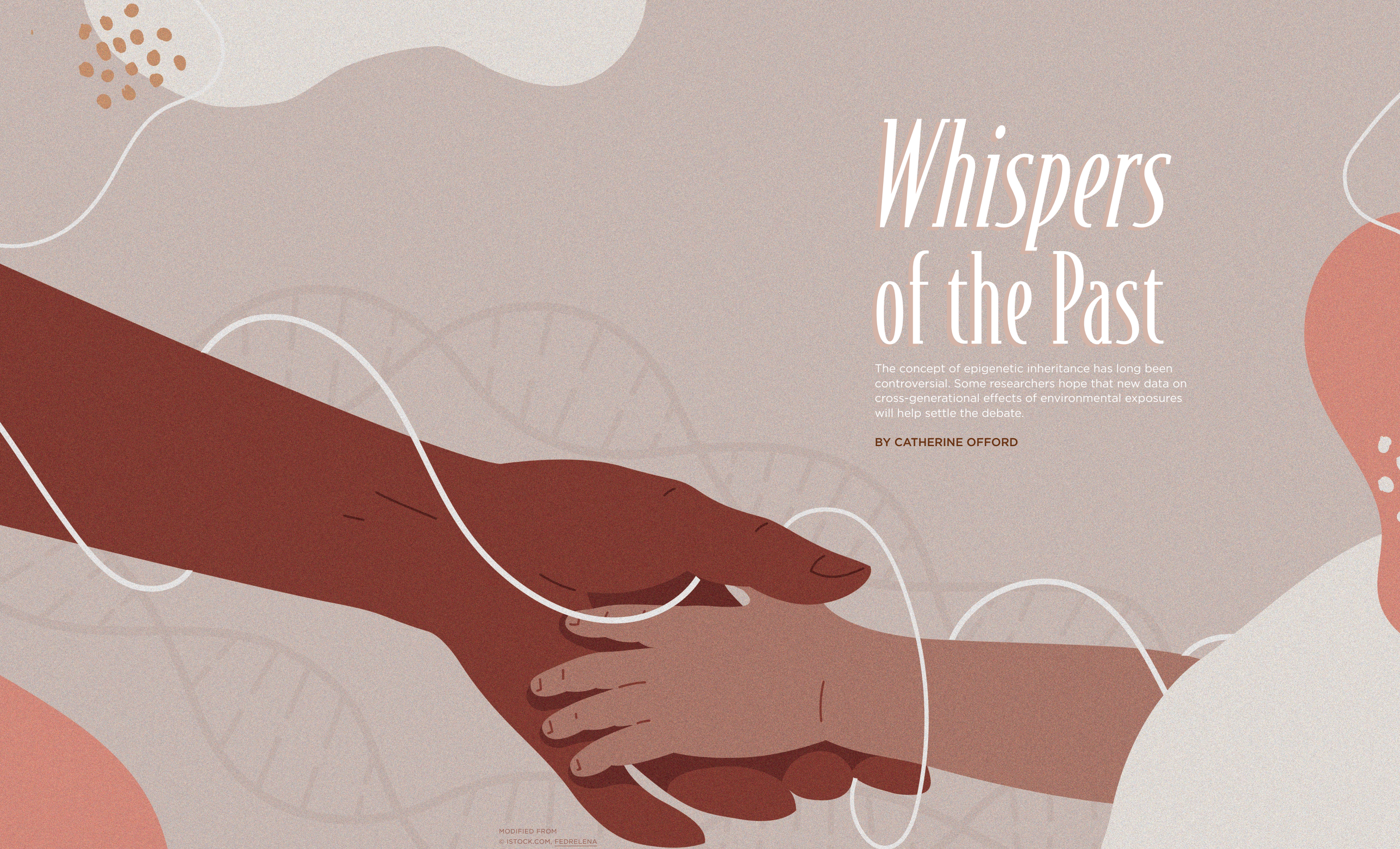
Whispers of the Past

The concept of epigenetic inheritance has long been controversial. Some researchers hope that new data on cross-generational effects of environmental exposures will help settle the debate.

