

Why the

y

Our X and Y chromosomes make an odd couple. The X resembles any other chromosome, but the Y—the source of maleness—is downright strange. How did the two come to differ so much?

by Karin Jeganian and Bruce T. Lahn

The human chromosomes that determine sex—the X and Y—are a bizarre pair. The other 22 sets of chromosomes in our cells consist of well-matched partners, as alike as twin candlesticks. One chromosome in each duo comes from the mother and one from the father, but both are normally the same size and carry the same genes. (Genes are the DNA blueprints for proteins, which do most of the work in the body.) In stark contrast, the Y chromosome is much smaller than the X; in fact, it is positively puny. It harbors no more than several dozen genes, far fewer than the 2,000 to 3,000 on the X. A number of the Y genes have no kin at all on the X. And the Y is riddled with unusually high amounts of “junk” DNA: sequences of code letters, or nucleotides, that contain no instructions for making useful molecules.

Until recently, biologists had difficulty explaining how the Y fell into such disrepair. They had various theories but few ways to test their ideas. That situation has now changed, thanks in large part to the Human Genome Project and related efforts aimed at deciphering the complete sequence of DNA nucleotides in all 24 distinct chromosomes in humans—the X, the Y and the 22 autosomes (the chromosomes not involved in

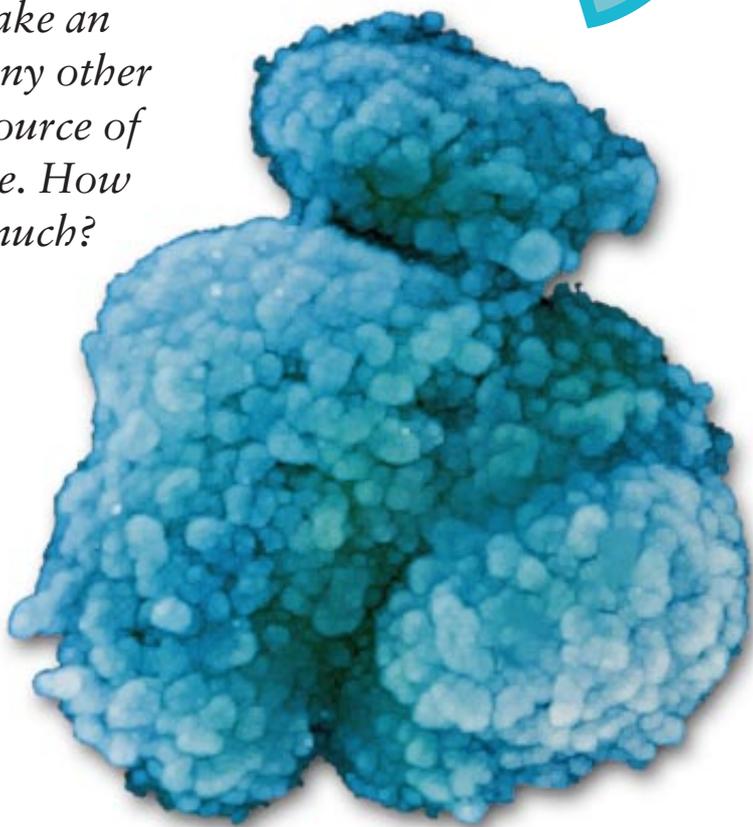
sex determination). Just as paleontologists can trace the evolution of a species by examining skeletons of living animals and fossils, molecular biologists have learned to track the evolution of chromosomes and genes by examining DNA sequences.

The new findings demonstrate that the history of the sex chromosomes has been strikingly dynamic, marked by a series of dramatic disruptions of the Y and by compensatory changes in the X. That interplay undoubtedly continues today.

Further, the Y chromosome—long regarded as a shambles, able to accomplish little beyond triggering the maleness program—turns out to do more than most biologists suspected. Over some 300 million years it has managed to

preserve a handful of genes important for survival in males and to acquire others needed for fertility. Instead of being the Rodney Dangerfield of chromosomes (as some have called the chronically disrespected Y), the male chromosome is actually more like Woody Allen: despite its unassuming veneer, it wields unexpected power.

