



## The Epigenetics Heretic

Michael Skinner's claim that chemicals can cause changes to gene expression that persist across multiple generations of animals has stirred excitement—and outrage

**MICHAEL SKINNER IS GLEEFULLY LISTING THE DISCIPLINES THAT HE'S** ruffled with his contention that, without altering the sequence of DNA, certain chemicals can cause harmful health effects that pass down generations. Toxicologists are so outraged that they have tried to block his funding, he says. Geneticists resist having their decades-old understanding of inheritance overturned. Then there are the evolutionary biologists, who have “the biggest knee-jerk reaction of all.”

Skepticism is to be expected, Skinner acknowledges: “This is probably going to be the biggest paradigm shift in science in recent history,” he declares.

Skinner is a polarizing figure in an already contentious area of biology—transgenerational epigenetic inheritance, or the notion that nonmutational changes to an individual's DNA, such as chemical coatings that alter a gene's activity, can persist in their great-grandchildren

and beyond. When he entered the fray 9 years ago, controversy was already emerging over more modest claims that environmental factors in childhood, such as stress or poor nutrition, could induce epigenetic changes that last into adulthood or into the next generation. Then Skinner's reproductive biology lab at Washington State University (WSU), Pullman, expanded the debate with a study in *Science* (3 June 2005, p. 1466). They reported that injecting pregnant rats with a common pesticide caused sperm abnormalities that persisted in the animals' male progeny for at least four generations—without any changes to the DNA sequence itself. Skinner, whose experiments have also implicated other common chemicals, even suggests that such changes may become a permanent part of our genetic inheritance.

To some scientists, Skinner is a pioneer who has uncovered a new and exciting potential driver of evolution, as well as a troubling route

by which one generation's exposure to chemicals could contribute to diseases such as obesity and infertility in their descendants. "He's demonstrating that this occurs for a wide variety of chemicals. This was a big shockeroo" for industry, says psychobiologist David Crews of the University of Texas (UT), Austin.

But skeptics—and there are many—point out that Skinner's original experiments have not been replicated, despite several attempts. They find unconvincing his evidence that specific epigenetic changes to DNA are transferred through the germ line. "People will find it hard to believe until there are defined mechanisms," says reproductive biologist Cheryl Rosenfeld of the University of Missouri, Columbia.

Some are also put off by Skinner's uncompromising personality, which has contributed to upheaval within his university. "He's sometimes a little cavalier in the way he presents," says reproductive biologist John McCarrey, an old friend and sometime collaborator at UT San Antonio. "I think he feels like, 'I've shown these things and people aren't listening.'"

### Man in black

Skinner seems to relish the role of maverick. He wears a suede Stetson and a long black coat during a recent interview in a downtown yogurt shop in Washington, D.C. He is in town to receive an "American Ingenuity" honor from *Smithsonian* magazine, awarded to 10 people who "are having a revolutionary effect" on their fields. A related profile in the magazine is the latest in a stream of favorable media articles recorded on Skinner's online curriculum vitae and lab website.

Skinner, whose family has deep roots in the Pacific Northwest, grew up on a ranch and started college on a wrestling scholarship. After earning a Ph.D. in biochemistry, he built a solid reputation as a reproductive biologist, studying the molecular biology of testes and ovary development and founding a center for reproductive biology at WSU with more than 100 faculty members.

His research took a turn around 2000 when a postdoc in his lab, Andrea Cupp, studied the insecticide methoxychlor, a so-called endocrine disruptor because it has hormonelike effects in the body. Cupp wondered whether the chemical would interfere with the formation of ovaries or testes in a pregnant rat's offspring if injected during a crucial window in fetal development. That did not happen, but as adults the male offspring had lower sperm counts and less motile sperm. By accident, Cupp bred these male offspring with the daughters of other pregnant rats that had been injected with the chemical. To her surprise, their male offspring—grandsons of the methoxychlor-treated pregnant rats—had the same sperm defects.

"I didn't believe her," Skinner says, because methoxychlor was not known to cause mutations that could account for the heritable effect. So he had Cupp repeat the experiment "about 15 times"—with the same result. Skinner's team saw the pattern again with another endocrine disruptor, the fungicide vinclozolin. Startlingly, the effects also showed up in subsequent generations of interbred rats, the so-called F<sub>3</sub> and F<sub>4</sub> generations.

The sperm problems were passed down to 90% of male offspring each generation, which suggested that some unexpected mutation could not be responsible. Mutations should be random and increasingly rare in each subsequent generation, Skinner says. Instead, Skinner's team identified a possible fingerprint of epigenetic changes in the rats' testes: methyl groups added to some genes, which could suppress their transcription into protein.

