiological, and other incompatibilities that are the reproductive barriers between new species? As a start, the many ways by which new species might arise by selection can be grouped into two broad categories: ecological speciation and mutation-order speciation. Ecological speciation refers to the evolution of reproductive isolation between populations or subsets of a single population by adaptation to different environments or ecological niches (*2*, *8*, *9*). Natural selection is divergent, acting in contrasting directions between environments, which drives the fixation of different alleles, each advantageous in one environment but not in the other. Following G. S. Mani and B. C. Clarke (*10*), I define mutation-order speciation as the evolution of reproductive isolation by the chance occurrence and fixation of different alleles between populations adapting to similar selection pressures. Reproductive isolation evolves because populations fix distinct mutations that

would nevertheless be advantageous in both of their environments. The relative importance of these two categories of mechanism for the origin of species in nature is unknown.

In this review, I summarize progress in understanding the general features of speciation by selection. I do not differentiate speciation by sexual selection here because natural selection drives the divergence of mate preferences, by either ecological or mutation-order mechanisms, in most theories of the process (*8*, *11*). I leave out discussion of sympatric and allopatric speciation but instead identify the likelihood of ecological and mutation-order speciation when there is gene flow. I ignore reinforcement, a special type of natural selection thought to favor stronger premating reproductive isolation once postzygotic isolation has evolved. I also ignore speciation by polyploidy, even though selection might be crucial in the early stages.

Speciation and Adaptation from Darwin to Dobzhansky

Appreciation of the connection between adaptation and speciation began with Darwin when a morphological concept of species largely prevailed. In *On the Origin of Species*, Darwin wrote that "I look at the term species, as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other..." and "The amount of difference is one very important criterion in settling whether two forms should be ranked as species or varieties" (*1*). Under this view, speciation is defined as the accumulation of sufficiently many differences between populations to warrant their classification as separate taxonomic species. Darwin understood the importance of reproductive barriers between species (*1*), but the study of speciation after the publication of this work focused mainly on the evolution of species differences, particularly of morphological traits but also of behavioral and other phenotypic traits.

Under this Darwinian perspective, linking speciation with adaptation was relatively straightforward, requiring only a test of whether phenotypic differences between species were caused by natural selection. For example, at the American Association for the Advancement of Science 1939 speciation symposium [the last major symposium on speciation before the biological species concept (*7*)], an extensive comparative and biogeographic study showcased instances in which derived morphological and life history forms of fishes had arisen over and over again from the same ancestral type (*12*). The repeated, parallel origin of nonparasitic lamprey in streams from the same migratory, parasitic ancestor showed that "Again and again the parasitic lampreys have evolved into nonparasitic forms...correlated with life in small streams, where a suitable food supply in the way of large fish is scarce or seasonal" (*12*). When correlated with environmental factors, such repetition is unlikely to result from chance; environmental selection pressures must therefore be the cause of speciation. "As a result of our recent studies on fishes...weight is constantly being added to the theory that speciation is...under the rigid control of the environment" (*12*). However, this case is only referring to the origin of morphological species.

The turning point for speciation studies came with the modern concept of speciation "Species separation is defined as a stage of the evolutionary process at which physiological isolating mechanisms become developed" (*6*) (here, "physiological" is interpreted to mean evolved reproductive isolation between populations, as distinct from geographical barriers to interbreeding). Subsequently, species were defined as "groups of interbreeding natural populations that are reproductively isolated from other such groups" (*7*). From this point on, the study of speciation was the study of the evolution of reproductive isolation (*3*). Progress up to then in understanding the link between morphological speciation and adaptation was largely forgotten, its contributions uncertain under the new concept.

The biological species concept must surely have made it more difficult to investigate any link between speciation and natural selection. T. Dobzhansky (*13*) suggested that the genes underlying differences between populations in ordinary phenotypic traits were unlikely to be the basis of reproductive isolation. He later changed his mind, but at the time this viewpoint, and the generally greater difficulty of studying reproductive isolation than morphology, must have discouraged many from pursuing the connection. Virtually no research effort followed that tested the role of adaptation in speciation.

Models of Speciation by Selection

The topic of natural selection in speciation is

once again receiving attention. The two most general hypotheses involving selection are ecological and mutation-order speciation. Ecological speciation is defined as the evolution of reproductive isolation between populations by divergent natural selection arising from differences between ecological environments (*2*, *8*, *9*, *14*). It predicts that reproductive isolation should evolve between populations adapting to contrasting environments but not between populations adapting to similar environments. The basic idea has been around for a while (*7*), although it was tested only recently. The agents of divergent selection are extrinsic and can include abiotic and biotic factors such as food resources, climate, habitat, and interspecies interactions such as disease, competition, and behavioral interference. Ecological speciation can lead to the evolution of any type of reproductive isolation, including premating isolation, hybrid sterility, and intrinsic hybrid inviability as well as extrinsic, ecologically based pre- and postzygotic isolation. Speciation by sexual selection is ecological speciation if ecologically based divergent selection drives divergence of mating preferences, for example by sensory drive (*15*).

In accordance with (*10*), mutation-order speciation is defined as the evolution of reproductive isolation by the fixation of different advantageous mutations in separate populations experiencing similar selection pressures. Whereas different alleles are favored between populations under ecological speciation, the same alleles would be favored in different populations under mutation-order speciation. Divergence occurs anyway because, by chance, the populations do not acquire the same mutations or fix them in the same order. Divergence is therefore stochastic but the process is distinct from genetic drift. It can occur in both small and large (though not infinite) populations. Selection can be ecologically based under mutation-order speciation, but ecology does not favor divergence as such. It can lead to the evolution of any type of reproductive isolation, with the exception of ecologically based pre- and postzygotic isolation.

Speciation resulting from intragenomic conflict such as meiotic drive or cytoplasmic male sterility (Fig. 1B) is likely to be mutation-order speciation because, by chance, the initial mutations causing drive and those countering it are unlikely to be the same in separate populations. Speciation by sexual selection is mutation-order speciation if divergence of mate preferences or gamete recognition occurs by the fixation of alternative advantageous mutations in different populations, as by sexual conflict (*16*). Divergence in song and other learned components of behavior under purely social selection, not molded by selection for efficient signal transmission (*5*), is the cultural equivalent of the mutation-order process. Additional scenarios are elaborated in (*5*).

Both models of speciation, ecological and mutation-order, are theoretically plausible, and only data can determine their relative importance in nature. The key is to figure out by which mechanism reproductive isolation first evolved (*3*). Once the earliest genetic differences have accumulated between populations by either process, subsequent mutations might be favored in one population and not the other because of epistatic interactions with genetic background (*10*). Hence, epistasis, including that producing Dobzhansky-Muller incompatibilities in hybrids between species (*3*), can result from either ecological or mutation-order speciation.

Speciation can be rapid under both speciation models, because alleles are driven to fixation by natural selection in both cases. However, under the mutation-order process, the same alleles, if present, would be favored in every population, at least in the early stages of divergence. For this reason, mutation-order speciation is difficult when there is gene flow, because gene flow increases the possibility that favorable mutations occurring in one population will spread to other populations, preventing divergence (*17*, *18*). Any process resulting in low levels of gene flow, including selection, facilitates subsequent divergence by the mutation-order process (*19*). In contrast, ecological speciation can proceed with or without gene flow, although it is easiest when gene flow is absent.

Experiments with laboratory populations of *Drosophila* and yeast demonstrate the plausibility of ecological speciation. In those instances when measurable pre- and postmating reproductive isolation evolved, it was greater between lines subjected to different environments than between lines raised under homogeneous conditions (*20*, *21*). Laboratory experiments on various microbes maintained under homogeneous conditions for many generations have detected genetic divergence consistent with the mutation-order process (*22*), but effects on reproductive isolation have not been explored.

Two approaches investigate the mechanisms of speciation by natural selection in nature. The bottom-up approach involves (i) genetic mapping of reproductive isolation between closely related species, (ii) testing whether discovered genes exhibit a genomic signature of positive selection, and (iii) identifying the phenotype and source of fitness effects of alternative alleles at selected loci. The approach has been hugely successful in identifying major genes implicated in hybrid inviability (*Hmr*, *Lhr*, *Nup96*), sterility
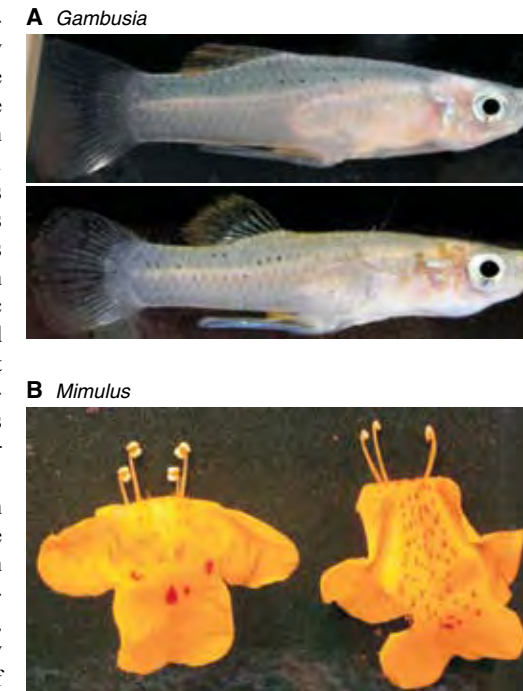

A *Gambusia*

B *Mimulus*

Fig. 1. (**A**) Example of ecological speciation. Repeatedly and independently, the mosquito fish, *Gambusia hubbsi*, inhabiting blue holes in the Bahamas has evolved a larger caudal region and smaller head in the presence of predators (top) than in their absence (bottom) (*29*). In laboratory trials, the probability of two individuals mating was higher when they were from different populations having the same predation environment (and similar body shape) than when they were from opposite predation environments. [Photo credit: Brian Langerhans (*29*)]. (**B**) Example of reproductive isolation evolving under the mutation-order mechanism. Male-fertile (left) and male-sterile (right) flowers of F2 hybrids between an Oregon population of monkey flowers (*M. guttatus*) having a cytoplasmic male sterility element and nuclear restorer and a closely related species (*M. nasutus*) having neither (*46*, *47*). Both flowers shown have *M. guttatus* cytoplasm. The flower on the left also has the nuclear restorer, whereas the one on the right, with undeveloped anthers, lacks the restorer. [Photo credit: Andrea Case (*47*)]

(*Odsh*, *JYAlpha*), and sexual isolation (*ds2*) between *Drosophila* species. Most of these genes show molecular signatures of positive selection, proving natural selection's role (*3*), provided that fixation occurred before complete reproductive isolation rather than afterward. The top-down approach involves identifying (i) the phenotypic traits under divergent selection, (ii) those traits associated with reproductive isolation, and (iii) the genes underlying traits and reproductive isolation. Step (iii) has been challenging under both approaches but is needed to understand how selection has led to reproductive isolation.

