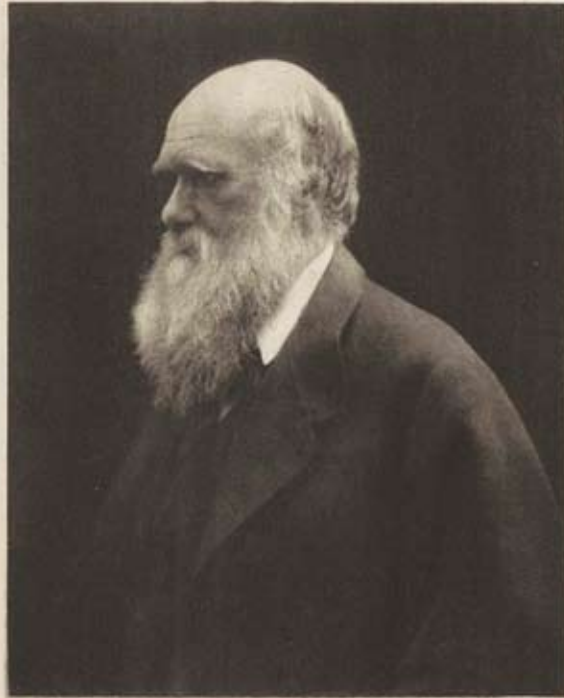


DARWIN *turns* 200



Two centuries ago, modern biology's founding father was born in England. He became the most celebrated scientist of his time, deciphering the records of life's history from creatures extinct and living and thereby explaining the genesis of life's diversity. Today his view of evolution by natural selection forms the core of the scientific study of life, and his mode of thought has earned its own addition to the lexicon of both scientific and popular discourse. Darwinian logic pervades the sciences of life, from the spread of viruses to interactions between and within human cultures, and has infiltrated other arenas as diverse as quantum physics and computer science. Far from a relic in textbooks, Darwinism breathes vitality into biology on a broad spectrum of research frontiers, inviting reflections on the life of, and the science made possible by, Charles Darwin.

Special evolution edition www.sciencenews.org/darwin

In commemoration of the bicentennial of the birth of Charles Darwin, this special web edition of *Science News* offers expanded versions of articles from the magazine's print edition plus two additional features. An opening essay describes Darwin's life and science, followed by five reports from the frontiers of research in evolutionary science.

- 2** Darwin's Evolution
- 6** Evolution's Evolution
- 13** Molecular Evolution
- 22** Step-by-step Evolution
- 27** Computing Evolution
- 32** A Most Private Evolution

By Tom Siegfried

Charles Darwin was born into a world that today's scientists wouldn't recognize.

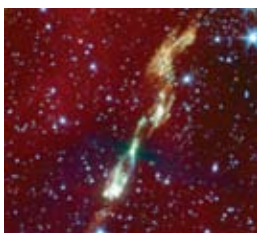
When baby Darwin arrived on February 12, 1809, modern science was also in its infancy. Dalton had just recently articulated the modern theory of the chemical atom, but nobody had any idea what atoms were really like. Physicists had not yet heard of the conservation of energy or any other laws of thermodynamics. Faraday hadn't yet shown how to make electricity from magnetism, and no one had a clue about light's electromagnetic identity. Geology was trapped in an antediluvian paradigm, psychology hadn't been invented yet and biology still seemed, in several key ways, to be infused with religion, resistant to the probes of experiment and reason.

Then came Darwin. By the time he died in 1882, thermodynamics possessed two unbreakable laws, chemistry had been codified in Mendeleev's periodic table, Maxwell had discovered the math merging electricity and magnetism to explain light. Lyell had established uniformitarianism as the basis for geology, Wundt had created the first experimental psychology laboratory, and science had something substantial to say about how life itself got to be the way it was — thanks to Darwin's perspicacious curiosity, intellectual rigor, personal perseverance and power of persuasion.

Superlatives are commonplace in accounts of Darwin's life. "An intellect which had no superior, and with a character which was even nobler than the intellect," wrote Thomas Henry Huxley, Darwin's champion in the original evolution debates. More recently Stephen Jay Gould called Darwin "the Muhammad Ali of biology." But all Ali did was fight. Darwin was more like Willie Mays — he could hit, hit with power, run, field and throw. Translated to science, Darwin could read, reason, experiment, theorize and write — all as well or better than any of his contemporaries. Several

From the beginning...

Milestones in the history of life and evolutionary science



4.6 billion years ago (bya)
Origin of solar system and Earth

3.9 bya
Meteorite bombardment of Earth ends. Evidence suggests presence of life.

3.5 bya
Evidence for presence of cyanobacteria, which conduct photosynthesis

2.7 bya
Disputed evidence for presence of eukaryotes, the first cells with DNA encased in a membrane-bound nucleus

Stromatolite formed by fossilized cyanobacteria communities



scientists before Darwin had expressed the idea of evolution, some even hinting about the role of selection. But none had the wherewithal to perceive the abundance of evidence for evolution, deduce its many nuances, explain its mechanism, foresee and counter the many objections, and articulate it so convincingly to the world.

And even had Darwin never written a word about evolution, he would be remembered today as one of the 19th century's premier botanists, a superb entomologist and prominent geologist. He was a leading authority on carnivorous plants and coral reefs, pigeons and bees, earthworms and orchids, beetles and barnacles (especially barnacles). And yet he was never educated to be a scientist and held no academic position. All he brought to the scientific table was his brain. What a brain.

Woe unto the beetles In his youth, Darwin was an average student but an avid reader. He had an early interest in observing and collecting, mainly beetles and butterflies. ("Woe unto the beetles of South America, woe unto all tropical butterflies," a friend wrote in advance of Darwin's famous sea voyage.) When it came time for higher education, Darwin headed to Edinburgh, a few hundred kilometers north of his birthplace in Shrewsbury, England, to study medicine. Soon discovering that he couldn't stand the sight of blood, Darwin headed back south to Cambridge, to prepare for the clergy, a profession in which blood wouldn't be such a problem.

His heart was not in religion, though, and his Cambridge years exposed him to other intellectual pursuits — lectures on botany, for instance, fieldwork with geologist Adam Sedgwick and friendships cultivated with biologists like John Stevens Henslow. Darwin's interest in science was most significantly stirred while reading books by the German savant Alexander von Humboldt and the English astronomer John Herschel, which imbued in him "a burning zeal to add even the most humble contribution to the noble structure of Natural Science," Darwin wrote decades later.

Henslow was perhaps the first to see in Darwin the makings of an uncommon scientist, and recommended him to serve as naturalist on the exploration voyage of the *Beagle*. During that ship's leisurely circumnavigation of the globe, Darwin spent five years observing the diversity of the planet's life, its sundry geological formations and rich fossil record of life long gone. Darwin's eye saw more than what met it. He remarked on the variations between fossils and

living forms, on the similarities of animals separated by vast distances and on the subtle differences and relationships among organisms on the South American mainland and the nearby Galápagos Islands.

By the time the voyage ended in October 1836, Darwin had amassed a mental catalog of life's diversities and subtleties never before held in one head. It gave him a lot to think about.

Sick at Down Darwin's dispatches to England during the *Beagle* trip made him a scientific celebrity by the time he returned, and he hobnobbed with the leading lights of London's elite. But soon ill health drove him southeast of London to a rural home (known as Down House) near the town of Downe.

For the rest of his life, Darwin suffered, almost daily, from a mystery illness something akin to repetitive food poisoning. Doctors of his day couldn't help him; modern diagnosticians have speculated on a variety of disorders, ranging from lactose intolerance to Crohn's disease.

Whatever it was, Darwin's illness, a curse to him, perhaps established the circumstances subserving his scientific success. Forced to live in the country, he had no job and few distractions. He could devote his time to investigating nature in his own way. He spent eight years studying every aspect of every species of barnacle, for instance. All that time he also read with a vengeance, compiling and indexing detailed notes from book after book. He read virtually all of every issue of the journal *Nature*, taking special delight in the physics and math articles that he admitted he could not understand. He read science and philosophy and history and even trashy novels (there should be a law, he said, against unhappy endings). When Darwin opined, he knew what he was talking about, and he knew what everybody else knew, too.

He knew so much that he could often see what others couldn't, and he could also reason about things without wondering whether his suspicions would be supported by observations — he knew what observations had already been made. If they were insufficient, he made his own, growing orchids, breeding pigeons, spying on earthworms.

... the most earthshaking ideas in the history of biological science remained unpublicized. Darwin was busy classifying barnacles.



Feeding goose barnacle



Balanus tintinnabulum barnacles

2.5 bya
End of the Archean eon. Free oxygen begins to accumulate in the atmosphere.

1.2 bya
First multicellular organisms

542 to 488 million years ago (mya)
(Cambrian period)
Time of the Cambrian explosion, when diversity of life-forms balloons



488 to 444 mya
(Ordovician period)
Invertebrates — especially arthropods and mollusks — dominate the sea. First land plants appear.

FROM TOP: DAVID SHALE; REPRODUCED WITH PERMISSION FROM JOHN VAN WYHE, ED., THE COMPLETE WORK OF CHARLES DARWIN ONLINE (DARWIN-ONLINE.ORG.UK); TIMELINE: QINGWA/ISTOCKPHOTO

Of all his reading, the most signal was the 1798 essay on population by Thomas Malthus, which Darwin perused “for amusement” in 1838. About 15 months earlier, Darwin had begun a systematic investigation of “the species question,” an issue at biology’s foundation. Conventional wisdom held that species had been created individually and were immutable (in much the way that astronomers assumed the universe to be everlastingly static). Some thinkers, though (including Darwin’s grandfather, Erasmus), believed otherwise. While on the *Beagle*, Darwin began to suspect that immutability could not be correct (though he had been unimpressed by grandpa’s book, finding it to contain an excessively high ratio of speculation to fact). But the idea of natural selection had not yet entered the grandson’s mind.

Malthus helped. Population, unchecked, would grow uncontrollably and run out of resources, he wrote. Scarcity kept populations in check; not all who were born could survive to reproduce. Darwin recognized in this account the “struggle for existence” he had observed in all manner of plants and animals. “It at once struck me that under these circumstances favorable variations would tend to be preserved and unfavorable ones to be destroyed,” he wrote in his autobiography. “The result of this would be the formation of new species. Here, then, I had at least got a theory by which to work.”

By 1842 he had prepared a rough 35-page outline (in pencil) of his evolutionary ideas, expanded by 1844 to a 230-page manuscript. In a letter to his wife, he allowed that his theory would be “a considerable step in science,” if it ever were to be accepted “even by one competent judge.” He asked in that letter that she be sure to publish the manuscript if he died before getting around to it himself. He did show it to a couple of colleagues, but otherwise the most earthshaking ideas in the history of biological science remained unpublicized. Darwin was busy classifying barnacles.

By 1854 he had begun spending most of his time on the species question, and in 1856 the geologist Lyell warned him to publish soon, before another naturalist anticipated him. Sure enough, two years later Alfred Russel Wallace, working in Indonesia, arrived at nearly the same notion — that species developed over time as small variations accumulated,

with favorable ones enhancing survival. For counsel and comment, Wallace sent his paper to — Darwin.

Dismayed, Darwin sought advice from Lyell. Wallace’s idea was sound, and deserved to be published. Could Darwin now dare publish himself, without appearing to be stealing Wallace’s discovery?

Lyell and Henslow brokered a compromise. Wallace’s paper would be read to the Linnean Society, and so would an extract of Darwin’s 1844 manuscript, at one session, with Lyell and Henslow vouching that they had indeed seen Darwin’s work years earlier. Wallace was acknowledged, but Darwin’s claim to priority was preserved.

That hardly mattered, though. It was Darwin’s artful reasoning and marshaling of the evidence

that established evolution by natural selection, as propagated in his masterwork, *On the Origin of Species*. Published in 1859, it electrified the scientific and intellectual world, evoking the prejudicial condemnation that afflicts most great new insights, but also filling the open-minded with food for centuries’ worth of future biological thought.

A simple solution For so momentous a problem, Darwin’s solution seems elegantly simple, although also so

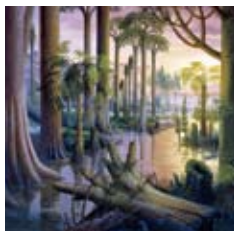
subtle that its exposition is often badly mangled. Offspring differ slightly from their parents and each other (descent with modification), making some “fitter” than others in the struggle for existence (survival of the fittest). Over periods of time unimaginably long, the small changes from generation to generation accumulate, mutating one species into others. On smaller scales, over shorter times, such accumulated changes can be seen in various breeds of dogs or pigeons or plants, often induced by the artificial selection of particular traits by human breeders. On evolutionary scales of millions of years, the selection driving the appearance of new species is natural.

Some scientists (such as Huxley) saw the truth in Darwin’s views immediately; others came to agree gradually. Many, of course, disagreed bitterly and attacked both Darwin and his book. But most of the “rebuttals” of evolution, even today, merely raise points that Darwin anticipated and countered. Gaps in the fossil record? To be expected, Darwin explained, because the geological record was so imperfect, as if only a

Some scientists saw the truth in Darwin’s views immediately; others came to agree gradually. Many disagreed bitterly.

359 to 299 mya

(Carboniferous period)
A time of forests, swamps, seed ferns, mosses and lycopods coincides with the origin of the amniote egg. Insects are abundant.



251 mya

Earth’s largest mass extinction event occurs at the end of the Permian period. Most marine and land vertebrate species are wiped out, along with many plants and insects.

251 to 65 mya

(Mesozoic era)
This era includes the age of gymnosperms (plants with seeds), the age of reptiles and the age of dinosaurs.

251 to 200 mya

(Triassic period)
Extinction survivors, including dinosaur ancestors, recolonize. Forests of gymnosperms and tree ferns are abundant.

few pages remained from only the most recent volume in the entire encyclopedia of the Earth's history. Complexity of the eye? The slightest sensitivity to light would aid in survival, and more versatile, focused organs should develop over a long enough time.

Besides explaining the vagaries of life-forms that nature presented, Darwin's work, in a sense, also made spectacularly successful predictions. One was the requirement for a mutable mechanism of heredity. Subsequent genetic research, from Mendel to Watson and Crick, produced just what Darwin ordered. The other was the need for a very old Earth, providing the eons of time necessary for natural selection's choices to accumulate. Prominent physicists of the day contended the planet was much too young for that, but Darwin's original intuition eventually proved accurate.

Darwin attributed his success to "love of science" and "unbounded patience" and "industry in observing and collecting facts." He understood fully the importance of his work, but his humility permitted only understatement. "With such moderate abilities as I possess," he wrote, "it is truly surprising that I should have influenced to a considerable extent the belief of scientific men on some important points."

As a scientist, Darwin was both chronicler and synthesizer, experimentalist as well as theorist. His power to unearth biology's secrets so successfully stemmed from his devotion to acquiring all the evidence and assessing it honestly. He compiled facts from all possible sources, arranging them to reveal the most logical general conclusions. He could explain all the subtle points of natural selection and its power by citing observations from the *Beagle* voyage, the writings of experts from around the world, or his own experiments in breeding pigeons, dissecting barnacles, tormenting ants. He could demonstrate how natural selection

reconciles observations otherwise irreconcilable if species had been created separately and remained immutable.

Today Darwin's original idea survives, although it has spawned many mutated forms, with nuances and complexities that make evolutionary science a constantly advancing field of research. And Darwin's logic has been borrowed by other investigators in diverse disciplines. Psychologists try to explain behavior based on what mental habits would have enhanced survival as human ancestors were evolving. Biomedical researchers grapple with evolutionary principles in fighting microbial resistance to antibiotics. Computer scientists mix and select segments of binary code to generate optimal computer programs. Even in physics, the word

"Darwinian" appears in papers on thermodynamics, quantum physics and black holes. Darwin would have been fascinated by such research and would no doubt have understood a lot of it, as so much of the underlying reasoning was his.

Darwin would also have been happy with the many modifications and adaptations to his ideas found in modern reformulations of evolutionary theory. Speciation isn't always gradual, change isn't always the result of selection, organisms are not the only units of selection, evolutionists now believe. Darwin foresaw some of these views, and he would have embraced them all — as a man of science willing "to give up any hypothesis, however much beloved ... as soon as facts are shown to be opposed to it," in his words. "If I know myself, I work from a sort of instinct to try to make out truth."

And in the battle to wrest truth from nature, none fought better than Darwin. "He found a great truth," Huxley wrote in Darwin's obituary, "trodden under foot, reviled by bigots, and ridiculed by all the world; he lived long enough to see it, chiefly by his own efforts, irrefragably established in science, inseparably incorporated with the common thoughts of men." ■



Notebooks full of writings and sketches, including this tree of life, document Darwin's ideas and inspiration.

200 to 146 mya
(Jurassic period)
Dominance of dinosaurs and conifers



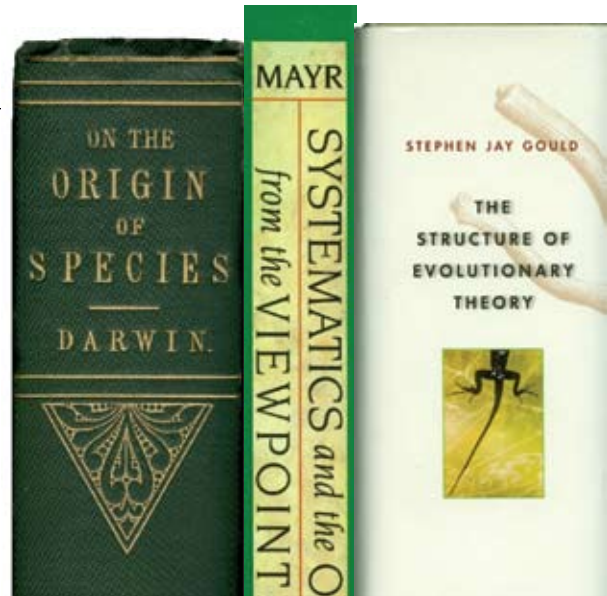
Allosaurus
skeleton

146 to 65 mya
(Cretaceous period)
End of the age of the dinosaurs, characterized by warm seas and named for chalklike rocks (right) that remain today. Flowering plants appear (130 million to 125 million years ago).



Evolution's Evolution

Darwin's dangerous idea has adapted to modern biology **By Rachel Ehrenberg**



Just a decade after he published *On the Origin of Species*, Charles Darwin was already worrying about the evolution of his idea. In an 1869 letter to botanist Joseph Dalton Hooker, Darwin lamented: “If I lived twenty more years and was able to work, how I should have to modify the *Origin*, and how much the views on all points will have to be modified! Well, it is a beginning, and that is something.”

