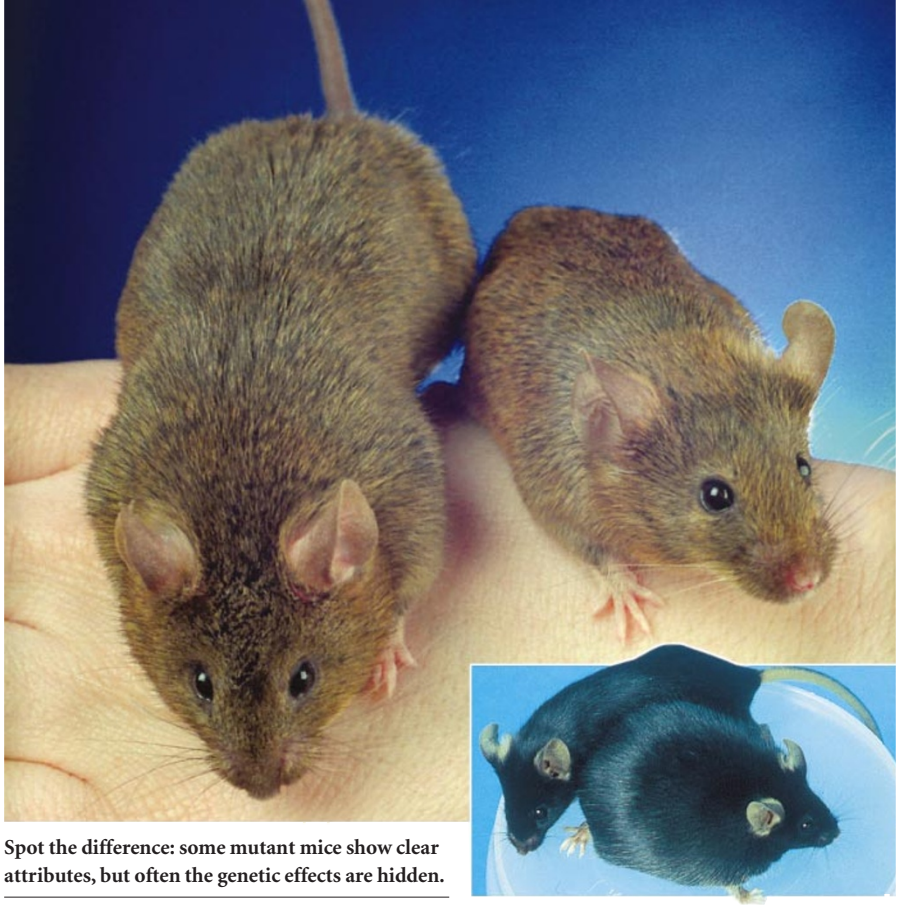
Surviving a knockout blow

Disabling a gene in one mouse strain can be fatal — but in another strain it can produce animals that seem normal. Making sense of such results requires stamina and skill, says Helen Pearson.



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Spot the difference: some mutant mice show clear attributes, but often the genetic effects are hidden.

Some mice should, by rights, be dead. At the very least, Teyumuras Kurzchalia expected his to be critically ill. But the most prominent symptom of his genetically engineered mice was a persistent erection.

The mice lacked a gene called *caveolin-1*, which is needed to make the flask-shaped pits that pockmark the surface of many mammalian cells and are thought to help assemble molecules that pass signals to the cellular interior. Disrupting the gene should have caused serious problems, reasoned Kurzchalia, who works at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden[1].

Kurzchalia's priapic rodents join a growing menagerie of mutant mice that are causing their makers some bemusement. Since the late 1980s, when 'knockout' technology to selectively disable target genes in mice was developed, it has proved a powerful tool for revealing gene function. "It's still a big dream — that knockouts will explain everything," says Josef Penninger of the Amgen Institute in Toronto, whose lab is one of the most productive in the knockout business.

But from the start, curious results have clouded this vision. In many cases, a mutant mouse does not show any obvious characteristics — or phenotype. In others, the phenotype disappears when the disabled gene is crossed into a different strain of mouse. Indeed, clear and consistent phenotypes now seem to be the exception rather than the rule.