Sheer curiosity has driven much of the research into the evolution of the human sex chromosomes. But a more practical pursuit has informed the work as well: a desire to explain and reverse male infertility. Discoveries of Y genes that influence reproductive capacity could lead to innovative treatments for



Y CHROMOSOME

Is So Weird

X CHROMOSOME

men who lack those genes or have defective versions [see box on page 61].

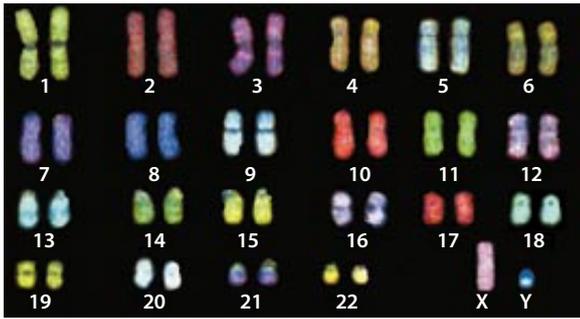
The recent advances have benefited from insights achieved beginning about 100 years ago. Before the 20th century, biologists thought that the environment determined sex in humans and other mammals, just as it does in modern reptiles. For reptiles, the temperature of an embryo at an early point in development tips some poorly understood system in favor of forming a male or female. In the early 1900s, though, investigators realized that chromosomes can arbitrate sex in certain species. About 20 years later mammals were shown to be among those using chromosomes—specifically the X and Y—to determine sex during embryonic development.

Clues Piled Up

In the ensuing decades, researchers identified the Y as the male maker and deduced that the X and Y evolved from matching autosomes in an ancient ancestor. By chance, sometime shortly before or after mammals arose, a mutation in one small part of the autosome copy that would become the Y caused embryos inheriting that changed chromosome (along with its mate, the future X) to become males. Embryos inheriting two Xs became females.

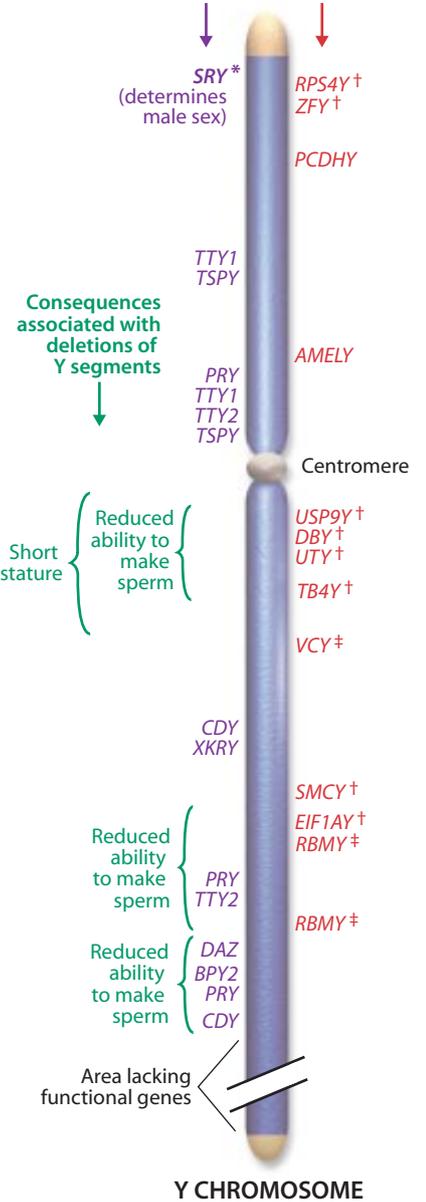
In 1990 geneticists pinpointed the part of the Y that confers maleness. It is a single gene, named *SRY*, for “sex-determining region Y.” The protein encoded by *SRY* triggers the formation of the

X AND Y CHROMOSOMES started off as a matched pair hundreds of millions of years ago. But the Y shrank to a nubbin, whereas the X maintained its integrity. How the pair came to diverge so strikingly is becoming clear. The micrographs show the chromosomes as they appear during the metaphase stage of cell division.