Calling the *Origin* a mere “beginning” is like saying the Beatles were just a rock band or that Shakespeare wrote some decent plays. Darwin's gifts to science were radical. He not only proposed that all of Earth's diverse beings shared a common ancestry, but also described an elegant mechanism to explain how all that diverse life came to be. Darwin was a master of merging data from different disciplines, painstakingly drawing from zoology, botany, geology and paleontology to build a solid foundation for evolutionary biology. Today, 150 years later, scientists continue to grapple with ideas descended from that foundation. Still, Darwin's central tenets survive, fit enough to frame the questions posed by modern biology.

“He had great intuition,” says Yale University's Michael Donoghue. “He's the guy we all envy.”

Darwin's powers of observation and reason extended from microflora to megafauna; he could see the whole forest while scrutinizing the branches on the trees. His ideas illuminated life's development in the Earth's deep past and foreshadowed many scientific developments that would come in the future, including the modifications and refinements to his theory that scientists are still exploring. Yet, were Darwin alive

today, his head might spin at the complexities entangling the expansion of his original ideas.

Evolutionary theory is not a well-preserved fossil in a dusty museum, but a thriving field of study pursued at lab benches, on beaches and in bogs. The exploding research program known as “evo-devo,” for instance, has wed evolutionary theory to embryology and genetics, helping to unravel the evolution of organisms' structures and forms. Scientists are also reformulating ideas about evolution's pace, showing that Darwin's idea that change happens gradually and incrementally doesn't always capture the whole story. Researchers are fleshing out Darwin's central idea of natural selection — discovering when it's the driver and when it takes a back seat. And along with investigating how selection operates on organisms — Darwin's unit of choice — scientists are also showing how it acts on groups, genes and behavior. Experts are still debating the very definition of a species.

If Darwin came back, “in some ways he would be mystified,” says evolutionary biologist Douglas Futuyma of Stony Brook University in New York. “Evolutionary biology has been radically changed — and deeply enriched.”

The ‘dangerous idea’ Of course, Darwin was familiar with radical change. In his day most biologists (or “naturalists,” then) believed that each species was individually created and forever immutable. But during his travels in the 1830s on her majesty's ship the *Beagle*, Darwin saw plants and animals and fossils — and distributions of all three — that just didn't square with the idea that species don't change.

“It was evident that such facts as these, as well as many

65 mya

Asteroid hits Earth. Mass extinctions of marine life and some terrestrial life, including dinosaurs (ancestors of modern birds). The age of mammals begins.



65 to 1.8 mya

(Tertiary period)
Birds, mammals, insects and flowering plants are widespread and flourish.



25 mya

Establishment of present-day forests; climatic cooling and restriction of broad-leaved evergreens to lower latitudes; prairie grasses

others, could only be explained on the supposition that species gradually become modified; and the subject haunted me," he noted in his autobiography.

Upon his return to England, Darwin pored over his notes and "collected facts." Eventually he accepted the unacceptable and wrote, in 1844, to his friend Hooker: "At last gleams of light have come and I am almost convinced (quite contrary to the opinion I started with) that species are not (it is like confessing a murder) immutable."

That year Darwin penned his idea in a manuscript that remained unknown to the public until portions of it were presented to the Linnean Society in 1858. Subversive as it was, Darwin's proposal that species can change was not the first. Naturalists and philosophers had long been contemplating life's diversity. By the late 1700s, French naturalist Georges Cuvier had established that after great environmental change, some organisms got snuffed out, went kaput, extinct. A little later, zoologist and philosopher Jean Baptiste Lamarck proposed the notion of adaptation, explaining variation among organisms as a response to their environments. But Lamarck saw the change in organisms through time as a one-way path to perfection, from simple to increasingly complex, with humans at the pinnacle. His environment-

caused variation was an excuse to explain why some organisms strayed from the "tendency toward perfection."

It took Darwin and Alfred Russel Wallace to recognize (independently) that variety was actually the spice of life, not its flaw. Both men had read the work of economist Thomas Malthus, who warned that food supplies could never keep up with growing populations. No matter what, some people would meet an early death. Darwin and Wallace both reasoned that beetles, birds and beech trees also have more babies than can survive and that variation among such offspring was important in determining who lived. Individuals who were better equipped for their environment than their siblings or neighbors would survive; the features that enabled their survival would be passed on to their kids.

Darwin called this process natural selection, and life evolved largely because of it, he argued in the *Origin*. (The word *evolved* appeared only once, the last word on the *Origin's* last page: "from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.") Evolution via natural selection, Darwin believed, could yield both life's incredible diversity and its striking similarities.

Those similarities are repeatedly and presciently remarked



“What can be more curious that the hand of a man, formed for grasping, that of a mole for digging, the leg of a horse, the paddle of the porpoise, and the wing of the bat, should all be constructed on the same pattern, and should include the same bones, in the same relative positions?”

— Darwin, *On the Origin of Species*

20 mya
Proconsul, one of the earliest apes, appears around this time.



5.3 to 1.8 mya
(Pliocene epoch)
The likely human ancestor *Australopithecus* lives at various African sites. The famous partial skeleton “Lucy” is thought to be from 3.2 million years ago.



2.5 to 2 mya
The first species of the genus *Homo* lives in southern and East Africa.

A model of “Lucy”

CLOCKWISE FROM BOTTOM LEFT: ANDREAS FEININGER/TIME & LIFE PICTURES/GETTY IMAGES; PHILIP DOWELL (C) DORLING KINDERSLEY; ROBERT CLARK; TIMELINE: PASCAL GOETIGHELUCK/PHOTORESEARCHERS; PHILIPPE PLAILLY & AELIER DAINES/PHOTO RESEARCHERS

upon by Darwin, who called morphology – the study of form – “the most interesting department of natural history, and may be said to be its very soul.” In the *Origin* he writes: “What can be more curious that the hand of a man, formed for grasping, that of a mole for digging, the leg of a horse, the paddle of the porpoise, and the wing of the bat, should all be constructed on the same pattern, and should include the same bones, in the same relative positions?”

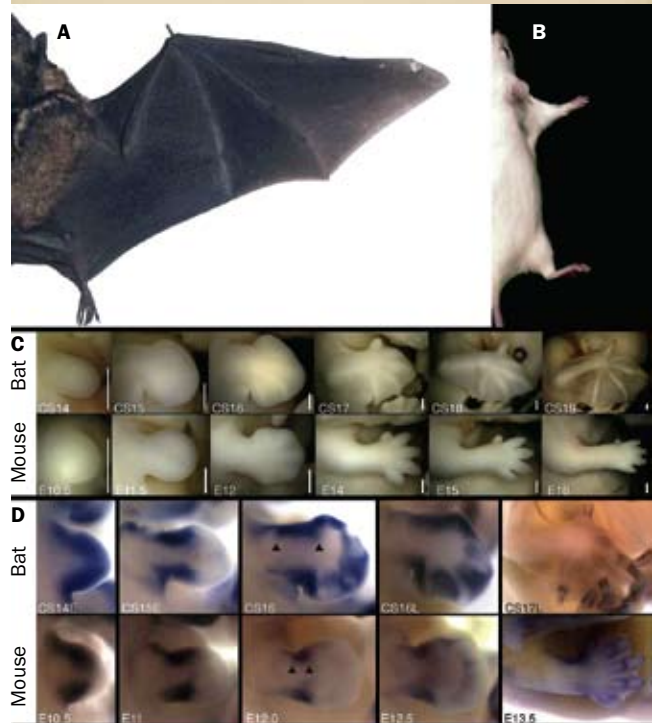
But only in recent years have evolution and embryology become integrated into a flourishing field dubbed “evo-devo,” for evolutionary development, a research program investigating how bodies – their size, shape, color and different parts – evolve.

Body building An early evo-devo milestone came in the 1980s when scientists learned that the genes for the body plan in fruit flies have counterparts in creatures as distantly related as humans, worms and yeast. As opposed to house-keeping genes that code for proteins involved in day-to-day living, these toolkit genes actually govern the construction of the house. Mutations in some fruit fly toolkit genes, for instance, transform a fly’s antennae into legs.

Scientists are finding more and more cases where toolkit proteins do the same jobs in animals separated by many millions of years of evolution. The toolkit proteins in charge of building the contractile muscles that eventually become a pumping heart, for example, appear to be shared by flies and fish and even mammals.

Mining the DNA record has revealed that regulation of gene activity – often by stretches of DNA previously thought of as junk – is critical in shaping development. These regulatory regions of DNA command genes to roar, keep quiet or merely murmur – making lots, none or a little of the molecules they encode. Several plant traits that aided domestication are associated with changes in where, when and how much genes are turned on. Mutations in genes linked to this regulation process helped enlarge tomato’s fruit, for example. Changes in regulation also get credit for the architectural shift from corn’s shrubby progenitor to the single-stalk version that now grows as high as an elephant’s eye.

While DNA is the ultimate forensic record of evolution, it hasn’t closed the case of how organisms develop. Scientists are still debating the precise role of regulatory DNA in large-scale morphological changes, but evidence is accumulating that the timing and location of gene regulation are as impor-



Going batty

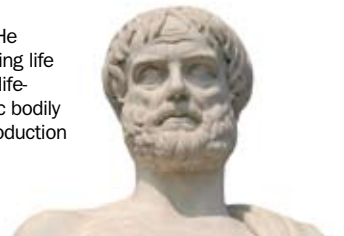
Darwin sought intermediates that would shed light on the evolution of novelties such as bat wings (top). By comparing development in related animals (C), scientists are closer to understanding how novelties arise. Tracking a gene linked to limb development (shown in blue) reveals cranked up activity in the developing bat limb (D). And when that piece of DNA was taken from a fruit bat (A) and stuck in a mouse (B), the mouse’s limb length increased significantly, scientists reported last year.

1.8 mya
Homo erectus lives in East Africa and eventually spreads throughout Africa and to Europe and Asia.

150,000 to 100,000 years ago
Appearance of first *Homo sapiens*, who migrate across Africa and Europe

100,000 to 40,000 years ago
Homo neanderthalensis, now extinct, lives in Europe and Asia.

384 to 322 B.C.
Aristotle’s lifetime. He defines an unchanging life hierarchy based on life-forms’ characteristic bodily activities, from reproduction to reasoning.



TOP: REPRODUCED WITH PERMISSION FROM JOHN VAN WYHE, ED., THE COMPLETE WORK OF CHARLES DARWIN ONLINE (DARWINONLINE.ORG.UK); GENES & DEVELOPMENT, JANUARY 2008; TIMELINE: SNEASKY/ISTOCKPHOTO

tant as changes in good old-fashioned protein-coding genes.

DNA also allows scientists to penetrate the smokescreen often presented by anatomy. Many cave-dwelling fish, for example, which spend their lives in perpetual darkness, have lost their eyes and pigment, which puzzled Darwin (he ascribed the fishes' loss of eyes as "wholly to disuse"). But scientists have recently shown that the loss results from the careful coordination of gene activity — the eyes are actively "killed" during development. Why remains unknown.

Exploring the gulf between genes and an organism's observable physical and biochemical traits (its phenotype) has revealed a much more complex picture of selection and inheritance than sketched by Darwin. In his view, natural selection was a grim reaper whose scythe was the external environment. As the late paleontologist Stephen Jay Gould put it, the organism proposes, the environment disposes. But many scientists now view the developing body as an environment in constant conversation with itself. Rather than a one-way street from DNA to organism, scientists now talk about U-turns, crosstown buses and roundabouts.

"It's much more complicated than what we thought," says biologist and philosopher Massimo Pigliucci of Stony Brook University. "Nonlinear interactions, branching, with lots of feedback. That's the new frontier."

For example, more and more scientists are investigating how environmental factors such as pH, diet or nurturing behaviors can change the way DNA is packaged. This packaging, which involves such features as the presence or absence of a chemical tag, can change gene activity, and these epigenetic patterns can be inherited. Such findings suggest "a bewildering increase in the complexity of the entire inheritance system," Pigliucci wrote recently in *Evolution*.

Other factors influencing the evolution of shapes and forms include the physical properties of cells.

"Take a pool of water — we're familiar with it having a still surface," says Stuart Newman of New York Medical College in Valhalla. "If we agitate it, we can get waves or vortices — but it can't do any old thing. It's hard to get variety — there's only a few things it will do based on its physical properties."

Similarly, the clusters of cells in a rudimentary embryo

can do only so much. One kind of perturbation might make them elongate, another might prompt a hollow cavity to form. Newman and his colleagues reported last year in *Developmental Biology* that when wings and legs begin to bud off a developing chicken embryo, a protein spurs the limb cells to become more cohesive than nearby nonlimb cells. This physical property of being differentially sticky can lead to the layers of tissues seen in embryos. Add some feedback loops and you can get repeating patterns, the kinds of patterns seen over and over again in animal body plans, such as the vertebrae of a backbone or a segmented abdomen.

Of course, even if physical properties can dictate some limits on form, you also need selection, says Newman. "There's still going to be a shakeout — you need selection on what can exist." But physics may have had much more to do with the evolution of innovations — the big leaps on the path of life — than Darwin had realized.

"Darwin wanted a worm to be an incremental worm, to build up little by little. But you don't have to put waves in the water one by one. If you use physics you can get segments in one generation with a feedback loop," Newman says. "You can get rapid transitions to novel forms with physics."

**"Nonlinear
interactions,
branching, with lots
of feedback.
That's the new
frontier."**

Massimo Pigliucci
Stony Brook University

Not always gradual Darwin was not a fan of rapid transitions. In his view evolution acted through the relentless accumulation of tiny changes through vast spans of time. These gradual transitions are sometimes found in the fossil record, but plenty of times they are not. And that record also reveals exuberant bursts of innovation, such as the Cambrian explosion, a period of roughly 20 million years beginning about 520 million years ago. The major body plans found in most modern animal groups, such as arthropods and chordates, were established by the Cambrian, available fossil evidence suggests.

In 1971 Niles Eldredge proposed an explanation for these moments of great change, an idea he later expanded with Gould. Rather than evolution always proceeding as an "easy and inevitable result of mere existence, as something that unfolds in a natural and orderly fashion," Eldredge and Gould argued that it can happen in fits and starts. Organisms remaining unchanged for long periods of geologic time — the

1651

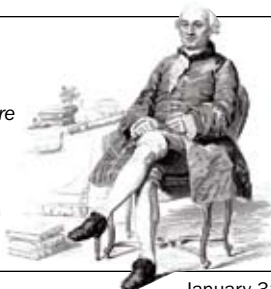
William Harvey publishes *On the Generation of Animals*, which describes how the embryo is built, pioneering modern embryology. Harvey also argues that all animals emerge from an egg.

1745

The work of Pierre-Louis Moreau de Maupertuis hints at the idea of natural selection.

1749

Georges-Louis Leclerc, Comte de Buffon (right) begins publishing *Histoire Naturelle*. It notes similarities between humans and apes and that the two may have a common ancestor.



1753

In *Species Plantarum*, Carolus Linnaeus classifies plants according to a binomial system of genus and species. He later does the same for animals.

stability so often seen in the fossil record — was actually the norm. This general state of equilibrium is then on occasion punctuated by the emergence of new species.

Punctuated equilibrium (or punk-eke) considered the limited clues left at the geologic crime scene. Say you are a paleontologist and observe the same snail fossil in layer after layer of rock. Then in the next layer up, a different snail fossil appears. What went down? Darwin's gradualism can't be excluded; the rock layers represent millions of years and Snail One might have gradually changed into Snail Two, but the transitional snails never fossilized.

Not necessarily, though. Say a barrier, such as a river, isolated a portion of the snail population. This smaller population might undergo intense selection quickly (in geologic time, where 5,000 to 10,000 years is a blink), becoming a new species. If the river then dries up, the new species is reunited with its sister species. The new species could outcompete its sister, which goes extinct, or both snail species might persist. In the fossil record, it might look like one species was replaced by a related species, or that a new species suddenly appeared.

Eldredge and Gould were familiar with the work of biologist Ernst Mayr and geneticist Theodosius Dobzhansky, who developed ideas on how species originate, laid out in Mayr's 1942 book *Systematics and the Origin of Species*. Punctuated equilibrium captured Mayr's idea of speciation — an isolated subpopulation accruing so many changes that it can no longer breed with its former population — and translated it into the language of the geologic record.

Mayr's ideas became a core part of the “modern synthesis,” the merging of Darwinian selection with Mendelian genetics and paleontology during the 1920s through the 1940s. In the early 1900s, after the rediscovery of Mendel's pea experiments, scientists such as Thomas Hunt Morgan began experimenting with fruit flies and established that mutations could be passed along to the next generation. The 1920s brought scientists such as J.B.S. Haldane, Ronald Fisher and Sewall Wright, who gave evolution a mathematical backbone. The field of population genetics was born; its tenets being that variation arises in populations through both random genetic mutation and recombination (sex),

and evolution occurs when the gene frequencies in a population change through time. Dobzhansky's and Mayr's ideas on speciation rounded out the mix, laying a rich foundation for exploring how evolution proceeds.

Selection and chance While adaptation was at the core of the modern synthesis, the mathematical musings of Fisher and Wright demonstrated that natural selection wasn't the only guest at evolution's cocktail hour. Chance also plays a role in determining the genes of the next generation.

The idea of natural selection “was brilliant, original, it was called the ‘dangerous idea’ because it was so powerful,” says Futuyma. “But is that going to explain everything? No.”

Recall the snail population that gets divided by a river and suppose that the original population was a mixture of red-shelled snails and brown-shelled snails. When the river runs through it and isolates a portion of the population, that new subpopulation — just by chance — might be mostly red snails.

Through time, the genes for red shells might dominate in this new population, or they might peter out — the ratio of brown to red snails will “drift” around. This genetic drift happens without selection — neither color gives either snail a

leg up in that environment — yet the gene frequencies for different shell colors in the population are changing through time. That genetic drift — often called the evolutionary equivalent of statistical sampling error — can be a mechanism of evolution. Drift can also reduce the amount of variation in a population, especially if that population is small, leaving natural selection less raw material on which to act.

While the idea of genetic drift arose out of the math of the modern synthesis, it was largely seen as a sideshow to selection's starring role. But following the discovery in 1953 of DNA's structure, molecules grabbed the attention of many scientists; intense investigations of enzymes, other proteins and amino acids, protein building blocks, ensued. In the late 1960s geneticist Motoo Kimura and others began making the case that most changes at the molecular level were neutral — imparting no benefit, or harm — suggesting that genetic drift, not selection, was the prevailing evolutionary force.

Many scientists found Kimura's “neutral theory” tough to

The idea of natural selection was “called the ‘dangerous idea’ because it was so powerful.”

Douglas Futuyma
Stony Brook University

1794

Charles Darwin's grandfather Erasmus Darwin writes that “warm-blooded animals have arisen from one living filament... possessing the faculty of continuing to improve by its own inherent activity.”



Erasmus Darwin

1795

James Hutton proposes the theory of geological uniformitarianism.

