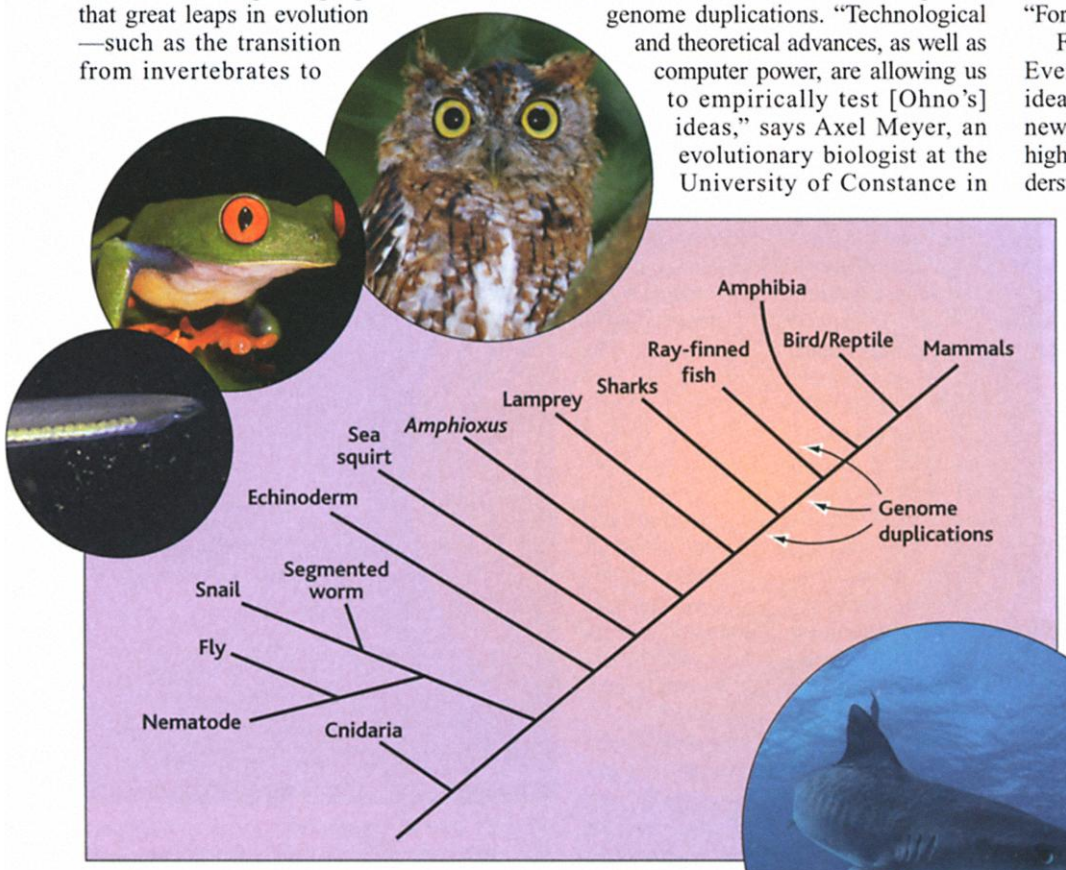
The controversial—and formerly unprovable—proposition that evolution moves forward through duplication of entire genomes is getting support from current advances in molecular biology

Genome Duplications: The Stuff of Evolution?

One of biology's greatest mysteries is how an organism as simple as a one-celled bacterium could give rise to something as complicated as a human. Thirty years ago, prominent geneticist Susumu Ohno of the City of Hope Hospital in Los Angeles put forth what many of his colleagues then considered an outrageous proposal: that great leaps in evolution—such as the transition from invertebrates to vertebrates—could occur only if whole genomes were duplicated.



Diversity explained. Genome doublings could have prompted the development of ever more complex organisms.

The resulting redundancy—each chromosome would have a twin—would enable the thousands of extra gene copies to evolve new functions, Ohno suggested. Thus the copies could become fodder for large innovations in body plans and other modifications, even as the functions of the original genes were maintained. At first, geneticists were either enthusiastic or appalled, but by the late 1980s, they had lost interest in the idea because they simply lacked enough data to support or refute Ohno's theory.

Now, however, an explosion of DNA sequence information, as well as software for finding sequence similarities, has made it easier for researchers to detect signs of genome duplications. "Technological and theoretical advances, as well as computer power, are allowing us to empirically test [Ohno's] ideas," says Axel Meyer, an evolutionary biologist at the University of Constance in Germany. He and increasing numbers of others are finding duplicated genes and chromosome segments that suggest Ohno might have been right after all.