Ecological Speciation

Evidence for ecological speciation has accumulated from top-down studies of adaptation and reproductive isolation [reviewed in (*2*, *8*, *9*)]. We now know of many real species that have, at least in part, evolved by divergent natural se-

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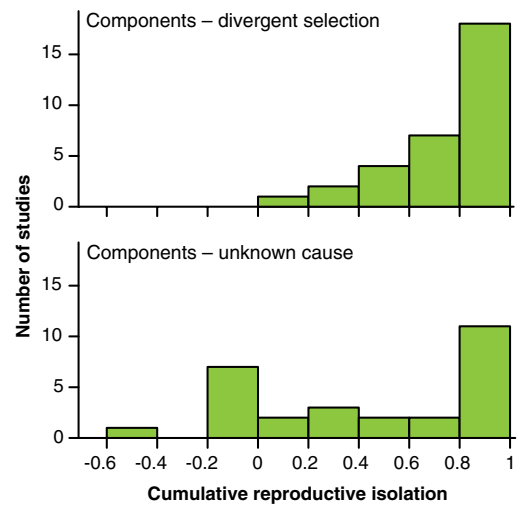
Fig. 2. Estimates of the magnitude of reproductive isolation resulting from divergent selection components (**top**), compared with other components lacking identifiable causes (**bottom**). Divergent selection components include those attributable to active selection on traits (immigrant inviability and extrinsic postzygotic isolation) and to trait-based assortative mating (habitat preference, floral isolation, and breeding time). The unattributed components include intrinsic hybrid inviability, sexual selection against hybrids, pollen competition, and reduced hybrid fecundity. Data were taken from (*32*, *31*) (table S1). A negative value indicates that hybrids had higher fitness than the parental species for at least one component of postzygotic isolation. One data value of –2.66 was left out of the bottom panel.

lection between environments. The connections between selection on ordinary phenotypic traits and reproductive isolation are often strong and straightforward. It follows that much of the genetic basis of reproductive isolation should involve ordinary genes that underlie differences in phenotypic traits. But we still know little about the genetics of ecological speciation.

One line of evidence comes from tests of parallel speciation, whereby greater reproductive isolation repeatedly evolves between independent populations adapting to contrasting environments than between independent populations adapting to similar environments (*20*, *23*). A major challenge in applying the test to natural populations is to eliminate the possibility that each ecotype has originated just once and has spread to multiple locales. This is difficult because gene flow of neutral markers between closely related but nearby populations can result in the false appearance of multiple independent origins of these populations when evaluated by phylogenies (*3*, *24*). However, multiple origins are supported in several examples of parallel speciation, including the sympatric benthic-limnetic species pairs of threespine stickleback in young lakes of British Columbia (*25*, *26*), the repeated origin of divergent marine and stream populations of threespine stickleback around the Northern Hemisphere (*27*), ecotypes of *Timema* walking stick insects living on different host

plants (*28*), *Littorina* marine snail ecotypes inhabiting different zones of the intertidal (*24*), and mosquito fish inhabiting blue holes with and without fish predators in the Bahamas (*29*) (Fig. 1A). In these studies, it was shown that males and females are more likely to mate if they are of the same ecotype, regardless of relatedness as indicated by phylogenetic affinity.

Ecological speciation is also supported by examples of premating reproductive isolation in which individuals choose or preferentially encounter mates on the basis of phenotypic traits that are under ecologically based divergent selection. Examples include assortative mating by host choice in insects, body size and coloration in fish, beak size in birds, pollinator preferences for specific phenotypic floral traits, and variation in flowering time—traits inferred to be under divergent selection between environments [see examples in (*8*, *30*, *31*)].

Ecologically based divergent selection has also been directly measured, as shown by reduced fitness of each ecotype in the environment of the other [immigrant inviability; reviewed in (*31*, *32*)] and by reduced fitness of hybrids in the parental environments [extrinsic postzygotic isolation (*33*)]. For example, each of the coastal perennial and inland annual races of the monkey flower (*Mimulus guttatus*) along the west coast of North America has low fitness when transplanted to the habitat of the other (*31*). This is an example of active selection on phenotypic differences, and it also constitutes direct reproductive isolation because it is an evolved barrier to gene flow between parental populations. Multiple traits are probably involved, including flowering time and tolerance of salt and drought. This type of reproductive isolation is context-dependent and is weakened in intermediate environments. On the other hand, active selection favors the evolution of ever-greater differences between populations, which may strengthen the barrier to gene flow (*20*).

It is unclear how much reproductive isolation typically evolves by ecologically based divergent selection in nature. We can approximate an answer from estimates of the combined contribution of active selection on traits and trait-based assortative mating, as compared with other forms of reproductive isolation (Fig. 2 and table S1). These estimates are incomplete because individual studies may lack data on components of reproductive isolation, separate components may not be independent, and the strength of barriers between species may not be symmetric (*34*). Nevertheless, compilation of the data shows that the amount of reproductive isolation attributable to active selection and trait-based assortative mating is at least as strong, on average, as the amount from components

of reproductive isolation lacking identifiable causes (Fig. 2). The unidentified component of speciation, if built by selection and not genetic drift, could be the result of either ecological or mutation-order mechanisms.

These examples indicate a growing knowledge of the mechanisms of selection and its consequences for reproductive isolation. At this point, the most glaring deficiency is our knowledge of the impact of selection on genes. Optimistically, progress is being made with genetic mapping to identify quantitative trait loci (QTLs) and genes or regulatory control regions that affect individual phenotypic traits on which components of reproductive isolation depend. Examples include the *yup* QTL, which affects flower color differences between the monkey flowers, *Mimulus cardinalis* and *M. lewisii* (*35*). Swapping alleles of this QTL between the species with repeated backcrossing resulted in shifts in pollinator preference and, hence, indirectly affected premating isolation. Survival and salt tolerance of second-generation hybrids between the sunflowers *Helianthus annuus* and *H. petiolaris* transplanted to the salt marsh habitat of their hybrid descendent species (*H. paradoxus*) mapped strongly to a QTL identified as the salt tolerance gene *CDPK3* (*36*).

Another form of investigation involves the analysis of genome scans of ecologically different populations and species. These scans compare allelic variation within and between populations at many marker loci spaced throughout the genome (*37*). Markers that show excessive differentiation between populations (outliers) may indicate selection on nearby genes. The method is particularly informative when applied to populations with ongoing hybridization, because outlier markers may identify points in the genome that resist the homogenizing influence of gene flow, perhaps indicating genomic regions under divergent selection. However, sets of genes that diverged under a mutation-order process can produce the same pattern (*17*, *18*), which makes analysis of such studies more difficult. Clues to whether ecologically based divergent selection is involved are gained if outliers at the same genomic locations turn up repeatedly in scans between populations that inhabit contrasting environments (*38*) and by identifying phenotypic traits under divergent selection that map to those locations in the genome (*36*, *39*, *40*). As genomic resources increase for more species, it will be possible to measure natural selection directly on genomic regions of interest by transplanting otherwise relatively homogenous experimental populations containing alternative alleles into the environments of the parent species (*35*).

Mutation-Order Speciation

Mounting evidence for divergent selection in speciation does not diminish the potential role

of mutation-order divergence. It may be that the mutation-order process is more difficult to detect, or perhaps we have not looked hard enough at species with only small ecological differences (*5*). We still do not know much about the selective factors causing mutation-order speciation.

Evidence for mutation-order speciation comes from instances in which reproductive isolation apparently evolved as a by-product of conflict resolution between genetic elements within individuals (intragenomic conflict), such as cytoplasmic male sterility in hermaphroditic plants (Fig. 1B), and genetic elements conferring meiotic drive. Under both mechanisms, a mutation arises that can distort representation in gametes and spreads in a selfish manner, even though such an element reduces overall fitness of the organism that bears it. This, in turn, places selection on mutations in other genes that counter the selfish element's effects and restore more equal genetic representation in gametes. Distorter and restorer mutations are unlikely to be the same in different populations regardless of environment; thus the process leads to divergence. The mismatch between the distorter in one population and the restorer in the other can result in hybrid sterility or inviability and, thus, reproductive isolation (*3*, *41*). Numerous examples of selfish elements, such as those observed in cytoplasmic male sterility of plants, support these hypotheses (*42*, *43*). In addition, partial reproductive isolation generated by meiotic drive has been identified in *Drosophila* [reviewed in (*3*, *41*)]. Sexual conflict is also expected to lead to mutation-order speciation, but there are few compelling examples (*3*). The contribution by these mechanisms to speciation is still uncertain, however. The alleles responsible for meiotic drive and cytoplasmic male sterility may be prevented from spreading to fixation because selection on such elements is frequency-dependent (*43*) and because restorer alleles arise and weaken selection on the distorter elements (*44*). Second, if divergent populations come into secondary contact, the alleles within each population causing cytoplasmic male sterility or meiotic drive (and the corresponding restorer alleles) will spread between the populations by gene flow, eliminating that component of reproductive isolation (*43*). Hence, for these mechanisms to contribute to speciation, the fitness of hybrids must be reduced to very low levels, or other incompatibilities must arise that interact with these genes to prevent their spread after secondary contact.