BY CATHERINE OFFORD



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Andrea Baccarelli loves black truffles—the fungi, not the chocolates. His parents, he says, are also fond of the delicacy. His grandparents liked them, too, as did his great-grandparents. Did he inherit the preference from previous generations through some biological mechanism? Or could this multigenerational appreciation for clumps of a subterranean fungus be related to the fact that all these people were constantly surrounded by black truffles at the family home in Umbria, the northern Italian region that happens to be a global black truffle hotspot?

This question of what's passed down from parent to child is complex and has often been socially and politically charged, says Baccarelli, who now chairs the environmental health sciences department at Columbia University's Mailman School of Public Health (and who attributes his truffle love to his childhood in Umbria). The nuances of inheritance are perhaps most commonly framed as the age-old debate of nature versus nurture, the pervasive idea of a tug-of-war between deterministic genetic sequences and changing environmental influences—a dichotomy that scientists have long criticized as an oversimplification, given the complex interactions between genetics and the environment. Over the last couple of decades, however, scientific and public conversations about inheritance have grappled with an apparently separate, non-genetic dimension of inheritance.

The shift was driven by an explosion of interest in epigenetics—broadly, the study of proteins and other factors beyond DNA sequences that influence how genes are expressed. In the early 2000s and 2010s, as researchers delved deeper into the molecular mechanisms of gene regulation, other papers accumulated supposedly showing that changes in DNA methylation, noncoding RNAs, and other elements might offer a mechanism by which a person's exposure to environmental factors—such as toxic chemicals, trauma, or, hypothetically, truffle-heavy diets—could have intergenerational (parent-to-child) and even transgenerational (grandparent-to-child and beyond) effects on health and behavior.

The findings tapped into an old and long-discredited idea—often associated with the writings of Jean-Baptiste Lamarck and Trofim Lysenko, among others—that traits acquired within one's lifetime can be inherited. As Oliver Rando of the University of Massachusetts Medical School wrote in *The Scientist* at the height of the epigenetic inheritance boom in the mid-2010s, scientists were discovering that this out-of-favor proposal "may not be completely off the mark." (See "Inheritance of Acquired Traits: From Lamarck to Now" on page 28.)

Now, just a few years after that peak in interest, it's become abundantly clear just how varied and complicated the different theories of epigenetic inheritance are, and how many often-controversial hypotheses they rest on. Critics have pointed out that, while there does appear to be evidence for some sorts of epigenetic inheritance in plants and non-human animals, it's uncertain how much the environment really affects the human epigenome—much of which is actually dictated by genome sequence—and to what extent changes in the epigenome affect gene expression and human biology. What's more, it's now thought that much, although not all, of the mammalian epigenome gets wiped out and reprogrammed twice per generation, once in a newly fertilized egg and again during the formation of egg and sperm cells, challenging the idea that epigenetic alterations could have a consistent influence from one generation to the next, let alone across multiple generations.

Some researchers question whether evolutionary biology even needs an additional inheritance mechanism, after genes, culture, and other existing factors are taken into account, to explain the persistence of characteristics across multiple generations. "Nah," is the assessment of Bas Heijmans, a biomedical data scientist at Leiden University Medical Center (LUMC) who studies within-generation epigenetic changes in people who endure famine and other environmental conditions. "I think that epigenetic inheritance does not exist, according to what seems logical, reasonable, and what data we have." The idea has lost its shine in the public sphere, too; where books, magazines, and news stories once hailed it as revolutionary, they now ask whether it's a "lost cause," a phenomenon for which evidence has "crumbled."

Despite this criticism, many researchers haven't given up on the possibility that one generation's environment can influence future generations via epigenetic mechanisms. While there isn't strong evidence for these kind of effects happening on a large scale in humans, particularly over two or more generations, several researchers who spoke to *The Scientist* expressed the view that more epidemiological and mechanistic data could yet swing the debate in favor of the phenomenon.

"I'm probably a bit skeptical we're going to have a lot of inheritance explained by epigenetics," says Carrie Breton, an environmental epidemiologist at the University of Southern California (USC) who recently coauthored a review of studies of epigenetic inheritance in humans and other animals.[1] "But when we talk about environmental exposure causing an epigenetic change that might affect health risk, and whether that individual effect can persist, I do think there is mounting evidence in support of this." Even if it happens only rarely, she adds, "all it takes is an environmental exposure to tweak a handful of loci in the system that might still be bad for you, and that effect might be carried forward."