The emerging data have not persuaded all of the skeptics, however. They maintain that evolutionary change could have been fueled by duplication of individual genes or perhaps segments of chromosomes—without invoking anything as dramatic as genome duplications. "You can't prove that [genome duplication] didn't happen, but [if it did], it didn't have a major impact," says Austin Hughes, a molecular evolutionist at the University of South Carolina, Columbia, who has done his own genome analyses. "For me, it's a dead issue."

For others, the issue is very much alive. Even biologists unfamiliar with Ohno's ideas are now combing genome data for new clues about how humans and other higher species came to be. "We want to understand where new genes came from," explains Kenneth Wolfe, a molecular evolutionary biologist at the University of Dublin, Ireland. Understanding gene evolution could have practical benefits as well, he adds. More insights about redundant genes could help geneticists sort out gene functions and perhaps even pin down disease genes.

Controversial proposal

The idea that duplication of existing genes provided the raw material for evolution actually predates Ohno's proposal by nearly 35 years. In the 1930s geneticists realized that evolving organisms had to have a way of maintaining old functions even as they gained new ones. Gene duplication fit the bill: One copy could stay the same while the other took on new functions as base changes accumulated.

But in Ohno's 1970 book *Evolution by Gene Duplication*, he went a big step further, proposing that not just genes but whole genomes duplicated. He thought that two rounds of duplication had occurred: one making possible the transition from invertebrates to vertebrates and the other leading to the diversity seen in vertebrates. Eventually this idea came to be known as the 2R hypothesis.

The idea was not well received. "Found

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throughout the book are inconsistencies, misrepresentations and errors of fact," the University of Chicago's Janice Spofford wrote in a review for *Science* in 1972 (*Science*, 11 February 1972, p. 617). She said, for instance, that Ohno misstated the amount of active DNA in bacteria and mice and failed to consider that the horseshoe crab, a primitive nonvertebrate, has far too much variation in protein makeup to fit his scenario.

But Ohno insisted that organisms could handle extra genomes far more readily than they could extra copies of a particular gene. A second copy of a single gene would likely translate into production of too much of that gene's protein, which could be deleterious to the organism. In contrast, duplication of the entire genome would mean that production of all proteins would increase proportionally.

2R revival

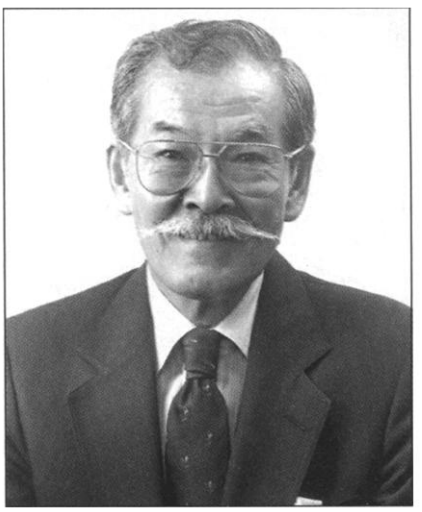
Only with the rise of comparative genomics have researchers been able to address Ohno's hypothesis seriously. Some have matched genomes against themselves to find stretches of DNA that were once identical and therefore represent gene, chromosome, or whole-genome duplications. Others have sought out the same genes in many species, expecting that an increase in the number of any one gene across species might signal ancient genome duplications. We now have "completely different data" than were available in the 1970s, says Meyer.

In 1993, for instance, Jürg Spring, a geneticist at the University of Basel in Switzerland, found that vertebrates have four copies of a gene for a cell surface protein called syndecan, whereas the fruit fly *Drosophila melanogaster* has but one. "I got stimulated to ask whether the fourfold duplication was general," Spring recalls. He found more than 50 instances in which the fruit fly has a single copy of a gene, whereas the human has multiple copies.

Researchers have also found signs of genome duplication at a key evolutionary crossroads: where vertebrates diverged from invertebrates. Some of this work comes from Peter Holland and Rebecca Furlong of the University of Reading, U.K., who are studying *Amphioxus*, the fishlike invertebrate thought to be most closely related to the vertebrates' ancestor. Recently, Holland and his colleagues compared some 84 gene families—groups of closely related genes—in *Amphioxus* to the equivalent families in other organisms.

In many, they found that where *Amphioxus* has one copy, vertebrates have more. These cases include genes for enzymes and for proteins that control gene activity or are involved in cell signaling. Although some genes have just two or three copies instead of four, Holland thinks they each had four at one time. "There are lots and lots of duplications, and [that] is very consistent with two rounds of vertebrate [genome] duplication in quick succession," he concludes.