1798

Georges Cuvier publishes studies of mammoth and Indian elephant anatomy. His findings suggest that species can go extinct.



swallow, seeing it as relegating selection to the sidelines. But today scientists generally accept that the evolution of molecules may differ in some ways from the evolution of organisms. Selection is still a star, drift certainly has its place, and which has the dominant role is often a matter of circumstance and which level of the hierarchy is being examined.

Acknowledging mechanisms other than selection didn't minimize Darwin's contribution; rather it signaled a larger view of evolution. This refreshing breadth vitalizes many subspheres of evolutionary theory, including the question of where in the biological hierarchy that selection really does its business.

Russian nesting dolls Darwin doggedly argued that selection acts on organisms, each individual engaged in a personal struggle for survival. Troubled by the sterile workers of a bee colony, he fumbled to explain how their existence did not “annihilate” his whole theory. Investigating “the” target of selection is still a productive if contentious field, but increasingly scientists are embracing a hierarchical view. Evolution can be a team sport, with selection acting above the level of individual, for the “good of the group.” Selection can also act below the level of the organism — on genes and cells.

Sterile workers of a bee or termite nest who live in dedicated service to their queen, or a vampire bat who regurgitates blood for colony members who haven't had a meal, are examples of altruism — their behavior benefits other organisms, often at a cost. In the Darwinian, organism-focused view, altruism shouldn't evolve. But if selection can act at the level of groups (an idea broached by Darwin in *The Descent*

of Man), a gang of altruists might have an advantage over a gang of selfish individuals.

However, if there is a cost to behaving kindly, then selfish individuals should have an advantage, which would eliminate altruism from the gene pool. In the 1960s, though, scientists started thinking about altruism in terms of kin. If altruistic behavior benefits relatives, then even if an individual doesn't get to pass on its genes, its siblings might. “Kin selection” says that organisms will behave altruistically toward close relatives, a prediction borne out by research, including recent work showing that related male turkeys work together to attract females, even though only the dominant male might sire offspring.

While debate continues over where and how selection acts, many scientists advocate the “Russian nesting doll” approach that allows selection at numerous levels, including species, groups, individuals, cells and, of course, genes, as popularized by British evolutionary biologist Richard Dawkins.

A similarly contentious (and productive) area of research focuses on the concept of “species” itself. Experts still debate whether Darwin concerned himself with actually defining species; many scientists argue that he viewed the category as an arbitrary point in the fuzzy, gradual divergence of lineages. In the past 50 years many species concepts have been proposed. A dominant approach, first championed by Mayr in animals (and later by botanist Verne Grant in plants), argues that

In a leafcutter ant colony the only female who mates is the queen. Scientists now understand that the sterile or celibate lifestyle could still evolve if kin get to pass on genes.



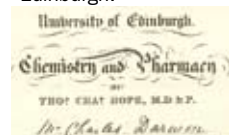
1809
Charles Darwin is born February 12 in Shrewsbury, England.



1809
Jean Baptiste Lamarck states in the *Philosophie Zoologique* that heritable changes could be forged by the environment during an organism's lifetime.



1825
Darwin matriculates at the University of Edinburgh.



species are real entities defined by their ability to interbreed. Yet some organisms snub this “biological species” concept. Among species with several populations over an extended range, it isn’t unusual that populations near each other can successfully interbreed, while populations at the opposite ends of the range (or ring) are so divergent that they are incompatible. (Recent work on *Ensatina* salamanders of the Pacific Coast, a noted example of a ring species, indicates both current and past hybridization between some of the more distant “species,” complicating matters further.)

While the ability to interbreed is certainly important in the maintenance of species, it does leave something to be desired as a definition (what about asexual species, for example?).

Some scientists have proposed phenetic species, which define organisms by their overall similarities. Many scientists now call for a phylogenetic species concept that recognizes groups descended from a common ancestor, as evidenced by the sharing of special derived characteristics, such as mammals having fur and mammary glands. Brent Mishler of the University of California, Berkeley, who with Donoghue was a framer of the phylogenetic species concept, has recently argued that hierarchical ranking, from subspecies to species and up through families and orders, is of little use intellectually or practically and that ranks on all levels, species included, should be done away with.

The tree of life has thousands of nested levels, Mishler writes in a chapter to appear in *Contemporary Debates in Philosophy of Biology*. Defining species — or any other rank for that matter — is in many ways arbitrary. For example, given a genus of moths and a genus of spiders, the rank of “genus” actually tells us almost nothing about the two groups, such as their evolutionary age, or the number of species. It would be better to recognize branches or “clades” on the tree of life — a fork and all the twigs that arise from it, which actually have meaning evolutionarily, Mishler says. For a conservation land manager comparing bird diversity in two canyons, for example, the meaningful information is how much of the bird section of the tree of life is represented in each canyon, not how many species.

Mishler points to similar problems with discrete definitions, biological or otherwise. Take the Gulf Stream, for

example. It is so distinct that you can see it from outer space. “This water comes up from Florida, crosses the Atlantic, and affects the weather in England — it is absolutely real,” Mishler says. “But if you were in a rowboat at the Gulf Stream’s edge and were trying to tell me which molecule of water is part of it and which isn’t — you’d be hard pressed.”

While humans crave discrete definitions, little in biology is tidy, and putting its parts together isn’t necessarily becoming easier. In making the tremendous progress since Darwin in documenting and exploring the mechanisms of evolution, scientists have become more and more specialized, says Pigliucci. That’s how novel contributions are made. But ironically, that specialization often comes at a cost — there’s a lack of integration

at higher levels — even though integration was Darwin’s claim to fame. His insights connected everything in biology, all life becoming related pieces of an integrated whole.

“Genealogy became the great problem of zoology and botany, of paleontology, and of all allied studies. The mighty maze of organic life was no longer without a plan,” scholar and writer Grant Allen wrote in Darwin’s obituary in April 1882.

Such integrated thinking is needed today as humans grapple with how eco-

systems will respond to climate change or invasive species, says Futuyma. Figuring out the genetic variation in a little alpine weed is one thing, but it doesn’t necessarily tell you whether that plant will be able to adapt to a warming world.

“It’s funny that evolutionary biology has not played much of a role in biodiversity — it’s been almost entirely seen as an ecological issue,” says Donoghue. “But evolutionary biology has a lot to say about these issues — oddly enough, evolutionary biology is all about diversity. We’re just starting to connect these dots.”

Darwin was all about connecting dots, says Pigliucci. “Today Darwin would be excited and bewildered by what we know, but would also probably push us to focus on the interdisciplinary aspects,” he says. Darwin was “an inherently interdisciplinary guy. But it took him years! The bulk of the *Origin* is painstaking examples from a variety of disciplines — in a sense we aren’t there now. We know a lot about molecular biology and development in model systems and we know a little about ecology and evolution, but we know almost nothing about how they all fit together.” ■

“The mighty maze of organic life was no longer without a plan.”

Grant Allen, writing in Darwin’s obituary

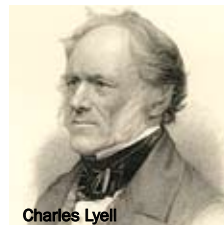
1827

Darwin is admitted to Christ’s College, University of Cambridge.



1829

Geologist Charles Lyell (right) publishes his *Principles of Geology*, promoting the idea that the surface of the Earth is gradually and continually changing.



Charles Lyell

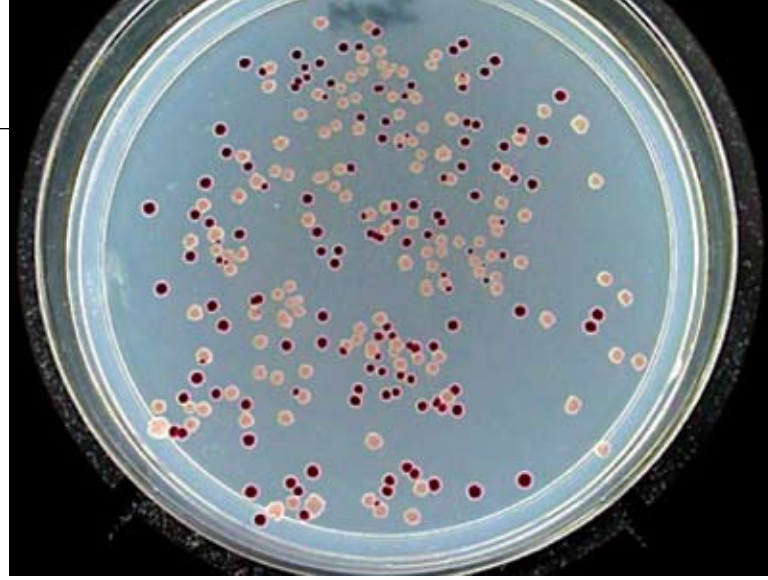
1831

Darwin earns his undergraduate degree at Cambridge.

Molecular Evolution

Investigating the genetic books of life reveals new details of ‘descent with modification’ and the forces driving it

By Tina Hesman Saey



To study evolution, researchers use color to distinguish ancestral *E. coli* from later generations in experiments gauging relative fitness (in this case, how well the bacteria use glucose).

Charles Darwin didn't know about genes and DNA. In fact, hardly anyone noticed when Gregor Mendel, a monk whose pea experiments eventually led to modern genetics, published his findings in an obscure journal a few years after Darwin's *On the Origin of Species* appeared in 1859. It would take nearly a century more before James Watson and Francis Crick deciphered the structure of DNA, the molecule that contains the manual for building an organism. Yet Darwin was still able to describe a mechanism — natural selection — for how evolution shapes life on Earth. That's like describing how a car works without knowing about the existence of internal combustion engines.

But while Darwin achieved his insights without molecular help, biologists today are intimately familiar with the molecules responsible for the diverse array of plants, animals and other organisms that populate the planet. The study of genes has revealed evolution as essentially a high-stakes poker game in which organisms draw randomly from a deck of genetic choices. At stake is the chance to pass along genes to the next generation. Sometimes the hand is good enough to get ahead in the game, but some hands are losers, perhaps to the extent of extinction. By studying the winners, scientists are learning how the forces of evolution work on DNA, the biochemical repository of an organism's entire natural history. DNA records the mutations that helped some animals to survive ice ages while others perished, the nips and tucks that make animals more attractive to mates, the big leaps that allowed plants to become domestic crops — they're all there, written out in a simple alphabet of four letters.

Each organism has its own book of life, but it's not a just-so

story. The genomes of living things are constantly undergoing editing and revision. And each individual has its own edition of its species's book, shaped by natural selection and the other, perhaps less-appreciated forces of genetic mutation, recombination and drift.

In recent years, the U.S. National Institutes of Health and private companies have sponsored programs to build a library of species' books, with projects to decode the genomes of humans, chimpanzees, bonobos, dogs, cats, cows, duck-billed platypuses, opossums, orangutans, bacteria, fungi, corn, wheat, bees, fruit flies, worms and a menagerie of other creatures large and small. Comparing the genetic records from these genomes will help researchers piece together a history of how Earth's current life evolved. But that work has barely begun, and many questions remain to be answered.

Scientists, for example, still don't know how cells evolved, including how former bacteria came to live inside cells as mitochondria and chloroplasts. (Mitochondria are tiny power plants that supply cells with energy, and chloroplasts are the organelles in plant cells that carry out photosynthesis.) Another mystery is how the complex structure of genes in eukaryotic organisms — in which the genetic material is encased in a nucleus — evolved. Researchers also debate how the shapes and forms of organisms came to look as they do. One of the biggest unanswered questions is whether life on Earth was destined to evolve the way it has.

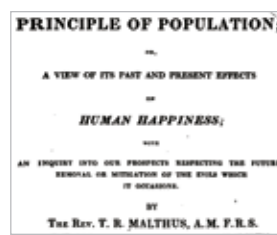
1831

Darwin departs on the voyage of the *Beagle* on December 27. He returns to England in 1836.



1838

Darwin reads economist Thomas Malthus' essay on population.



Limits on evolution At some time in your life you've probably asked yourself if, given a chance to do it all again, you would do it the same way.

Scientists have been asking the same question about evolution, but they've been getting different answers.

Play a poker game, rewind it to the beginning, start again and see what happens. Would the game play out the same every time? Stephen Jay Gould, the late evolutionary biologist, didn't think so. If you replay the game, the shuffled cards will turn up a little different each time, and the order in which the cards are drawn can have profound consequences for the outcome. Replaying the "tape of life" from some point in the past would produce very different life-forms than the ones we have today, Gould thought.

Other scientists disagree. Organisms are dealt a finite number of genes and so must choose from a limited menu of evolutionary options, narrowing the directions the organisms can go in a particular environment. "The evolutionary routes are many, but the destinations are limited," says Simon Conway Morris, a paleontologist at the University of Cambridge in England. As a result, disparate organisms often end up independently developing the same sorts of structures to solve a particular problem. Take eyes. Although the details of how eyes work vary between species, the basic structures are similar.

But since it's impossible to turn back time (no matter how easy Superman makes it look) and replay all of evolution again, scientists have devised other ways of investigating the issue.

Richard Lenski, an evolutionary biologist at Michigan State University in East Lansing, is among the scientists hitting the rewind button on evolution. Meter-high letters taped to the windows of his lab spell out the lab's motto: EVOLVE. In the center of the "O," the face of Charles Darwin peers out toward the football stadium.

Inside the lab, a dozen glass flasks containing what looks like clear liquid swirl in a temperature-controlled incubator. Although the naked eye can't see them, millions of *E. coli* bacteria grow in the flasks, doing what the window exhorts. Lenski started the cultures in 1988, intending to follow the course of natural selection for several hundred generations. Now, 20 years later, the cultures are still growing and have produced more than 45,000 generations of bacteria each.

Lenski inoculated each of the 12 flasks with bacteria from the same ancestor, so they all started out with the same genetic deck of cards. Only one gene differed among the bacteria at the beginning — six flasks contain a marker gene that makes the



Michigan State University graduate student Zachary Blount sits in front of a tower of petri dishes. Blount used the assembled dishes to test trillions of bacteria to see which had evolved the ability to eat a chemical called citrate.

bacteria red when grown on certain media while bacteria in the other six grow white. The marker gene doesn't affect the strains' fitness — the term biologists use to describe the capacity of an organism to compete against others to pass on its genes — but it does help researchers distinguish between two lineages of the bacteria during competition experiments.

Each flask contains media with only the minimum requirements for survival — some glucose (a sugar that bacteria use for food) and a few other nutrients. The bacteria replicate, or divide, six or seven times daily, creating a new generation with each round. Each division shuffles the cards and produces genetic changes and mutations, some of which may help or hurt the bacteria's ability to compete for glucose and win the evolutionary poker game. The next day, a dilution is done, with

1839

Darwin marries Emma Wedgwood.



1842

Darwin settles at Down House, in the village of Downe in Kent. He prepares a rough sketch (a 35-page outline) of his evolutionary ideas.



10 percent of the culture within a flask transferred to a new flask, and a new hand is played. (Every 500 generations or so, the remaining 90 percent is frozen and stored for later experiments.) The dilution acts as a population bottleneck, randomly selecting a subset of the bacteria (and so a subset of accumulated genetic changes) to continue the experiment.

These 12 flasks “represent the stripped-down bare essentials of evolution,” says Zachary Blount, a graduate student in Lenski’s lab. The environment never changes. No new genes enter the system from migrating microorganisms. And the scientists take no action to affect the course of evolution within the flasks. Only the intrinsic, core processes of evolution influence the outcome, Blount says.

Lenski and his colleagues have watched the game play out, occasionally analyzing DNA to peer over the players’ shoulders and find out what cards they hold. On the surface, the populations in the 12 flasks seem to have traveled similar paths — all have grown larger and become more efficient at using glucose than their ancestors. And many of the strains have accumulated mutations in the same genes. Notably, though, no one strain has developed exactly the same genetic changes as another.

Randomness is an important part of the evolutionary equation, as the experiment illustrates. During the first 2,000 generations, all of the 12 populations rapidly increased in size and fitness. But then cell size changes and reproductive and glucose efficiency gains began to slow down, hitting the evolutionary equivalent of a dieter’s plateau.

After 10,000 generations, it became apparent that not all the flasks were on the same trajectory. Though the cells in all the flasks became larger, each population differed in the maximum size the cells reached. The populations also differed in how much fitter they were than their ancestors, when the researchers grew them in direct competition. The “experiment demonstrates the crucial role of chance events (historical accidents) in adaptive evolution,” Lenski and his colleague Michael Travisano wrote in a 1994 paper.

The experiment has progressed, and several of the flasks now contain mutator strains, bacteria that have defects in their DNA replication system. Such defects make mistakes more likely to happen every time those bacterial strains copy their DNA to divide. Sometimes a mistake can have lethal consequences, damaging a gene crucial for survival. But other times coloring a bit outside the lines creates opportunity for advancement.

Even within a given flask, some bacteria take slightly different paths. One flask now contains two separate strains — one that evolved to make large colonies when grown on petri dishes, and one that makes small colonies. The large- and small-colony strains have coexisted for more than 12,000 generations. The large-colony producers are much better at using glucose so they grow quickly, but they make by-products that the small-colony producers can eat. Each of the populations, both large and small, have improved their ability to use glucose over the generations. By at least one measure, the two populations could constitute separate species, Lenski and his colleagues proposed in 2005 in the *Journal of Molecular Evolution*.

Still, it seems that Conway Morris was basically right: Though the details were different, replaying evolution in a dozen flasks produced very similar outcomes in each. But then something completely unexpected happened.

After about 31,500 generations, glucose-eating bacteria in one of the flasks suddenly developed the ability to eat a chemical called citrate, something no other *E. coli* do, the researchers reported last June in the *Proceedings of the National Academy of Sciences* (*SN Online*: 6/2/08).

“They’ve been eating the main course for thousands of generations,” Lenski says. “They didn’t realize that there was a dessert tray around the corner.”

The switch was clearly a radical change of destination for the bacteria. The inability to eat citrate is a biochemical hallmark of the *E. coli* species, so by some definitions, the citrate eaters in that flask are no longer *E. coli*, but a different species.

But a single change did not a citrate eater make. The researchers found that the bacteria went through a series of steps before evolving the ability to use citrate. One initial mutation happened at least 11,000 generations before the citrate eaters appeared. Lenski and his crew don’t yet know which specific DNA changes led to citrate use, but the researchers have enough evidence to say that the ability to use citrate is dependent, or contingent, upon those earlier changes. And even the bacteria that have undergone those initial changes are still not guaranteed to find the dessert cart. Blount tested 40 trillion bacteria from earlier generations to see if any could evolve the ability to eat citrate. Fewer than one in a trillion could.

The profound difference between the citrate eaters and the other 11 strains, as well as the dependence of the citrate change on earlier mutations, seems to suggest that Gould was

1844

Darwin finishes a 230-page manuscript on the origin of species, but does not publish it.

