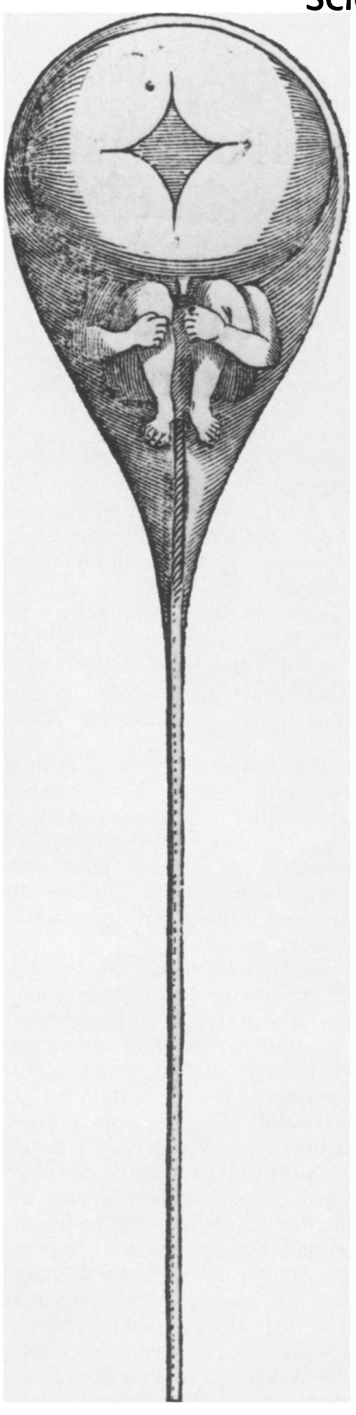
A Genetic Gender Gap

Scientists discover that not all genes are created equal



This 17th-century engraving of a human sperm portrays a belief of that era that sex cells contained a tiny version of a fully developed person. New work suggests the genetic contents of sex cells are less explicit but equally strange.

By RICK WEISS

Every high-school biology student knows the story: In a small monastery garden in the 1850s, Gregor Mendel tallied the offspring of smooth and wrinkled peas, giving birth to the modern science of genetics. The monk-scientist observed that pea progeny inherit the traits of both parents equally — and that these traits display themselves in statistically predictable ways — laying the groundwork for the basic principles of heredity that survive to this day.

But was Mendel right? In the past five years, experiments have confirmed that he — and generations of scientists since — systematically overlooked a baffling exception to these otherwise straightforward laws of inheritance. Today, outside the research limelight that surrounds such topics as cancer and AIDS, a few scientists are using modern genetic techniques to understand a subtle but fundamental biological mechanism that runs counter to more than 100 years of scientific dogma.

The mechanism is called genomic imprinting. It says, in essence, that contrary to classical genetic theory, a gene contributed by a mother to her offspring may differ functionally from an identical gene contributed by the father. That is, while two inherited genes may have exactly the same DNA sequence, their expression may be very different depending on which parent provided each.

Researchers know almost nothing about how imprinting works, and they know even less about why it evolved. But geneticists are beginning to see imprinting as one of the most fundamental molecular manifestations of "maleness" and "femaleness." They say an understanding of genomic imprinting could tell a lot about an organism's evolutionary "decision" to use sexual reproduction. It may also solve some of the most basic riddles about embryonic development and gene expression — including the immunological mystery of why a mother doesn't reject her fetus as a foreign body.

Imprinting has clinical relevance as well. Increasingly, researchers invoke the poorly understood process to explain unusual inheritance patterns of certain diseases, including some leukemias, juvenile diabetes and Huntington's disease. With further insight into imprinting's molecular biology may come improved means of screening for genetic abnormalities.

"It took sort of heroic efforts to show that imprinting even existed," says Carmen Sapienza of the Ludwig Institute for Cancer Research in Montreal. "And the fact that things went on for so long with just Mendelian genetics, and that everything seemed just fine, may indicate that imprinting is not very important — that it doesn't affect many genes. I think that's probably wrong, and that it is important and it does affect many genes and we did see the effects of imprinting, but that we failed to recognize them for what they were."

The belief that male and female contributions to the embryo may not be equal is by no means new. Long before anyone actually saw individual sperm and eggs, Aristotle speculated that the mother provided "matter" and the father provided "motion." Microscopy put this notion to rest. But the subsequent observation that an unfertilized egg can be as much as 1 million times larger than a typical sperm led to new assumptions about gender-based genetic inequity.

