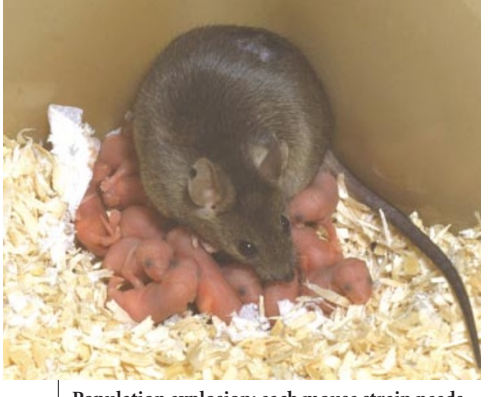
Full house

Across the world, animal facilities are overflowing with mutant mice. Jonathan Knight and Alison Abbott consider a logistical nightmare that is reaching crisis point, thanks to the revolution in genomics.

The new mouse facility at Baylor College of Medicine in Houston, Texas, is state of the art. It has robotic cage washers and separate rooms for experimental procedures. Opened in September 2000, it holds 40,000 cages. But by next spring, all those cages will be full.

It's the same story at research centres all over the world, with mice filling up the space as fast as the bricks are laid. The GBF, the National Centre for Biotechnological Research in Braunschweig, Germany, opened a new facility for 8,000 mice less than two years ago, and it's already full. A second mouse house, which will nearly double capacity, is scheduled to open in October. "And that will be full by the end of next year," says Werner Müller, who runs the centre's mouse facilities.



Population explosion: each mouse strain needs several hundred individuals to keep it going.

Overworked animal technicians can blame genomics. On 6 May, an international sequencing consortium posted 95% of the mouse genome sequence on the Internet. And on 31 May, Celera Genomics of Rockville, Mayland, published a paper on one chromosome of its rival mouse genome sequence (R. J. Mural *et al. Science* **296,** 1661–1671; 2002). But turning raw sequence data into functional information means creating many thousands of mutant mice in which individual genes are disabled. And mice take up a lot more space than fruitflies or nematode worms — a single shoebox-sized cage holds only four or five animals, and has to have space around it for ventilation and easy access by handlers.

The number of labs making and studying knockout mice — in which a particular gene is targeted and disabled — has risen steadily since the technique was developed in the late 1980s. Around 3,000 knockout strains are now available, and the number is growing exponentially.

Yet this flood of new mutants is barely a trickle compared with the inundation that is to come. As well as creating knockouts, researchers can feed the chemical *N*-ethyl-*N*-nitrosourea (ENU) to male mice to cause small random mutations in their sperm. These often don't completely disable the gene but change the properties of the protein it encodes. The treated males are bred and their offspring, each of which could carry a unique mutation, are screened for interesting characteristics, or phenotypes. Very large mutagenesis screens are in progress on four continents. The first, and still the biggest, is in Munich, where 28,000 mutants have been screened since 1998 for phenotypes ranging from allergy to changes in behaviour.

No limits

Geneticists agree that the two approaches are complementary. Whereas knockouts are the faster way to find out what a given gene might do, random mutagenesis is more efficient at finding genes involved in a particular phenotype. Mutagenesis screens can also turn up genes that a computer algorithm designed to scan the genome for sequences that look like genes would miss. And mutagenesis can generate different small mutations in the same gene, which enables researchers to investigate subtler phenotypes than those produced by the sledgehammer knockout. Mouse geneticists may eventually get what they've always wanted — one or more mutants for every gene in the genome.

Supposing an average of five useful mutations per gene, and assuming that the mouse has 30,000 genes, that would mean 150,000 distinct genotypes. Pierre Chambon, director of the Institute for Genetics and Molecular and Cellular Biology in Strasbourg, France, estimates that breeding and working on this many strains of mice would need at least 60 million animals.

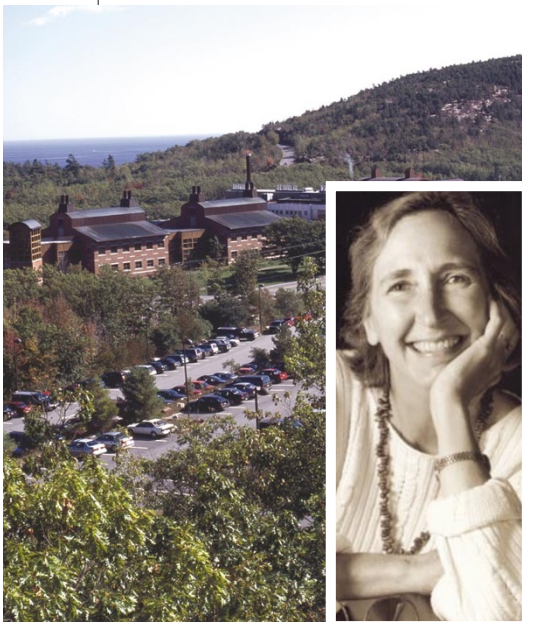
The logistics become even worse, because the effects of a knockout or mutation may need to be studied in different mouse strains. Mouse geneticists also use extra tricks to turn

their mutants into better models of human disease. Since the 1980s, they have been making transgenic mice by inserting foreign genes into fertilized mouse eggs. This approach is particularly useful when combined with knockouts; one can see if a human version of the gene can substitute for the knocked-out mouse version. And, since the early 1990s, 'conditional' knockouts have let researchers disable a target gene in a particular tissue at any time in the mouse's life. "There is really no limit to the number of strains that could be generated — you can expand to infinity," says Chambon.