Y genes (or gene families) not found on the X and active only in the testes

Y genes having counterparts on the X



because the tips of the X and Y have remained twinlike and able to engage in a process called recombination. During meiosis (the cell division that yields sperm and eggs), matching chromosomes line up together and swap segments, after which one copy of every autosome plus a sex chromosome is distributed evenly to each reproductive cell. Even though most of the Y now bears little resemblance to the X, the tips of those chromosomes align during meiosis in males and exchange pieces as if the X and Y were still a matching set. (Such alignment is critical to the proper distribution of chromosomes to sperm.)

Other evidence that the X and Y were once alike came from the part of the Y that does not recombine with the X. Many of the genes scattered through this nonrecombining region still have counterparts on the X.

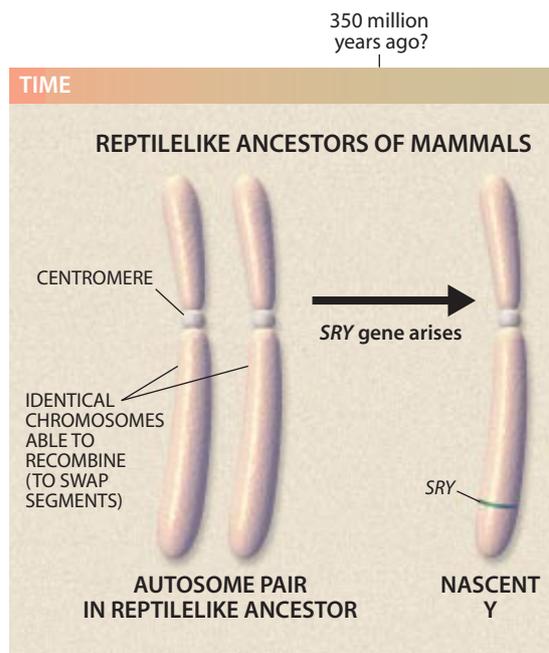
The existence of the nonrecombining region, which makes up 95 percent of the Y, offered a clue to how that chromosome became a shadow of its original self. In nature and in the laboratory, recombination helps to maintain the integrity of chromosomes. Conversely, a lack of it causes genes in nonrecombining regions to accumulate destructive mutations and to then decay or disappear.

- "Pseudoautosomal" regions, able to swap DNA (recombine) with the X
- * *SRY* causes the testes to form. It derived from the gene *SOX3* and resembles *SOX3* on the X, but the two have different functions
- † Housekeeping genes
- ‡ Genes that have X counterparts but are active only in the testes

CHROMOSOMES from a normal male cell (*photograph*) include 22 pairs of autosomes (those not involved in sex determination), plus an X and a Y; one member of each pair comes from the mother and one from the father. Genes in the NRY, or nonrecombining region of the Y (*blue in diagram*), have helped reveal the evolutionary history of the X and the Y. The region is so named because it cannot recombine, or exchange DNA, with the X. Only genes that still work are listed. About half have counterparts on the X (*red*); some of these are "housekeeping" genes, needed for the survival of most cells. Certain NRY genes act only in the testes (*purple*), where they likely participate in male fertility.

testes, apparently by activating genes on various chromosomes. Thereafter, testosterone and other substances made in the testes take over the molding of maleness.

Scientists concluded that the human sex chromosomes started life as a matched pair in part



