



David Page has spent his career studying the mysteries of the Y chromosome.

Photo: Stanley Rowin

AN UNFINISHED STORY ABOUT THE GENESIS OF MALENESS



By Delia K. Cabe

If a book about the human Y chromosome were to make the best-seller list, it might be titled *The Masculine Mystique*. Like a biological counterpart to Betty Friedan's *The Feminine Mystique*, the book would explore the genesis of maleness. Some of its themes might even echo Friedan's classic: sexual identity, evolution and self-expression. But as the story of Y unfolds, the book would read more like a mystery, a murky tale that reveals long-held secrets about a diminutive protagonist who veers toward degeneracy.

Not that our central character is powerless. After all, Y can take credit for about half the human population. (The other half are, of course, women.) But when it comes to chromosome allocation, there is no equality of the sexes.

Consider this: Women carry 23 pairs of chromosomes, each set matched like a new pair of socks. In females, the 23rd pair is an XX. Males carry 23 sets also, but their 23rd pair is an XY twosome—one sock and its severely frayed partner, the Y chromosome, which is actually an X cropped at one end. The maimed chromosome has only 20 or 30 genes compared with roughly 2,000 to 3,000 genes on the mighty X.

Scientists identified the X and Y chromosomes at the turn of the last century. Not until 1959, however, did they realize that Y carries the code for maleness. Having discovered the male chromosomal headquarters, they could now figure men out—or so they thought. During the ensuing manhunt for maleness genes, which began in earnest in the 1980s, scientists sought to answer a handful of questions. How did the Y come to be? Might the source of male infertility lie hidden within it? Are X and Y related? Which genes on the Y chromosome instigate diseases?

Then came a map to guide researchers through the wilds of the human Y. Published in 1992, the rough map showed the chromosome's genetic landmarks, which would help scientists pinpoint the locations of genes. The map emerged from the laboratory of HHMI investigator David C. Page, a seasoned veteran of Y chromosome research at MIT's Whitehead Institute for Biomedical Research. Now, Page and his lab team are about to release the next chapter in the book of Y: The completion of a map of all the DNA on Y.

Reprinted from the September 2000 *HHMI Bulletin*, Vol. 13, No. 3, pp. 20-25.

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A House of Mirrors

As Page and his group have readied their findings for their next series of articles, set to begin publication later this year, their workplace itself looks like a map. Three long sheets of white paper hang on a huge bulletin board, creating a paper trail of the group's journey along the Y chromosome. At several points, the word "GAP" is scrawled inside circles in yellow highlighter next to stretches of terra incognita: DNA still undefined. Some of the circles are X'ed through—simple shorthand that marks the group's progress.

Other researchers around the world, though not many, are also intrigued with Y and by the details on those wide ribbons of paper. "It's a smallish field," says Bruce T. Lahn, a former graduate student of Page's who recently was named an HHMI investigator at The University of Chicago. And, no wonder. Y has a small number of genes, and the genes are not implicated in many human diseases. The chromosome's small size might suggest that it's a fast read, but short books aren't always the easiest to grasp. Mapping and identifying the chromosome's active genes has been maddening, in fact, as investigators have found themselves hampered by the presence of multiple gene copies and many inert genes. "Mapping the Y chromosome," says Page, "is like walking into a house of mirrors for a few minutes and, when you leave, being asked to draw a floor plan."

Figuring out this confusing layout and understanding the design is a tantalizing puzzle for Page, whose office is lined on three walls with shelves filled with textbooks and binders carefully organized by subject and with journals clustered according to date and publication—a setup that would make a librarian beam. Gazing out his window toward an area of Cambridge where several biotechnology companies involved in genomics are headquartered, Page rattles off a series of questions that hint at semantic implications: "Why do we call sex chromosomes sex chromosomes? What puts the sex in sex chromosomes? What do the sex chromosomes have to do with sex, being male and female?"

His phrasing is no idle wordplay; nor are these the same question. The Y chromosome's idiosyncrasies need to be examined simultaneously from slightly different perspectives. "In the same breath, we can think about the gene's relationship to making eggs and sperm, the role of mutations in that gene to infertility, and that gene's arrival on the sex chromosome in evolution," Page says. All of these are roles that researchers began exploring in the early 1980s and still haven't fully explained.

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Sperm Production and More

Page and Finnish geneticist Albert de la Chapelle embarked on their research in the early 1980s—not by studying typical XY men but rather by seeking out so-called sex-reversed individuals. XY women and XX men, individuals who appear to be one sex but have sex chromosomes of the opposite sex, provided clues to where the sex-determining gene might dwell on the Y chromosome. The tip-off was a snippet of Y attached to an X on the XX males as well as a segment of Y chromosome absent in the XY females. Page and de la Chapelle deduced that those bits of Y housed the critical gene. They produced an early map of the crucial area of where the sex-determining genes reside. The map would help researchers take the critical step in honing in on the exact address.

