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The Renaissance rat

Thanks to the availability of its genome sequence, and the promise of new genetically engineered strains, the rat is restoring its reputation as researchers' favourite lab animal. Alison Abbott hails a remarkable rodent.

Rats tend to provoke strong reactions. Many people, repulsed by the rodents' tendency to feast on refuse and to spread disease, hate them. But physiologists, pharmacologists, toxicologists and pathologists are among the rat's biggest fans. For them, the rat is the most important experimental animal — and one for which they can't hide their affection.

"I love them," admits John Fozard, a pharmacologist with the drug company Novartis in Basel, Switzerland. "They're wonderful little beasties: easy to house, nourish and handle, and scientifically very reliable." Today's lab rats have a rich scientific pedigree (see 'Roll of honour', opposite), and their value is now being enhanced by the availability of the rat genome sequence, described in this issue of *Nature*[1].

Not before time, say the rat's scientific cheerleaders, who have met regularly since the 1970s under the unofficial banner of the 'Rat Pack'. For in recent years, superior genetic techniques used on mice — and information they have generated — have seen the rat's smaller cousin rise to prominence.

The rat's contributions go to the heart of biomedical research — quite literally, as it is the most significant model for human cardiovascular disease. It's a similar story for diabetes and arthritis, and many behavioural disorders. When it comes to major health issues, rats are similar enough to humans to provide useful experimental data. And pharmaceutical companies use the animals for a large proportion of their mandatory toxicity testing.

Researchers particularly appreciate the rat's relatively generous proportions, which make it easy to carry out detailed physiological measurements. Yet once the human genome project was nearing its end, it was the mouse that became the next mammal in line to have its genome sequenced[2].

It's a knockout

That decision was influenced by the views of geneticists, who have always favoured the faster-breeding mouse. But even those researchers who complained that little was known about mouse physiology conceded that mice had gained a formidable technical advantage in the form of gene 'knockout' technology. Pioneered in the late 1980s, this allowed strains to be produced that lacked a single, specific gene.

Since then, the technique has been refined to allow genes to be 'knocked in' as well as out. Today, geneticists can even make 'conditional' mouse mutants, in which a gene can be switched on and off at will in different tissues. Largely as a result of these technical advances, mice became more attractive both for genetic studies and as experimental models of human disease. During the 1990s, the ratio of scientific publications based on the rat or on the mouse decreased from 2:1 to 3:2 (ref. 3).

Until very recently, it seemed that the rat would not allow itself to be genetically manipulated in the same precise way. Knockout mice are made by performing targeted genetic modifications on mouse embryonic stem cells, which are then injected into mouse embryos. By crossing the resulting animals over several generations, you can derive a line of mice bearing the desired modification in all of their tissues.

For reasons that aren't completely understood, it has proved impossible to isolate stem cells from rat embryos. Over the past couple of years, scientists working with sheep and pigs managed to sidestep this problem in their mammals by using cloning[4,5]. Instead of modifying embryonic stem cells, the researchers manipulated a

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gene in the nucleus of adult cells, and then fused these cells with egg cells that had been stripped of their own chromosomes. The resulting clones bore the genetic modification made to the original cells.

But cloning proved exceptionally difficult in rats. At the first hint of physical disturbance, rat egg cells spontaneously activate, and then cannot be made to implant in the uterus and develop to term. This was overcome when a team led by Jean-Paul Renard of INRA, the French agricultural research agency, in Jouy-en-Josas, worked out how to delay egg activation using a chemical that inhibits an enzyme involved in the process. His team reported the successful production of rat clones last autumn[6] — and genOway of Lyon, the company that owns the technology, says that it will offer licences to academics wanting to make knockout or knock-in rats.

Under development

Others researchers, including Philip Iannaccone at Northwestern University's Feinberg School of Medicine in Chicago, Illinois, have recently succeeded in controlling the activation of rat eggs using different approaches, which they hope will also lead to successful cloning. Iannaccone wants to work with genetically manipulated rats to investigate a gene called *Gli1*, which is involved in a key developmental signalling pathway, and has also been associated with cancer in humans. Mice have not been so helpful to him, because knocking out the mouse version of the gene doesn't cause any noticeable biological changes.

It will take some time for the Rat Pack to catch up with their mouse colleagues, but the advent of genetically engineered cloned rats should help to level the playing field. And just as the mouse genome provided a host of new gene targets to knock in and out, so too will its rat counterpart. "Scientists are going to be able to choose the animals they want to work on based on biological rather than technical considerations," predicts Howard Jacob, a rat geneticist at the Medical College of Wisconsin in Milwaukee. Being able to compare both rat and mouse genomes with the corresponding human sequence will also provide a sound basis to relate the results of experiments conducted in rats and mice to human disease. "You can do the physiology in the rat and the genetics in the mouse," says Jacob.

Until now, much of the research done on rats has used outbred strains such as the Sprague–Dawley or the Wistar, which are relatively diverse genetically. But even before the advent of cloning, physiologists had access to some rats with defined genetic characteristics. Over the years, breeders have generated more than 300 inbred strains — all members of each are genetically identical. The Brown Norway, the strain whose genome has now been sequenced, has been the most popular. Other inbred strains model human disease.

Roll of honour

1621: Sexual confusion
Theophilus Müller and Johannes Faber of Italy's Accademia dei Lincei in Rome perform the first recorded rat dissection (right). Their pregnant wild specimen appears to have a uterus, penis and testes, and they describe it as a hermaphrodite. The penis was actually a clitoris, and the testes were vaginal glands.

