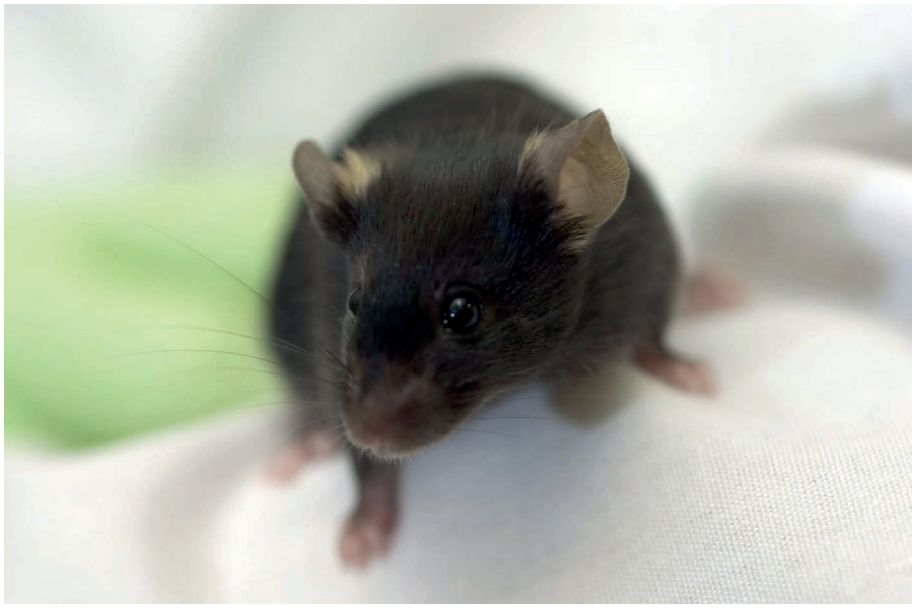

ANNE CHADWICK WILLIAMS/*SACRAMENTO BEE*/ZUMA PRESS/ALAMY

The genomes of lab mice can shift in subtle and unpredictable ways over generations of breeding.

REPRODUCIBILITY

Genome of mouse 'Eve' sequenced

Effort aims to help scientists understand how generations of inbreeding have altered the genetics of research rodents.

BY SARA REARDON

Adam and Eve, a pair of black mice, lived for less than two years and never left the Jackson Laboratory (JAX) in Bar Harbor, Maine. But since they were bred in 2005, their progeny have spread around the globe: the pair's living descendants probably number in the hundreds of thousands. They are members of the most popular strain of mice used in biomedical research, which was created nearly a century ago.

Now, researchers at JAX are reconstructing Eve's genome in the hope of better understanding — and compensating for — the natural mutations that occur in lab mice over generations. These genetic changes can cause unanticipated physiological effects that can confound experiments. Researchers suspect that such differences between supposedly identical mouse lines have hampered some areas of research.

The scientists who founded JAX created Adam and Eve's breed, which is called C57BL/6, in 1921. To keep the mice genetically similar, researchers have repeatedly bred brothers with sisters — and sold the resulting offspring to customers around the world. But this strategy created a genetic bottleneck: every generation, between 10 and 30 new mutations pop up and are passed to offspring. This 'genetic drift' quickly accumulates over the years, says Laura Reinholdt, a geneticist at JAX. The genomes of the C57BL/6 mice that the lab sells today have thousands of genetic differences from the mouse reference genome, which was created in 2002 from three mice from the substrain C57BL/6J. The genome is used as a template for developing genetically modified mice.

Other suppliers have inadvertently created divergent substrains of C57BL/6 mice when they've bought rodents from JAX and bred them over several generations. In 2016, mouse supplier Envigo in Somerset, New Jersey, found that C57BL/6 mice at 6 of its 19 breeding facilities around the world had acquired a mutation in a gene related to the immune system. The company notified the researchers who had bought these mice, and asked customers to specify which location they preferred to source mice from in the future, given that the company's stocks were no longer identical.

And although it is easy to spot a mutation that changes fur from black to white, for instance, some changes are discovered only if researchers are investigating a particular trait. One substrain of C57BL/6 mice that the US National Institutes of Health bred for 50 generations is uninterested in alcohol, whereas another bred at JAX's facility display a preference for alcoholic beverages.

GENETIC REBIRTH

In 2005, a team at JAX decided to reset the genetic clock by selling only C57BL/6J mice descended from two chosen mice: Adam and Eve. The researchers froze hundreds of embryos of the duo's grandchildren, enough to last for 25–30 years. Every five generations, the company thaws some of the embryos and raises them as new breeding pairs.

"We've not stopped general drift, but we've slowed it considerably," says Michael Wiles, the lab's senior director of technology evaluation and development, who led the project. Once the stockpiled embryos run out, however, JAX will have to start again with new breeding pairs from a much later generation.

Yet Eve's genome is very different from the 2002 mouse reference genome. In a presentation last month at the American Society of Human Genetics meeting in Orlando, Florida, JAX computational scientist Anuj Srivastava spoke about the company's effort to reconstruct Eve's genome in great detail, using three different sequencing methods. Wiles says that the genome will be finished by the end of November, and that JAX plans to publish it early in 2018.

Other mouse breeders have started their own efforts to account for genetic drift. Taconic Biosciences, a mouse distributor in Hudson, New York, restarts its C57BL/6 line every ten generations from its stash of frozen embryos. Because Taconic has bred its line separately from the JAX line for decades, the Eve genome won't necessarily reflect the genetic make-up of Taconic's mice.

Ana Perez, Taconic's global director of genetic sciences and compliance, says that the company plans to publish the genome of its own Eve. "From my perspective, each particular breeder should have their own reference genome to follow," she says.

But most researchers don't think about the differences between the various substrains of C57BL/6 mice, or how they can affect reproducibility in research, says Cory Brayton, a pathobiologist at Johns Hopkins University in Baltimore, Maryland. It is impossible to quantify how often experiments or entire research programmes are wasted when researchers realize that their supposedly identical mice have diverged genetically from the ancestor they bought from a vendor, but Brayton suspects it is common.

"If you use [inbred mice] wisely, they can be highly informative," she says. "If you use them stupidly, they may really confound your studies." ■