Longtime skeptic Wolfe also has new evidence that supports genome duplication in the prevertebrate lineage. Wolfe and his Dublin colleagues searched the draft sequence of the human genome for clusters of genes that exist on more than one chromosome. They located 80 pairs of clusters of varying sizes. That's "far more than you would expect by chance," Wolfe says. What's more, the largest cluster pair, located on chromosomes 2 and 12, matched up across 34 million bases. Genome or chromosome—and not gene—duplications are the most likely explanation, he concludes.



Wild idea. Susumu Ohno (1928–2000) said genomes were duplicated in evolution.

CREDIT: CITY OF HOPE HOSPITAL, LOS ANGELES

Next, Wolfe's team tried to estimate when these copies first appeared. These data showed that "there had been a burst of duplication events around 330 million to 500 million years ago," he says. That's not exactly the time that Ohno predicted but is still in the ballpark, Wolfe adds.

Ohno would be pleased, because "these data are consistent with the 2R hypothesis," comments John Postlethwait, a genome researcher at the University of Oregon in Eugene. Indeed, says Wolfe, "I've moved from being skeptical to being more open-minded" about Ohno. Others, however, have yet to be convinced, although new evidence of yet another genome duplication, this time in the fish lineage, is helping Ohno's cause.

Going another round

Late in his career, Ohno added a third—and much later—round of genome duplication to his original two. He decided that there might also have been a genome duplication in fish, occurring just after the divergence of the lobed-fin fishes that led to land-based organisms. In 1998, Postlethwait's team found evidence for that third round of duplication (*Science*, 27 November 1998, p. 1711), and he and others postulated that these extra genes might have fueled the evolution of fish diversity.

Postlethwait and his colleagues were looking at the *HOX* genes, which play a key role in regulating the development of higher organisms. They found that the zebrafish has seven *HOX* clusters, not the three or four known to exist, say, in mammals. More recently, other researchers discovered similar numbers of *HOX* gene sets in the puffer fish, in a Japanese species called medaka, and in cichlid fishes. Because these fish are distant piscine cousins of zebrafish, the findings imply that this duplication occurred in their common ancestor.

Despite this accumulating evidence, Manfred Schartl, a geneticist at the University of Würzburg in Germany, and others who want to accept this scenario are still cautious. He points out, for example, that it would be very difficult for the first tetraploid fish—those with four rather than the usual two copies of each chromosome—to engage in sexual reproduction. He also says that other work, in which researchers have traced the histories of individual genes, points to duplication of single genes in fish, not the doubling of whole genomes. "The problem [with gene duplication] really is that you can find examples that are in agreement with one or the other hypothesis," Schartl notes.

Marc Robinson-Rechavi and his colleagues at the École Normale Supérieure de Lyon in France, for example, determined the lineages of related genes from three fish species to see whether the genes duplicated before or after the fish groups diversified. These duplications "did not occur all together at the origin of fishes, as would be expected if they are due to an ancestral genome duplication," Robinson-Rechavi says. Although Meyer and his colleagues take issue with Robinson-Rechavi's analysis, others are not so sure how to interpret these findings.

Duplication rebutted

Indeed, the same problem plagues efforts to pin down those earlier genome duplications proposed by Ohno. In one study, South Carolina's Hughes pieced together the histories of 134 gene families in the *Drosophila*, *Caenorhabditis elegans*, and human genomes by looking at how the sequences of these related genes changed through time. If Ohno was right, Hughes says, the family history of any gene should show that an ancestral gene gave rise to two descen-

dents simultaneously and that later in time, each of those two yielded two more—again at the same time. But almost three-quarters of the gene families he examined, including the *HOX* family, had different histories.

Hughes also took a close look at the order of genes on supposedly duplicated chromosomes, an analysis that he says also failed to support Ohno's hypothesis. If a whole chromosome was copied, then most or all of the genes should be more or less in the same order in both. But often they are not. "Everything we've looked at [fails to] support the hypothesis," Hughes concludes. He proposes instead that the genes occurring on multiple chromosomes moved to these different locations as a group and then stayed together because it was advantageous to the genome.

But Holland isn't giving up that easily, and his scenario could be a way of reconciling Hughes's findings with Ohno's proposal about the two duplications early in vertebrate evolution. He thinks the inconsistencies highlighted by Hughes might be resolved by assuming that the time between the two rounds of duplication was much shorter than Ohno imagined.