Conclusions

Our understanding of the role of natural selection in speciation has come a long way since Darwin's time. If he were here to witness, he would most likely be staggered by the discoveries of genes and molecular evolution and astonished at the prospect that evolutionary conflict

between genes could generate reproductive isolation (*45*). Mostly, I expect that he would be chuffed by mounting evidence for the role of natural selection on phenotypic traits in the origin of species. This is really what *On the Origin of Species* was all about. Between 1859 and the present, the general acceptance of the biological species concept altered the focus of speciation studies. Yet, the discovery that reproductive isolation can be brought about by ecological adaptation in ordinary phenotypic traits bridges Darwin's science of speciation and our own.

The most obvious shortcoming of our current understanding of speciation is that the threads connecting genes and selection are still few. We have many cases of ecological selection generating reproductive isolation with little knowledge of the genetic changes that allow it. We have strong signatures of positive selection at genes for reproductive isolation without enough knowledge of the mechanisms of selection behind them. But we hardly have time to complain. So many new model systems for speciation are being developed that the filling of major gaps is imminent. By the time we reach the bicentennial of the greatest book ever written, I expect that we will have that much more to celebrate.

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The Bacterial Species Challenge: Making Sense of Genetic and Ecological Diversity

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The Bacteria and Archaea are the most genetically diverse superkingdoms of life, and techniques for exploring that diversity are only just becoming widespread. Taxonomists classify these organisms into species in much the same way as they classify eukaryotes, but differences in their biology—including horizontal gene transfer between distantly related taxa and variable rates of homologous recombination—mean that we still do not understand what a bacterial species is. This is not merely a semantic question; evolutionary theory should be able to explain why species exist at all levels of the tree of life, and we need to be able to define species for practical applications in industry, agriculture, and medicine. Recent studies have emphasized the need to combine genetic diversity and distinct ecology in an attempt to define species in a coherent and convincing fashion. The resulting data may help to discriminate among the many theories of prokaryotic species that have been produced to date.

The species debate in microbiology is not only about a human desire to catalog bacterial diversity in a consistent manner, but is also a fundamental argument because of what it reveals about our ignorance of how evolutionary forces form, shape, and extinguish bacterial genetic lineages, of the mechanisms of differentiation between subpopulations sharing common descent, and of the process of adaptation to new niches and changing environments. Animal species are defined by their morphological and behavioral traits and by their ability or inability to interbreed, but such categories cannot easily be applied to the Bacteria or Archaea (or indeed to many eukaryotic microbes). Instead, taxonomists have been forced to rely on biochemical tests and limited morphological characteristics for this purpose. Naturally, biochemical characters have been selected for the convenience of taxonomists; they reflect only a tiny subset of those characters that allow bacteria to use different resources in the environment, and only capture a small fraction of the true diversity in this superkingdom of life. More recently, molecular methods [particularly DNA-DNA hybridization and ribosomal RNA (rRNA) sequencing] have helped to define species, but these methods have serious limitations and cannot reliably assign a large collection of similar strains to species (e.g., rRNA sequences are too conserved to resolve similar species). rRNA sequence surveys have, however, revealed the ex-

traordinary variety of microbial life, much of it uncultured (1). Beyond this, taxa too similar to be distinguished and circumscribed by rRNA sequences have revealed further diversity through multilocus sequence analysis (MLSA) (2) and metagenomic studies (1), and this diversity needs to be explained by theory. Thus, practical difficulties, lack of theory, and observations of vast amounts of as yet unclassified microbial diversity have all fueled the controversy of how one defines a bacterial species (3–8).

Genetic Clustering

Darwin commented that "all true classification is genealogical" [(9), p. 404]. Taxonomists have thus used sequence relatedness to define cutoff values that place two bacterial isolates into the same or different species. The overall genetic relatedness of isolates may be measured by the extent of DNA hybridization between them, and those that show 70% or more DNA hybridization are defined as the same species (2, 10). Such cutoffs imply that sequences that cluster together with a certain amount of similarity must be from the same species, and moreover that this cutoff value is applicable to all groups of bacteria or archaea. Recent MLSA studies, which use the concatenated sequences of multiple housekeeping genes to discern clustering patterns among populations of closely related taxa, suggest that species defined by taxonomists in many cases correspond to well-resolved sequence clusters. However, these studies also show that there is no universal cutoff or descriptor of clusters that characterizes a species. Furthermore, inspection of the clusters does not always clearly reveal which level in the hierarchy is more fundamental than any other (Fig. 1) (7).

As an example, Fig. 1A shows the relationships among multiple isolates of three closely related streptococcal species. *Streptococcus*

pneumoniae is a major human pathogen, *S. mitis* is a commensal bacteria with a history of taxonomic uncertainty (11), and *S. pseudopneumoniae* is a recently described organism of uncertain status that nonetheless corresponds to a distinct cluster in these data (12). There are striking differences in the amount of sequence diversity observed within homologous housekeeping genes in these named species, ranging from 1.2% for *S. pneumoniae* to 3.0% for *S. pseudopneumoniae* and up to 5.0% for *S. mitis*. The distance between two randomly selected *S. mitis* genotypes is similar to the average distance between *S. pneumoniae* and *S. pseudopneumoniae* genotypes (5.1%) (2). This implies that the use of a fixed level of sequence divergence for differentiating species would tend to either rejoin *S. pneumoniae* and *S. pseudopneumoniae*, or break up *S. mitis* so that nearly every isolate was a species of its own. This is clearly unsatisfactory.

Habitats and Ecological Differentiation

A clear natural criterion to identify clusters of evolutionary importance, which we might want to call species, is to find ecological features that distinguish them from close relatives. Among pathogens, the ability to cause a distinctive disease has historically been used to define species, but pathogens constitute only a minute fraction of overall bacterial diversity. Mapping of bacterial diversity onto environmental resources indicates that closely related groups of bacteria can be ecologically divergent. For example, fine-scale resource partitioning has been observed among coastal *Vibrio* populations coexisting in the water column (13). Partitioning was discovered because strains were collected from distinct, ecologically informative samples, and the phylogenetic structure of the ecologically differentiated populations was superimposed on their habitats. Habitats were defined using an empirical modeling approach. This analysis revealed high levels of specialization for some populations (e.g., *V. ordalii* is only found as single free-swimming cells), whereas others are more generalist (Fig. 1B) and can colonize a wide variety of surfaces, including organic particles and zooplankton in the water column (13). Most of the predicted *Vibrio* populations are deeply divergent from each other, and in many cases are congruent with named species; however, *V. splendidus* is a notable exception and splits into numerous closely related groups with distinct ecological preferences, presumably indicating recent ecological radiation from a sympatric ancestral population (13). Thus, genetic clusters that correlate with ecology can be discerned.

What do the genetic data tell us about mechanisms of population differentiation and the evolutionary history of the microbes in question? That bacteria are organized in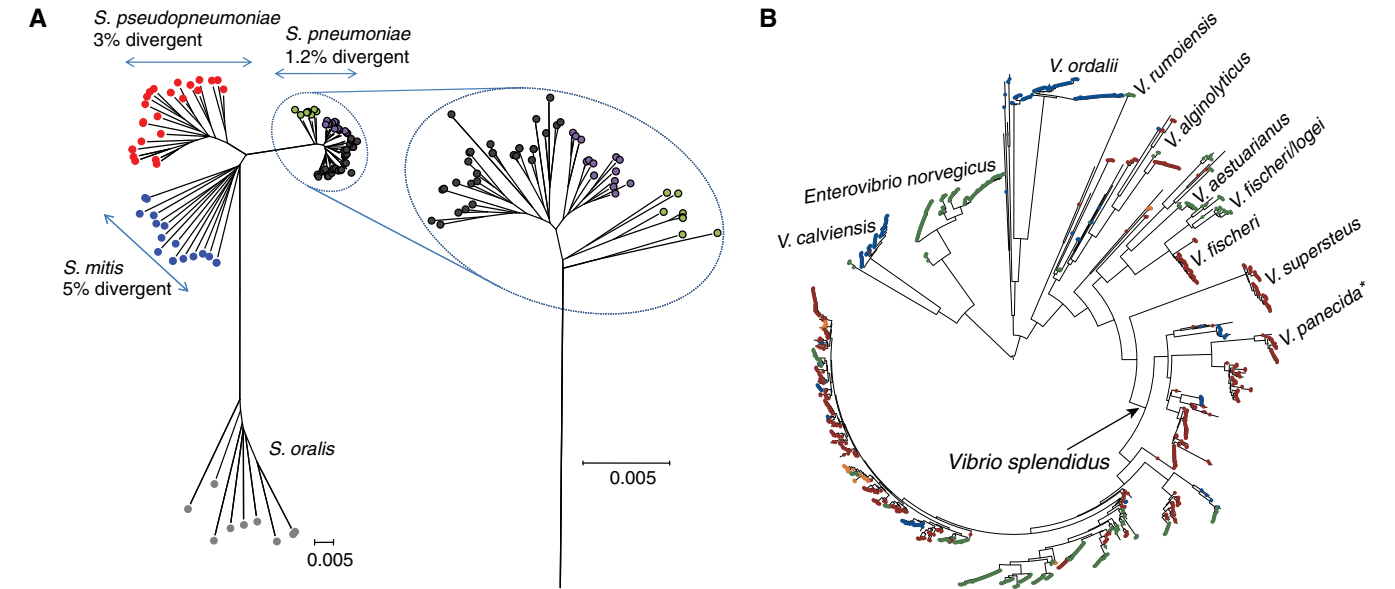to genetic clusters is not, per se, a very interesting observation; many or most models of a population reproducing with a small amount of mutation will eventually produce populations consisting of clusters of related organisms, irrespective of the details of the evolutionary forces or ecological differentiation. A more substantial observation is that there is very little neutral diversity in many populations of microbes, from which we may infer some features of the selective landscape. Neutral diversity is the amount of polymorphism that is evident in noncoding regions or results in synonymous substitutions. One common measure of neutral diversity is the effective population size N_e, defined as the size of a population evolving in the absence of selection that would generate as much neutral diversity as is actually observed. Estimates of N_e for bacteria range from 10^5 to 10^9 (14–18). To put this into context, the numbers of *Vibrio* cells per cubic meter of seawater in temperate coastal regions range from 10^8 to 10^9 (19), which suggests vast census population sizes (>10^{20}). This observation—a mismatch of many orders of magnitude between effective population size and census population size (true of most bacteria studied to date)—was originally used to counter claims of neutrality and instead argue that all genetic variation was adaptive (20, 21). However, there are several different mechanisms that can explain this mismatch (Fig. 2).