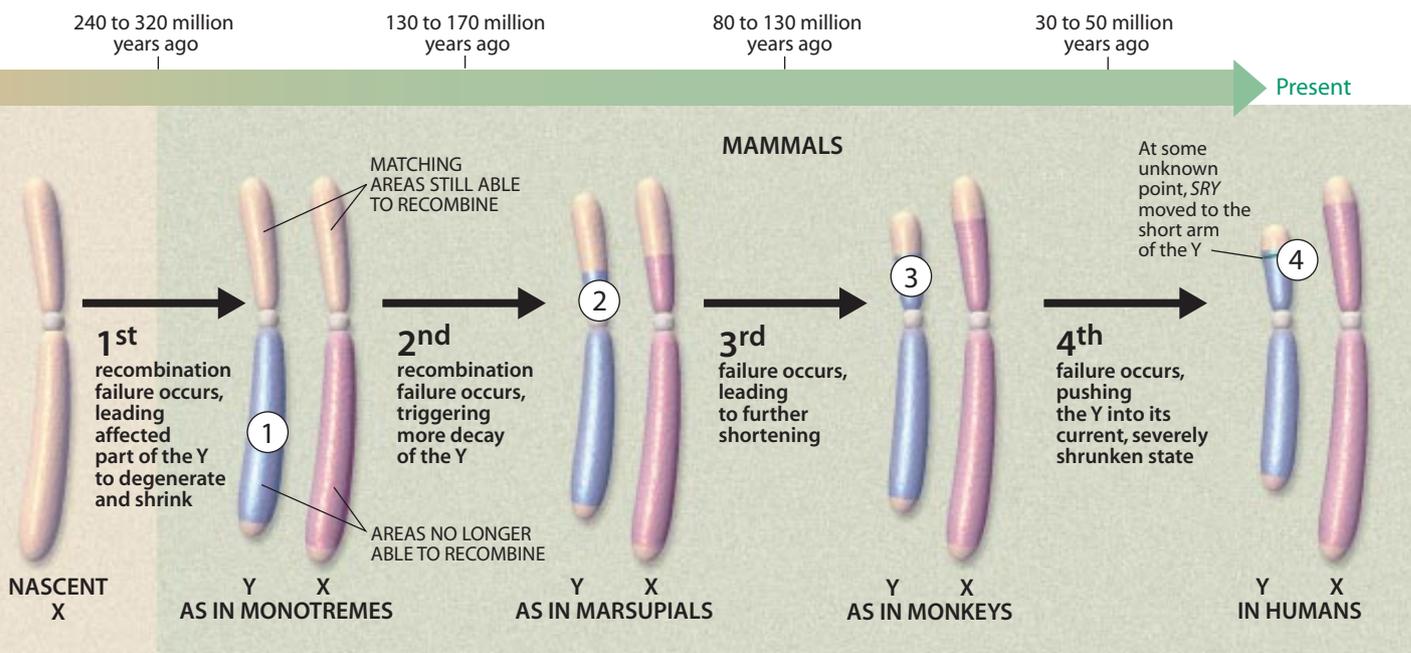
It seemed reasonable to think, therefore, that something caused DNA exchange between large parts of the X and Y to cease, after which genes in the nonrecombining region of the Y collapsed. But when and how recombination stopped after the Y emerged remained uncertain for decades.

Shaped in Stages

Work completed in the past five years has filled in many of the gaps. For instance, in 1999 one of us (Lahn) and David C. Page of the Whitehead Institute for Biomedical Research in Cambridge, Mass., showed that the Y lost the ability to swap DNA with the X in an unexpected, stepwise fashion—first involving a swath of DNA surrounding the *SRY* gene and then spreading, in several discrete blocks, down almost the full length of the chromosome. Only the Y deteriorated in response to the loss of X-Y recombination, however; the X continued to undergo recombination when two copies met during meiosis in females.

What could account for the disruption of recombination between the X and the Y? As the early X and Y tried to trade segments during meiosis in some far-distant ancestor of modern mammals, a part of the DNA on the Y probably became inverted, or essentially flipped upside down, relative to the equivalent part on the X. Because recombination requires that two like sequences of DNA line up next to each other, an inversion would suppress fu-

HESED PADILLA-NASH/National Institutes of Health (micrograph); ALFRED T. KAWAJIAN AND HEIDI NOLAND (illustrations at left and above)



ture interaction between the formerly matching areas of the X and Y.

We discovered that recombination ceased in distinct episodes when we examined the nucleotide sequences of 19 genes that appear in the nonrecombining region of both the X and the Y. (Some of the Y copies no longer function.) In general, if paired copies of a gene have stopped recombining, their sequences will diverge increasingly as time goes by. A relatively small number of differences implies recombination stopped fairly recently; a large number means it halted long ago.

Most of the X-Y pairs fell into one of four groups. Within each group, the X and the Y copies differed by roughly the same amount, indicating that recombination stopped at about the same time. But the groups clearly varied from one another. The Y copies that began diverging from their counterparts on the X at about the time the *SRY* gene arose differed from their partners the most, and the other groups showed progressively less divergence between the X and Y copies.