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Page thought he had pinpointed the area on the Y itself and published his findings in 1987. But in 1990, a British team led by Peter Goodfellow nailed down the region, calling it *SRY* (short for sex-determining region Y). The protein produced by *SRY* may regulate other genes that cajole an embryo's fetal genital ridges into boyhood and prod the formation of testes. Once on the scene, the testes secrete hormones that instigate the male reproductive tract's development.

At puberty, sperm development and production, or spermatogenesis, are triggered within the seminiferous tubules in the testes by the hormone testosterone from the testes and gonadotropin hormones from the pituitary gland. Researchers wondered whether other genes on the Y chromosome orchestrated spermatogenesis. Again, the answers turned up in men who departed from the norm. In 1976, Y was first implicated in male infertility by Italian researchers who found deleted portions of the chromosome in men with spermatogenic failure. They labeled the region *AZF*, short for azoospermia factor. Two decades later, Page uncovered a chromosomal pattern in infertile men with azoospermia—an absence of sperm from semen, or oligozoospermia—a very low sperm count. In analyzing blood cells from these two groups, Page's team found that some men were missing a region of the Y chromosome, a deletion that was the same in each case. Later, Page's group found *AZF* deletions in the Y chromosomes of sperm isolated from men with oligozoospermia and evidence of *AZF* in blood cells. Within the *AZF* region, they discovered one gene with fertility connections, which they branded the *DAZ* gene (for deleted in azoospermia). *DAZ* is expressed only in the testes, where the gene is active in the very earliest stage of spermatogenesis. Last year, the group uncovered a mutation in *USP9Y*, a gene also within the *AZF* region, that results in infertility.

"One of the striking things is that at least half of the genes on the Y chromosome appear to be active in sperm production," Page says.

The discovery that gene mutations can cause infertility brought another question to the fore. Obviously, azoospermia is not inherited since men who produce no sperm cannot have children. Azoospermia shows up *de novo* in some males. But could men with oligozoospermia pass on the mutation to their sons? Researchers could not even approach this puzzle until 1992, when a revolutionary

method of assisted reproduction enabled men with low sperm counts to father children, even if the oligozoospermia resulted from genetic defects. (Through intracytoplasmic sperm injection, or ICSI, physicians first retrieve sperm from the testicles or epididymis and then inject a single sperm into an egg.) By studying the fertility of sons conceived with ICSI, Page's group and researchers at the Infertility Center of St. Louis found that men with oligozoospermia do, in fact, produce sons likely to be infertile.

The inheritance of mutations other than those that cause infertility also captivates scientists who study the human male chromosome. For example, Page and his colleagues reported findings this summer in *Nature* concerning children with genetic diseases caused by a newly identified mutation. His group was interested in determining the proportion of new mutations that were inherited from fathers as opposed to mothers. Since the 1940s, biologists thought that the majority of new mutations came from the father. However, Page's lab found that the rates of inheritance between mothers and fathers are similar.

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Evolutionary Divergence



Bruce T. Lahn worked with David Page to determine Y's curious evolution.

Photo: Mark Segal

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While tracking genes in human offspring, Page and other investigators are also pondering where Y itself got its genes. To trace Y's lineage, researchers could not ignore this chromosome's heftier counterpart, the X. The two chromosomes have quite a history, coexisting as equal partners without specialty some 240-300 million years ago, only to part ways around 170 million years ago—dates arrived at by Page and Lahn. The Y chromosome map that Page's lab released in 1992 aided the investigators' trip back in time. An article in *Science* late last year detailed how they determined the time intervals and the incidents that caused two autosomes—chromosomes not involved in sex determination—to differentiate into the mismatched set of sex chromosomes we have today.

The project originally started in a different direction, Lahn recalls. He was looking at the degree of sequence similarity and dissimilarity between genes on the Y chromosome and those on the X and other chromosomes. The high degree of correlation between X and Y got his attention. "Lo and behold, a striking pattern emerged that could readily explain the progression of X-Y divergence," Lahn says.