Whatever mechanisms are driving the differentiation of bacteria into clusters, they must restrict the accumulation of neutral

diversity. The first proposed mechanism was based on artificial selection experiments with bacteria grown for extended periods under stable conditions in chemostats, which showed repeated selective sweeps in which the whole genome was thought to hitchhike to fixation along with an advantageous mutation (periodic selection) (22). Selective sweeps can purge almost all genetic diversity in the population and thus constitute a candidate mechanism for reducing neutral variation (23).

Niches and Ecotypes

To extend this model, one can consider multiple ecological niches characterized by the selective advantages they confer to specific genes. This is the ecotype model, where genes adapted to specific niches cause selective sweeps within those niches but not in other niches. In this way the population will undergo adaptation and differentiation while maintaining relatively low levels of neutral diversity, as selective sweeps confined to each ecotype regularly purge the population of any diversity that might have accumulated (Fig. 2A). Crucially, what neutral diversity we do observe is predicted to be associated with adaptive traits. The ability of such selective sweeps to limit the effective population size has been recognized for some time (17, 23), and this model has been substantially developed by Cohan and colleagues (4, 16, 24). Because it links patterns of genetic differentiation with adaptation, and

makes reference to the unifying biological principles of selection and niche partitioning, the ecotype has rightly become popular as a framework within which to discuss bacterial evolution, speciation, and ecology.

The ecotype model (4, 16, 24) predicts that common ancestry will be preserved among bacterial populations within niches (which should be monophyletic), and thus predicts that ecotypes are coherent self-contained gene pools. As a result, it has been suggested that ecotypes should be considered as putative or actual species, depending on the level of genetic differentiation from the ancestral population. This model therefore has the advantage of providing a mechanistic understanding of the evolutionary processes, as well as an organizing principle for classifying species, that is based on experimental observations of bacterial populations.

However, these observations of repeated selective sweeps were made in chemostats, whereas natural environments are markedly unstable and diverse. How would one detect the presence of selective sweeps in natural bacterial populations? The most conclusive examples come not from bacteria but from RNA viruses, which mutate at much higher rates than DNA-based life forms. It has been established that sequences collected over many years that the population structure of the human influenza virus is predominantly driven by repeated selective sweeps (25) and that the resulting effective population size N_e (<100) is very much smaller

Fig. 1. Multilocus sequence analysis of closely related species. (**A**) Radial minimum evolution tree constructed using MEGA4, showing clusters among 97 isolates of four *Streptococcus* species identified as indicated. The tree was built using concatenates of six housekeeping loci, resulting in a total of 2751 positions in the final data set (2). Distances were calculated as the percentage of variant nucleotide sites. The mean distance within the clusters, calculated by MEGA4, is shown. To the right, the pneumococcal cluster is shown at larger scale, and putative subclusters are indicated in dark gray, purple, and green. (**B**) Ecological associations of Vibrionaceae sequence clusters (13). Habitats (colored dots) were estimated as differential distributions of groups of closely related strains among samples (size fractions enriched in different environmental resources). Clusters associated with named species are evident, and in most cases species show a clear predilection for one of the habitats. The exception is *V. splendidus*, which breaks up into many closely related ecological populations. Asterisk denotes that trees based on additional loci indicate that the placement of *V. panecida* within *V. splendidus* may be an artifact of horizontal gene transfer at the Hsp60 locus.
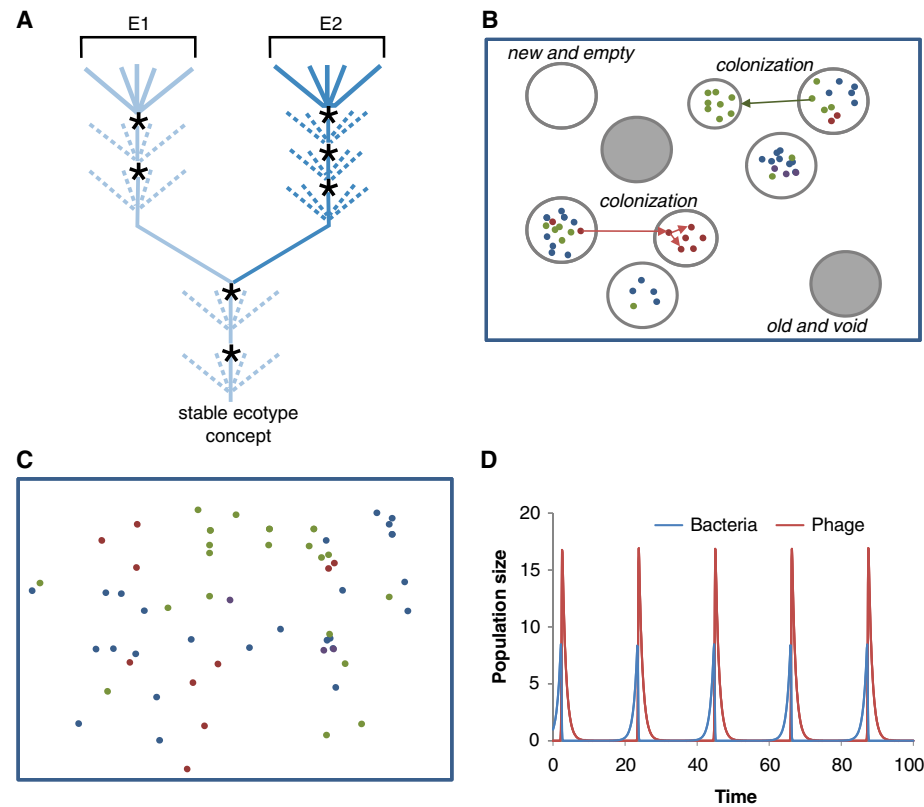
Fig. 2. Different models of microbial evolution that lead to low values of N_e. (**A**) The ecotype model of bacterial population differentiation. The tree shows a single bacterial lineage that differentiates into two sublineages (E1 and E2) that differ in some aspect of their ecology. Periodic selection (a selective sweep) occurs at the points marked by asterisks and eliminates almost all of the diversity that has arisen since the last episode of periodic selection, which is shown by the dashed branches (diversity purged by periodic selection) or solid branches (existing diversity) on the tree. As the two populations are ecologically distinct (i.e., ecotypes), periodic selection in one sublineage does not influence diversity in the other sublineage and vice versa. Each ecotype can therefore diverge to become separate species. Reproduced from (*24*) with permission. (**B**) A metapopulation. Patches of varying size (gray circles) are vacant (empty) or may be colonized by a single genotype randomly acquired from another patch. Strains may diversify within a patch (as shown by different colors representing distinct genotypes), which may colonize empty patches as described above. A characteristic of this sort of metapopulation is patch turnover, in which patches occasionally become unable to support colonization and their inhabitants are removed (solid gray circles). (**C**) A neutral model with small population size. Different genotypes (different colors) arise by mutation or recombination and increase or decrease in the population by random drift. For some purposes, this simple model is an adequate effective description of the more complex evolutionary processes represented in (A), (B), and (D), and of other more complex evolutionary models not described in this review. (**D**) Predator-prey dynamics and population bottlenecks. Regular population bottlenecks can drastically shrink the effective population size. In this case, bacteria-phage predator-prey dynamics are simulated with a classical Lotka-Volterra model, which can generate oscillations in population size of any amplitude. Population sizes and time axes are in arbitrary units for illustrative purposes only.

than observed for bacteria. The use of longitudinal ecological and genetic data to distinguish between competing models of evolution has a long pedigree in eukaryotic biology (*26*). On the basis of these analogies, any inference of a population structure driven by selective sweeps would require good longitudinal data from natural bacterial populations, as well as observations of episodic crashes in diversity causally associated with genetic changes and not associated with changes in ecological covariates.

Bottlenecks, Metapopulations, and Local Extinctions

The essential element of the ecotype model with respect to limiting neutral diversity is not niche adaptation per se, but rather the effective bottleneck caused by the replacement of the whole population by descendants from a single individual and the resulting extinction of all other lineages (Fig. 2A). Other mechanisms that induce or involve regular population bottlenecks will also restrict neutral diversity.

Metapopulation structure, in which the population is divided into patches and where individuals disperse between patches, can generate very low effective population sizes if patches turn over (i.e., if patches are only intermittently able to support bacterial growth, and if a small number of bacteria are dispersed to colonize empty patches) (Fig. 2B) (*27*). This structure well describes the situation for parasites, which can colonize a host but are then forced to move on because the host develops immunity or dies (*17*). It also describes any situation where bacteria use a limited resource intensively for short bursts, followed by dispersal to new resource patches (e.g., colonization of organic particles in seawater by *Vibrio* populations). This metapopulation model is fundamentally different from the ecotype model because it does not predict an association between neutral diversity and adaptive traits.