Only with this century and the discovery of chromosomes — the gossamer strands of DNA inside every living cell — came today's accepted notion that male and female sex cells contribute equally to genetic inheritance. The 23 chromosomes in a sperm are more tightly packed than are the 23 in an egg, and eggs contain more nonchromosomal ingredients than do sperm. However, analyses of chromosomal DNA appeared to settle the question once and for all: A gene for blue eyes is a gene for blue eyes — whether contributed by the father or the mother.

But a growing number of researchers disagree with this modern, egalitarian view. They suspect that a biochemical modification of the DNA in male or female sex cells somehow results in variable expressions of otherwise identical genes. Until recently, the mechanism's apparent complexity precluded scientists from proving its existence. But the recent development of new biological tools, including gene transfer methods and par-

ticularly the use of transgenic mice, has changed all that, says Joachim Messing, director of the Waksman Institute at Rutgers, the State University of New Jersey, in Piscataway. "These methods are particularly interesting since they appear to show that genes and chromosomes are not equivalently passed on to the offspring as we always have believed from Mendel's laws of inheritance," Messing says.

The first good experimental evidence of such genetic nonequivalence came in 1984, in experiments performed by James McGrath and Davor Solter of the Wistar Institute of Anatomy and Biology in Philadelphia. They took a freshly fertilized, single-cell mouse embryo containing sperm- and egg-contributed bundles of DNA that had not yet had time to fuse into a complete nucleus within the cell. Using microsurgery, they replaced half this DNA with DNA from another sperm or egg so that the fertilized embryo now contained genetic material from either two eggs or two sperms. The embryos failed to develop normally. But embryos whose sperm DNA had been replaced with other sperm DNA, or whose egg DNA had been replaced with other egg DNA – thus leaving the embryo with a normal complement of "male" and "female" DNA – did develop normally.

"On the basis of our results, we conclude that maternal and paternal genomic contributions are not functionally identical and that both are essential to complete embryogenesis," the Wistar researchers wrote in the May 1984 CELL.

Similar experiments by British researcher M.A.H. Surani and his colleagues, published in NATURE later that year, confirmed those findings. Their research suggested that during sex-cell formation, some genes in sperm and eggs are subjected to a specific molecular "imprint." Only a proper combination of imprinted and nonimprinted genes can add up to normal embryonic development, they concluded.

Since those early experiments, molecular biologists have accumulated additional evidence that in mammals and some other organisms, eggs and sperm indeed undergo a biochemical process that results in some of their genes being "turned off" – at least temporarily. Imprinting, they find, is really a form of gene inactivation.

Because having a turned-off gene is like having no gene at all, a sex-linked process of gene inactivation can lead to some surprises in the rules of inheritance. But unlike other types of sex-linked genetic traits, such as hemophilia or color blindness, whose manifestation depends on the sex of the inheriting offspring, these traits may show up in offspring of *either* sex. An imprinted gene's expression depends not on the gender of the inheriting individual but on the gender of the *parent* contributing that gene.

To explain a simple case of imprinting, Solter uses the example of red and white flowers. In this model, without imprinting, two white flowers will produce white offspring, two red flowers will produce red offspring, and a red and a white flower together will produce pink offspring.

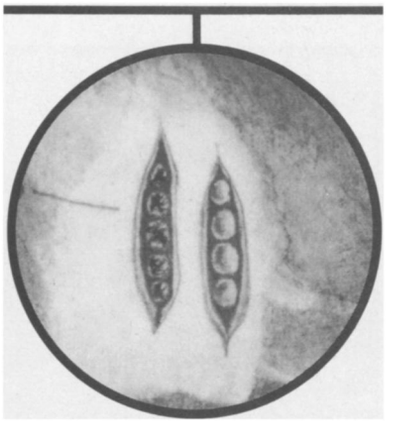
Now consider, says Solter, a cross between a red and a white flower. "Mendelian genetics would say that it's totally irrelevant whether the white flower is male or the red flower is male. The progeny of red and white flowers are going to be pink." However, he says, "imagine that the color gene is imprinted [inactivated] in females. Then if the female is red, this gene will be silent, and when crossed with a white male, the progeny will be white." Similarly, if the female is white, then *that* gene will be imprinted and the male's red genes will come through undiluted, making red progeny. Depending on which parent is red and which is white, he concludes, you will get either white or red progeny.

"You might say, 'Wow, that really doesn't fit with Mendelian laws,' " Solter adds. But in fact, he notes, the color-determining genes have still segregated evenly among progeny as Mendel predicted. With imprinting, "you still inherit two genes, but one of them is silent and may not become visible again until the next generation."