These variable results often reflect the fact that genes acting in parallel pathways can compensate for the one that is missing. In such cases, strain differences reflect the different genetic background in which the disrupted gene finds itself. In other cases, phenotypes go undetected because the techniques used to look for them are too crude. "We've all been a bit naive," concludes Howard Jacob, who studies the genetics of complex phenotypes at the Medical College of Wisconsin in Milwaukee.

But geneticists argue that with painstaking work, these problems can be overcome — and even be made into a virtue. Variation in results from different strains, for example, provides a starting point from which to track down the compensatory genes.

Better knowledge of the characteristics of common lab strains will also help researchers to decide which mice to conduct their knockout experiments on. And techniques to find subtle phenotypes are being refined — none too soon, because knockout technology is now being joined by huge 'mutagenesis' screens in which chemical mutagens are used to derive thousands of mice in which genes have been altered at random. The first two screens published preliminary results last year[2,3], and a dozen or so similar projects are in progress.

Breeding hell

It was Terry Magnuson of the University of North Carolina at Chapel Hill who opened many mouse geneticists' eyes to the influence of the rest of the genome on knockout experiments. In 1995, his team disabled the gene for the epidermal growth-factor receptor. In one mouse strain, CF-1, the knockout embryos perished at around the time of implantation in the uterus. But in the CD-1 strain, they survived for up to three weeks after birth[4]. "From that time on, everyone started paying much more attention to the genetic background," says Magnuson.

Ideally, experiments on knockout mice would routinely include work on multiple strains. In practice, most researchers in the field argue that this is not realistic — creating a single knockout strain can take up the majority of a three-year PhD project.

But where strain differences are uncovered, there is the potential to use them to track down the 'modifier' genes that enhance or suppress a characteristic. The principle was demonstrated in mice carrying a chemically induced mutation in the tumour-suppressor gene *adenomatous polyposis coli* (*APC*). In the C57BL/6J strain, this mutation causes mice to develop numerous polyps — growths in the colon that can become cancerous. But in the AKR strain, the same mutation caused mice to develop only a handful of polyps. By crossing the two strains together, Bill Dove of the University of Wisconsin in Madison and his colleagues identified marker genetic sequences that were inherited along with the AKR mice's milder phenotype. This narrowed the search for the modifier gene to chromosome four[5]. It was eventually shown by other researchers to encode an enzyme called phospholipase A2, which is involved in the inflammatory response to polyps and is inactive in the C57BL/6J strain[6].

So far, there have been few successful attempts to follow Dove's lead using knockout mice. The problem is that the experiments are time-consuming and expensive. And if several genes are all influencing the phenotype, each with a relatively small effect, mapping them to a region of the genome becomes extremely difficult[7].

But interpreting strain variation should

be aided by attempts to collate information on the natural phenotypic differences between commonly used mouse strains. The Mouse Phenome Project, based at the Jackson Laboratory in Bar Harbor, Maine, was launched in 2000 and aims to collect baseline physiological and behavioural data, such as blood glucose levels, growth rate and daily rhythms of activity for 40 different inbred mouse strains. "It was way overdue," says project leader Molly Bogue.

In addition, the project should help researchers planning knockout or mutagenesis projects to choose the most appropriate strain to work with. Attempts to investigate genes that may be involved in narrowing of the arteries, for instance, may best be done in mice with naturally high blood cholesterol.

Character assassination

But even with such information, knockout experiments will continue to throw up mice that show no obvious phenotype. Many mouse genes belong to families whose functions overlap, and this 'redundancy' may mean that a clear phenotype only emerges when two or more genes are removed.

For example, knocking out the mouse gene *Uch-L3*, which codes an enzyme involved in breaking down regulatory, misfolded or damaged proteins, creates mice that are indistinguishable from their genetically intact relatives. But mice also lacking the related gene *Uch-L1* develop walking difficulties, paralysis and eventually die early from degeneration of nerve cells in the spinal cord[8].

Although such examples do get reported, many knockout experiments in which no phenotype could be found never see the light of day. "A lot of those things you don't hear about," says Barbara Knowles, director of research at the Jackson Laboratory. To address the problem, the journal *Molecular and Cellular Biology* has, since 1999, given over a section to knockout and other mutant mice that seem perfectly normal.