The relevance of the metapopulation model to the species question is that, although highly idealized and simplified, it may capture some of the effects of complexity and instability of actual ecosystems on population structure. Selective sweeps are predicted to be inevitable in simple, stable environments but not in complex metapopulations [a point partly addressed in (*28*)]. A metapopulation may evolve, differentiate, and adapt without global selective sweeps. Diversity lost by a local selective sweep in one patch may be rescued and reintroduced from other patches. The ecotype model, with its predicted monophyletic relationship between niche and genotype, may therefore not be an appropriate model of speciation in complex ecosystems.

Choosing Between Models

It has proven difficult to discriminate between models of population differentiation that focus on ecotypes or metapopulations. For example, the ecotypic structure of a soil *Bacillus* has been modeled to predict a priori which sequence clusters were ecotypes, and hence which ones should be associated with specific ecological properties (*16*). Some clusters are associated with certain phenotypic traits, such as a propensity to grow on shady north-facing slopes or sunny south-facing slopes. However, this model fitted no better (and in fact slightly worse) than a version of the model with several subpopulations and diversity generated only by neutral drift. This version of the model was dismissed because of its association with a very low estimate of population size (*14*). However, estimates of effective population size N_e are often grossly disconnected from census population sizes. It has proven very challenging to find models that successfully explain low estimated values of N_e while providing better predictions than models based on simple neutral drift. The analysis of *Bacillus* partly did this by predicting more ecotypes in the model than were observed

using established ecological criteria, a hypothesis that can be tested.

This problem of low power to detect selection (or, more accurately, to reject neutrality) is a very general problem in population genetics that does not negate the importance of adaptation in evolution, but rather suggests that more work is needed if we want model-based methods to discriminate among different biologically plausible explanations of genetic data. In Table 1 we propose a scheme for performing analyses that could be used to test, develop, and validate different competing models more systematically.

Homologous Recombination

One specific challenge to models that invoke ecotypic structure involves a feature of bacterial evolution—homologous recombination—that we have not yet discussed. Bacterial reproduction does not involve the obligate reassortment of genetic material observed in most higher organisms. However, recombination does occur in bacteria and archaea (*29*) and typically involves the replacement of a short piece of DNA with the homologous segment from another strain. Recombination becomes less probable with increasing sequence divergence between the donor and the recipient (*30, 31*), which reduces but does not eliminate recombination between closely related species. Because of such interspecies recombination, any given isolate within a species is almost certain to contain at least some genetic material that is characteristic of other closely related species. Hence, whereas it was once thought that bacteria do not form species in the eukaryotic sense because they do not recombine at all (*32*), one current view is that they do not form species because they recombine too much (*5*).

In asexual clonal organisms, even in the absence of any selective pressure, clusters will spontaneously split into multiple lineages or "daughter" clusters (*15*). However, under certain circumstances recombination can prevent this, and we can hence divide the bacteria into "sexual" and "nonsexual" species. This effect, described at greater length elsewhere (*15*), is summarized in Fig. 3, which shows the rate at which two clusters diverge over time—that is, the increase in the mean genetic distance between them. If this becomes negative, then the two clusters will stop diverging and instead converge. The three examples shown in Fig. 3 differ only in the rate of homologous recombination between the clusters, all other parameters being held constant. As recombination increases, we see a distinction between a "clonal" organism in which clusters are predicted to diverge (the green line) and a "sexual" organism (the blue line) in which they are predicted to be held together by recombination. For "sexual" species, the divergence of clusters requires a
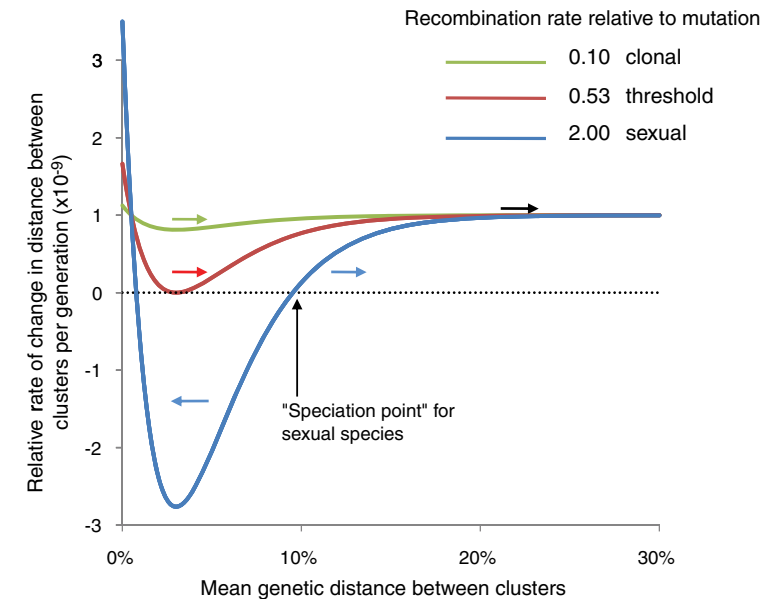

Fig. 3. The dynamics of cluster divergence. The figure summarizes some key results from (*15*) in a phase-space plot of the genetic dynamics of two populations, with recombination occurring between them at a rate that is varied for the three different simulations. The *y* axis shows the rate of change of genetic distance between the clusters as a function of the genetic distance itself (*x* axis). When the rate of change is positive, the populations will diverge genetically; when negative, they converge. The direction of change for each scenario is shown by arrows color-coded to each scenario. For low recombination rates, the populations are effectively clonal and always diverge (green line). As the recombination rate increases, the cohesive effects of recombination slow the rate of divergence, until a threshold is passed (red line) and the populations become effectively sexual in the sense that the populations no longer diverge. For recombination rates above this level, the fate of the two populations will depend on how genetically distinct they are at the outset. If they are within the "speciation point," then recombination will cause them to merge. If they are farther away than this "speciation point," they will continue to diverge from each other. These curves are derived using the model described in (*15*).

process that reduces the rate of recombination between them—for example, a period of allopatry or ecological differentiation. The speciation point is the amount of divergence between clusters that needs to accumulate to prevent them from returning to a single cluster if the barriers to recombination are removed. A recent study hypothesized that two related *Campylobacter* species are currently undergoing this process of merging into a single species as a result of changes in their environment (*33*).

The above insights were reached using models based on the assumption that genetic variation is neutral. Although this is obviously not always an appropriate assumption, it is plausible that the number of loci explicitly involved in adaptive ecological differentiation will be small, and thus that in an unstable landscape, genomic barriers to recombination will depend more on the accumulation of differences at neutral loci than at adaptive loci. The models also assumed a homogeneous distribution of polymorphisms across the genome, and violation of this may alter the tempo and mode of these processes (*34, 35*).

Illegitimate Recombination and Gene Content Variation

Illegitimate recombination or gene acquisition is another unusual feature of bacteria. In this case, genes or clusters of genes are acquired that typically have no homolog(s) in the recipient strain. The importance of this phenomenon is evident in the clear and ubiquitous signature of such events in the growing body of genomic data. These are identified by differences in the characteristics of the acquired DNA and that of the host strain, for example, in base composition or codon usage; in most cases, the donor of the DNA in question is unknown. Gene acquisition leads to genomes being punctuated by stretches of foreign DNA. The largest of these (which may be many kilobases in length) were initially termed "pathogenicity islands," because the new functions encoded by the imports were often involved in virulence, but a better term is "genomic islands" as the phenomenon is far from limited to pathogens (*36, 37*). Although it is hard to quantify the selective impact of importing any given gene(s) into a new background, the occasional ability to gain a new adaptation in this fashion—such as a new metabolic capa-

bility or a new mode of transmission for a pathogen—may be of enormous importance in terms of speciation.

Perhaps even more striking is the amount of variation in gene content revealed by multiple genomes from the same species, which implies that gene acquisition occurs at a surprisingly high frequency. It is now commonplace to speak of the "core" genome, which encodes fundamental functions shared by all members of a species (and, it should go without saying, other related species), onto which is bolted the "auxiliary" or "accessory" genome, composed of genes and operons that may or may not be present in all isolates. It seems likely that such auxiliary genes help to determine the specific ecological properties of the organism. For example, a group of related *Leptospirillum* has recently been hypothesized to adapt to different areas of an acid mine drainage system by shuffling of chromosome segments enriched in noncore genes (*38, 39*). We should, however, be aware that changes in core genes may also lead to ecological differentiation, a phenomenon well documented in experimental studies of bacteria growing in structured environments (*40*).

Estimates vary, depending on the genomes that are available, but as little as 40% of genes may be present in all sequenced genomes of a named species (*41*). We may consider genes within a named species as being characteris-

Ecological factor b

Ecological factor a
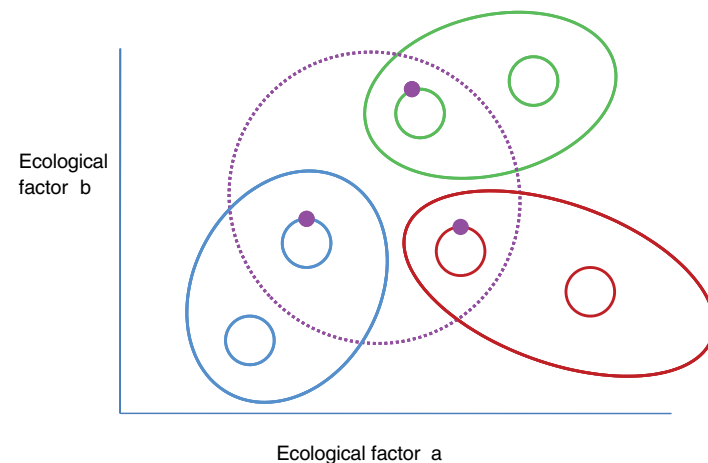
Fig. 4. Differences between core and auxiliary genes. This schematic illustrates the relationships between three species in "ecotype space," shown here in two dimensions, and a mobile gene common to all three. The areas occupied by the species are shown as solid lines in red, blue, and green. The part of the ecological space where the shared mobile gene is selected in each species is shown by a dashed purple line and overlaps all three species ranges. Examples of (circular) genomes from each species with and without the purple mobile element are also illustrated. Note that for each species, the locus is not selected for all isolates, and its evolutionary fate is uncoupled from that of each host species, because if one undergoes a selective sweep or goes extinct, the mobile gene may be reintroduced from one of the other species. Examples of such distributed loci include drug resistance determinants in pathogens (e.g., β-lactamase genes) and heavy metal resistance in environmental organisms. These genes may be transferred among strains and species by conjugative plasmids or other mobile elements (including transducing phage).

tic of different levels of ecological specificity, ranging from highly conserved core functions that are essential for growth in all environments to loci that are involved with adaptation to a specific habitat. Some narrow niche-specific genes may be distributed across species, being transferred between them by mobile elements. The evolutionary fate of such genes may hence be only loosely coupled with that of any par-

Table 1. A proposed strategy for developing and validating models of bacterial evolution that might eventually be used to classify genetic diversity data and provide a firm foundation for a bacterial species concept.