1856

On Lyell’s advice, Darwin begins writing a projected “big book” called *Natural Selection*.

1858

Alfred Russel Wallace (right) sends a paper, which mirrors Darwin’s idea, to Darwin. Both of their ideas are presented at the Linnean Society.

**1859**

On the *Origin of Species* is published in London November 24.

also right: Replaying evolution will result in some surprise endings. “The long-term evolution experiment with *E. coli* provides some of the best evidence for both Conway Morris and Gould that one could ever hope to see,” Lenski says. “Conway Morris ‘wins’ based on the number of changes that fit his pattern, but Gould might prevail if weighted by the profundity of change. Both perspectives are important contributions, and they are not mutually exclusive.”

Now the researchers are watching to see if citrate-eating bacteria will evolve in other flasks, and if citrate eaters will eventually reject glucose and feast only on citrate. Such a transformation would probably herald the birth of a new species. “It would be amazing,” says Blount. “It would be like teenagers who no longer like to eat pizza — they prefer broccoli.”

When the game changes While Lenski’s experiment takes place in a constant environment, natural evolution must cope with a messier reality. In Steven Finkel’s lab at the University of Southern California in Los Angeles, a long-term experiment is showing how evolution plays out in a closed and ever-changing environment, more like the real world. This allows Finkel to focus on how evolution and environment are interwoven.

Finkel didn’t start out to test evolution in changing environments. The experiment was prompted by a graduate stu-

dent’s casual remark that *E. coli* will live a long time. Finkel asked, “How long?”

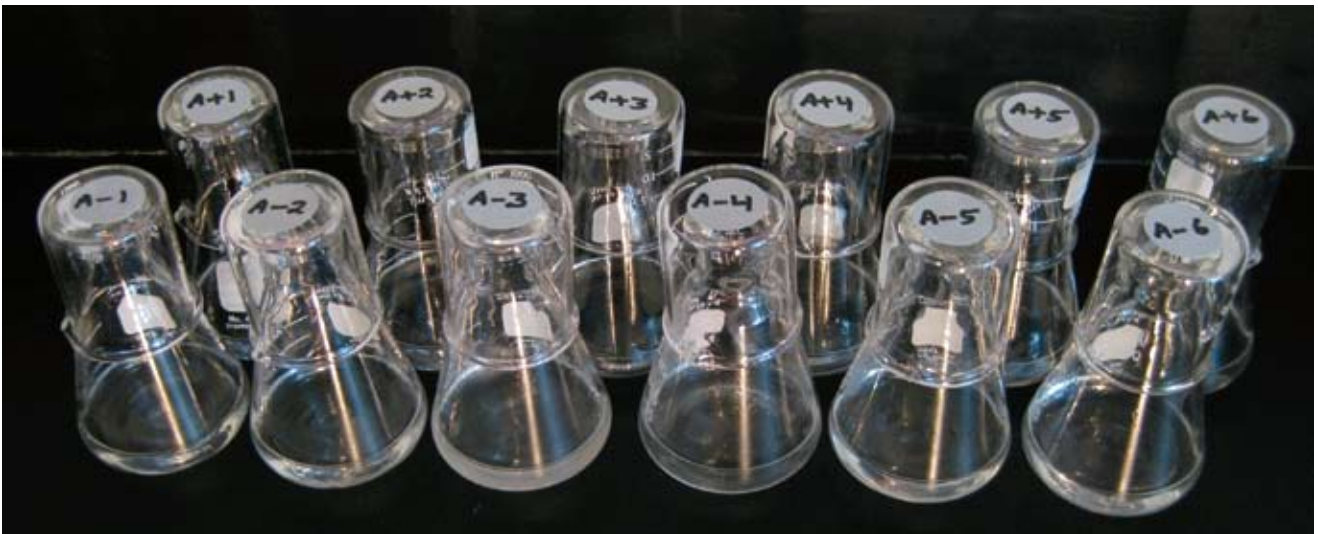
“A long time,” the student responded.

“So we set up some experiments to see how long they would live, and they just would never die,” Finkel says. The immortal bacterial cultures are teaching scientists a few things about how organisms change their environments and adapt to changes wrought by outside forces.

Some of the cultures in Finkel’s laboratory have been growing for more than five years. The bacteria started out with the same genetic background, or so the researchers like to tell themselves. In reality, each flask started with a single bacterial colony, containing perhaps 50 billion individuals. Given that DNA replication systems aren’t perfect, one in every 10,000 cells probably carries a typo in at least one letter of its instruction manual. Such DNA typos are known as point mutations.

Finkel and his colleagues placed the bacteria in a rich broth full of sugars and many other nutrients and then just let them grow. After a short initial lag, the bacteria began growing like gangbusters, a phenomenon known to microbiologists as “log phase” because the bacteria increase their numbers logarithmically. Once the nutrients start to run out, the bacteria stop growing so quickly and settle into a senescent state. After a few days, millions of bacteria die, spilling their guts into the surrounding media and providing food for survivors.

These twelve flasks contain separate populations of *E. coli* bacteria, all evolved from a single ancestor in Richard Lenski’s long-term replay of evolution. Only bacteria in flask A-3 evolved into citrate eaters, possibly making them a new species.



1862

Darwin predicts that there must be a moth with a tongue long enough to pollinate the orchid *Angraecum sesquipedale* (the Christmas Star orchid).



1865

Gregor Mendel prescribes experiments on here hybrid pea plants (unknown to Darwin).



1869

Johann Miescher isolates a molecule rich in phosphorus and nitrogen that he calls nuclein, later revealed to be DNA.

It's the postapocalyptic survivors that interest Finkel. As 99 percent of their comrades die off, the surviving bacteria feed on the carcasses of the dead and on metabolic by-products of other survivors. Thus the bacteria change the environment in which they live. It doesn't take long for the cultures in each flask to go their own ways. Within a month, the bacteria in the various flasks convert the light honey color of the broth into a spectrum ranging from light amber to dark amber, Finkel says. And his nose tells him the cultures are different as well. Microscope examinations reveal that the originally rod-shaped bacteria take on a wide variety of shapes; in one flask, some of the cells never cut the apron strings during cell division, forming long strands resembling linked sausages.

Yet as different as the bacterial populations appear, they also have something in common. All of the cells that have gone through the valley of death and come out the other side are tougher than naive bacteria. And the older the cells get, the more competitive they are, so that 20-day-old cells will drive 10-day-old cells to extinction, and 30-day-old cells beat 20-day-olds. Finkel calls that phenomenon "growth advantage in stationary phase," or GASP.

On the surface it appears that the number of surviving cells stays constant. But underneath, different mutants rise and fall in number, like waves crashing on the beach, Finkel showed in a 2006 review published in *Nature Reviews Microbiology*.

The ability for older cells to compete better has been traced to mutations in four genes. Three of the genes allow the bacteria to feast more readily on certain amino acids. One of the genes encodes a key protein, RpoS, needed to turn on stress-response genes. The protein gives the green light to turn on genes under certain conditions. When cells are under stress — for bacteria, stress means high salt, low or high temperatures, broth that is too acidic or alkaline, or other environmental extremes — RpoS turns on genes that help the bacteria cope. But the protein is not necessary when cells aren't under stress. In fact, it takes resources away from the cells' main "go" signal, RpoD, a protein critical to normal function. Inactivating or handicapping RpoS makes more resources available for other genes.

Many of the GASP cells contain changes in *rpoS*, the gene

for RpoS, but they don't all have the same change, Finkel and colleagues reported in 2003. Nearly all of the changes reduce activity of RpoS to 1 percent or less of its normal activity but don't abolish it entirely. Low levels of RpoS are a fixture in bacterial populations that have GASP.

But just because a mutation serves an organism well under some conditions doesn't mean it's always beneficial. Thomas Ferenci, a microbiologist at the University of Sydney in Australia, reviewed what happens to *rpoS* mutants under a variety of environmental conditions in the May 2008 *Heredity*. Depending on a cell's genetic background, an *rpoS* mutation might give the strain a big boost in fitness or make an undetectable difference. And even if the mutations are beneficial under most conditions, the changes hold the bacteria back when the environment changes. If salt concentrations go up, the temperature drops, bacteria lack oxygen or encounter a toxin, then *rpoS* mutants, less able to cope with certain types of stress, don't become established members of the community as quickly as they do under other conditions.

Natural selection works for *rpoS* mutants in some environments and against them in other conditions. "Selection is a deterministic force pushing relentlessly in one direction," says Michael Lynch, an evolutionary biologist at Indiana University in Bloomington. That direction is toward ever-greater adaptation for the environment in which a population finds itself. But most environments are in a constant state of flux and, as Darwin was careful to point out in his introduction to the *Origin of Species*, selection isn't the only evolutionary force at work.

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Sex, chance and genes Random genetic drift is an evolutionary force to be reckoned with too. And, as with selection, molecular biologists are helping to reveal its workings.

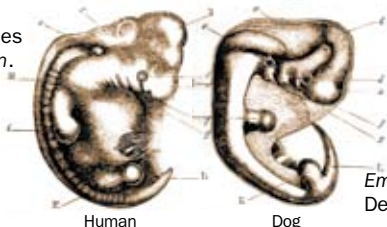
Drift by any other name would be known as chance. The number of individuals that carry a specific genetic variation within a population — what scientists call the frequency of a gene variant — can change at random, bobbing along like driftwood on the ocean. The indiscriminate nature of drift doesn't always work to organisms' betterment.

"Drift doesn't care about fitness," Lynch says.

Drift can haphazardly make a detrimental gene prominent

As Darwin was careful to point out in his introduction to *Origin of Species*, selection isn't the only evolutionary force at work.

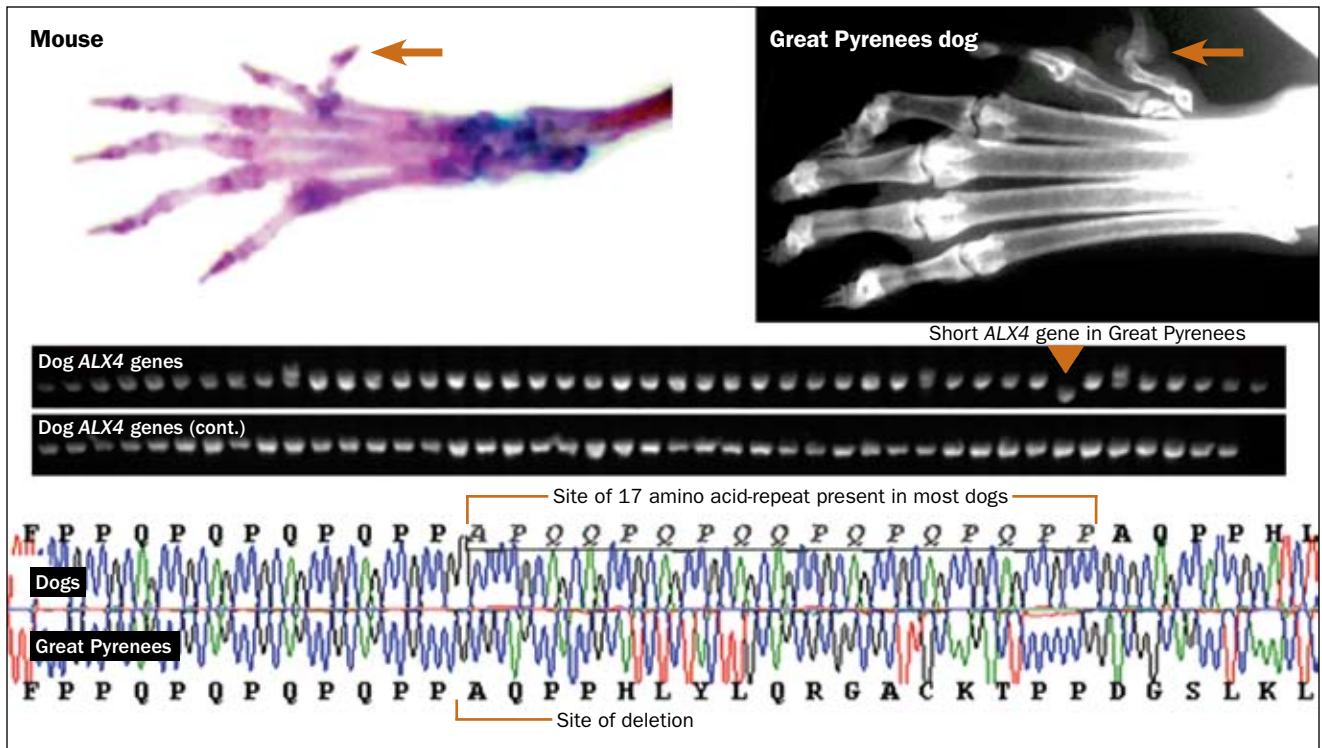
1871
Darwin publishes
Descent of Man.



1882
Darwin dies April 19 at
age 73. He is buried in
Westminster Abbey.

Embryos illustrated in
Descent of Man





in a population, or accidentally eliminate beneficial mutations — especially in small populations. Imagine two versions, or alleles, of a gene as the head and tail of a coin. Every time an organism reproduces, the coin is tossed to see which allele will be passed to the offspring. In a large population, coins will flip many times and the number of heads and tails will be roughly equal. But in a small population, runs of heads or tails can skew the outcome in favor of one or the other allele, maybe even eliminating one version altogether.

That’s a simple example of what drift can do, but Lynch thinks it also accounts for some complex traits, such as the complicated structure of genes in eukaryotic organisms — including multicellular beings like people and plants and unicellular life such as yeasts.

Drift causes noise in the evolutionary process, says Lynch. But there is yet another force that mixes things up — genetic recombination. Recombination is an essential element of sexual reproduction. In general, each parent contributes a single copy of each chromosome to its offspring. Before mom and dad divvy up the genetic goodies to hand down to the children, the two copies of each chromosome are lined up and matched

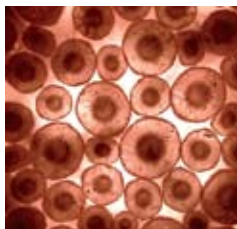
Recipe for an extra toe

Mice with a defect in the gene *ALX4* grow extra toes on their back feet (top left). Back feet with extra digits are a hallmark of the Great Pyrenees dog breed (top right). Scientists have associated the extra toe with a deletion of repeated amino acids in the breed’s *ALX4* protein. In tests of 89 dog breeds, researchers found that the *ALX4* gene was shorter only in Great Pyrenees dogs with an extra toe (gels, middle, compare *ALX4* from 89 breeds; arrow shows Pyrenees’ short version). The short gene results in the loss of a 17 amino-acid repeat (highlighted in the amino acid sequence from other dog breeds) from the *ALX4* protein in Great Pyrenees (site of deletion marked on sequence, bottom).

like pairs of socks. When the two chromosomes are zipped together, they swap chunks of DNA, giving each egg or sperm a different combination of the parents’ genes.

Genes follow each other along a chromosome like freight cars on a train follow the lead engine, unless recombination happens. So if one gene develops a lethal mutation it may doom the other genes on the chromosome, like a train car that gets unbalanced and derails the train. Similarly, a beneficial mutation might get trapped on a slow train to nowhere if not for recombination shuffling the mutated gene’s position on the chromosome. Or a particularly good mutation may

1892
August Weismann puts forth the germ-plasm theory, proposing that germ cells (sperm and eggs) carry the heritable material that’s passed on to the next generation.



1900
Working independently, Hugo de Vries, Carl Correns and Erich von Tschermak rediscover and confirm Mendel’s work on heritable traits.

1903
Walter Sutton publishes a paper making the first clear case for the chromosome theory of heredity.



create such a powerful engine that natural selection can't resist taking along whatever's attached — like an engine dragging a decrepit train.

Low rates of recombination enhance the effect of drift because “beneficial alleles could get trapped in bad backgrounds,” Lynch says. Natural selection would derail some trains, taking “good” genes along with the bad. “That’s simply because you are a victim of the surrounding genetic material,” he says.

Recombination allows the cars to uncouple and switch around, creating faster, more efficient trains. Once removed from a bad neighborhood and pasted in a beneficial or neutral stretch of chromosome, an allele's attributes can shine, and natural selection can act on the allele without any worries about the company it keeps. In this way, the process helps to increase the efficiency of natural selection, Lynch says.

Researchers are still debating the details of how selection works together with mutation, recombination and drift to shape genes and help organisms adapt to their environments, producing the abundance of species around today.

“We’re peeling back the onion of the evolutionary process,” says Sean Carroll, a developmental and evolutionary biologist at the University of Wisconsin–Madison. “The question is no longer ‘does adaptation happen?’ but ‘how does it happen?’”

Evolutionary tweaks In the past few years, scientists have learned that genes work together in vast networks to regulate every process in the body. Of special interest to many evolutionary biologists are transcription factors, proteins that are important for controlling the timing and placement of gene activity during development (and later). Each transcription factor may govern multiple genes, even hundreds of genes.

“We’re into scores of direct targets,” Carroll says. “More than we imagined. More than we even have an explanation for.”

Implications of such vast gene regulatory networks are clear for Carroll. Altering the structure of a transcription factor to better regulate one gene could have effects on hundreds of other genes. Tinkering with a transcription factor doesn't just alter the shape of a fin, add a horn or move a spine. No. These molecules are so important and work in so many different parts of an organism that changing the transcription factor itself is likely to affect nearly everything about the living thing. Most of the time, such far-reaching

changes are bad, even lethal. Carroll and others have been working to understand how body shapes and coloring morph in animals. If changing transcription factors could be catastrophic, organisms must make molecular tweaks elsewhere to create a new look.

Carroll is a leading proponent of an idea called the *cis*-regulatory theory. (*Cis* refers to a region adjacent to the gene or on the same chromosome.) The theory holds that altering the control region of a gene to change some feature of an animal or plant would produce fewer side effects than tinkering with the proteins that direct construction of the features. So an organism can change one part of its body without affecting the rest simply by adding a few more switches and buttons to its control panel (or taking some away), or by rewiring a switch to work at a different time or govern development in a new location.

Fish called three-spined sticklebacks have provided some of the most direct evidence that the *cis*-regulatory theory could be correct. The fish live in saltwater but swim into rivers to spawn. That habit led to isolation of many of the fish in inland freshwater lakes at the end of the last ice age. Over the past 10,000 years the fish have adapted to their new homes, says David Kingsley, an evolutionary biologist at Stanford University.

In the ocean, sticklebacks wear armor and sport pelvic spines that protect them from sliding down the throats of predators. Fish stranded in freshwater lakes found themselves without the fishy predators they knew in the ocean, but some encountered deadly insects, such as dragonflies.

Dragonflies grab the sticklebacks by their pelvic spines and eat the fish sideways, so the feature that once offered protection became a liability. Over time, some populations of fish have shed their armor and their pelvic spines.