Baccarelli, who studies how environmental toxins could trigger changes in DNA methylation and gene expression, also takes an agnostic view. Many studies from the early 2000s, particularly those relating to transgenerational inheritance in animal models, haven't been replicated, he says, and it's often difficult to interpret human studies. Taken together, he says, while "we have enough evidence to do more research, I'm not sure we have enough research to say this is a thing."

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Finding connections

With a nearby Arctic moose farm and prime views of the aurora borealis, the municipality of Överkalix is in many ways a typical little Swedish Lapland township. But in scientific circles, this forested area of northern Scandinavia is famous for a data set assembled from historical records, including birth and death dates for several generations bracketing the turn of the 20th century, cause-of-death information, genealogy records, grain prices, and harvest statistics. In the early 2000s, Swedish scientists used data from several hundred people in this cohort to tackle a deceptively simple question: Can a young person's diet shape the health of their grandchildren decades later?

The study—which reported more deaths from diabetes among people whose paternal grandfathers had lots of food available when they themselves were kids—decided that yes, it can.[2] And because it's unusual to see large-scale genetic changes in the course of just three generations, the authors speculated in their paper that the mechanism behind the observed link could be epigenetic, perhaps via some sort of diet-driven change to the grandfather's germline. The findings were expanded on a few years later, and in more-recent follow-ups by the same and other groups, all of which reported a relationship between a grandparent's nutrition and at least one health outcome in their grandchildren. The results seemed to jibe with animal studies published during the same period, showing, for example, that feeding mice high-fat diets promoted metabolic or body-size effects one, two, or even sometimes three generations later.

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The Överkalix studies and their follow-ups provide compelling evidence for epigenetic inheritance in people—or an illustration of the pitfalls associated with this type of research, depending on your perspective. Critics of the original study pointed out that the small cohort likely contained multiple sibling and cousin pairs, for example, meaning that individual grandparent-child observations weren't really independent. And the study authors had analyzed multiple health outcomes simultaneously, increasing the likelihood that at least one would appear to have a statistically significant relationship with grandparent diet.

More generally, the data weren't exhaustive. Although several of the studies collected information on parental income, education, and other sociocultural factors, the researchers may still have missed the real reason for the association they identified. This caveat applies broadly to any study drawing links between environmental factors and health outcomes in people, says Breton. "We try to measure as many of the things that we think are the most important. . . . But undoubtedly, we can't measure everything that's important," she says. "And even the things we measure, they may not be measured well, so there's still a lot of noise."

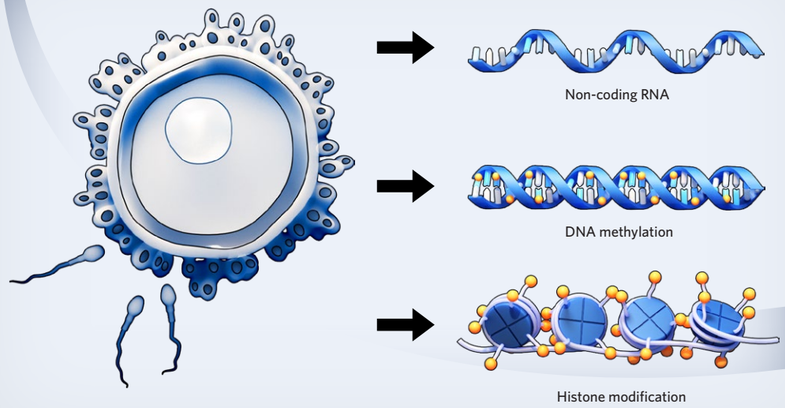
Environmental epidemiologists are now trying to develop better datasets by gathering more information—and in some cases, biological samples—from large, multigenerational groups of people. While still observational, such studies could support, or refute, particular connections between health outcomes and previous generations' exposures that have cropped up repeatedly, says Breton, and so help researchers decide which to pursue. (See "What's Passed On?" on page 26.)

One potential future source of data is the Environmental Influences on Child Health Outcomes (ECHO) program, a network funded by the National Institutes of Health (NIH) that comprises more than 70 cohorts of children plus their family members—and more than 50,000 children in total—across the US. Researchers started following some of these cohorts decades ago and in a few cases are already collecting data from a third generation, which could be used to investigate whether a parent's early life affects their child or grandchild, says Breton, who directs ECHO's USC site. "Some of us who have older cohorts think that that could be a really interesting path

WHAT'S PASSED ON?