Although such methyl tags are known to pass down generations in plants and some other organisms, biologists didn't think this happened very often in mammals. That's because in the formation of sperm and eggs and in early embryos, cells go through a reprogramming stage believed to wipe away most methylation marks, except on a few genes crucial to early development. But the results from Skinner's team suggested that methylation marks on additional genes escape this reprogramming, even in generations that had no direct exposure to the toxin. (Skinner defines transgenerational effects as those in at least the F<sub>3</sub> generation, the great-grandchildren of the original animal. That is because treating a pregnant animal may also expose her embryos and the germ cells in those embryos to the toxin—see graphic.)

In their original study, Skinner's group did not hold back on the implications. "The ability of an environmental factor (for example, endocrine disruptor) to reprogram the germ line and to promote a transgenerational disease state has significant implications for evolutionary biology and disease etiology," they wrote.

The resulting *Science* paper became the most cited paper in reproductive biology for 2005; by now, it has more than 1200 citations, according to Google Scholar. But it also drew skepticism at toxicology meetings. Questions about the paper did lead to a lengthy clarification in 2010 explaining that key data from the original study were not published in that paper but elsewhere. (Skinner says the data were omitted because of *Science*'s space constraints.)

More concerning to some, in three published papers, the latest last year, two labs at companies that make vinclozolin or a similar fungicide tried to replicate the vinclozolin rat experiment but found no effects beyond the first-generation offspring. An Environmental Protection Agency research group has reported similar results at meetings. "Doubt in the scientific community likely arises as a result of these

conflicting reports," Rosenfeld says.

Skinner says these studies were negative because they "didn't even come close" to following his protocol. In some cases, the researchers fed rats the chemical instead of injecting it, as he did. Or they used an inbred strain of rats instead of the outbred animals Skinner had studied. Toxicologists have long known that strains differ widely in their sensitivity to chemicals, he notes.

Some scientists attacked his work from behind the scenes, Skinner says. "I've had people try to get my [National Institutes of Health] grants revoked," he says. Others blocked further funding, he complains. He says that he has struggled recently to support his lab. "My funding has dramatically declined because we're pushing the envelope," he says.

### Bumps in the road

Meanwhile, problems arose within Skinner's university. In 2008, he stepped down as director of WSU's Center for Reproductive Biology and later moved to another school within WSU because of what he labels "political battles" over a campus reorganization involving his center. Michael Griswold, dean of WSU's College of Sciences at the time, says he removed Skinner because the center needed a change in leadership after 12 years. Skinner also had "some disagreements" with members of his original school, Griswold adds.

Another shadow appeared on Skinner's professional record in 2010. Federal officials found that a Taiwanese postdoc in his lab had fabricated data in a 2006 *Endocrinology* paper. Skinner's group had retracted the paper in 2009 because they could not find some of the

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underlying data. “I thought we had all the checks and balances in place, but clearly we didn’t,” Skinner says.

Yet Skinner has pressed ahead with his research. With Crews and his wife Andrea Gore, also at UT Austin, he reported in 2007 that when a female rat was caged with two different males—the  $F_3$  male offspring of vinclozolin-treated pregnant rats and a control animal—the female shunned the male descended from a treated rat. The sperm abnormalities Skinner’s team had documented did not affect reproductive success, but this behavioral change could bias reproduction, suggesting that such multigenerational effects could play a role in evolution, Crews says.

Since then, Skinner has examined other chemicals, largely funded by a specific allocation—an earmark—within a Department of Defense (DOD) spending bill. His local congresswoman, Representative Cathy Rodgers (R-WA), and others earmarked \$3.7 million over 4 years to support his search for transgenerational effects from chemicals that soldiers might encounter. These studies, published over the past 2 years, showed that the insecticides DDT and permethrin, jet fuel, plastic additives known as phthalates and bisphenol A, and dioxin can all trigger transgenerational health effects in rats such as obesity and ovarian disease. Each resulted in a different pattern of methylation marks in the DNA of sperm, Skinner says. The DOD funding ended in 2011 when House Republicans banned earmarks.

Although his papers dominate the literature, Skinner notes that a handful of groups have also reported similar effects. For example, Emilie Rissman’s lab at the University of Virginia reported in 2012 that exposing pregnant mice to bisphenol A can cause changes in social behaviors and in behavior-related hormones, such as vasopressin, in their  $F_4$  offspring. Several labs have suggested that diet and stress can also cause epigenetically controlled health effects that pass through to the  $F_3$  generation. The weight of evidence has convinced some. “I’ve gone from skeptic to provisional believer,” says toxicologist Kim Boekelheide of Brown University.