Kingsley and his colleagues discovered that a protein called *PITX1* is responsible for building the pelvic spines. The protein is made in the hind limbs of many different animals, including humans. A group of researchers from Washington University in St. Louis showed that a mutation in *PITX1* in humans caused clubfoot in members of a family. The team published the research in the Nov. 7 *American Journal of Human Genetics*. The protein also controls development of the pituitary gland and facial development. Defects in *PITX1* can lead to cleft palate.

But when Kingsley and his colleagues examined the gene encoding *PITX1* in stickleback fish with and without spines,

1903

A moth predicted to exist by Darwin is discovered in Madagascar and named *Xanthopan morgani praedicta*, in Darwin's honor.

1908

Godfrey Harold Hardy and Wilhelm Weinberg independently derive a formula for gene allele frequency in populations.

1910

Thomas Hunt Morgan discovers *Drosophila* mutant with white eyes, a sex-linked trait he relates to Mendel's recessive traits.



1913

Alfred Sturtevant, a student of Morgan's, publishes the first rudimentary map of a fruit fly chromosome, establishing that genes are real.

the researchers found no differences. That led them to believe that the defect must lie in the control panel for *PITXI* and not in the gene itself. But the scientists had no direct evidence that changes in the control panel were responsible for the missing spines.

Recently, Kingsley's group did discover that some of the stickleback species that have lost spines also lost a portion of the control panel that turns *PITXI* on in the pelvis. Restoring the lost switch also restored spines, Kingsley told science journalists gathered in Palo Alto, Calif., in October at a conference sponsored by the Council for the Advancement of Science Writing.

"That's an 'i' that has needed to be dotted," says John "Trey" Fondon III, an evolutionary biologist at the University of Texas at Arlington. "We've had some really great circumstantial evidence for *cis*-regulatory evolution, but the data have been lacking. It's been a little, what we call, 'empirically challenged.'"

Fish aren't the only animals providing evidence for how evolution works in genes. Fondon and others have turned to man's best friend to figure out how genes influence body shape and size, behavior and other traits. Dogs come in an astounding number of variations, with the smallest dogs, Chihuahuas, weighing under six pounds and the largest breeds weighing more than 100 pounds.

A group of scientists from the National Institutes of Health and collaborators traced body size in dogs to a variation of the insulin-like growth factor gene (*IGF1*). Within the gene itself, researchers found no differences between large dogs and small dogs. But dog breeds that weigh less than 20 pounds had a common change in the *IGF1* control panel, altering how much of the protein is produced, the researchers reported in 2007.

Kingsley cites the *IGF1* finding as further evidence that changes in control regions can account for surprisingly large differences in body shapes and sizes. But Fondon says he isn't ready to swallow the "*cis*-regulatory Kool-Aid" just yet.

In contrast to the *cis*-regulatory theory, Fondon and his colleagues have found evidence that tampering with transcription factors can change specific features without having disastrous consequences for the whole organism. The team focused on repeats of amino acids within proteins. Proteins work a bit like Swiss Army knives with various tools tied

together in a single package. The repeated amino acids are often found between the stretches of amino acids that form each of the tools. Fondon reasoned that slightly altering the number of repeated amino acids, each of which are encoded by repeated three-letter DNA sequences, might subtly change the function of the protein, creating a variant that could be put to an evolutionary test.

But Fondon realized that hypothesis also had its weak point: Repeats in DNA tend to mutate at high rates. The machinery that replicates DNA loses its place when reading the same letters over and over and over again. Sometimes it slips up and skips a repeat or adds an extra. High mutation rates can be dangerous because of a higher chance of catastrophic error. In some families, extra DNA triplet repeats in the gene for the huntingtin protein can lead to Huntington's disease. Often the number of repeats grows with each generation, causing people to develop the disease at younger and younger ages.

"I thought selection wouldn't tolerate this kind of crap in our genes," Fondon says.

But when he created a computer program to find genes in dogs that contain such repeats, he found a surprising number. "The top half of the list was a who's who of development," he says. This list includes genes that control bone development and the homeobox genes, which encode transcription factors that direct construction of an animal's body, distinguishing head from tail and back from front, and guiding the positions of limbs and appendages. These genes are found in almost every type of animal on Earth, from sponges to people. Even fungi and plants have some forms of homeobox genes.

Expanding and contracting the number of amino acid repeats in certain homeobox genes seems to give dog breeds some of their distinguishing characteristics.

"If what the protein does is a verb, a repeat is an adverb," says Fondon. The repeats don't change what the protein does; they just make it happen more quickly, slowly or frequently.

For instance, in Great Pyrenees, deletion of a repeat in the *ALX4* gene leads to the formation of an extra toe on the back feet, a hallmark characteristic of the breed. Mice with defects in *ALX4* also grew extra digits on their back feet.

A protein called RUNX2 governs genes that help control facial development in dogs. Fondon and his colleagues found

"I thought selection wouldn't tolerate this kind of crap in our genes."

John Fondon III
University of Texas at Arlington

1925

In a high-profile trial, a Tennessee court convicts teacher John Scopes of violating a state law against teaching that people descended "from a lower order of animals."

Scopes trial

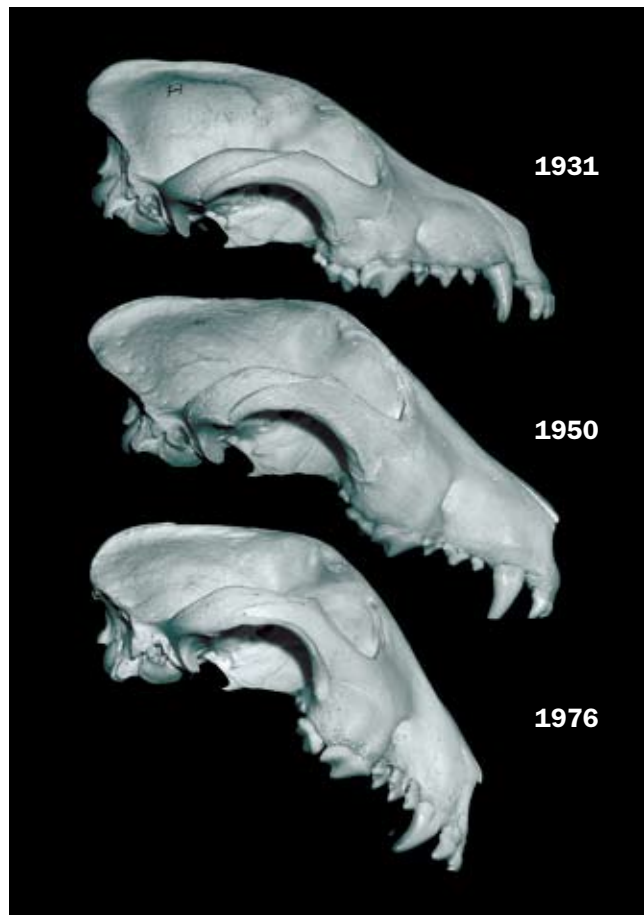


John T. Scopes

1925

Raymond Dart publishes his discovery of a fossilized skull from a new species, *Australopithecus africanus*, a key member of the human evolutionary tree.





Skulls from purebred bull terriers show development of ever-flatter faces over a span of just 45 years. The change may be due, in part, to a changing number of repeated amino acids in RUNX2, a protein that helps control facial development. A bull terrier from 1931 had a different number of repeated amino acids than its modern counterparts.

that changing numbers of repeats within RUNX2 are associated with ongoing exaggeration of certain face traits. Scientists have documented such changes in the bull terrier's RUNX2 protein between 1931 and today. Modern bull terriers have fewer repeats of a certain amino acid sequence than members of the breed did in 1931. That doesn't sound like a big change, but could be one factor contributing to the flatter faces seen in today's bull terriers.

Carroll doesn't deny that mutations happen in transcrip-

tion factors too, but he thinks such mutations probably affect genes that play a more limited role in development. Regulatory changes are more likely in genes that govern development of many different parts of the body, he says.

But others don't think it has to be all regulatory mutations or all protein changes that create novel traits in animals. Vincent Lynch, an evolutionary biologist at Yale University, and his colleagues discovered that both types of changes were needed for pregnancy to evolve in mammals.

Control issues When most female mammals get pregnant, the embryo attaches to the wall of the uterus. Normally something burrowing into the body causes the immune system to take up arms and oust the intruder, but that would make pregnancy impossible. So placental mammals turn up production of prolactin, a protein that calms the immune system and does other jobs that allow an embryo to develop safely.

Yale's Lynch and his colleagues discovered that the evolution of pregnancy probably happened in several steps. The first step was that a jumping gene, called a transposon, hopped into the control panel of the prolactin gene. The transposon brought with it a switch operated by the homeobox protein HOXA11. Over time, HOXA11 developed changes that allowed it to work with other proteins to more precisely control prolactin production, Lynch and his colleagues reported in the Sept. 30 *Proceedings of the National Academy of Sciences*. Only HOXA11 from mammals turns on prolactin, the researchers showed. HOXA11 from chickens, platypuses and opossums (all animals without a placenta) failed to turn on production of the pregnancy-associated protein.

Such complementary changes to proteins and their genetic control panels help evolve a toolkit that organisms can use for every occasion, Lynch says.

"A hammer in a toolbox can be a ball-peen hammer. It can be a hammer that pulls nails. It can be a mallet, but it's still a hammer. It evolves to its own context," he says.

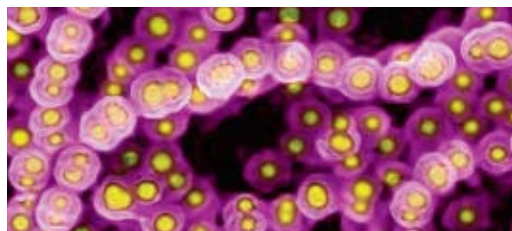
Animals, plants, bacteria, archaea, fungi and all organisms on Earth evolve to their own contexts as well. Scientists are now beginning to learn how tweaks and major changes on the molecular level enable adaptation to environments. The picture is painted in DNA, but it's far from a completed masterpiece. Changing environments coupled with the forces of natural selection, mutation, recombination and drift are continually reworking the painting. Only time will tell how the landscape will morph — and its inhabitants with it. ■

1927

H.J. Muller uses X-rays to experimentally create genetic mutations in *Drosophila*.

1928

Frederick Griffith's transformation experiments with streptococcus bacteria suggest that the genetic material cannot be a protein.



1930

Ronald Fisher publishes a mathematical analysis of how natural selection can change the distribution of genes in a population.

Step-by-step Evolution

Mining the Gaps: Transitional fossils are the hardest to find, but sometimes tell the best stories **By Sid Perkins**

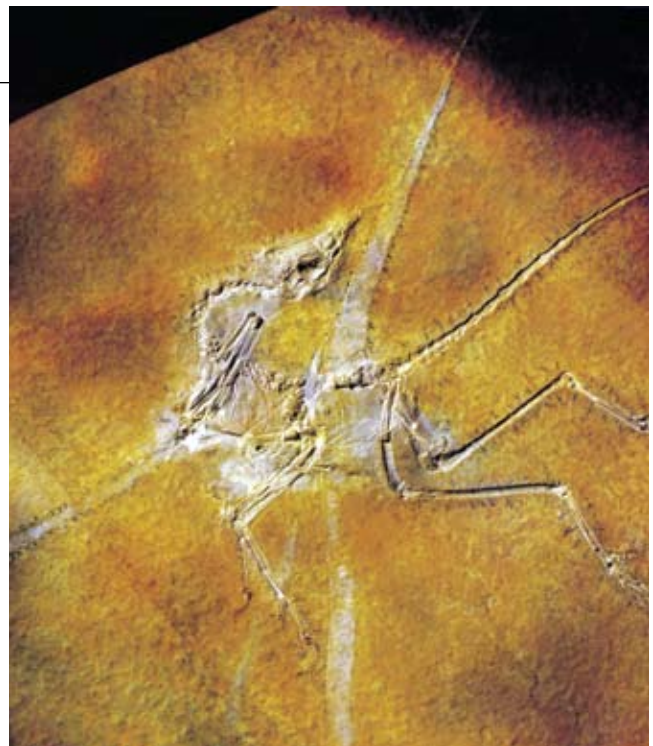
When Charles Darwin proposed the idea of evolution in *On the Origin of Species*, he wrote “if my theory be true, numberless intermediate varieties, linking most closely all the species of the same group together, must assuredly have existed.” At the same time, he bemoaned the dearth of such transitional fossils as perhaps “the most obvious and gravest objection which can be urged against my theory.”

Surely it was serendipity when, just two years later, quarriers unearthed fossils of *Archaeopteryx*. This creature, now recognized by many scientists as the first known bird, has a mosaic of features that links it with the disparate groups of species on either side of it in the fossil record: While its teeth, tail and overall body shape are distinctly reptilian, its feathers have the same complex structure as the lift-generating feathers of modern birds. In other words, it is just one of the “numberless intermediate varieties” that Darwin predicted must have existed.

“It was the right discovery at the right time,” says Richard Fortey, a paleontologist at the Natural History Museum in London.

Darwin blamed the lack of transitional fossils in part on the poorness of the paleontological record. It’s a rare accumulation of fortuitous events when a creature is fossilized, its remains are preserved over millions of years and then those remains are discovered.

In many cases, that critique still holds true: Researchers have yet to discover fossils of a creature that fits in the gap between bats – which seem to appear suddenly in the fos-



Archaeopteryx (fossilized skeleton shown) is a transition species between ancient reptiles and modern birds.

sil record about 54 million years ago – and their mammalian predecessors (*SN: 5/14/2005, p. 314*). The gap in the fossil record between *Archaeopteryx* and its reptilian ancestors also remains unoccupied, although several discoveries of feathered dinosaurs in China have given researchers clues about what these still undiscovered intermediate creatures may have looked like.

Many of the gaps in the fossil record that remained unfilled in Darwin’s time now throng with creatures, such as the ones used to chronicle the 48-million-year series of evolutionary changes between whales and their predecessors (*SN: 9/22/01, p. 180; SN: 1/5/08, p. 5*). And particular biomarkers – chemical fossils, if you will – in rocks more than 240 million years old have provided clues about the evolution of flowering plants (*SN: 4/21/01, p. 253*).

Paleontologists still randomly stumble across transitional fossils these days, such as a creature found in Texas that falls in a 50-million-year gap in amphibian evolution and helps pin down when the groups that include salamanders and frogs arose.

As often as not, however, transitional fossils are found when researchers head into the field with a specific target in mind: By focusing on rocks deposited during an interval

1931

Harriet B. Creighton and Barbara McClintock, working with maize, and Curt Stern, working with *Drosophila*, provide the first visual confirmation of genetic crossing-over.



1931

Sewall Wright begins to publish work showing that “random drift,” or chance fluctuations in a population’s gene frequencies, could be a significant factor in evolution.

1941

George Beadle and Edward Lawrie Tatum propose the one gene/one enzyme hypothesis.

1942

Julian Huxley publishes *Evolution: The Modern Synthesis*.

where gaps in the fossil record exist, scientists can boost the chances of making a critical discovery. That's how researchers unearthed *Tiktaalik*, a 2.7-meter-long beast that plopped into a 9-million-year gap in the chronicle of vertebrates' transition from water to land (*SN*: 6/17/06, p. 379).

Techniques such as CT scanning, used to reinvestigate fossils collected decades ago, have revealed new insights about the anatomy of semiaquatic creatures that preceded *Tiktaalik*. Even genetic analyses of living creatures can provide insight into the fossil record: The evolutionary changes observed in fossil fish deposited over a time period of 20,000 years in an ancient lake can be linked to a particular gene often studied in that species' modern-day kin.

Amphibian enigma Gaps in the fossil record can be large in terms of time — sometimes many millions of years — and in the extent of the evolutionary changes seen when comparing creatures before and after the gap. When *Archaeopteryx* was discovered, for instance, the fossil record was sparse and the disparity between known fossil reptiles and birds was vast.

Until recently, the gap in the fossil record separating frogs and salamanders from their amphibian ancestors was similarly huge. About 290 million years ago, a diverse assemblage of primitive amphibians walked the land, says Jason Anderson, a vertebrate paleontologist at the University of Calgary in Canada.

But in rocks documenting the 50 million years or so that followed, amphibian fossils are few and far between.

***Gerobatrachus hottoni* lived about 290 million years ago. The species fits into a 50-million-year gap in the amphibian fossil record between primitive amphibians and the frogs and salamanders that subsequently evolved. Paleontologists have unearthed just one example of the species (shown below).**



1943

Salvador Luria and Max Delbrück report that random mutations, not just selection, can confer resistance in bacteria.



1944

Oswald Avery, Colin MacLeod and Maclyn McCarty suggest that nucleic acids are the material of heredity.

1952

A blender experiment by Alfred Hershey and Martha Chase demonstrates that DNA, not protein, is the genetic material.

Only in rocks deposited after 240 million years ago do such fossils — and specifically, those of frogs and salamanders — appear. These two groups of creatures are distinct both from each other and from their ancestors, and they apparently evolved during an interval for which few fossils have been discovered.

Recently, however, Anderson and his colleagues unearthed *Gerobatrachus hottoni*, a species whose genus name means “elder frog.” The single specimen unearthed so far is about 11 centimeters long, the size of most modern-day salamanders. It was found in a two-foot-thick knob of 290-million-year-old, fine-grained siltstone in north-central Texas. Even though the fossil was found in rocks deposited just before the start of the lengthy gap in the fossil record, the remains have features characteristic of the frogs and salamanders that presumably descended from it or others like it, Anderson says.

A main clue is that some of the bones in the first and second innermost toes on each of *Gerobatrachus*' feet are fused together, a trait characteristic of salamanders but rarely found in other creatures. Because some of the other bones in the fossil aren't fully developed, Anderson and his colleagues suggest that the creature was a juvenile, indicating the fusion of the toe bones occurred even before adulthood — a stronger sign that it betrays an evolutionary link to salamanders.

But like frogs, *Gerobatrachus* has a broad skull and a shortened tail, the researchers reported last May in *Nature*. The shape and configuration of bones in the creature's skull, and particularly those in its palate, are very froglike. Therefore, “this fossil seals the gap” between primitive amphibians and the frogs and salamanders that evolved later, Anderson says.

On the amphibian family tree, *Gerobatrachus* and its kin are ancestors to salamanders and frogs, the researchers contend, and the evolutionary split between those two groups probably occurred between 260 million and 270 million years ago.

Gerobatrachus was “quite advanced” compared with other amphibians of its era, he adds. Another way to look at it, he notes, is to consider the amphibians appearing 290 million years ago to be evolutionary holdovers best representing species that first evolved long before.

Getting a foot on land The series of gradual anatomical changes that enabled semiaquatic creatures to completely leave the water and conquer dry land is one of the most important chapters in the tale of evolution. Among other changes, creatures had to develop limbs to support their

weight and develop a way to extract oxygen from the air.