1. Collect samples according to systematic ecological stratification. Focus on longitudinal studies, geographical studies, and measurement of physical and chemical gradients affecting bacterial growth. Consider biotic factors such as the presence of other competing bacteria or parasitic phage.
2. For each isolate, sequence as much as possible and affordable (16*S* rRNA, MLSA, auxiliary genes, full genomes, etc.).
3. Use empirical classification algorithms that use genetic and ecological data to jointly map isolates.
4. To guide model formulation, use population genetic tests on observed clusters, focusing on tests for selection, population structure, and gene flow.
5. Generate evolutionary models and simulate populations.
6. Test, then reject or adapt, evolutionary models according to agreement between simulations and real populations; if necessary, return to step 1.
7. For successful models, develop model-based methods for interpreting pure genetic data (without ecological covariates) and test on new data.
8. If one or more validated models emerge, use these to classify genetic data and to develop bacterial species concepts.

ticular species or strain in which they are found, and they are maintained through selection by the habitat to which each host strain is adapted. In the case of very mobile elements—for example, plasmids encoding resistance to antibiotics or heavy metals—the ecological specificity determined by these accessory loci may have no link to the sequence we observe using housekeeping genes (Fig. 4).

Identifying Mechanisms and Delineating Species

What do we want from bacterial species? Do we need theoretical consistency even at the expense of taxonomic practicality, incorporating both "clonal" and "sexual" populations into a single theoretical framework? One unifying theoretical concept is to consider species as the arena within which individuals are similar enough, or interbreed enough, that individual variant genes compete directly for reproductive success. Practical advances building on this or other theoretical concepts will only come when these are developed into explicit models and model-based algorithms that are tested and refined on a wide range of data. Alternatively, it may be sensible to suggest an ad hoc application of principles to different genera on the basis of their specific characteristics, including the extent of variation in gene content and recombination. In any case, no biologist would deny the importance of ecology to what we observe, but it may not be easy to incorporate it in a fashion that is convenient for taxonomists. Nonetheless, population geneticists may have little choice but to tackle the question of defining bacterial species or, at the very least, populations. Whether we are estimating effective population size from neutral diversity or choosing an appropriate set of strains to test for positive selection at a locus of interest, species definitions are implicit in much of the analytical toolkit of population genetics.

Distinguishing among mechanisms of population differentiation in bacteria ultimately comes down to testing the ability of different models to explain highly variable patterns within and between genetic-ecological clusters (Fig. 1). It is still unclear whether these patterns are maintained by gene flow or selection, and what the effect of population structure is. The joint distribution of genetic and ecological data can be used, as described above for *Vibrio* species (*13*), to define populations without making a strong theoretical commitment to either of

these alternatives. One clear result from all of the studies discussed here is that the underlying theoretical questions concerning species will not be answered in the absence of more detailed genetic-environmental mapping. Moreover, some guidelines for the types of ecological studies that will be most informative are emerging. Most important, the ecological data collected must be relevant to the niche boundaries of the populations studied. And if genetic groups do not map exclusively onto sampling categories (as is likely to be the case), more complex statistical models will be needed to identify and describe the underlying niche structure. Longitudinal studies that measure the dynamics of ecological associations over time will also be helpful to determine how transient natural habitats are, and thus how likely bottlenecks are to result. Finally, whole-genome sequences from entire populations of environmental bacteria will be useful in dissecting the roles of the auxiliary and core genome in ecological differentiation. If after this process it emerges that some model or models are consistently validated for different study systems, these would inevitably form a good basis for identifying fundamental levels of clustering, or species.

In the foregoing we have emphasized ecotype and metapopulation models, but there are others that deserve consideration—notably the epidemic clonal model (*42*) and the impact of phage epidemics causing classic Lotka-Volterra boom-bust dynamics (*43*) illustrated in Fig. 2D—and it is possible, even likely, that more than one of these mechanisms may be relevant to any given problem in speciation and cluster formation. Distinguishing among these mechanisms is the bacterial species challenge (Table 1), described in 1991 by John Maynard Smith as follows: "Ecotypic structure, hitch-hiking, and localized recombination can explain the observed patterns of variation. The difficulty, of course, is that the model is sufficiently flexible to explain almost anything. To test the hypothesis of ecotypic structure, we need to know the distribution of electrophoretic types [i.e., genotypes] in different habitats" (*17*).

Much research on bacterial species to date has come from studies on pathogens, where the correct identification of species is crucial for accurate clinical diagnoses. However, for pathogens the identification of the multiple ecological

niches within (for example) the nasopharynx or gut is difficult, and studies of the relationships between bacterial populations and ecology may be more fruitful for some environmental species where the categorization of niches is a more tractable enterprise. Hopefully, we will soon obtain richer data sets that map bacterial diversity onto ecology and provide a way to distinguish among various models of population differentiation and speciation, including those based on ecotypes or metapopulations.

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Stability Predicts Genetic Diversity in the Brazilian Atlantic Forest Hotspot

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Biodiversity hotspots, representing regions with high species endemism and conservation threat, have been mapped globally. Yet, biodiversity distribution data from within hotspots are too sparse for effective conservation in the face of rapid environmental change. Using frogs as indicators, ecological niche models under paleoclimates, and simultaneous Bayesian analyses of multispecies molecular data, we compare alternative hypotheses of assemblage-scale response to late Quaternary climate change. This reveals a hotspot within the Brazilian Atlantic forest hotspot. We show that the southern Atlantic forest was climatically unstable relative to the central region, which served as a large climatic refugium for neotropical species in the late Pleistocene. This sets new priorities for conservation in Brazil and establishes a validated approach to biodiversity prediction in other understudied, species-rich regions.

Late Quaternary climate fluctuations helped to shape present-day diversity in temperate and boreal systems (*1*), providing a general context for understanding current patterns of endemism. In the tropics, Pleistocene refugia models have been dismissed because of conflicting evidence (*2*, *3*) or circularity in identifying putative refugia (*4*), but historical processes must be invoked to explain regions of high endemism (*5*, *6*). Recent studies from subtropical biomes have usefully employed post hoc palaeoclimate models of species and habitats to provide insights about processes shaping genetic and species diversity (*5*, *7*). Building on them, we first map the palaeodistribution of endemic species to identify temporally stable (refugial) and unstable (recently colonized) regions for species occurrence, which are then validated with multispecies molecular data. Going beyond the traditional species-by-species approach, the molecular analyses contrast the fit of assemblage-level data to the spatially explicit demographic scenarios suggested by the climate-based models.

We apply this approach to one of the world's most species-rich, yet notoriously endangered and understudied ecosystems: the Brazilian Atlantic rainforest. Originally extending for 1,300,000 km^2 along the Brazilian coast and reaching into Paraguay and Argentina, this biome has been reduced to less than 8% of its range (*8*). Today's fragments harbor one of the largest percentages of endemic species in the world, with many species and even genera of vertebrates still

being described (*8*, *9*). Our ultimate goal is to pinpoint regions for inventory work and habitat protection before we lose a substantial fraction of described and undocumented diversity. The approach differs from previous methods by directly modeling historical processes, as opposed to observed biodiversity patterns (*10*), with the aim of informing conservation.

We use molecular genetic data from multiple, largely codistributed species to test whether spatial modeling of species-specific Late Quaternary refugia sheds light on historical processes and hence improves prediction of genetic endemism and diversity in tropical Brazil (*11*). We focus on three common species of tree frogs that are widely distributed along the Brazilian Atlantic forest: *Hypsiboas albomarginatus, H. semilineatus*, and *H. faber*. Given their life history traits, amphibians are useful indicators of environmental changes through time (*12*). Whereas *H. albomarginatus* and *H. semilineatus* occur in low and mid altitudes and are mostly restricted to the evergreen or semideciduous components of the Atlantic Forest in eastern Brazil, *H. faber* has a broader altitudinal range and also inhabits mixed and deciduous areas, occupying interior and coastal sites in the Atlantic Forest south to Paraguay and Argentina (figs. S1 and S2) (*13*). The comparative phylogeographic approach is a powerful test of assemblage-scale responses to former environmental change and thereby provides a means for critical assessment of the scenarios produced by modeling of species' distributions under palaeoclimates (*7*).

The palaeomodeling method intersects predicted species' distributions under current conditions and climatic extremes of the Late Quaternary (6000 years before present, or 6 kybp, and 21 kybp) to predict areas of stability (regions in which species are predicted to occupy irrespective of time period) and unstable areas (*7*, *14*). Because the stability maps raise specific hypotheses about regional differences

in persistence and hence diversity, they lead to phylogeographic predictions for both individual species and assemblages (codistributed taxa; Fig. 1). Field sampling is driven by the model predictions to cover both predicted refugia and unstable (recently colonized) areas, particularly emphasizing previously undersampled areas. If the approach correctly predicts current patterns of biodiversity at the regional scale, species should consistently show (i) higher genetic diversity within and among populations in refugia relative to unstable areas, because of long-term persistence and population structure; (ii) genetic signature of population expansion in unstable areas, reflecting multispecies colonization from adjacent refugial regions after the Last Glacial Maximum (LGM, 21 kybp); (iii) absence of genetic patterns of isolation-by-distance in unstable areas, given that colonization has been too recent to permit restoration of equilibrium between migration and genetic drift (*15*); and (iv) strong phylogeographic structure between refugia, reflecting assemblage-wide, long-term population persistence in isolated areas.