Despite a growing body of evidence that parentally determined gene inactivation occurs frequently in nature, and that it represents an important, if long overlooked, genetic phenomenon, researchers remain puzzled about how it might actually occur. Any such mechanism must fulfill certain unusual requirements. The most important criterion: If a silent, imprinted gene is to maintain the potential to show up loud and clear in a subsequent generation – depending on the parent through which it later is passed down – then gene inactivation by imprinting must be reversible.

Imagine, for example, a classic case of maternal imprinting, in which a particular gene is routinely imprinted in an egg while its equivalent gene in the father always remains active in sperm. What if that maternal, silenced gene, now resting quietly in an egg, finds itself part of a growing embryo that develops into a male? Cell division by cell division the young male grows, and in every cell there is a silent, mother-derived gene and an active, father-derived version of the same gene. But later in life, some of these cells will divide to make sperm. Since all sperm are supposed to have an active copy of that gene, at some point the imprint must be "erased" from the maternally derived gene – that is, the gene must be reactivated – before it becomes incorporated into a sperm.

Nobody knows how this works. But a series of gene transfer experiments has left many researchers believing that the simple addition of a few methyl groups (four-atom carbon-hydrogen complexes) during sex-cell formation may be the key difference between a gene being active or imprinted.



Researchers know that methylation and demethylation – the enzyme-mediated processes of adding and subtracting methyl groups – occur periodically in the course of sex-cell formation and embryonic development. Exposure of male- or female-derived gametes (sperm and egg) to different patterns of gene-targeted methylation could account for many of the observed differences in expression of otherwise equivalent genes, they say.

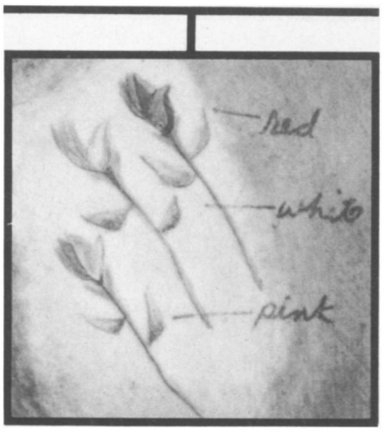
"It's probably not a simple quantitative difference in methylation but different patterns of methylation," says Marilyn Monk of the MRC Mammalian Development Unit of the University College London in England. "These [patterns of methylation] may perhaps control the initial patterns of gene expression in early development. Then I think we probably have a dynamic situation where different patterns of gene expression themselves determine later modifications in [DNA] structure and methylation."

Sapienza, of the Ludwig Institute, notes that imprinting implies the existence of yet another level of genetic regulation. Patterns of imprinting are themselves inherited, he points out. So, in addition to the genes that are themselves methy-

lated, or imprinted, there must also be genes that program for the production of enzymes or other products that actually *do* the imprinting. The bottom line: "There must be genes that imprint and genes that are imprinted. And we really don't have any idea how they do it."

If researchers remain baffled about how imprinting works, they are equally hard pressed to explain why it has gone almost completely unnoticed.

"Most people will right off the bat argue that there cannot be many genes that are imprinted," Solter says. "Because if there



were many, then it would have been very hard for Mendel to make his rules of genetics and we would see this phenomenon all the time, and we don't."

Apparently one reason the phenomenon went unnoticed for so long – at least in mammals – stems from a quirk in the way researchers traditionally conduct mammalian genetics experiments.

When researchers want to understand the relationship between two mammalian genes – a common line of inquiry among geneticists – they often take a male mouse featuring both genes of interest and breed that mouse with many females genetically similar to each other. This provides, in a short period of time, a large number of offspring from essentially identical crosses. The researchers can examine these offspring and determine, for example, how often the two traits are inherited together, providing important information about how closely the two genes are "linked."

But geneticists rarely perform the reverse experiment – crossing a female with many males – because a female can only produce so many litters in her lifetime and the total number of offspring available for analysis will be much smaller than those from crosses done in the other direction.

Had scientists performed more experimental crosses in both directions over the years, Solter and others say, researchers might well have noticed direction-dependent inequities in inheritance patterns. Indeed, says Solter, "I would argue that the presence of imprinting can be found in some old CELL papers from 1946, but you have to know what you're looking for. You have to look at the tables and say: 'Look, statistically he did enough crosses and there should have been 30 animals and there's only three.' "

In fact, such discrepancies occasionally have been noted, researchers say, but they were incorrectly attributed to other causes, such as hormonal influences. Only recently have experiments shown that such factors are not involved. Still, Solter concedes, "it's true that if thousands and thousands of genes are really imprinted and visibly expressed, one would have noticed [in more than a few experiments] that there is a huge discrepancy between what one would expect and what one was seeing, and this has clearly not been the case."