By comparing DNA sequences across species, biologists can often calculate roughly when formerly matching genes (and hence the regions possessing those genes) began to go their separate ways. Such comparisons revealed that the autosomal precursors of the X and Y were still alike and intact in reptiles that existed before the mammalian lineage began branching extensively. But monotremes (such as the platypus and echidna), which were among the earliest to branch

DEGENERATION OF THE Y occurred in four discrete episodes starting about 300 million years ago, after a reptilelike ancestor of mammals acquired a new gene (*SRY*) on one of its autosomal chromosomes. Each of the episodes involved a failure of recombination (DNA exchange) between the X and the Y during meiosis, the cell division that yields sperm and eggs. If recombination is blocked, genes in the affected regions stop working and decay. The sequence shown is highly simplified. For instance, the Y actually expanded temporarily at times (by stitching autosomal DNA into areas still able to recombine), before failures of recombination led to a net shrinkage.

off from other mammals, possess both the *SRY* gene and an adjacent nonrecombining region. These differences implied that the *SRY* gene arose, and nearby recombination halted, close to when the mammalian lineage emerged, roughly 300 million years ago.

We gained more information about the timing by applying a “molecular clock” analysis. Biologists can estimate the background rate at which DNA sequences are likely to change if they are under no particular pressure to stay the same. By essentially multiplying the extent of sequence disparity in X-Y pairs by that estimated rate, we deduced that the first recombination-halting inversion took place between 240 million and 320 million years ago.

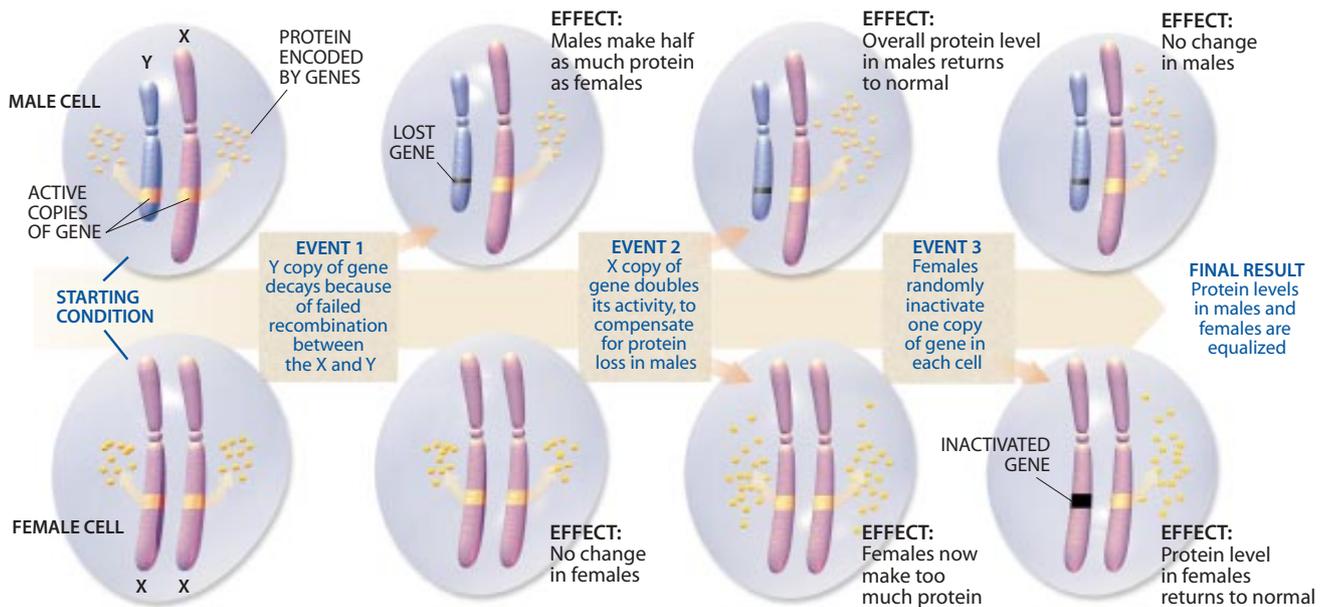
Similar analyses imply that the next inversion occurred 130 million to 170 million years ago, shortly before marsupials branched off from the lineage that gave rise to all placental mammals. The third struck 80 million to 130 million years ago, before placental mammals diversified. And the final inversion rocked the Y roughly 30 million to 50 million years ago, after monkeys set off on their own evolutionary path but before apes and hominids parted company.