“This whole transition is known from quite a few [species],” says Neil Shubin, a paleontologist at the University of Chicago. Nevertheless, new discoveries — both in the field and in the lab — are still fleshing out the details of these evolutionary developments.

Members of one species considered to be an important part of the water-to-land transition, a lobe-finned fish called *Panderichthys*, lived in what are now Latvia and Scotland about 385 million years ago. Until recently, the next known member of the evolutionary sequence was a land-adapted creature called *Ventastega*, which lived on land that is now in the Northern Hemisphere about 365 million years ago. Earlier this decade, Shubin and his colleagues braved the cold of northeastern Canada’s Ellesmere Island to search for fossils to fill that 9-million-year gap. Besides being located in

the right place, Ellesmere Island has substantial outcrops of rocks of the right age to hold such fossils, Shubin notes.

He and his team struck paleontological pay dirt with *Tiktaalik*, which lived about 382 million years ago. Like some fish of the day, the animal had fleshy limbs that ended in fins. But, like land-adapted tetrapods, it had sturdy ribs and a neck (*SN Online: 10/15/08*). Its fossils also suggest that *Tiktaalik* had both gills and lungs. Altogether, this blend of features spurred the researchers to dub the creature a “fishapod.”

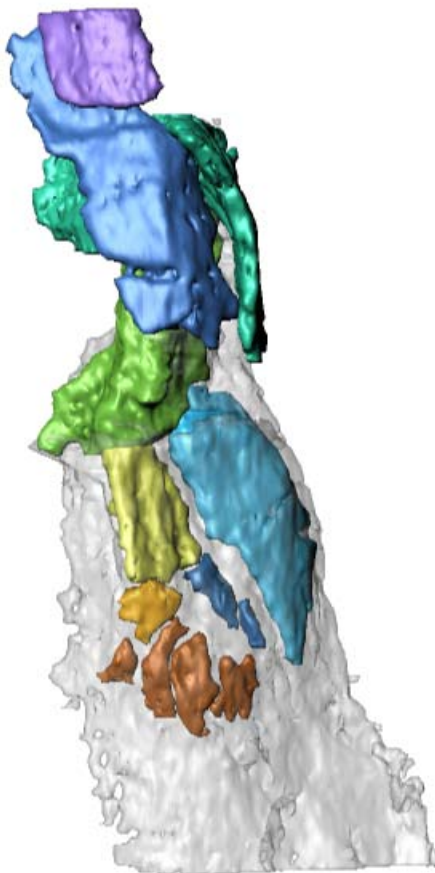
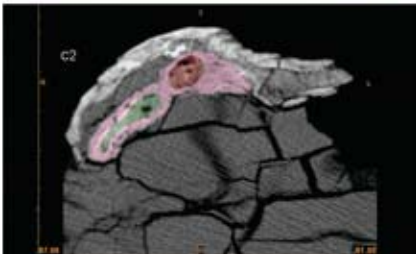
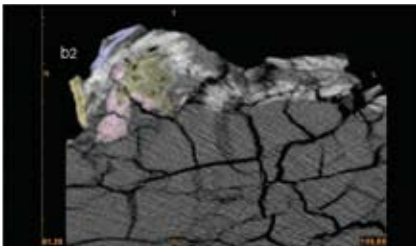
Fins fringing the end of *Tiktaalik*’s protolimbs also include bones that are analogous to those in human wrists and fingers. At the time *Tiktaalik* was found, scientists hadn’t yet discovered similar bones in the fins of predecessors such as *Panderichthys*, in part because that creature’s fossils are so fragmentary.

In the 1990s, analyses of fossils of *Panderichthys* and the lobe-finned fish of their era didn’t reveal bony features in the fins. Scientists interpreted this lack as a sign that digits were an evolutionary novelty that only arose later in land-adapted creatures such as *Acanthostega*, says Per Ahlberg, a vertebrate paleontologist at Uppsala University in Sweden. Lab studies of some modern-day fish such as zebra fish backed up that notion: The second wave of *HOX* gene activity that leads to the development of digits in tetrapod embryos didn’t occur in those fish, a sign that ancient fish may not have been genetically equipped to make fingers and toes.

However, new analyses of a near-complete specimen of *Panderichthys* — specifically, a CT scan of a fossil still partially encased in rock — do in fact suggest that those semiaquatic creatures had such bones after all, Ahlberg and colleagues reported last year in *Nature*. And recent studies in other fish species such as paddlefish and Australian lungfish suggest that a second wave of *HOX* gene activity can occur during embryonic development in fish. To create a foot, one of the key features of land vertebrates, Ahlberg notes, “all that evolution did was reshape and

Another way to view a fossil

Previous analyses of fragmentary fossils of *Panderichthys*, a lobe-finned fish that lived about 385 million years ago, hinted that the creature didn’t have bones analogous to those in human wrists and digits. But CT scans of a lump of rock containing a nearly complete specimen (two individual CT slices are shown below) indicate that the creature indeed had those bones (dark blue, gold and bronze in the reconstruction at right). The presence of these bones makes the fish-to-land transition “a little less dramatic than we thought it was,” one researcher notes.



1953

James Watson (left) and Francis Crick (right) determine the double-helix structure of DNA. They use Rosalind Franklin’s X-ray diffraction images.



1958

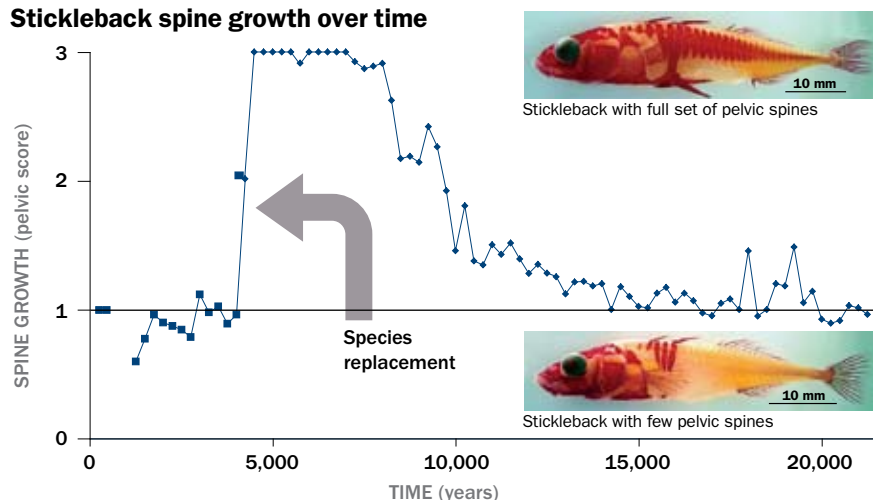
Crick enunciates the legendary “Central Dogma,” that the transfer of information flows from nucleic acid to protein but not vice versa.

1958

Matthew Meselson and Franklin Stahl show that when DNA replicates, the new helix comprises one old strand of DNA and one new strand.

CT SLICES: BOISVERT ET AL.; RECONSTRUCTION: C. BOISVERT, P. AHLBERG; TIMELINE: BETMANN/CORBIS 1959; RIGHT: GECKO763/ISTOCKPHOTO

Stickleback spine growth over time



About 10 million years ago, stickleback fish living in a lake void of its usual predators in what is now west-central Nevada sported few if any pelvic spines (modern example at bottom; researchers assign these spineless fish a “pelvic score” of 1 or less). Then, the fossil record suggests that something, possibly a significant change in environmental conditions, led to a sudden shift. Less than 500 years later, sticklebacks with a full set of pelvic spines (top) dominated deep waters in the lake for about 3,000 years. Then, a gradually increasing proportion of fish in the lake lost their pelvic spines. After 8,500 years or so, most of the sticklebacks had lost their spined defenses, returning the species’ average pelvic score to around 1. One change agent may have been *PITX1*, a recessive gene that influences the size of pelvic armor in modern sticklebacks.

repattern a structure that was already there.... It didn’t have to build a novel extension of the body from scratch, so the transition from fish to land vertebrate becomes a little less dramatic than we thought it was.”

Hidden genes, big changes Modern genetic tests are also shedding light on evolutionary changes chronicled in the fossil record of stickleback fish that lived about 10 million years ago in a lake that was in an area now in west-central Nevada. During a 21,500-year interval, one stickleback species — equipped with a full set of pelvic spines — suddenly replaced a species that lacked such protection, only to gradually lose its spines a few millennia later. Because these changes can be tracked from one generation of fish to another throughout a relatively short period, all of the fossils can be considered, in essence, transitional.

Local topography and geology suggest that the lake in question was several kilometers across and that rivers flowing through the region occasionally provided a connection to the Pacific Ocean, says Michael Bell, an evolutionary biologist at Stony Brook University in New York. For the most part, the lake was free of predators: At the site that Bell and colleagues studied, paleontologists have unearthed the fossils of about 20,000 minnow-sized sticklebacks but have found remains of only two trout and one freshwater catfish. The condition of the trout and catfish fossils hints that those fish had lived elsewhere and had been washed to the site as carcasses. Bell and biologist Matthew Travis of Rowan Uni-

versity in Glassboro, N.J., reported their findings in October in Cleveland at the annual meeting of the Society of Vertebrate Paleontology.

Bell and his team focused on the stickleback fossils entombed in one band of rock six meters thick. That stratum clearly shows varves, sublayers of sediment each a little less than one-third of a millimeter thick and each, somewhat like a tree ring, preserving the amount of sediment deposited during one year, Bell says. Those varves enabled the scientists to track changes in stickleback anatomy over the 21,500 years. The researchers grouped fossils into 250-year-long periods and categorized the sticklebacks according to the presence or absence of the components that make up the pelvic spine assembly.

At the beginning of the interval, most of the sticklebacks living in this part of the lake had no pelvic spines, but they did have the bony plate on which the spines are attached. Only a few stickleback fossils had a full complement of pelvic spines.

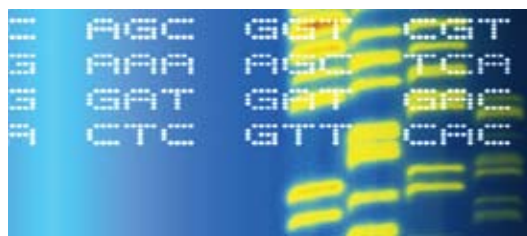
Then, about 4,000 years later, relatively sudden change came to the lake — possibly because of some as-yet-undefined environmental catastrophe — and the sticklebacks that lacked pelvic spines were supplanted by those that did have pelvic protection. For about 3,000 years, these spine-sporting fish dominated the ecosystem, but then individuals that lacked pelvic spines began to account for an ever-increasing portion of the stickleback population. Eventually, after another 8,500 years or so, most of the sticklebacks in this part of the lake again lacked pelvic spines.

1964

Louis Leakey identifies fossils of and names *Homo habilis* (skull at right).

**By 1966**

Francis Crick, Sydney Brenner and Alan Garen succeed in working out the genetic code.



A stickleback's pelvic spines, like other body parts, require an investment of energy to grow and maintain. If not in danger from predators, an individual benefits if its genetic makeup allows it to forgo those spines, says Bell. That could explain the eventual loss of pelvic spines, he notes, but it doesn't explain why it took 3,000 years for that phaseout to begin.

Modern genetic studies provide a clue, however. Scientists have identified at least six genes that influence the presence and length of a stickleback's pelvic spines. Most of those genes have little effect, but one — a recessive gene known as *PITX1* — has a significant influence. Not only that, in modern-day sticklebacks, as the expression of the *PITX1* gene declines, the spines on the creature's left side shorten more slowly than those on the right side.

That same pattern of asymmetry shows up in this lake's fossil record, says Bell. During the 8,500-year period when the sticklebacks were losing their pelvic spines, about 75 percent of the fish fossils with pelvic girdles — precisely the percentage expected in a population with such a recessive gene — had larger remnants on the left sides of their bodies.

Even before the decline in growth of pelvic spines for the sticklebacks kicked in, however, subtle evolutionary changes were taking place, Bell says. Measurements of the spines indicate that during the 3,000 years when all the sticklebacks retained all of their pelvic spines despite danger from predators, the spines were becoming shorter as generations passed. That trend suggests that anatomical changes were happening via one of the other genes known to have an effect on pelvic spines — or possibly via a different gene yet to be discovered in modern relatives.

Genetic studies help explain the changes seen in the fossil record but also offer a cautionary tale for interpreting that record, says Bell. The delayed decline in growth of pelvic spines for the Nevada sticklebacks can be explained by an initially low frequency of the recessive *PITX1* gene in that population, Bell and Travis propose. For example, if 1 percent of fish in a group have two copies of such a gene, then the chances of two of them mating and having offspring that also have two copies of the recessive gene are only one in 10,000.

The case of the Nevada sticklebacks illustrates the difficulty in selecting for an extremely rare recessive gene, the researchers propose. “For thousands of years, genetic diversity of a population can be hidden,” Bell notes. For creatures that take many more years to mature and breed than sticklebacks do, the physical manifestation of recessive genes could go undetected in the fossil record for even longer.

Plugging holes Critics of evolution delight in a simple irony: When paleontologists discover a creature that fills one gap in the fossil record, they create yet another — one that precedes the newly found intermediate species, and one that follows it. Much to evolutionists' delight, however, paleontologists have remained busy “creating gaps in the fossil record” in recent years. Before the 1970s, scientists discovered an average of 12 new dinosaur genera per decade; since 1990, the rate of discovery has been 10 times higher. (*SN*: 11/20/04, p. 334).

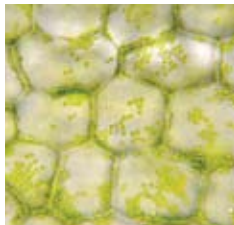
But post-Darwin discoveries haven't been limited to large, lumbering land creatures: Scientists have assembled several well-documented evolutionary lineages of foraminifera, single-celled organisms whose distinctive and intricate shells help pin down the era when sediments containing them were deposited. “This is on-the-ground evidence that Darwin wouldn't have had,” says Fortey of the Natural History Museum in London.

And many stretches of the fossil record poorly represented in Darwin's day — such as the Precambrian, an era before the Cambrian period (which began about 542 million years ago and is when much of life's diversity apparently evolved) — are now more thoroughly populated. Fortey notes: “For Darwin, the Precambrian was a complete mystery, whereas now we have a tremendously detailed narrative” for that era, much of it gathered in the past few decades.

As such discoveries pour in, evolutionary trends almost invariably become clearer. “As you find more and more fossils, you close the gaps with more new species,” Fortey adds. In essence, the ever-increasing number of paleontological discoveries is converting a crude connect-the-dots sketch of evolution into a richly detailed pointillist painting. ■

Before the 1970s, scientists discovered an average of 12 new dinosaur genera per decade; since 1990, the rate of discovery has been 10 times higher.

Lynn Margulis proposes that some internal structures in eukaryotic cells originated as independent organisms.



Chloroplasts inside plant cells

1971/1972
Niles Eldredge proposes “punctuated equilibrium,” later developed further with Stephen Jay Gould.

1977
Carl Woese and George Fox publish a paper in *PNAS* identifying the third domain of life, the archaeobacteria (now commonly called Archaea, shown at right).



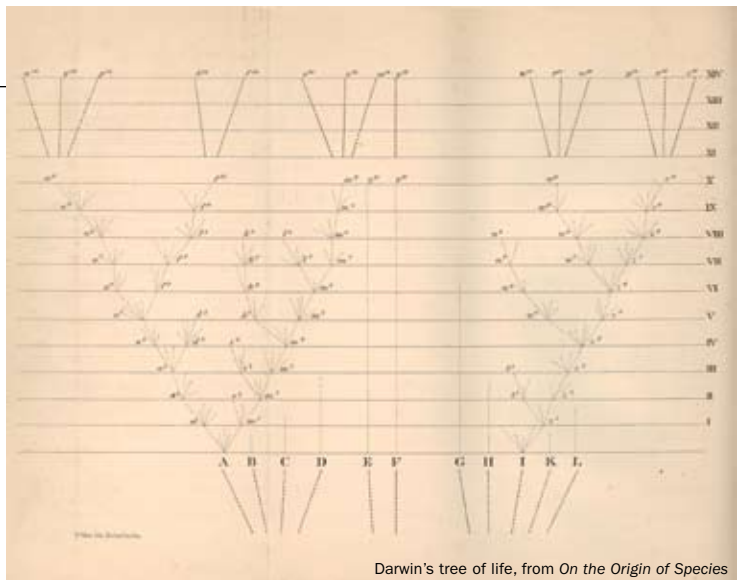
Computing Evolution

Scientists sift through genetic data sets to better map twisting branches in the tree of life **By Patrick Barry**

Among its many prose-filled pages, Charles Darwin's *On the Origin of Species* includes only one illustration. It's a diagram of short lines leading upward from the base — a few lines at the bottom branch out repeatedly as they extend up. Darwin meant for the image to depict what he dubbed the “tree of life.” This figure embodied Darwin's vision for how the tremendous diversity of life on Earth arose. A few species — the base of the tree — mutate and evolve over time, sometimes branching to form new species. An ancient species of bird might colonize a chain of islands and slowly evolve narrower beaks or other features specialized for the birds' new habitats. Eventually, groups in different habitats become separate species, and each species continues to evolve and adapt, perhaps branching again. In this way, the first fishlike land animals gave rise to the great diversity of amphibians, lizards, insects, rodents, marsupials, primates and birds.

It was a sweeping vision of life, revealing it to be a giant family with a vast genealogy. Branches of the tree show the kinship among creatures and the history of change and adaptation. Darwin toiled for much of his life to understand the relationships among species, the branches of this immense tree, by gathering countless specimens and scrutinizing their similarities and differences — a longer neck, a brighter-colored shell. Expanding this tree has been the painstaking work of generations of naturalists, biologists, taxonomists and paleontologists during the 150 years since Darwin published his seminal book.

Now that slow slog has quickened to an all-out sprint. Rather than divining clues to an organism's evolutionary history from observed traits, scientists are going straight to the genetic



Darwin's tree of life, from *On the Origin of Species*

ledger sheet. Modern tools for rapidly reading species' DNA are laying bare those species' genetic inheritances, the patterns of genetic code shaped by eons of mutation and natural selection. And ever more powerful computers are churning through gigabytes and gigabytes of this genetic data to decipher which species are like sisters and which are only distant cousins.

“We've really learned more about relationships [among species] in the last 10 years than we did in the previous 200 years,” says Doug Soltis, an evolutionary biologist at the University of Florida in Gainesville. “This is definitely going to be viewed as a golden era in our study of biodiversity. And it's just now taking off.”

Already, large branches of the tree are being redrawn as scientists compare the DNA of dozens or hundreds of distantly related species. Within years, rather than decades, this computational excavation of life's past will achieve an important milestone in the history of science: a highly accurate map of the major branches in Darwin's tree of life.