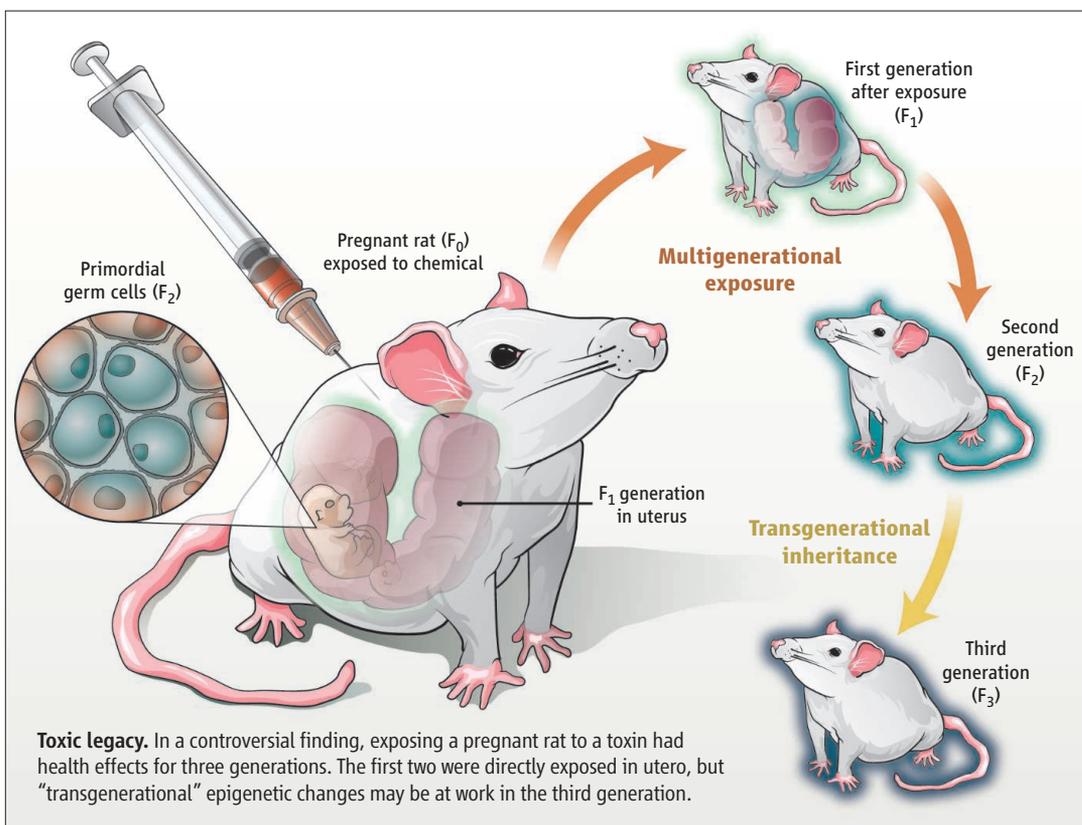
### Lingering doubts

Some of the remaining skeptics speculate (often off the record) about factors other than epigenetic changes that could explain the effects Skinner’s lab has observed. For example, some kind of change in how the  $F_1$  generation behaves or in the wombs of the chemical-exposed animals might alter the offspring’s health in a way that in turn influences the health of their descendants, says reproductive endocrinologist Richard Sharpe of the University of Edinburgh in the United Kingdom.

Skinner’s claim that he has identified permanent methylation changes in key genes, capable of resisting the normal erasure process in germ cells, has not won over the doubters. “I find the methylation differences unsatisfying,” says epigenetics researcher Oliver Rando of the University of Massachusetts Medical School in Worcester. He and others say methylation patterns vary widely among Skinner’s animals, so it’s hard to find a clear signal in the noise.

Skinner agrees that more data would help allay the controversy. If he can win funding, he wants to demonstrate that the specific methylation marks in a developing  $F_3$  or  $F_4$  generation male embryo match the methylation patterns in the adult animal’s sperm, which would support his claim that these marks are protected from the usual erasure process.

But he dismisses an approach that many have suggested could solidify his claims—that he artificially add methyl tags to spe-



cific genes to see if he can reproduce the effects he observes from exposures to pesticides and other chemicals. That’s impractical, he says, because his data suggest that “hundreds or thousands of epigenetic sites” are involved, and some affected genes may compensate for others. It is yet another example of the gulf between his views and those of geneticists, he says: They are “reductionists,” while “I am a systems biologist.”

To those who don’t flatly dismiss Skinner’s findings, he has raised a tantalizing glimpse of a new phenomenon, one that should be explored further. Transgenerational epigenetics “is either going to be blown away or it’s really going to be confirmed and expanded on and that’s what I find exciting” says epigenetics researcher Wolf Reik of the Babraham Institute in Cambridge, U.K.

Skinner doesn’t expect answers anytime soon. “I suspect that for the rest of my career, there will be skeptics,” he says.

—JOCELYN KAISER