Multiple studies have reported associations between child health and parental or grandparental lifestyle, while a number of animal and human studies hint at connections between environmental exposures and epigenetic changes in eggs or sperm. However, evidence to support causality in these correlations is lacking in humans. Below are some of the factors frequently studied by researchers interested in the idea of epigenetic inheritance.

1 ENVIRONMENTAL EXPOSURES
A multitude of environmental factors have been proposed to influence a person's health and potentially that of their children. These include exposure to harmful chemicals from cigarettes or pollution, lifestyle circumstances such as exercise and diet, and other factors including age and experience of stress or trauma.



2 EPIGENETIC MECHANISMS?
Reported epigenetic changes in gametes, especially sperm cells, in response to environmental factors include changes in the level of certain RNAs, in DNA methylation patterns, and in modifications to histones. The effects of these changes on gene expression and their persistence after fertilization are largely unknown.

forward," she says. Currently, she and her colleagues are analyzing cheek swab samples to assess consistency in DNA methylation patterns—still the most-studied part of the epigenome—across three generations of people. "Overall, we're seeing a very low percentage of loci correlated across these generations," she says. "It's just one study . . . but I think in the end, it's not going to be a high number of loci that are sort of written in stone and passed on in a concrete way from one generation to the next."

Some cohort networks investigating inter- and transgenerational health effects have already begun publishing data. The Pregnancy and Childhood Epigenetics (PACE) consortium, set up in 2018 by NIH researcher Stephanie London and colleagues, aims to bring together nearly 40 ongoing studies of children and parents. Following mouse studies suggesting that males fed high-fat diets had daughters with impaired insulin secretion and particular DNA methylation signatures in certain pancreatic cells, for example, the PACE group combined a chunk of its own data with information from other cohorts to dig into a possible association between a father's body mass index (BMI) and a child's DNA methylation patterns. Analyzing nearly 7,000 samples of newborn cord blood and blood from older children, the researchers found "little evidence" of any associations with paternal BMI. Because DNA methylation patterns vary among cell types and tissues, the authors emphasize in their paper that the results don't rule out a link altogether.[3]

Smaller observational efforts, such as the Avon Longitudinal Study of Parents and Children in the UK and the Norwegian Mother and Child Cohort Study, continue to put out data too. While some semi-consistent patterns have emerged from these studies—an association between grandparent smoking and grandchild asthma, for example, and, more controversially, a link between parental early-life trauma and child mental health—most have acknowledged that confounding factors such as prenatal exposure, upbringing, or other influences on child health have not been ruled out. As such, researchers

who spoke to *The Scientist* agree that it remains unresolved what might underlie these associations in people.

A working theory

Richard Pilsner started pondering sperm epigenetics around the time he and his wife started planning a family a little over a decade ago. Having trained in environmental health sciences, Pilsner recalls warning his wife about the risks of smoking and other behaviors that might affect conception or fetal development. That made them wonder whether he, too, might inadvertently affect his future child through his own lifestyle. After joining the faculty at the University of Massachusetts Amherst, Pilsner decided to dig into this potential link between parental exposure and child development. In 2014, he and colleagues launched the Sperm Environmental Epigenetics and Development Study (SEEDS), a cohort that uses leftover samples from IVF clinics, with participants' consent, to look at the relationship between a father's environment and his sperm epigenome—plus perhaps one day, his child's epigenome and health outcomes.

Focusing on sperm has advantages from the perspective of studying how parental environments influence future generations. For starters, because fathers and their babies are physically separated, it bypasses some of the confounding effects of in utero exposure that lead to a common criticism of studies in the field—that they conflate prenatal with inter- and transgenerational effects. Additionally, it could help researchers home in on what's physically transmitted across generations, rather than trying to infer it from parent-lifestyle and child-health associations. The sperm-centric approach has taken off in the animal literature, too—investigations of sperm epigenetics in rodent models have found that both negative experiences (exposure to harmful chemicals or trauma, for example) and purportedly positive ones (exercise) are associated with differing levels of DNA methylation and of RNA modification in sperm, and with measurable changes in offspring phenotype.