Bucking the overall trend for X-Y

pairs, some genes in the nonrecombining region of the Y code for proteins that differ remarkably little from the proteins encoded by their X counterparts, even in the regions that underwent inversion earliest. Their preservation is probably explained by a simple evolutionary law: if a gene is crucial to survival, it will tend to be conserved. Indeed, the Y genes that have changed the least are mainly “housekeeping” genes—ones critically required for the integrity of almost all cells in the body.

Making up for Losses

Logic—and a large body of research—indicates that the failure of recombination between the X and the Y, and the subsequent deterioration of many Y genes, must have been followed by a third process that compensated for the degeneration. The reasoning goes like this: Not all genes are active in every cell. But when a cell needs particular proteins, it typically switches on both the maternal and paternal copies of the corresponding genes. The amount of protein generated from each copy is fine-tuned for the optimal development and day-to-day operation of an organism. Therefore, as genes on the Y began



EVOLUTION OF X INACTIVATION, the silencing of most genes on one X chromosome in female cells, apparently occurred in a piecemeal fashion—one gene or a few genes at a time—to compensate for losses of genes on the Y chromosome (*diagram*). One effect of X inactivation can be seen in calico cats (*photograph*). The gene determining whether fur color is orange or black (that is, not orange) resides on the X. Females that carry the orange version on one X and the black version on the other X will end up with some orange areas and some black ones, depending on which X is shut down in each cell. A different gene accounts for the white areas.

to disappear, the production of the associated proteins would have been halved disastrously in males unless the affected genes evolved compensatory tricks.

Many animals, such as the fruit fly, handle this inequity by doubling the activity of the X versions of lost Y genes in males. Others employ a more complex strategy. First they increase the activity of X genes in both males and females, a maneuver that replenishes protein levels in males but creates an excess in females. Some animals, such as the nematode worm, then halve the activity of X genes in females. Others, including mammals, invoke a process called X inactivation, in which cells of early female embryos randomly shut off most of the genes in one of their two X chromosomes. Neighboring cells may silence different copies of the X, but all the descendants of a given cell will display its same X-inactivation pattern.

Although X inactivation has long been thought to be a response to the decay of Y genes, proof for that view was lacking. If degeneration of Y genes drove X inactivation, then X genes having functional counterparts in the non-recombining region of the Y would be

expected to keep working in females (that is, to evade inactivation)—so as to keep protein levels in females equivalent to those in males. In analyzing the activity levels of surviving X-Y pairs from two dozen mammalian species, one of us (Jegalian) and Page found a few years ago that the X copies of working Y genes do escape inactivation. Those analyses also revealed that X inactivation, although it happens in an instant during an animal's development today, did not evolve all at once. Instead it arose rather diffidently—patch by patch or perhaps gene by gene within a patch, not all at once down the chromosome.

Emerging Themes

Curiously, the nonrecombining region of the Y possesses not only a handful of valuable genes mirrored on the X but also perhaps a dozen genes that promote male fertility. The latter code for proteins made solely in the testes, presumably to participate in sperm production. Some seem to have jumped onto the Y from other chromosomes. Others have apparently been on the Y from the start but initially had a different purpose; they acquired new functions over time. Degeneration, then,

is but one theme prominent in the evolution of the Y chromosome. A second theme, poorly recognized until lately, is the acquisition of fertility genes.

Theorists disagree on the forces that turned the Y into a magnet for such genes. The species as a whole may benefit from sequestering in males genes that could harm females or do nothing useful for them. It is also possible that being on the Y protects male fertility genes by ensuring that they go from male to male without having to detour through females (who could discard them without suffering any direct consequences).

Another mystery is how fertility genes can thrive in the absence of recombination, under conditions that corrupted most of the Y's other genes. An answer may lie in the observation that nearly every male fertility gene on the Y exists in multiple copies. Such amplification can buffer the effects of destructive mutations, which usually afflict just one copy at a time. As some copies accumulate mutations and eventually fail, the remaining ones continue to preserve a man's reproductive ability and to serve as seeds for their own multiplication.