What, then, explains this well-kept secret of parentally determined genetic regulation? Researchers point to two probable culprits: genetic leakage and mosaicism. Mosaicism is a common genetic phenomenon whereby only a sprinkling of cells in an organism express a given genetic trait – in this case, gene inactivation. With enough cells unaffected, the overall effect of having a few cells inactivated may not be obvious.

The concept behind genetic leakage is, in essence, that although imprinting may happen to all or many cells, it is too temporary or incomplete in each cell to be noticed. "Say the gene is imprinted in all the cells but this imprinting is not very strong – it leaks," explains Solter. "So in most of the cells the gene that is supposed to be silent is not silent. Maybe only a few cells are really well imprinted and are really silent," but enough remain sufficiently active to behave normally.

If either leakage or mosaicism is applied to the example of red and white flowers, most offspring would appear pink after all – as Mendel observed. But inactivation of a few red or white genes, while not necessarily obvious in the adult pink flower, may have been crucial during that flower's fertilization or early development – and may still be important in certain key cells or tissues.

Recent research suggests the same may hold true for a variety of imprinted genes in mammals, including humans; patterns of gene inactivation appear most important during early development and become less obvious as the organism grows. Moreover, say researchers, the persistence of even a few, key imprinted cells in adulthood seems to have major implications for the manifestation of certain hereditary diseases.

Imprinting results in unique and somewhat complicated patterns of inheritance that geneticists today increasingly recognize in some genetically linked diseases. Among the simpler examples are cancers resulting from the inactivation of a tumor-suppressor gene.

A normal individual has two of every gene – one from each parent – residing at equivalent locations, or loci, on paired chromosomes. Each gene is called an allele. In the case of a tumor-suppressor gene – a gene that protects against cancer – a normal individual can afford to lose one copy of it through mutation or some other genetic accident because the second copy can still do the job of preventing the cancer it protects against. Geneticists believe that such "single hits" occur frequently and go completely unnoticed; only when both alleles become disabled will a cancer occur.

Children can inherit a single defective allele from one parent or the other, and if a mutation someday occurs on the second allele, cancer may result. When researchers perform genetic tests on such affected individuals, one would expect them to find an equal number of cases in which the new mutation is on either the maternally or paternally derived chromosome. But for some cancers and other genetically linked diseases, this is not the case. Researchers find, for example, that in certain diseases the new mutation rests almost invariably on the maternal chromosome. This implies that the paternal cancer-suppressor allele may be among those genes routinely inherited in an already inactivated, or imprinted, state. In other diseases, the reverse is true: The new mutation is generally on the paternal chromosome, suggesting a preexisting inactivation of the maternal allele.

Scientists say imprinted cancer suppressors may be surprisingly commonplace. But because of genetic leakage or mosaicism, few cells actually contain *strongly* imprinted suppressor genes. And of the few that do, only a minuscule portion are likely to get the "second hit" required to initiate a cancer. But when such a cancer does occur, researchers note, it generally will follow an inheritance pattern linked to that of the imprinted gene.

"There are lots of diseases that are more commonly linked to either the father or the mother" and that are probably associated with imprinted genes, says Solter. "Probably the most common one is juvenile diabetes, which seems to be

more severe and occurs earlier when it's inherited from the father."

Similarly, says Sapienza, imprinting appears to play a role in the age of onset of Huntington's disease – an inherited, fatal neurological disorder that varies greatly in its age of onset. Of the 10 percent of cases described as "juvenile onset," Sapienza notes, 90 percent are inherited from the father. Although researchers still know little about the genes that control such complicated events as disease onset, a growing number now believe that imprinting best explains these parental influences.

Making matters more complicated, a single imprinting gene may inactivate several different genes, including a wide variety of cancer-suppressor genes, says Sapienza. "If you have an imprinting gene that acts on a bunch of loci, we conclude that you could get multiple disease phenotypes [expressions] within a single family," he says. "The families that fit those criteria best are what are called megagenetic cancer families, where you can look at pedigrees and find that half the offspring have cancer but sister Sue has breast cancer, brother Billy has rhabdomyosarcoma, somebody else has osteosarcoma and somebody else has a brain tumor." The various tumor-suppressor genes thought to be involved in those different types of cancer don't all necessarily map to the same chromosome, Sapienza says. But a single gene may imprint them all. In this way, he adds, the interplay of genes that imprint and those that are imprinted wreaks havoc for researchers trying to link certain types of cancers with causative genes.