Distribution models developed under current climatic conditions accurately predict distributions of each of the target species along the Atlantic rainforest domain [area-under-the-curve (AUC) values (*16*) 0.968, 0.989, and 0.994; maximum Kappa (*17*) 0.81, 0.925, and 0.94 in *H. albomarginatus, H. faber*, and *H. semilineatus*, respectively (fig. S2)]. Stability maps, depicting the intersection of distribution models for each taxon under current, 6 kybp, and 21 kybp climates, predict for all species a large central refugium throughout the Late Quaternary ("Bahia refugium") (Fig. 2). A second, much smaller refugium is predicted in the northeasternmost portion of the forest ("Pernambuco refugium"). In *H. faber*, a third, southeastern refugium of intermediate size is also predicted ("São Paulo refugium"). This is not surprising, given that this species occupies a broader environmental niche. In contrast to the central and northern regions, populations south of the Bahia or São Paulo refugia appear much less stable, despite the more extensive (preclearing) range of the forest in southern and southeastern Brazil. We hypothesize that these areas received a significant influx of migrants from adjacent, large refugial populations after the LGM. These palaeomodel results are congruent with the fossil pollen record, which documents a replacement of forests by grasslands in the southern Atlantic forest during the LGM (*14*, *18*) and suggests the occurrence of small forest refugia in the southernmost range of the putative Bahia refugium (*19*). The results also agree generally with forest models published previously (*14*), although the central refugium extends farther south in the frog-based models. Such differences are expected because the forest and its associated species may differ slightly in their climatic tolerances and realized

niches. In *H. albomarginatus* and *H. faber*, the extension of the predicted São Paulo refugium westward into the neighboring Cerrado biome reflects model overprediction (fig. S2) (*14*).

Models of habitat stability through fluctuating climates correctly predict patterns of phylogeography in the Brazilian Atlantic rainforest (Fig. 2 and figs. S3 to S5). In all species, high levels of divergence and population structure are observed across refugia (Tamura-Nei corrected distances (*20*): 4 to 7% between Bahia and Pernambuco refugia, 1% between the nearby Bahia and São Paulo refugia in *H. faber*). Similarly, in all taxa there are multiple, divergent clades within the Bahia region, agreeing with model-based predictions of a large refugium in this area. In *H. faber*, divergent clades are also represented in the São Paulo region, matching predictions of a mid-sized refugium in this area. All taxa show low genetic diversity across the southernmost range of the forest, an area predicted to be less stable by the palaeomodels. Furthermore, mitochondrial DNA (mtDNA) lineages found in this region are shared with adjacent refugia (one in *H. albomarginatus* and *H. semilineatus*, two in *H. faber*).

Metrics of genetic diversity confirm the above patterns (Table 1). In *H. albomarginatus* and *H. semilineatus*, genetic diversity (*21*) is an order of magnitude larger in the central (Bahia) refugium relative to the less stable (southern) portion of the forest. Diversity of *H. faber* in this southern area is higher than the other species because of the presence of two lineages that co-occur in the adjacent refugia. In all species, average net nucleotide differences across localities (*22*) reflects high geographic structure within refugia (2.6 to 6.2% divergence). In contrast, sites located outside (south of) the refugia are genetically more similar to each other, although to a lesser extent in *H. faber* (0.1 to 1.6%). Signatures of population expansion (*23*) are found in the unstable area for *H. albomarginatus* and *H. faber*, as well as in the Bahia refugium area for *H. faber* and *H. semilineatus*. The lack of signature of population expansion in the southernmost localities of *H. semilineatus* may reflect low statistical power because of the exceptionally low levels of diversity observed in this species. As predicted, isolation by distance is not observed in unstable regions, but is detected within refugial areas for *H. albomarginatus* and *H. faber*.

The hierarchical approximate Bayesian computation (HABC) method (*24*) allows us to use data from all three species at once to test for assemblage-wide responses to Late Quaternary climate change. These analyses support both model-driven hypotheses of (i) simultaneous, multispecies colonization of unstable areas from adjacent refugial populations since the LGM, as opposed to long-term persistence of populations in unstable areas, and (ii) assemblage-
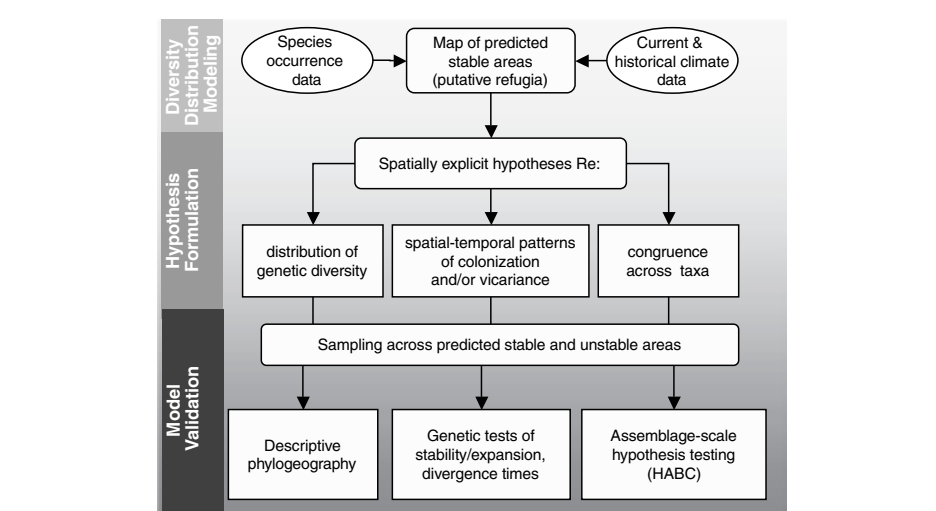
scale, long-term persistence of populations in isolated refugial areas, as opposed to post-LGM colonization of refugial regions.

To test for assemblage-wide colonization of predicted unstable areas, we group mtDNA sequences from the southernmost refugial sites [population 1 (Fig. 3A)] and from localities in unstable areas south of the refugium [population 2 (Fig. 3A)] to contrast two alternative historical models across the three codistributed species, while allowing the taxon-specific demographic parameters to vary. In H$_1$, the long-term persistence model, two contemporary populations split from an ancestral population prior to the LGM (120,000 to 1.2 million years before present, or Mybp, Fig. 3A). In H$_2$, the recent colonization model, population 2 is modeled as being colonized from refugial population 1 subsequent to the LGM (0 to 20 kybp; Fig. 3A). The results indicate that all three species colonized the southern (unstable) areas after the LGM ($Z_2 = 3$, the number of species evolved under H$_2$), even when allowing for postisolation migration (Fig. 3, B and C). When Bayes factor is used (*25*), there is strong support for recent colonization in all three species ($Z_2 = 3$) under the no-migration model [$B(Z_2 = 3, Z_2 < 3) = 35.16$], and moderate support under a postisolation migration model [$B(Z_2 = 3, Z_2 < 3) = 5.70$].

Using the same framework to test for long-term persistence of refugial populations, we compare mtDNA sequences between the predicted Pernambuco refugium [population 1 (Fig. 3A)] and adjacent (northern) populations from the Bahia refugium [population 2 (Fig. 3A)] to contrast alternative historical models H$_1$ and H$_2$. In this case, the HABC results infer long-term persistence of populations in isolated refugia for all three species ($Z_2 = 0$, i.e., $Z_1 = 3$), even when allowing for postisolation migration (Fig. 3, D

and E). Using Bayes factor (*25*), we also detect evidence for stability in both areas under the no-migration model [$B(Z_2 = 0, Z_2 > 0) = 4.89$], as well as under a postisolation migration model [$B(Z_2 = 0, Z_2 > 0) = 4.84$].

Relative to nuclear loci, mtDNA data are more variable and readily collected and often provide key insights into biological response to environmental modification (*1*). Although single-locus inference can be imprecise in the face of coalescent variance and the possibility of selection (*26*), our method benefits from a multitaxon approach, while explicitly accounting for the stochasticity of a single-locus coalescent across taxa. Combining data sets from several codistributed groups into a single hierarchical Bayesian analysis allowed us to estimate congruence across species, while borrowing strength from the full comparative phylogeographic sample (*24*). This can translate into higher analytical power and be more informative than qualitative comparisons of species-specific analyses. By capturing the historical signal that emerges from larger, combined multispecies molecular data sets, HABC will offer the possibility of looking at patterns of historical community assembly in codistributed nonmodel organisms for which barcode-type DNA sequence information (e.g., mtDNA data) can be feasibly collected.

Collectively, the results identify the central region as a hotspot within the Atlantic rainforest hotspot and a refuge for biodiversity during climatic extremes of the Late Pleistocene. This is not to say that southern areas entirely lacked forested habitats in the late Pleistocene: The existence of species and genera endemic to the southern forests (*27*), as well as some palaeoecological and genetic evidence (*28*), offer evidence to the contrary. Rather, the phylogeographically validated palaeomodels

Fig. 1. Proposed method of biodiversity prediction. Three stages are involved: biodiversity distribution modeling (top), model-based hypothesis formulation (middle), hypothesis testing and model validation (bottom).