The evolution of the sex chromosomes has been studied most thoroughly in humans. But together with cross-species comparisons, that research has

identified general principles operating even in creatures that evolved sex chromosomes independently from mammals. Some of those animals, such as birds and butterflies, use the W-Z system of sex determination. When inheritance of a single copy of a specific chromosome leads to the formation of a male, that chromosome is termed the Y, and its partner is termed the X. When inheritance of one copy of a chromosome leads to the formation of a female, that chromosome is called the W, and its mate is called the Z.

One notable principle is that sex chromosomes derive from autosomes. The affected autosomes can vary, however. W and Z chromosomes in birds evolved, for example, from different autosomes than those that gave rise to the mammalian X and Y. And the X and Y in fruit flies derived from different autosomes than those enlisted by mammals.

In most sexually reproducing species, once sex chromosomes arose, they became increasingly dissimilar as they underwent one or more cycles of three sequential steps: suppression of recombination, degeneration of the nonrecombining parts of the sex-specific chromosome (the Y or W) and, finally, compensation by the other chromosome. At the same time, the sex-specific chromosome in many instances became important for fertility, as happened to the Y in humans and insects.

It is reasonable to wonder what the future holds for our own species. Might the cycle continue until it wipes out all recombination between the X and the Y and ultimately destroys the Y, perhaps thousands or millions of years from now? The new discoveries suggest males are able to protect Y genes that are critical for male survival and fertility. Nevertheless, total decay of the Y remains a theoretical possibility.

Research into genes is often undertaken with an eye to understanding and correcting human disorders. Some in-

NEW ANSWERS FOR MEN

Y Am I Infertile?

Beyond revealing the history of the sex chromosomes, genetic studies of the Y are helping to explain some cases of infertility. In about half of all affected couples, the problem rests fully or partly with the man, who occasionally produces insufficient numbers of sperm or even none at all. Often the roots of these abnormalities are obscure. New findings suggest, though, that the Y contains a number of fertility genes and that disruption of one or more of them accounts for about 10 percent of men who make little or no sperm.

Investigators first inferred a role for the Y in infertility in the 1970s, when they saw through a microscope that many sterile men lacked small bits of the Y normally present in fertile men. Today scientists know that deletions in any of three specific regions on the Y can cause infertility, and they have learned that each of these regions—referred to as AZF (for azoospermia factor) a, b and c—contains multiple genes.

Most of those genes are highly active in the testes, where sperm is made (that is, the genes yield abundant amounts of the proteins they encode). This behavior strongly suggests that the genes in the AZF regions are important for sperm manufacture, although their exact contributions, and their interactions with fertility genes on other chromosomes, remain to be determined.

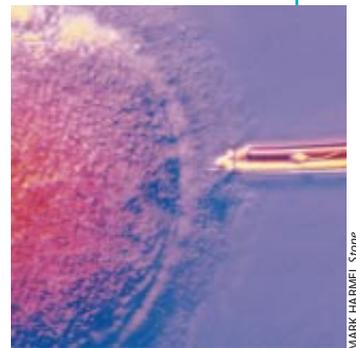
Some infertility specialists are now assessing Y chromosome deletions as part of their diagnostic workups. If men found to have such deletions produce at least some sperm, they might

be offered a therapy called intracytoplasmic sperm injection (ICSI), in which sperm is retrieved from the

testes and inserted into eggs in the laboratory. Regrettably, sons conceived in this way will inherit their fathers' defective Y chromosomes and so will probably face the same fertility challenges.

Once researchers decipher the exact functions of the proteins encoded by AZF-area genes, they may be able to reverse infertility in men possessing Y deletions by replacing the missing proteins, perhaps by restoring the lost genes themselves. On the flip side, such information should make it possible to devise drugs that purposely disrupt the sperm-production machinery—thereby providing new male contraceptives.

DELIVERING SPERM (*visible in microneedle*) directly into an egg may overcome infertility in some men afflicted by mutations of the Y chromosome.



MARK HARMEL/Stone

vestigations into the Y chromosome began with just such a goal in mind—understanding male development and correcting infertility. But many studies were less focused on therapy. As more and more genes on the X and Y were identified by medical research and systematic

sequencing efforts, evolutionary-minded scientists could not resist asking, on a more basic level, whether those genes had anything new to say about the distant past of the strangely mismatched X and Y chromosome. As it turns out, the genes had a rich tale to tell.

The Authors

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Further Information

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