“It's Darwin come full circle,” Soltis says. “Starting from his tree figure [in the *Origin*], we're now putting together a basic tree of life for a large portion of known species. It's just incredibly exciting.”

Such genetic comparisons have already overturned long-held ideas about the evolution of birds and have shed new light on the origins of animals. Scientists are also getting close to mapping the rapid diversification of the first flowering plants — which happened so quickly and recently, on a geologic timescale, that Darwin called it an “abominable mystery.” And studies are refining ideas about the roots of all life, the initial emergence of the three superkingdoms: bacteria, archaea and

1983

Homeobox genes are discovered. The homeobox proteins turn on other genes in precise patterns at certain times during development to determine an animal's body plan.

1996

Dolly the Sheep is the first mammal ever cloned from an adult cell.



1990

The U.S. federally funded Human Genome Project begins.

1998

Celera Genomics, a private company headed by J. Craig Venter, announces it will also sequence the human genome.

eukaryotes, the group that includes all plants and animals.

For the past five years, the National Science Foundation has allocated \$12 million each year for these genome comparisons in a program called Assembling the Tree of Life, or AToL. Its goal is producing a tree that maps the evolutionary relationships among all the roughly 1.7 million known species. The Human Genome Project pales in comparison.

As with mapping the human genome, which led to the enormous task of understanding *how* the genome works in health and disease, completing a basic tree will mark the fulfillment of one challenge and the beginning of larger ones. Filling in all the twigs and leaves — every genus and species — will probably take decades. And in the near term, having the major branches of the tree and many of its leaves in hand will point biologists toward another set of questions to answer: Once scientists know what evolution did, they can ask better questions about how it did it.

“We can sit down and say, how did these species evolve? Why did they evolve this way instead of that way?” says Rebecca Kimball, an evolutionary biologist at the University of Florida. “When we’re not certain if a chicken is related to a duck, that limits us from looking at this bigger picture. As we begin to get definitive trees of life for many groups, then maybe we can understand better how evolution works.”

Ducks in a row The concept behind these data-intensive comparisons is simple: The genomes of two closely related species should be very similar to each other, while genomes of species that have evolved separately for a longer time will have accumulated more differences.

It seems easy enough. First put chromosomes from each species through a DNA sequencer to read the genetic catalog: the long sequences of A’s, T’s, C’s and G’s that represent how information-carrying chemicals in DNA are strung together. Then line up the matching parts of those data strings and note all the spots along the strings where the letters differ. Organisms that share large segments of genetic coding or a given mutation in their DNA are more closely related than organisms that don’t.

But actual comparisons are a lot more complex. Just lining up the matching fragments of many genomes can be a tremendous challenge. Random mutations to DNA that drive evolutionary change sometimes come in the form of wholesale photocopying of large sections of DNA, or by the loss of a segment containing an entire gene. Species often have different numbers or types of chromosomes. And available genome sequences for infrequently studied

Avian Branches: Building a tree of life

Instead of relying on observable traits to guess evolutionary relationships among species, scientists can now go straight to the source: The DNA that is marked by evolution. Here’s one simple way to reconstruct the history of bird species using genetic sequences. (Although the evolutionary relationships shown here are real, the specific genetic codes and mutations shown are representative and are not based on actual DNA data.)

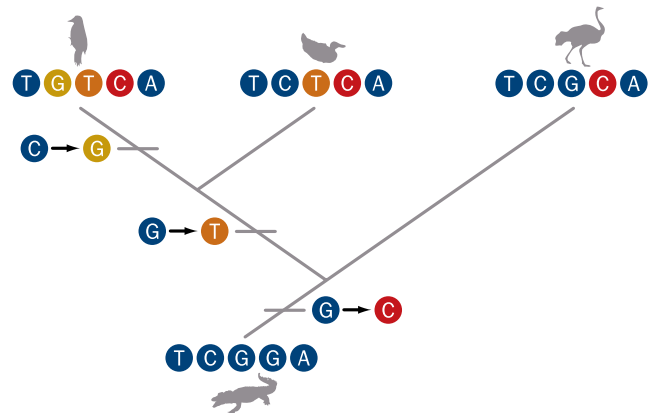
To construct a tree of related species, scientists need to compare these species to one that is not in the group of interest, but is closely related (the “outgroup”). Here, the outgroup is an alligator species that roots the tree by serving as a reference point for all of the species.



1. Researchers pick a region of DNA that has counterparts in all of the species including the outgroup. Usually, many different regions of DNA are compared to make the most accurate tree (just one such segment of DNA is shown here).



2. The strings of DNA from different bird species and the outgroup are lined up and compared. Differences in the letters of DNA code, shown here in color, are identified. Then the species are ordered from fewest to most differences.



3. Scientists assume that the tree with the fewest number of evolutionary changes best represents the species relationships.

Constructing evolutionary trees is complicated. Missing DNA sequences, repeated stretches of DNA and a daunting amount of information all confound the task of generating accurate species histories.

1999
Human Genome Project completes first sequence of a human chromosome.

2001
Working drafts of the human genome sequence are published in *Nature* (mainly reported by the Human Genome Project) and *Science* (mainly reported by Celera Genomics).



2004
Human Genome Project reports the near-complete sequence of the human genome. Later, private companies announce full sequences for individual genomes.

species are usually fragmented and incomplete.

“There aren’t that many genomes available,” says Jonathan Eisen, an evolutionary biologist at the University of California, Davis. Public databases contain partial genomes for more than 140 plants, 250 mammals, 390 invertebrates and 1,600 microbes — a sliver of life’s astonishing diversity.

Even in a well-studied group like mammals, scientists have found only about 2,000 genes having counterparts across the whole group that can be lined up for comparison. And limited budgets for fast computers and DNA sampling mean that in these kinds of comparison studies, dubbed by Eisen as “phylogenomics,” scientists typically compare only a few hundred or a few dozen genes.

Then there’s the question of how to translate the differences among those fragments into maps of the branches in the tree of life. Some genes accumulate changes faster than others, so comparing one gene might tell a different story about the species’ histories than comparing another gene would. And genes can sometimes jump from one organism to a distantly related one, mixing up the genetic clues. This “noise” in the data poses a challenge to scientists trying to draw the correct evolutionary tree for a certain set of organisms from a dizzying number of possibilities.

“When you’re trying to build trees, once you get over a few hundred organisms there are more possible trees than there are atoms in the universe,” Soltis explains. “So it’s a huge problem.”

Even on fast computers, crunching the numbers for this problem can take months of continuous calculation.

“We managed to crash a few computers with the size of our data set,” Kimball says. “We had an analysis running for two months on a computer one time and then a power outage hit. Although we joked about it, it was frustrating at times.”

Kimball and her colleagues were analyzing about 32,000 letters of genetic coding from each of 169 bird species to decipher the early branches in the evolution of birds. The results, reported last June in *Science*, confirmed some long-held ideas about bird evolution, but upended others. Surprisingly, perching birds such as the house sparrow are actually closely related to parrots. Flamingos are indeed closely related to water-loving grebes — a relationship that had been disputed — though neither is part of the main branch of waterbirds.

Beyond an aquatic lifestyle, several other traits that might outwardly suggest kinship also evolved more than once in separate groups, according to the team’s analysis. An order of

daytime birds that includes hummingbirds actually evolved from nocturnal ancestors, which shows that being active during the day must have re-evolved in this lineage. And as Kimball’s team reported last September in *Proceedings of the National Academy of Sciences*, flightlessness among birds such as ostriches, emus and kiwis evolved not just once, as scientists had thought, but at least three times.

Similar studies have begun to unravel the “abominable mystery” of flowering plants’ rapid emergence. Comparisons of plant genomes show that these diverse plants arose between 140 million and 180 million years ago — earlier than suggested by the oldest known flowering plant fossil, which is only 132 million years old, Soltis and his colleagues noted last year in the *Annals of the New York Academy of Sciences*. Also, water lilies appear to be one of the first lineages to diverge. Although more evidence is needed, this research could settle a long-standing debate about whether flowering plants began as forest shrubs or aquatic herbs.

Soltis says unpublished research by his team takes this work further, outlining many of the major branches of flowering plants’ evolutionary history. “We’ve now got most of [these branches], and we’re getting the last papers out now on most of those deep-level relationships,” he says.

Within four or five years, Soltis says, scientists are likely to have a complete, basic tree for the roughly 15,000 genera spanning 300,000 to 400,000 species in this diverse family of plants.

Such in-depth studies can flesh out the tree’s details piecemeal. That’s part of the design of large projects such as AToL: All the work need not be done in a single, giant effort. Individual teams can riddle out parts of the tree and then snap those parts into the master tree like pieces of a jigsaw puzzle.

To reveal the largest branches that form the overall framework of this master tree, scientists use a broader set of DNA samples that includes a wider range of species. Lineages for species as different as slime molds and squirrels diverged hundreds of millions of years ago, so with enough genomic data from diverse species such as these, researchers can map those ancient branches.

For example, illuminating the oldest and largest branches of the animal kingdom required crunching the data for nearly 40 million letters of genetic code from 29 animals representing 21 major groups. The results shook scientists’ ideas about how the first animals evolved. Biologists have long believed that the ancestors of sea sponges, which have very simple bodies, were the first to branch off from the rest

2004

Geologists ratify naming the Ediacaran period, a time just before the Cambrian period that hosts fossils suggesting significant diversity before the Cambrian explosion.



Ediacaran fossil

2006

Benjamin Voigt and colleagues publish data showing that, within human history, a large portion of the human genome has changed in response to “selective pressures.”

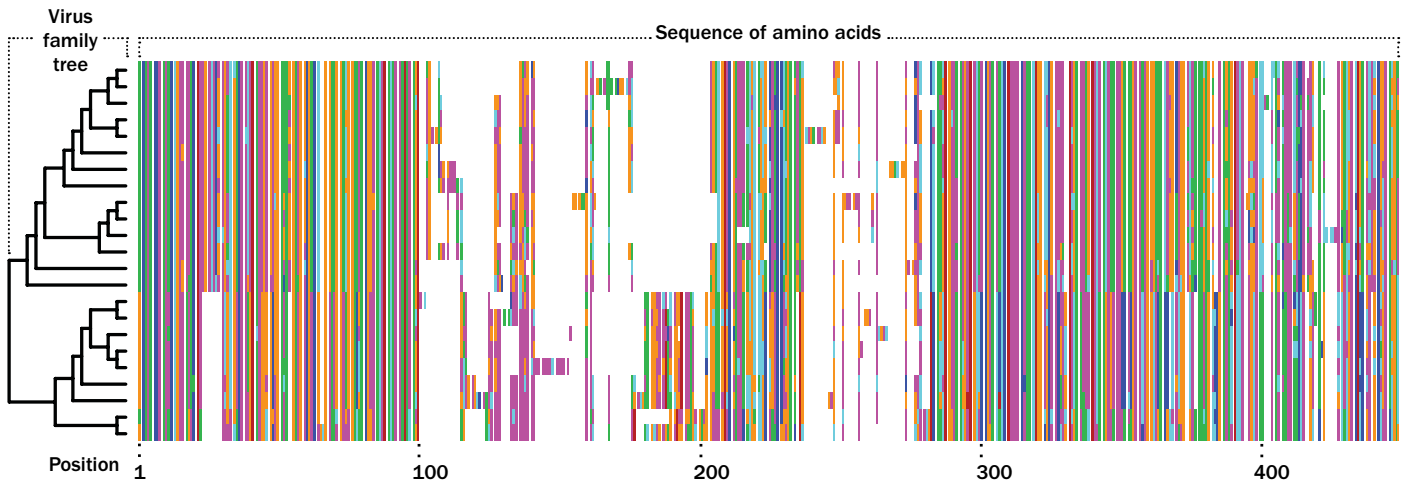


2008

Scientists report the first complete sequence of a Neandertal mitochondrial genome, showing no evidence of Neandertal interbreeding with humans.



Then Darwin and others collected specimens and scrutinized the similarities and differences among the species' bodies and behaviors. From these comparisons, scientists inferred the evolutionary histories of species. The insects shown here are from Darwin's personal collection.



Now Rather than comparing animals' bodies and behaviors, scientists today can directly compare genetic codes. Computer-aided analysis of reams of genetic data reveals which species share segments of code or certain mutations, allowing scientists to infer the evolutionary history of life with high accuracy. The branched diagram (far left) represents a family tree of viruses. A section of each virus's genome, depicted here in terms of the string of amino acids encoded by the genetic sequences, reads from left to right. Matching amino acids are the same color. At position 1, for example, viruses in the top section of the tree share code for a particular amino acid (green). Viruses at the bottom section have code for a different amino acid (yellow) except for one strain (green).

FROM TOP: ENGLISH HERITAGE PHOTO LIBRARY; A. LÖYTYNOJA, ET AL. SCIENCE, P. 1632-1635, 20 JUNE 2008

of the animal tree and start evolving independently. But the new work, reported by evolutionary biologist Casey Dunn of Brown University in Providence, R.I., and his colleagues last year in *Nature*, suggested that comb jellies, which have more complex bodies, branched off first instead (*SN*: 4/5/08, p. 214). If so, this discovery would imply that the last shared ancestor of sponges and comb jellies either had evolved a complex body already — in which case sponges' bodies must have become simpler over time — or that the common ancestor had a simple body, implying that complex body plans evolved separately in the comb jelly lineage and in the branch containing the rest of the animal kingdom.

Finding the last common ancestor of plants, animals, fungi and protozoa — all of which are called eukaryotes and all of which have much larger and more complex cells than bacteria — is more difficult. Eukaryotes, bacteria and single-celled organisms called archaea constitute the three largest, most fundamental branches in the tree of life, diverging billions of years ago. No consensus yet exists on when and how eukaryotes branched off from the other two superkingdoms, but studies are beginning to illuminate even this deep history.

A team led by Takao Shinozawa, a visiting professor at Waseda University's campus in Saitama, Japan, compared the genomes of 46 species: 36 bacteria, eight archaea and two eukaryotes. The analysis, reported in the August *Genes & Genetic Systems*, suggests that the main DNA in the nucleus of all eukaryotes descends from an archaea. But the DNA in mitochondria, energy-producing organelles in eukaryotic cells, has a different origin. Mitochondria were once free-living cells that became incorporated into eukaryotic cells long ago, most biologists believe. Shinozawa's comparison suggests that the free-living forebears of mitochondria belonged to a group of ancient bacteria called alphaproteobacteria.

"The bacterial taxonomy has been totally changed in recent years," says Bernard Labedan, an evolutionary biologist at the University of Paris-Sud 11 in Orsay, France. "Before it was a mess, and now it's clearer and clearer."

However, Labedan adds, "there are still a lot of things to make more precise." Pinning down these earliest branches with confidence will take more gigabytes of genomic data, more computer horsepower and more time.

Braiding the branches Microbes, in particular, will be hard to deal with, in part because they swap genes like 13-year-old boys once traded baseball cards. Though direct

exchange of genes among distantly related species is fairly rare in large, multicellular organisms such as plants and animals, single-celled microbes are masters of the gene trade. Snippets of DNA can float out of one cell, let's call it Alice, and get picked up by a cell of another species that we'll call Bill. Scientists who base their comparisons on this snippet of DNA will get the false impression that Bill is close kin to Alice and her relatives.

Such gene swapping braids the evolutionary branches, so that the collection of genes in a microbe's DNA may descend from many far-flung species. Some scientists argue that, for this reason, the evolutionary history of microbes is better imagined as a heavily crisscrossed web, rather than a branching tree. This braided genetic past doomed earlier studies that attempted to find a tree-shaped history based on a single gene shared by many species.

But recent work that compares larger swaths of DNA can partially overcome this problem. Some regions of a microbe's genome — parts involved in cell division and other essential functions — are resistant to this lateral swapping of genes, and so follow more predictable rules of inheritance.

"The cores of these microbial genomes do have a tree," Eisen says. "There didn't seem to be any hanky-panky going on." However, this stable core represents only 5 to 10 percent of the microbes' genes, forcing scientists to study these microbes' evolutionary histories as if looking through a keyhole.

For ancient microbes, these lingering genetic data are usually the only clues available. Larger creatures occasionally leave behind fossils that scientists use as a reality check, showing when certain adaptations arose and calibrating the

timeline suggested by the DNA. Microbes aren't so helpful.

More genomic data from more species will eventually bring the picture into clearer focus, even if some details of the tree will never be known with 100 percent certainty.

"Could there still be some fuzziness? You bet — that's how science works," Soltis says. "Fuzziness is not always bad. Sometimes the areas of fuzziness are telling you that something else is going on here, something that you might want to look at in more detail."

Despite a few lingering spots of uncertainty, having a highly accurate map of the historical tree of life that is freely available will be a boon for biology, and perhaps for education too. "This is my vision," Soltis says, "where schoolkids are going to be able to navigate the tree of life by clicking on one branch, and they can go down that branch and navigate and explore life's history." ■

"Could there still be some fuzziness? You bet — that's how science works. Fuzziness is not always bad. Sometimes the areas of fuzziness are telling you that something else is going on here, something that you might want to look at in more detail."

Doug Soltis

A Most Private Evolution

Dumb Designs for Sex: Evolutionary biology walks on the weird side

By Susan Milius

Maybe female seed beetles have their own what-the-bleep exclamation. Even for insects, it's difficult to imagine any other reaction to a male *Callosobruchus maculatus* beetle's sex organ, which has spikes.

"It jumps to mind as something quite dumb," says evolutionary biologist Göran Arnqvist of Uppsala University in Sweden, who for much of the past eight years has studied seed beetle sex.

Male beetles of several *Callosobruchus* species have sharp edges on their sperm-delivery organs. The females' ducts grow a bit of extra toughening but not enough to make sex safe from the risk of injury. After many tests, Arnqvist has concluded that the genital excesses aren't good for the species as a whole. These seed beetles would have less-damaging sex — and would produce more babies — if males lost their edges.

Discussions of evolution often glorify the beautifully apt forms: orchids with nectar recesses just the right length for the tonguelike structure of a certain moth, or harmless butterflies with the same wing colors as a poisonous neighbor. Yet the most dramatic examples of the power of evolutionary theory may come from the strange and ugly stuff — biology too dumb to have been designed.

Trying to understand counterintuitive sexual parts and habits follows in the best of scientific traditions. As Charles Darwin worked up his ideas on evolution, he pondered male phenomena that looked useless, or even harmful, for surviving. Outsized horns on male beetles puzzled him, as did male birds with gorgeous plumage.

Out of this consternation came his insight into a process he called sexual selection, which he distinguished from natural selection. There may be survival of the fittest, but there's also survival of the sexiest.

Today the sex-related selection process doesn't get much attention outside scientific circles, but it's a powerful tool for making sense of downright peculiar stuff. Arnqvist and other biologists are expanding Darwin's framework, exploring the counterintuitive aspects of sex from flirtation to family life. And theorists are discussing female behavior that Darwin never recognized, or perhaps just didn't care to discuss in print.