By Ohno's thinking, the first round produced two copies of each chromosome, or four total, because the chromosomes exist as pairs. At first those copies randomly paired off, but eventually they became different enough to have preferred partners, and each set of four became two sets of two, restoring diploidy, the typical chromosomal arrangement. Only after that had happened, which Ohno proposed would take many millions of years, would the second round of duplication have taken place.

At a meeting* in April in Aussois, France, Holland suggested instead that the second duplication occurred before the four chromosomes produced by the first duplication diverged, thus producing eight roughly equivalent chromosomes. If that had been the case, then the recombination and switching of parts of chromosomes that typically takes place between chromosome pairs would have involved all eight, with different genes moving around at different times. Thus, gene order would vary from chromosome to chromosome, and neighboring genes could appear to have duplicated at different times instead of all at once. This scenario would confound analyses such as that done by Hughes.

Other molecular events may change the genome in ways that obscure its true evolutionary history. Hiccups in DNA replication can spit out extra copies of genes or additional pieces of chromosomes. Mobile genetic elements can move genes and gene pieces around. And frequently, one copy of a gene loses its function and becomes unrecognizable as a gene. Sorting through all this to get a clear picture of how each organism's genome reached its present state will be hard, perhaps even impossible, says Meyer. Improvements in dating genes and identifying what instigates changes in a genome can help, however.

But if the work resolves how the evolution of genomes prompts the evolution of new organisms, it will make possible a much better understanding of our own recently sequenced genome. If researchers can figure out the histories of families of genes, they will be in a much better position to sort out which genes are equivalent between, say, human and mouse or human and zebrafish. Knowing that will help tremendously as researchers try to pin down the functions of human genes in mice or other organisms that are more amenable to genetic manipulations than humans. No matter what, says Hughes, "we have to really understand how the genome is arranged." And that is one thing that he and Ohno would agree on.

–ELIZABETH PENNISI

* The Jacques Monod Conference on Gene and Genome Duplications and the Evolution of Novel Gene Functions, Aussois, France, 26 to 30 April.

PATENT INFRINGEMENT

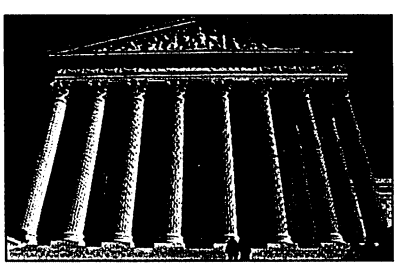
High Court Asked to Rule on What Makes an Idea New

Ten years after a U.S. company sued a Japanese firm for patent infringement, the Supreme Court will hear "the biggest patent case in decades"

When is imitation innovation—and when is it piracy? The U.S. Supreme Court will hear conflicting answers to those questions early next month in a patent infringement case that is being watched closely by academic and industrial groups.

The case, referred to as *Festo*,* centers on a 150-year-old legal concept known as the "doctrine of equivalents." The doctrine is designed to prevent businesses from making minor changes to a patented technology and then claiming it as a new invention. Companies that have patented proteins, for instance, have invoked the doctrine to prevent competitors from marketing molecules that have slightly different amino acid sequences but perform the same biological function.

Last year, however, a federal appeals court stunned many experts by ruling that the doctrine doesn't apply to any patent claim that was narrowed during the review process before the patent was issued. Because that happens to most patent claims, the ruling could have a broad impact—especially, some experts claim, in the biotechnology industry. The business, biotechnology, and patent law communities have filed dozens of friend-of-the-court briefs since the high court agreed in June to hear the case (see table); oral arguments are scheduled for 8 January.



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THE FIGHT OVER *FESTO**

Let the decision stand
Genentech; Applera (Celera); MedImmune; IBM; Kodak; Ford; DuPont; Intel; Cypress Semiconductor; United Technologies

Reverse it
20 major research universities and higher education groups; Celltech; Chiron; Bose; U.S. Chamber of Commerce; American Intellectual Property Law Association; Association of Patent Law Firms; Minnesota Mining and Manufacturing

Other positions
U.S. Solicitor General; Institute of Electrical and Electronics Engineers

* Selected parties

Supporters say that last year's ruling clarifies the law and should prevent nuisance lawsuits while it fosters better written patents and greater innovation. But many major research universities disagree, joining critics who predict that it will open the door to wholesale copying and undermine thousands of patents. With billions of dollars in licensing revenues potentially at stake, "this is the biggest patent case in decades," says Susan Braden, an attorney at Baker & McKenzie in Washington, D.C.,

* *Festo Corporation* v. *Shoketsu Kinzoku Kogyo Kabushki Co. Ltd.* (a.k.a. SMC Co.). U.S. Supreme Court Docket 00-1543.