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Fig. 2. Genetic diversity in putative refugial (stable) versus unstable areas in the Brazilian Atlantic rainforest. (**Top**) Species-specific stability maps; modeled refugia in black. (**A**) *H. albomarginatus*, (**B**) *H. semilineatus*, (**C**) *H. faber*. Note the absence of large stable regions in the southern portion of the forest (south of the Bahia and São Paulo refugia) relative to the central and northern areas. Asterisks denote refugia inferred beyond the current ranges of the target species. Symbols indicate localities sampled for molecular analysis. Scale bar, 400 km. (Bottom) The 50% majority-rule consensus Bayesian phylogenetic trees, rooted with sequences from the other two congeneric species studied (root not shown). Thick internodes denote clades with posterior probability greater than 90%. Percentages indicate Tamura-Nei corrected distances between clades (*20*).
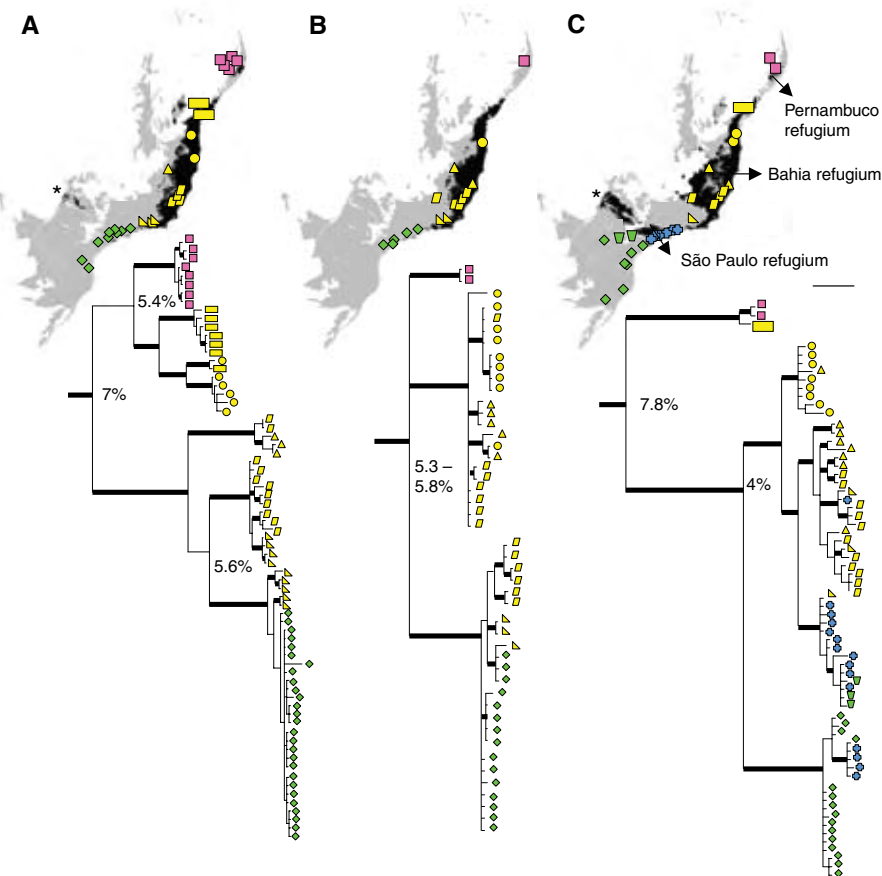
Table 1. Population genetic summary metrics used in model validation. *n*, Sample size; *S*, number of segregating sites. The diversity parameter θ and mean D_a across localities are given per base pair (bp). *Hs* test (*23*) is used to detect population expansion. BA, Bahia; SP, São Paulo refugia. Because predicted refugia were often larger than predicted unstable (recently colonized) areas, *n*, *S*, θ, and average D_a values of the former were obtained not only from the total number of samples, but also from all possible combinations of spatially contiguous localities distributed within the geographic extension of the unstable area. Parentheses encompass minimum and maximum values from subsamples. *P* values in bold highlight statistical significance at 0.05 probability level.

Species	Area	*n* (min.; max.)	*S* (min.; max.)	θ (min.; max.)	Mean D_a (min.; max.)	*Hs* (*P* value)	Mantel's corr. coef. (*P* value)
H. albomarginatus (970 bp)	Stable (BA)	36 (13; 23)	207 (81; 155)	0.076 (0.034; 0.072)	0.062 (0.020; 0.082)	−20.546 (0.141)	0.499 **(0.001)**
	Unstable (south of BA)	27	22	0.003	0.001	−11.498 **(0.004)**	−0.140 (0.580)
H. semilineatus (718 bp)	Stable (BA)	28 (6; 13)	71 (14; 58)	0.031 (0.009; 0.034)	0.036 (0.007; 0.041)	−17.778 **(0.029)**	0.054 (0.460)
	Unstable (south of BA)	15	9	0.003	0.004	0.114 (0.357)	0.436 (0.248)
H. faber (771 bp)	Stable (BA)	28 (13; 23)	94 (42; 80)	0.018 (0.012; 0.022)	0.026 (0.001; 0.044)	−38.111 **(0.003)**	0.803 **(0.0003)**
	Stable (SP)	15	48	0.023	0.028	−5.981 (0.115)	0.305 (0.221)
	Unstable (south of SP)	18	40	0.015	0.016	−13.255 **(0.014)**	0.0001 (0.456)

presented here show that the central region had much higher stability relative to the south. Forest lizards (*14*, *29*) and birds (*30*) also show high diversity in the central portion of the biome relative to southern areas, and provide evidence for population expansion in southern regions. This reassures us that the processes uncovered by the amphibian data may be generalized to and help to explain patterns of diversity in other,

much more distantly related groups of Atlantic forest endemics.

Because collection efforts, molecular studies, and conservation priorities have been heavily biased toward southern and southeastern Brazil (*8*, *9*, *31*), we predict that genetic diversity and narrow endemism in the central corridor of the biome have been substantially underestimated. This is serious, given the higher rate of defor-

estation in this region relative to the more extensive forests in São Paulo and southern Brazil (*9*, *31*). Not only could much unique diversity be lost, but ongoing habitat destruction could quickly erase the signature of the historical processes that led to it, preventing a full understanding of the mechanisms underlying local endemism and, therefore, impeding more effective conservation measures.

Fig. 3. HABC analyses. (**A**) Simulated models H_1 (long-term persistence) and H_2 (recent colonization). In both cases, each species was modeled as two contemporary populations with mutation-drift parameters θ_1 and θ_2 that split from an ancestral population at a time τ in the past. Ancestral population sizes are represented by $(\theta_\tau)_1$ and $(\theta_\tau)_2$; ybp, years before present. (**B** to **E**) Hyperposterior (bars) and hyperprior (dashed) densities of Z_2 (number of species evolved under H_2) given data from three codistributed frog species. (B) and (C) Models of refugial sites (population 1) and unstable, southern areas (population 2). (D) and (E) Models of Pernambuco refugium (population 1) and Bahia refugium (population 2). (B) and (D) Postisolation migration not included in model; (C) and (E) postisolation migration included in model.
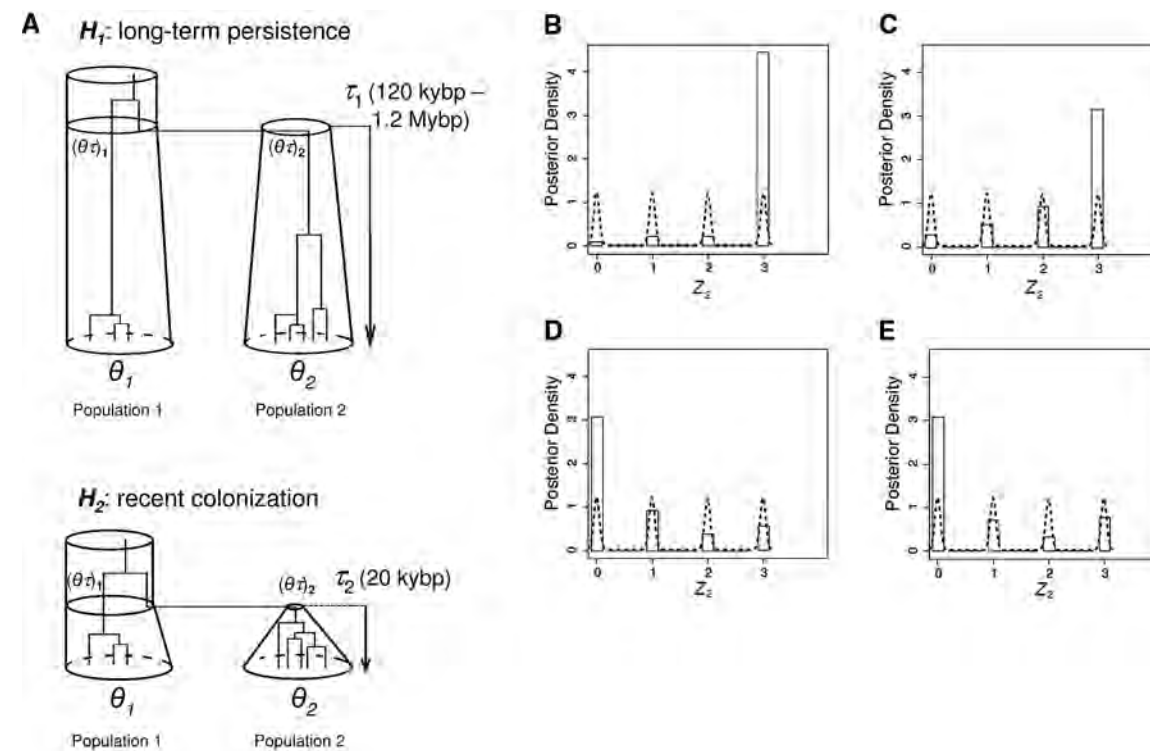
At a broader level, the congruence between model-based demographic hypotheses and joint, multispecies analyses of mtDNA diversity shows that palaeoclimatic niche models and assemblage-scale molecular genetic analyses can be used to forecast spatial patterns of diversity in poorly explored, highly threatened ecosystems. In a world of ever-accelerating environmental changes, this approach can help to guide research and conservation in other global hotspots or similarly complex tropical ecosystems.

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Here's to another 200 years of evolutionary thinking.

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