Except that the space available is far from infinite. In practice, only a fraction of mutants would be under investigation at any time; the rest would be in storage as frozen sperm or embryos. But university mouse houses and central storage facilities are already creaking under the strain. "There is a chronic shortage of storage for mutants," says Steve Brown, director of the UK Medical Research Council's Mammalian Genetics Unit at Harwell, near Oxford, which houses the world's second-largest ENU screen. "It is a worldwide problem — we'll soon be swamped and forced to lose mutants, which is a pity."

This already seems to be happening. The Jackson Laboratory in Bar Harbor, Maine, which serves as the principal repository for mutant mice in the United States, is accepting a smaller percentage of applications for storage each year. Currently, Jax — as the lab is known — can accept about 70 new knockout strains a year, says Barbara Knowles, Jax's director of research. Last year, the lab turned down 50 applications. Although most of these were rejected for scientific reasons, such as duplication of an existing strain or unpublished supporting data, a few couldn't be accommodated simply because of lack of space and funding. Tak Mak, who studies knockout mice at the University of Toronto's Ontario Cancer Institute, says that he stopped trying to store animals at Jax years ago. "I got rejected too many times."

Steve Brown (left) fears losing mutant strains, and Barbara Knowles at Jax (below) is already turning away storage applications.



Deep freeze

As a result, academic researchers are having to store their mutant mice on campus. One consequence of this is that mouse strains tend to be lost when a project ends or a postdoc or graduate student moves on. "We've stopped keeping old mice around," says Richard Flavell, an immunologist at Yale University in New Haven, Connecticut. He says it's not unusual to receive a request for a mouse mutant that is mentioned in a publication but is no longer being kept.

So what are the solutions? Greater use of cryopreservation is one possibility. Frozen embryos take up very little space. But to get the 500 embryos needed to preserve a strain reliably means mating around 200 females, limiting the number of strains that can be archived each year. Jax now keeps most of its strains frozen, transferring embryos to surrogate mothers when it receives an order, which means that biologists have to wait up to six months to get their mice.

Sperm can also be frozen and, in theory, just a few males can produce enough sperm to preserve the strain. But freezing and thawing weakens sperm, making *in vitro* fertilization less successful. Thawed sperm have trouble penetrating the egg's protective coat, the zona pellucida. Possible solutions include injecting the sperm directly into the egg or weakening the zona with a laser beam or solubilizing chemicals. But there is still a way to go. "It's still not as efficient as we would like," says Larry Mobraaten of Jax.

With no great scientific advances on the horizon, researchers argue that funding agencies will have to spend more money on expanding and upgrading animal facilities. Adriano Aguzzi, who works on mouse models of prion disease at the University of Zurich in Switzerland, says that his Institute of Neuropathology spends more than 400,000 euros (US$377,000) a year maintaining its mouse colony. The university pays half, but the rest must be found from agencies that seem reluctant to invest in infrastructure. "The problem is getting bigger every day," says Aguzzi. "The pressure on space and money is a nightmare."

The US National Institutes of Health (NIH) is now responding to the impending crisis. In May, it agreed to pay for the storage and distribution of the mutants produced by an ENU screen for neurological mutants coordinated from Northwestern University in Evanston, Illinois. The size of the grant is still under negotiation, but it will include money for a centralized ordering facility and for cryogenic storage at Northwestern, Jax and the Oak Ridge National Laboratory in Tennessee.

Fertile ground: Larry Mobraaten is trying to boost the effectiveness of frozen sperm for IVF.

The NIH is also providing more than $3 million per year to sponsor the Mouse Mutant Regional Resource Centers, a collection of small academic and privately operated facilities for storing and distributing mutants. Meanwhile, the European Mouse Mutant Archive, a distributed network of centres with its hub at Monterotondo, near Rome, is on its way to becoming the European Jax.

But in the long run, the pressure may only be eased by technologies that silence genes at will, allowing functional studies to be carried out without the need for thousands of mutant strains. For example, RNA interference, a technique that silences genes by using short stretches of double-stranded RNA to block their messenger RNAs, was initially thought not to function in mammals. But recent developments have raised hopes of making it work reliably in mice.

Other researchers are banking on the identification of small molecules that selectively disable the protein produced by the gene of interest. The humble mouse, it seems, is already being lined up for a key role in biology's next revolution — that of proteomics. ■

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Jackson Laboratory
www.jax.org
European Mouse Mutant Archive
www.emma.rm.cnr.it