In an even more unusual twist, at least one genetic disease may result from a specific error in imprinting on the gender-related X chromosome itself. Fragile X syndrome, a chromosomal disorder characterized by mental retardation, occurs about as frequently as Down's syndrome. Because the defect occurs on the X chromosome, for which there is no double in males, one might expect it to manifest in all males with an affected X chromosome. But it doesn't.

"The fragile X has very, very unusual genetics," says Charles Laird, a geneticist at the University of Washington in Seattle. "It's the most complex pattern of inheritance I've ever seen." Laird has published evidence that fragile X syndrome results from an imprinted gene that fails to become un-imprinted when it should.

Researchers say that with a better understanding of genomic imprinting, a host of complex, inherited diseases may finally begin to make genetic sense. Most important, a compilation of the various genes that affect – directly or indirectly – the occurrence of various hereditary diseases should allow molecular biologists to develop genetic "probes" for screening individuals whose family histories indicate they are at risk for a disease.

But while such applications should prove a boon to geneticists and clinicians in coming years, the most fundamental questions about genomic imprinting may remain unanswered for a long time. Perhaps most intriguing: Why did the mechanism first evolve?

"There are several evolutionary ways that imprinting could have happened," Solter says. For example, some researchers theorize that it represents a mechanism of "gene dosage regulation." Many lower animals, such as fruit flies, have independent mechanisms that regulate the amount of protein a given set of genes produce without having to turn alleles on or off. But "mammals are very bad at that; they never work on that principle," Solter says. "If they need more [gene product], they try to duplicate many copies of that particular gene or they inactivate a gene that's blocking production of the needed product."

Given that reality, imprinting can be seen as a crude way of regulating genes, he says. "You have two genes, but you want only one of them to work. It's a very convenient way to do it, especially if you want to have it only for a while and then subsequently you want to have both."

Moreover, he says, even if not designed to do so in the first place, imprinting may be the key to a relatively recent evolutionary development: the mammalian system of internal gestation and an embryo's ability to remain in its mother's uterus without being immunologically rejected despite its display of "foreign" proteins from the father (SN: 10/11/86, p.234). Studies by British researcher Surani and others have demonstrated that in the newly developing embryo, maternal genes are especially important for proper embryo formation and the paternal contribution is key to proper development of tissues outside the embryo, such as the placenta. In evolutionary terms, says Solter, "perhaps implantation in a womb was crucial to mammalian development. And in order to achieve that implantation, there is a need for a specific tissue that can interface between the mother and the fetus." He says some research suggests that the differential distribution of maternal and paternal proteins on the surface of a developing fetus and its placenta may modify the mother's immune response and prevent rejection.

Sapienza believes imprinting's evolutionary roots run even deeper. "While imprinting certainly has effects on development, I don't know whether it's designed to do that. My personal opinion is that imprinting is designed to control sexual reproduction exclusively and that all the other effects at all the other loci are just a consequence" of that basic function.

He notes that researchers have identified imprinting mechanisms in an extremely wide variety of organisms, including yeast, corn and humans. In yeasts, which come in two "mating types" (scientists don't refer to them as "male" and "female"), imprinting clearly serves to ensure that only yeast cells of opposite mating types can go on to reproduce. And

yeasts, being unicellular, have no embryonic development at all. "What that says to me is that the ability to recognize genetic components that have gone through one type of gametogenesis [sex-cell production] or another . . . predates any complicated developmental program," says Sapienza. "It's a very old process."

Whatever the original, evolutionary motivation, scientists agree that imprinting had one clear effect: It left affected organisms forever dependent on sexual reproduction. "Imprinting mandates the existence of male and female sex," Solter says. "It mandates that genomes, before they unite, must undergo oogenesis and spermatogenesis."

Beyond that simple fact, hidden in its complex mechanisms of gamete-of-origin gene inactivation, may rest fascinating clues about the birth of sexuality and fundamental insights into genetic regulation.

"Until tumors came into this, it was just science for the fun of it. It didn't really matter, as long as it eventually got known," says Solter. But now, "one could envision learning how to switch some of these genes on. It could change our understanding of genetic diseases and genetic diagnosis." □