Pilsner's group, now at Wayne State University in Michigan, has completed several studies with the SEEDS data set. In a small-scale study a few years ago, the team followed up on other groups' reports that exposure to chemicals found in many everyday plastics altered DNA methylation in rodent sperm. Using urine samples to measure nearly 50 men's exposure to phthalates, Pilsner and colleagues identified more than 130 regions of the genome that were differentially methylated in the sperm of people who were exposed to the chemicals Many of these regions were found around genes involved in growth and development.[4]

More recently, the researchers explored another proposed risk factor for certain health conditions in children: advanced paternal age. While scientists have proposed multiple mechanisms, including accumulated mutations and the decreased structural integrity of DNA in sperm, to explain reported connections between advanced paternal age and risk of certain cancers and neurodevelopmental disorders, Pilsner's group published data suggesting that sperm methylation may also play a role. Specifically, the researchers found that male age was associated with particular epigenetic patterns at genes involved in embryogenesis and neurodevelopment.[5]

Whether epigenetic changes seen in sperm persist past fertilization or have biological effects in offspring is harder to gauge from these studies. Indeed, some of the rodent research that inspired the SEEDS phthalate study suggested the DNA methylation alterations in exposed males' sperm were completely reverted in the next generation. But a recent mouse study by Pilsner and colleagues reported that while DNA methylation patterns did differ between sperm and the embryonic cells of newly conceived offspring, the latter varied consistently in relation to the former.[6] "We see what we call an amplified effect—we see many more changes in the embryo than we see in the sperm," says Pilsner, who holds provisional patents related to age-associated epigenetic changes in sperm. "There's some sort of signal that's being passed." He adds that he's now working with Sarah Kimmins, an epigeneticist at McGill University in Quebec whose team has shown that changes in the methylation of histones, proteins that package DNA into chromosomes, can make it from sperm to embryo in certain mouse models. With their colleagues, the researchers are now investigating these and other types of epigenetic modifications.

This more holistic approach to epigenetics is one that's gaining traction among researchers interested in this type of inheritance, says Isabelle Mansuy, a neuroepigeneticist at the University of Zurich and ETH Zurich. Mansuy, who recently summarized more than 100 studies on potential inter- and transgenerational effects of environmental exposure, says it's likely that the pathways involved will be complicated, and not, say, a strict one-to-one copy of DNA methylation.[7] "I wish that people would avoid thinking that there is only one mechanism."

Romain Barrès, an epigeneticist at the University of Copenhagen and the Université Côte d'Azur who heads up the Gametic Epigenetics Consortium against Obesity (GECKO), is of a similar opinion. "We think that the epigenetic modifications talk to each other," he says. The full picture "may be missed if you're studying DNA methylation only, in only a set of tissues [such as] blood." Failing to find a conserved signal across generations or within a single person during their lifetime "doesn't mean that the signal is totally gone. Perhaps it is integrated into another epigenetic mark, like small RNA or chromatin conformation."

Conscious of the fact that the epigenome could change over time, Barrès's group has been trying to study specific life events rather than lifelong exposures. A few years ago, his team tracked changes in DNA methylation patterns in the sperm of people undergoing bariatric surgery for obesity. Using another data set, the team found that slim and obese men showed differences in sperm DNA methylation patterns and noncoding RNA levels, even when controlling for genetic sequence variation. Using the new cohort, the researchers found that morbidly obese men showed remodeling of sperm DNA methylation just a week after undergoing surgery to reduce

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INHERITANCE OF ACQUIRED TRAITS: FROM LAMARCK TO NOW

Although theories of epigenetic inheritance have drawn new interest in the last 20 years or so, the ideas they tap into have been around for centuries.

1800s

Jean-Baptiste Lamarck hypothesizes that traits an animal acquires during its lifetime—an extended neck after years of stretching up to reach high leaves, for example—can be inherited by future generations. He proposes that this idea, versions of which have been circulating since ancient Greek times, explains how species evolve.

1920s

Soviet agriculturalist Trofim Lysenko rejects decades of genetics research while pushing his own theory of how traits that organisms acquire in their environments can be inherited. He'll use the idea to develop disastrous agricultural policies that contribute to crop failure and famine.

1940s

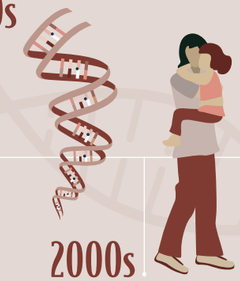
Embryologist Conrad Waddington coins the term "epigenetics" to describe the developmental processes that connect an organism's genotype to its phenotype. The term will later be coopted to describe work in other disciplines, including research on the regulation of gene expression.