The split between X and Y took place over millions of years. The chromosomes became less and less alike in four instances of genetic reshuffling on the Y. Most of the genes on our modern X existed before the breakup, but their counterparts on Y gradually degenerated. In mammals, the 22 other pairs of chromosomes swap genetic material, through the process called recombination which occurs during meiosis, the cell division that takes place in ovaries and testes before half of each pair is sequestered into either a sperm or an egg. In human females, XX pairs do this without problem. During sperm formation in males, X and Y still exchange some DNA, but with only a few genes. These reside in Y's "pseudoautosomal" region—the address for genes that Y has in common with X. The rest of Y's genes belong to the genetic equivalent of a boys-only club.

Proof exists that genes came from elsewhere to join this all-male chromosome. One such gene is *DAZ*. In the same study that identified *DAZ*, the Page group identified a cousin called *DAZL* on chromosome 3. Evidence points to *DAZL* as the original gene, a copy of which made its way to Y. Page and the other investigators wrote in their research report that the existence of a Y-gene precursor challenges theories that X was the source for most or all of Y's genes. While many of Y's genes do have homologs on X, the researchers suggest that Y's evolution had other outside influences.

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Save the Males

Such exclusivity by the male chromosome could engender many jokes about guys being guys—and does. For example, Page's Tuesday morning lab meetings break up into laughter in response to occasional wisecracks of the sort, "If, by any chance, we find the gene that gives males big teeth..." The researchers have heard them all: Genes for remote control use, unwillingness to ask for directions, selective hearing. The Borscht Belt it's not; the quips distract for only a moment during the weekly updates.

During one recent meeting, Page sipped juice from his teal-colored mug imprinted with "Save the Males." In some ways, that slogan is no joke. Scientists agree that the Y chromosome is degenerating and might eventually disappear—though not for another 100 million years or so. Unable to recombine or repair themselves, the Y's clannish genes are left to fend for themselves. Autosomal genes may repair themselves during mitosis when they couple with the opposite member, but Y genes have no chance for repair. So, in the course of millions of years, mutated Y genes with no more reason for being will fall out of service. Y's arms will dwindle. The chromosome will lose genes faster than it gains.

That fact, though, helps researchers develop other evolutionary insights. "It tells us that there's something special about recombination for the long-term persistence in genes or species," says William R. Rice, an evolutionary geneticist at the University of California, Santa Barbara. "The Y chromosome is a model system to study the adaptive significance of recombination." For example, asexual species become extinct because of a lack of recombination. Rice says that a suppression of recombination is behind Y's degeneration and eventual demise. "Species that don't recombine decay over time," he says. "If you stop recombining, you have a limited future."

This history has given the Y chromosome a reputation as a wasteland, essentially ineffective save for its sex-determining gene. Because that imputation arose from anecdotal data, Page and Lahn took inventory of Y's nonrecombining region and published their results in 1997. They found a dozen genes that, combined with previously discovered genes, brought the total number of known genes on the Y at the time to about 20. The two researchers observed that about half of the known human Y genes appear to be engaged in functions needed by all cells in the body. And this housekeeping crew—around ten genes, including the five reported in this study—does have complementary genes on the X.

Page and Lahn think their discovery suggests that these genes are not inactivated in one member of a pair of sex chromosomes. Rather, everyone may require two working copies for cell health. When both Xs are present, as in normal females, one X is silenced except for these five genes. In the condition known as Turner's syndrome, which affects one in 3,000 females, only one X chromosome is present. The absence of half of these housekeeping genes may help explain the short stature, infertility, webbing of the neck and lack of female secondary sex characteristics associated with the syndrome. The rest of the recently identified genes probably function within the testes and are involved in

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spermatogenesis, the investigators say.

Researchers know that Y holds more genes. This quirky little character still possesses many secrets, and it will be years or decades before investigators can write the final chapters of the story. "The X and Y represent a very peculiar experiment of *Nature*, " Page says. "The experiment isn't over."

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Another Genetic Battle of the Sexes: Imprinting



Shirley Tilghman says genomic imprinting is rapidly evolving.
Kay Chernush

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The genetic power struggle between the sexes extends beyond the X and Y chromosomes to the level of individual genes.

Called genomic imprinting, this intricately orchestrated give-and-take plays out within a developing embryo, although the stage is set much earlier, when sperm and eggs are formed. About 40 genes have now been identified that are distinctively marked, or imprinted, so that the embryo will recognize its parental origin. As the embryo grows, the genes act differently depending on whether they came from the mother or the father.

Embryonic genes interact like performers in a jazz ensemble. A pianist introduces a passionate melody, the bassist starts up a few bars later and the saxophonist and drummer slip in, embellishing harmony and tempo. The genes from both mother and father cooperate with one another to create a perfect embryo. Imprinted genes, in contrast, go solo, such that only one copy is expressed, while the same gene inherited from the other parent remains mute because of chemical silencers, known as methyl groups, on the DNA. But, unlike musicians playing together, the genes are not cooperating with one another: They are at war, with the maternal genes trying to slow down growth and the paternal genes trying to promote it.