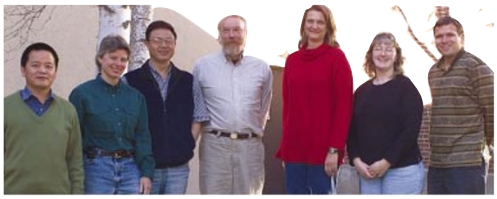
Many of these animals might reveal their phenotypes — if only researchers knew how to look for them. "I don't believe there is a single mouse that doesn't have a phenotype," says Mario Capecchi of the University of Utah in Salt Lake City, who shared a 2001 Lasker award for his pioneering work in developing the knockout technique. "We just aren't asking the right questions."

Hidden traits

In some cases, a phenotype only becomes apparent when a mouse is exposed to particular environmental conditions. Mice made by Shoichi Kado, for example, developed cancer only when their intestines were colonized by bacteria. He and his colleagues at the Yakult Central Institute for Microbiological Research in Tokyo made mice lacking both the tumour suppressor gene *p53* and *TCRβ*, a gene that regulates the immune system in the intestine. Mice reared in a standard, dirty mouse house developed intestinal tumours, they found, whereas those brought up in germ-free conditions had none[9].

The large mouse-mutagenesis projects now under way are highlighting the need for more sensitive techniques for assessing phenotypes. Rather than disrupting an entire gene, as in knockouts, the mutagens used in these screens usually create changes in a single DNA base. This may favour the creation of correspondingly faint phenotypes, which can easily be missed. "Those subtle phenotypes will be the next wave of biology," predicts Mark Fishman of Harvard University, who works on both zebrafish and mouse mutants.

Some groups are improving the sensitivity of mutagenesis screens by tweaking their genetic strategy. Bill Stanford and his colleagues at the Samuel Lunenfeld Research Institute in Toronto, for instance, are crossing randomly mutagenized mice with animals that have a mutation in a single copy of the gene *c-kit*, which is involved in the production of blood cells and has been implicated in blood cancers. The team looks for mutations that suppress or enhance this phenotype by screening mice for altered levels of blood cells. "You will pick up things you would normally miss," says Stanford.



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Taking the strain: Molly Bogue (left, in red) is heading an effort at the Jackson Laboratory to track the characteristics of various lab mice.

P. FETTERS/HHMI

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>
> **Mario Capecchi**

Sensibly, most of the big mouse-mutagenesis projects are concentrating on particular conditions, such as neurological or heart defects, and developing phenotypic screens that are optimized for the purpose. At Mount Sinai Hospital in Toronto, for instance, Janet Rossant hopes to use mouse-sized versions of medical imaging technology to identify mutants with defective growth or anatomy. The Mouse Imaging Centre will feature 16 magnetic resonance imaging scanners for high-resolution, high-throughput imaging of internal organs, and ultrasound to measure heartbeats from tiny embryos.

But even with such technological aids, phenotyping thousands of mutant mice is a formidable task. "We're going to have freezers filled with mutagenized sperm and not enough scientists to study the mice," Stanford predicts.

As experience with knockouts has shown, mouse genes often do not give up their secrets without a fight. In identifying the phenotype associated with an altered gene, and then working out why it only emerges against certain genetic backgrounds, there is no substitute for hard graft. ■

Helen Pearson works in *Nature*'s science writing team.

1. Drab, M. *et al. Science* **293,** 2449–2452 (2001).
2. Nolen, P. M. *et al. Nature Genet.* **25,** 440–443 (2000).
3. Hrabé de Angelis, M. *et al. Nature Genet.* **25,** 444–447 (2000).
4. Threadgill, D. W. *et al. Science* **269,** 230–234 (1995).
5. Dietrich, W. F. *et al. Cell* **75,** 631–639 (1993).
6. MacPhee, M. *et al. Cell* **81,** 957–966 (1995).
7. Nadeau, J. H. *Nature Rev. Genet.* **2,** 165–174 (2001).
8. Kurihara, L. J., Kikuchi, T., Wada, K. & Tilghman, S. M. *Hum. Mol. Genet.* **10,** 1963–1970 (2001).
9. Kado, S. *et al. Cancer Res.* **61,** 2395–2398 (2001).

Mouse Phenome Project ➧ **www.jax.org/phenome**