1960s

Research on chromatin structure takes off, with DNA methylation and histone modifications becoming associated with variation in the expression of particular DNA sequences. It will be many years before the research becomes known as epigenetics.

1990s

The discovery of imprinted genes—sequences that are methylated at an organism's birth and whose expression depends on which parent they were inherited from—launches the idea that DNA methylation carries information from parent to child.



2000s

The number of papers including the term epigenetics soars, and the idea that the field offers an alternative to genetic explanations for inheritance gains traction in the public eye. News stories claim that the field is "rewriting the rules" of heredity.

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weight, and to a greater extent after one year, particularly at genomic regions associated with appetite control. Some of these regions were the same ones that differed between slim and obese men, the authors noted in their paper.[8]

Barrès is now working with researchers in Australia to launch a rare experimental study in humans: pairs of adult male identical twins will be split to receive either a processed or unprocessed diet, and then asked to give sperm samples for researchers to analyze DNA methylation, small non-coding RNA levels, and chromatin structure. "There's a lot of things we cannot address looking just at the sperm itself," Barrès acknowledges. "But if we find a common denominator of an epigenetic signature in gametes in response to nutritional stress, and we identify that in these men we have more of this signature, and these men have children that themselves have specific traits, then we can build a model where we can appreciate whether this association is likely to be causative or not."

Moving toward more experimental approaches would be good for the field, agree researchers who spoke to *The Scientist*. In animal models, researchers are increasingly trying to modulate the epigenome rather than simply observe it. Mansuy and others have reported that injecting RNAs from mouse sperm cells into eggs or embryos elicits physiological or behavioral changes in the animals. Baccarelli notes that new technologies derived from DNA-editing enzymes could allow researchers to edit DNA methylation patterns too. "I think the big opportunity for epigenetics now is to use epigenetic editing to actually get to see what happens when you . . . edit a certain methylation site, to see whether the gene turns on and off—that's not something we can take for granted—and then to see what is the phenotype that changes."

Living with uncertainty

As data trickle in, arguments about which, if any, aspects of epigenetic inheritance hypotheses are likely to apply to humans continue to simmer in the literature. Many scientists still lament what they view as hype, misreporting, and an unhelpful blurring of definitions in the field, particularly when it comes to distinguishing between inter- and transgenerational effects. Some researchers in this field, meanwhile, say they feel their work's been unduly maligned. Many people don't appreciate how much effort it's taken to get research on epigenetic inheritance recognized, Mansuy says over email, adding that "collecting data and publishing [in this discipline] require more efforts and time than in more classical fields." She also points to struggles that she and some of her colleagues have had obtaining funding for projects on epigenetic inheritance in mammals in recent years.

Other scientists say they're still prepared for concepts of epigenetic inheritance to fail, either because the relevant mechanisms turn out to be vanishingly rare in humans or because their effects end up being negligible compared to everything else influencing development. "I don't think we'll ever point to a single study across multiple generations [and say], 'They finally showed it!'" Breton says. "I think it's going to end up being the cumulative evidence. The more papers that show the same set of relationships, that's where we're going to end up saying, 'OK, I think we start to believe this'—or maybe we don't. Maybe in the end it was all the other life stuff that was getting in the way that really made it look like an association, and really it isn't."

For LUMC's Heijmans, reduced interest in epigenetic inheritance now as compared to several years ago offers a welcome opportunity for epigeneticists to focus efforts on more-fruitful research directions, he says, noting that "there are more-relevant nuts to crack." He has been studying how prenatal or early-life environments might influence the epigenome, and whether epigenetic alterations can be used as biomarkers to predict disease risk within a person's lifetime. This could in theory "help us in identifying vulnerable individuals, and also monitoring [their] health," he says—in other words, "helping them using epigenetics. That's where I think it can be quite relevant."

Perhaps, he speculates, the field is ready for the sort of transition that many scientific disciplines have to go through. "Sometimes, the first question is not even answered by the field, and then we all jump to the next question. It's like when small children play football, you see them all where the ball is, not spread out across the field. . . . I think in science it's also a bit like that." ■

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