Sometimes, an imprinted gene misses a cue and either toots its own horn inappropriately or remains silent at the wrong time. Since there's no backup to fill in for the aberrant gene, the slip-up can result in a devastating disease such as Prader-Willi or Angelman syndrome. "We've become slightly more vulnerable because of this imprinting process," says Marisa S. Bartolomei, an HHMI investigator at the University of Pennsylvania. Bartolomei did her postdoctoral studies in the laboratory of another HHMI investigator, Shirley M. Tilghman of Princeton University, a leading expert in genomic imprinting.

Genomic imprinting certainly did not catch Gregor Mendel's attention in the mid-19th century when he was experimenting with crossing garden peas. His peas in a pod inherited their traits in a predictable pattern, helping Mendel figure out how genes are transmitted between generations. Mendel concluded that crosses are the same whether a dominant trait is maternal or paternal in origin. Perhaps he should have studied mammals and used his leisure time for gardening—a pastime that Tilghman throws herself into, but not for

genetic insights.

Scientists didn't recognize the phenomenon of mammalian imprinting until the 1980s. In 1991, Bartolomei says, the concept of imprinting underwent a molecular revolution when scientists discovered that the gene for insulin-like growth factor 2 (*Igf2*) is imprinted. Scientists had previously conducted experiments to show that genetic imprinting occurs, but now they had actual genes responsible for the process, genes whose molecular mechanisms they could study. Additional genes have since been identified, and researchers have discovered that these tend to cluster together. But investigators in the field are still trying to answer some fundamental questions: What purpose does imprinting serve? Why are so few genes imprinted? Exactly how does an imprinted gene silence its complement?

A Genetic Arms Race

David Haig of Harvard University compares gene imprinting to a genetic arms race in polyandrous species, including certain types of mice, in which females mate with several partners within the same litter. According to Haig's theory, paternal genes that are expressed and lobby for fetal growth duke it out with maternal genes that limit growth. In evolutionary terms, such a parental tug-of-war is good for a species. While large offspring put more of a strain on the mother's resources, they have a better chance of survival. But the mother has a worthy argument for smaller offspring: Females need to conserve their nutritional resources within one litter to ensure that they themselves will survive to produce more litters.

Tilghman and colleagues sought to test Haig's theory about polyandrous mammals in monogamous species in which both parents have an equal interest in the offspring's survival. They chose *Peromyscus polionotus*, a wild breed of mouse. Imprinting, they found, occurs in this monogamous species—an observation that didn't fit with Haig's theory. Moreover, when crossed with *Peromyscus maniculatus*, a distant polygamous relative, the hybrids failed to imprint. Tilghman's team concluded that imprinting is rapidly evolving and could contribute to a high rate of speciation in mammals.

The same researchers published a study in the May 2000 issue of *Nature Genetics* that provides more insight into what happens when closely related species are crossed. "What my recent paper shows is that imprinting can contribute to the rapid acquisition of genetic incompatibilities between two related species that are no longer breeding," Tilghman says.

What end, then, does imprinting serve? Tilghman suggests that it may be that imprinting did not arise to benefit mammals. Rather, genomic imprinting is the consequence of the differences in parental strategies that optimize the number and fitness of their offspring.

Preventing the expression of a gene makes the most sense in the X chromosome, which in fact carries the greatest number of clusters of imprinted genes. Females inherit two X chromosomes while males get an X and a Y. If both X chromosomes were expressed, females would get a double dose of the protein encoded by the genes. Males would get only half the amount because they have only one X. Gene expression, however, logically should be at the same levels in both sexes of a species. Thus, jettisoning one of the female's sex chromosomes would allow for equality of the sexes.

Investigators would like to find the genetic switch that shuts off one copy of a gene for life and lets the other one replicate often and at a very active rate. Tilghman is studying six imprinted genes residing in one chromosomal region. DNA methylation is acting as a genetic muffler in one of these genes, but not in the others. She and Bartolomei have suggested that the imprinting of one gene,

Igf2, depends on the methylation of another gene, *H19*, rather than on its own methylation. *H19*, which encodes a highly expressed RNA and not a protein, is expressed in embryonic and neonatal tissues, but its function is unclear. "The bottom line is that there is not a single mechanism for silencing genes in imprinting," Tilghman says.

She and her fellow researchers in this field are hard at work trying to understand the various genes that seek solos during embryonic development. The arrangement works nicely, to be sure—but its driving force and ultimate purpose remain unclear.

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