CHAP. XIII.

DECORATION.

77

obscurely coloured and destitute of all ornaments, whilst the males are probably the most highly decorated of all birds, and in so many ways, that they must be seen to be appreciated. The elongated and golden-



FIG. 49. *Spathura underwoodi*, male and female (from Becken).

Ridiculously long tail feathers don't make sense for survival of the fittest, so Darwin developed the idea of sexual selection to explain features such as the impractical plumage of some male birds (hummingbird pair illustrated in *Descent of Man*).

Not-so-natural selection When Darwin first put his full idea of natural selection into print, he knew it wasn't enough.

In 1859, he argued in *On the Origin of Species* that organisms best adapted to their environment survive in greater numbers and leave more offspring than do their less fit neighbors. Thus more suitable traits gradually replace clunkier versions.

Yet antlers on stags and tails on peacocks could hardly be adaptations to the environment. Both antlers and tails may be so familiar that it takes a minute to summon a sense of their absurdity. They're huge. They must drain energy to produce. There's no way they improve agility in locomotion or foraging.

"The sight of a feather in a peacock's tail, whenever I gaze at it, makes me sick!" Darwin wrote in a letter to the botanist Asa Gray, albeit in a whimsical paragraph. Nauseated or not, Darwin was willing to step beyond survival of the fittest.

He devoted a few pages in the *Origin* to introduce sexual

selection as a sort of wild oats younger brother of natural selection. Sexual selection, as Darwin formulated it in the sixth edition of *Origin*, depends “not on the struggle for existence in relation to other organic beings or to external conditions, but on a struggle between the individuals of one sex, generally the males, for possession of the other.”

Antlers evolved as stag-on-stag weaponry for fights over a female, he argued. Males also compete in contests “of a more peaceful character,” he wrote. Extravagant plumage, singing and what he called “strange antics,” such as bird acrobatic displays, bedazzle a female into choosing one male over his rivals.

What’s good for bedazzling can be bad for survival, of course. Darwin made a glancing allusion to the conflict in his 1871 work, *The Descent of Man, and Selection in Relation to Sex*. There he admits that peacock tails wow the peahens but could be slightly harmful to the male.

Today’s tales Harm may be part of the charm, although debate continues on how supersized, shimmery tails evolved. The year 2008 proved a lively one for peacock studies, as a long-term line of research met a challenge from a new one.

Three independent studies in the past 20 years have found that tails matter. For example, Marion Petrie of Newcastle University in England and a colleague turned the same birds from hotties to notties and back again by clipping some of the eyespots out of the males’ tails and then reattaching the finery. The females probably weren’t counting male spots, but were choosing males that displayed a greater density of spots, according to similar tests by Adeline Loyau, now at France’s CNRS Moulis station.

Peahens’ interest in eyespots could have arisen for no particularly practical reason, Petrie and Loyau speculate. Their idea draws on the concepts of sensory bias and sensory exploitation, which deal with an apparent arbitrary silliness at the heart of sexy traits. Sure, a blue spot now burns hot with allure. But biologists puzzle over why a purple stripe didn’t evolve instead.

In this scenario, basic arbitrary-looking evolutionary directions (blue not purple, long tail not wide eyes) actually were arbitrary as far as mate choice goes. For some reason that had nothing to do with reproduction, females might have tended to notice a particular color or shape or motion.

Let’s imagine it was a blue spot. Males exploit that predisposition as guys with even a modest dot attract extra female attention. If the female bias gets inherited along with male coloring, then off go the males in an evolutionary race for bigger, better, bluer blues.

That was the beginning in the peacock’s tale. At some point, the story goes, tails grew so fancy they posed a handicap for males. Growing the best tail or keeping it flossy or managing a little sprint despite its weight demanded energy or vitamin-rich food or something otherwise limited. And in animal communication, that’s when fashion starts to mean something.

What’s called the handicap principle comes from the Israeli biologist Amotz Zahavi, now retired from Tel Aviv University, who thought about how creatures judge each other’s quality.

Suppose the peacock’s tail signals, “Hey, honey. I’m the best bird, and you need me right now.” Such a tail stays reliable as a badge of quality across generations only if good tails present a handicap that not all individuals can overcome, Zahavi suggested. A robust bird can pay the cost and still look good. A puny bird can’t compensate for the loss, and looks like a second-rater. The tail signal honestly indicates quality.

A signal with no cost, Zahavi argued, means anybody could waggle a full rainbow rear. Everybody could signal “best bird.” The signal would lose its utility and fade away over generations, or never evolve to begin with.

Darwin said the peacock’s tail is at least slightly harmful. Maybe it has to be.

Petrie and others have been taking this signaling idea further, testing to see whether the tail might signal good genes or some true benefit for a female who mates with a showy male. It sure isn’t help with the chores and the chicks. Peacocks do only the most basic task of fatherhood.

In a jolt after years of research linking female preferences to tail feathers, readers of the journal *Animal Behaviour* were startled to learn in April that a seven-year study of feral peacocks in a park near Shizuoka, Japan, found no sign that females were

Male and female seed beetles engage in evolutionary arms races that appear to harm the species as a whole. Species with spinier, more dangerous male genitals (most extreme of three species at left) also have tougher walls in the female reproductive tracts (inset, cross section for each species).



choosing males based on their tails. Neither eyespot number, tail symmetry nor tail length correlated with a male's success or his health, reported Mariko Takahashi of the University of Tokyo and her colleagues.

Loyau, Petrie and two other researchers responded in the November issue with ideas about why the new study doesn't agree with old research. For one thing, the researchers point out, the studies took place on opposite sides of the world. Other animal studies have recently detected what's called adaptive plasticity in mate choice, or differences in how various groups of females of the same species choose mates. What's a useful signal in one environment may not matter much in another. Also, Loyau says, "If we really want to understand, we need to study peacocks in the wild."

One commentary isn't going to settle a matter that's been under study since it nauseated Darwin, though. The Japanese experiment's challenge to years of experiment, theory and assumption is "sure to prove controversial," predicts Louise Barrett, one of the journal's editors.

For kicks Plausible explanations for a dazzling but impractical tail don't make sense for injurious genital spikes. Beetle genitalia look more like instruments of war. The latest research suggests warfare may be the point.

In Darwin's writings, males fought males. Now researchers recognize that males and females clash too.

As Arnqvist puts it, "Unless you have perfect monogamy, there are conflicts of interest." When a male and a female can take different strategies in mating, their best interests often differ. What's good for the goose in terms of how often to mate, with whom and for how long probably won't be best for the gander.

Thus human scientists confront the question of how to spot battles of the sexes in other species. In a 2000 paper in *Nature* titled "Genital damage, kicking and early death," two researchers reported evidence that seed beetle mating might have more conflict than concord. Helen Crudgington and Mike Siva-Jothy of the University of Sheffield in England timed beetle mating that takes place on black-eyed peas. After about three minutes, females start slamming their hind legs against the male. A typical mating encounter lasts about four minutes.

When the researchers removed females' legs so they couldn't kick, males persisted around six minutes. The sexes appear to disagree about how much is enough.

Female beetles' kickoffs probably are not a way of reducing contact with wimpy males that can't stand a drubbing, Siva-Jothy says. Female seed beetles look as if they have genuine cause to minimize mating. The longer an encounter lasted, the more rips and tears Siva-Jothy and Crudgington found in

the female reproductive tract. And as additional evidence of harm, females that mated only once during the experiment lived longer than females that mated twice.

Those harmful male sex organs in the beetles "look like medieval torture instruments," Arnqvist says. Yet such a device may not have evolved through any direct benefit of its power to injure. Instead, injuries are probably side effects, Arnqvist contends. He and his colleagues have tested for potential direct benefits for the male, including what's called the "terminal investment."

In a terminal investment, a mauled creature facing an uncertain or shortened life span throws all resources and effort into the current batch of young. A dad with no guarantee he'll sire one of mom's future clutches will certainly benefit if he can get her to make an all-out investment in his offspring right then.

It's not an easy idea to test. Exactly mimicking the damage of mating isn't possible, so Arnqvist and his colleagues inflicted other injuries, including body punctures or cuts on wings, after a group of females had mated. The injured females actually laid fewer eggs than intact moms, so Arnqvist dismissed the idea of a terminal investment bonus for the males. Also the damaged females tended to mate again sooner than usual, so the damage doesn't look like a roundabout way of foiling rivals.

To explain how the sharp edges of a seed beetle arose without direct benefits, Arnqvist proposes that some quirk of male

physiology, such as an irregular surface to improve anchoring, injured females incidentally. The risk of such injuries favored females with tougher plumbing, which in turn favored spikier males. So seed beetle anatomy, he argues, could derive from an ongoing arms race between the sexes. Even if the conflict harms the species.

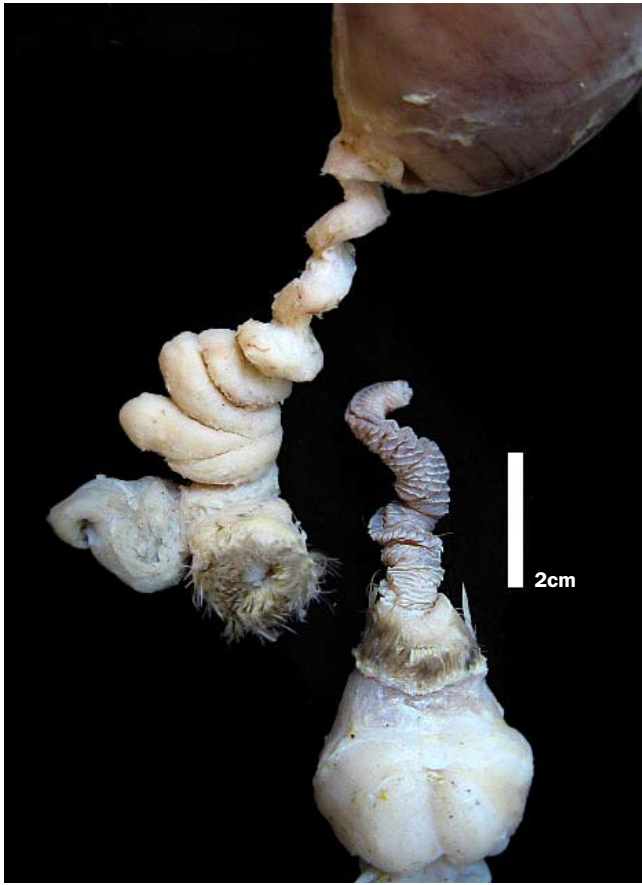
Similar harm, and possibly arms races, could be smoldering far beyond seed beetles. "Being an entomologist, I know of hundreds of insect groups with male genitalia that have this appearance," Arnqvist says.

Some male insects deploy bundles of spines, knives and even full-fledged swords. Male bedbug organs look like a stiletto, and "they literally use it as a stiletto," Arnqvist says. Females' reproductive tracts do have external openings, but male bedbugs usually just stab through some spot in the body wall and let the sperm swim from there.

Birds have evolutionary arms races too, says Patricia Brennan of Yale University.

Most birds don't have insertable parts, achieving fertilization by the so-called cloacal kiss. It's just his-to-hers contact of cloacae, the all-purpose openings of reproductive and excretory systems. Male ducks, however, belong among the 3 percent of male bird species with a phallus, and some duck

Researchers now recognize that males and females clash too.... Thus human scientists confront the question of how to spot battles of the sexes in other species.



Mallard duck anatomy shows signs of an escalating battle of the sexes. The male has a long phallus (bottom), but the female's genitals (top) corkscrews in the opposite direction.

organs extend a full 40 centimeters.

In the mallard and long-tailed duck, males deploy an unusual length “what looks like a weird tentacle with bumps and ridges,” Brennan says.

Female duck anatomy hadn't received as detailed a look until Brennan spent some time in Tim Birkhead's lab at the University of Sheffield. Female mallards and long-tailed ducks have a correspondingly intricate reproductive tract “like a maze,” Brennan says.

In studying 16 species, she found that if the male had a long and elaborate phallus, the female had intricate genitals too. The sexes' intricacies seemed at odds with each other, however. Males spiraled counterclockwise (from the base) but female reproductive tracts antagonistically curved clockwise (from the outer opening). Blind pouches along the female tracts looked like traps for sperm.

A classic arms race is what Brennan and Birkhead proposed in *PLoS ONE* in May 2007 to explain the mismatched genitalia. Males of the extra-long species are more likely to try forcing themselves on females than are less elaborated males. Thus females might have benefited from countermeasures against unsuitable matings. A maze that proves navigable only

when a female cooperates and relaxes could have provided some control, but it would also favor the evolution of even more extreme males.

“These kinds of evolutionary races are costly,” Brennan says. “You would have been better off without this conflict in the first place, but you can't stop investing because you're already in the war.”

There's chemical warfare too, says William Rice of the University of California, Santa Barbara. Male fruit flies dope their seminal fluids with a cocktail of additives that revs up the female so she devotes extra resources to the eggs. Never mind that it shortens her life and therefore shrinks the total number of offspring she can produce.

Possible high quality offspring won't make up for the loss in quantity, Rice and his colleagues report in the November *Journal of Evolutionary Biology*. They tested the idea that mating with a male carrying superb genes might, over the course of generations, give a female enough extra grandkids and great-grandkids to compensate for her initially small brood.

Yet breeding experiments showed that good genes don't help enough, the researchers conclude. At most, females mating with a superior male might get a modest increase in the number of their offspring's descendants. The uptick isn't big enough to compensate for the downside of drugged sperm. This evidence and earlier work show fruit flies paying a toll for their battle of the sexes. “It's clearly bad for the species,” Rice says.

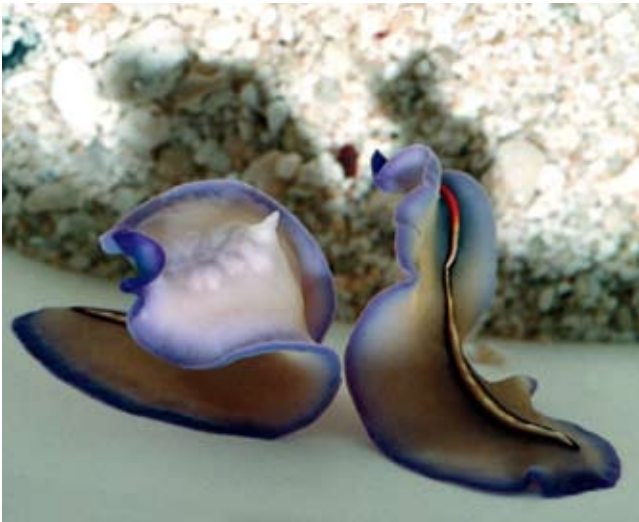
Even hermaphrodites can have battles of the sexes. Conflicts arise when everybody tries to play the guy instead of the girl, according to ongoing work by Nico Michiels and Nils Anthes of the University of Tübingen in Germany.

Just why it would be better to be “male” has inspired much theorizing about sperm being energetically cheaper than eggs to produce. Anthes, though, does the accounting in different terms. He sees conflicts looming if one sex, usually the male, benefits from virtually unlimited matings while the other sex rapidly reaches some limit. Females, for example, might be able to produce only so many eggs in a lifetime, so matings to fertilize even more eggs wouldn't be useful.

Whatever drives the conflicts, researchers see what looks like a lot of antagonism out there. In the small marine flatworm *Pseudoceros bifurcus*, two flatworms stand up on the hind parts of their bodies, stick out both their penises (each worm has two) and jab them at each other. Worms bend and dodge as any duelists would, trying for a hypodermic strike that injects sperm anywhere on the opponent's body. Bouts sometimes last 20 minutes.

In the flatworm *Pseudobiceros bedfordi*, ejaculate dissolves its way through skin and can leave scars. A full frontal splash can dissolve the recipient into two pieces, although the flatworms do regenerate lost body parts.

The latest battle that Michiels and Anthes have documented “turned out to be quite spectacular,” Anthes says. Hermaphroditic *Siphopteron quadrispinosum* sea slugs stab at each other with a sharp spike on the side of the penis. When one



Combining both sexes in one body doesn't eliminate conflict. Two hermaphroditic marine flatworms (*Pseudoceros bifurcus*) struggle for the male role in a duel (top) to be the first to inject sperm into the opponent's body (middle). Sperm from *Pseudobiceros bedfordi* can dissolve through skin, but too big a splash corrodes the recipient into fragments (bottom).

slug gets spiked in the head region, it slows down and stops dueling. "They look pretty sleepy," Anthes says. The spiker is apparently injecting some kind of sedative that allows unilateral insemination, Anthes and Michiels reported in 2007 in *Biology Letters*.

Counterintuitive reproductive strategies continue even into parenthood. Consider the penduline tits (*Remiz pendulinus*). In any given nest, the mother and/or the father often desert and start a second family, says Tamás Székely of the University of Bath in England. A single parent can still raise chicks to adulthood, given the right location, but sometimes both parents desert. In this case, the chicks starve. In populations across Europe, about a third of egg clutches die from abandonment, Székely and his colleagues have found.

To make sense of this, Székely describes a competitive desertion arms race between male and female tits. Each sex can increase its number of offspring by starting another nest with a new partner, as long as the old partner stays around to care for the previous clutch.

As the optimal time for desertion nears, when all eggs have been laid, female tits behave as if they're trying to keep their current mate from seeing the true number of eggs. Females confront a male at the nest opening and fuss at him furiously.

Whether this loss of a third of clutches ends up as a bad thing for the species overall will take more research, says István Szentirmai at Órség National Park in Hungary. But he speculates that the strategy limits the species to insect-rich places like wetlands, where a single parent can catch all the necessary baby food.

Mothers certainly didn't run off with other males in *On the Origin of Species*. Darwin acknowledged that males of various species take more than one mate but said hardly anything about such shocking behavior (to mores of the era) in females. So one of the biggest developments in the theory of sexual selection has been the recognition that females in many species aren't monogamous, says Jeanne Zeh of the University of Nevada, Reno.

"It's molecular genetics," says David Zeh, also at Reno. Once DNA analysis could identify the true fathers of offspring, biologists could see widespread challenges to old ideas of females as the choosy, monogamous sex. That idea opens the way for much entertaining science.

And another major shift, as illustrated in the arms races, has been the recognition that sexual competition continues into the depths and details of the reproductive tracts. "[Darwin] spoke only about mating," Arnqvist says. Now scientists have created a whole discipline called sperm competition that takes the struggle for access even further.

Reproduction in the modern view isn't particularly pretty. With medieval torture instruments, mazes and corkscrews, drugged sperm and arms races everywhere, reproduction looks more like war than love. All in all, it's easy to wonder if sex itself was such a great idea.

But that's another story. ■