Early nineteenth century: Into the lab
The brown rat becomes the first animal species to be domesticated for scientific use. Early physiological studies concentrate on the effects of food and oxygen deprivation.

1877: Well bred
Twenty-three years before the monk Gregor Mendel's work on inheritance comes to prominence, researchers carefully interbreed rats with different coat colours and note their results.

1900: Model behaviour
Rat intelligence and learning is tested in the 'Small maze', a labyrinth based on the maze at the former royal palace of Hampton Court in England. Mazes (below) and other behavioural tests are refined over the next century to study learning, memory and the brain.

1906: Setting the standard
Researchers at the Wistar Institute of Anatomy and Biology in Philadelphia interbreed albino rats to produce the first Wistar rats. More than half of all laboratory rats used today are thought to be direct descendants of these rodents.

1920: The big C
Rats fed on tapeworm eggs develop liver tumours within eight months, becoming the first rat model of cancer. Subsequently, researchers develop inbred strains that are prone to particular cancers. Fischer 344, for instance, spontaneously develops leukaemia and prostate cancer.

G. BLOT/RMN

1963: Under pressure
Researchers inbreed Wistar rats and produce the Spontaneously Hypertensive rat. These animals are still used to test and develop drugs to treat hypertension, and to help find genes that regulate blood pressure.

1971: Short of breath
Rats are given small amounts of a protein such as egg albumin to stimulate their immune systems. When the treatment is repeated, their lungs become inflamed, mimicking human asthma.

1980: In the swim
The Morris water maze is developed to test rat learning and memory — the animals must learn to find a platform in a murky pool of water. Easy to use, the test is widely adopted to study topics including stroke and neurodegenerative disease.

1980s onwards: Spare-part surgery
Rats have donated and received transplants of skin, heart, bone marrow, liver, small bowel, pancreas and brain tissue, allowing researchers to identify key molecules involved in rejection, and to combat this process.

2002: Remote control
Need a replacement for your robot? The radio-controlled rat could be the answer. A computer sends electrical signals to electrodes embedded in its brain, guiding the animal to turn left or right. Another signal stimulates the brain's reward centre — providing an incentive to follow these instructions. **Helen R. Pilcher**

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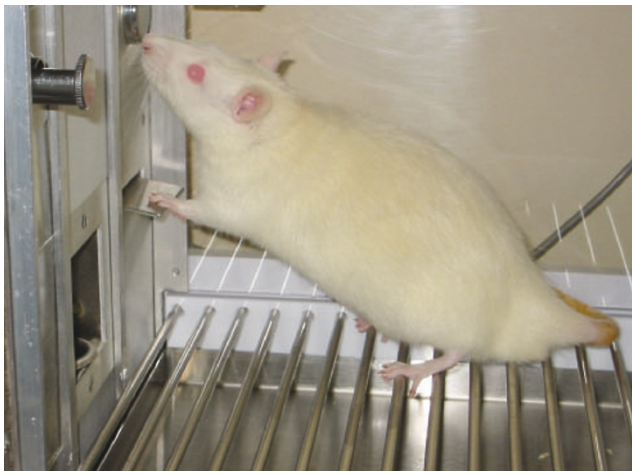
Rats of particular strains may develop metabolic diseases such as high blood pressure and diabetes — or even the rodent equivalent of depression.

And although knockout and knock-in rats are only now about to become available, it has for some time been possible to genetically engineer rats by the cruder technique of injecting a single gene into one of the unfused nuclei of a newly fertilized egg. The first transgenic rat was developed using this method in 1990 by Detlev Ganten at the Max Delbrück Center for Molecular Medicine in Berlin[7]. This rat contains an additional gene for the enzyme rennin, which triggers a sequence of events leading to rampant high blood pressure.



Model students: rats are ideal for behavioural studies because they learn tricks, such as pressing a lever to get alcohol, more readily than mice.

Bred for success

By laborious breeding programmes, scientists have also developed inbred strains that differ by a single chromosome, or just a small region of one chromosome. Called consomic and congenic strains, respectively, these rats can help to locate areas of the genome housing genes that increase the risk of succumbing to a complex disease such as diabetes. Jacob, for example, is leading a programme called PhysGen, funded by the US National Institutes of Health (NIH), which is developing consomic strains systematically and documenting their detailed physiology and pathology.

Jacob is also treating rat sperm with chemical mutagens in an attempt to generate rats with mutations in genes that change their susceptibility to disease. Other researchers have already used this method to generate a couple of rat strains in which genes that suppress breast cancer are inactivated[8]. The genomics company Ingenium Pharmaceuticals in Martinsried, Germany, began a similar project a year ago. It is making profitable use of the rat genome sequence, which has been available online for 18 months. "It was a big step forward that the rat genome, so well annotated, was made public," says Reinhard Sedlmeier, Ingenium's director of genomics.

In addition to supporting the development of new rat strains, the NIH is funding efforts to get them into the hands of researchers across the United States. In 2001, the agency began providing $1.5 million per year for the Rat Resource and Research Center at the University of Missouri in Columbia. The centre currently maintains and distributes about 30 different strains — a figure that is expected to grow rapidly. "The number of useful rat strains is exploding," says John Critser, the centre's principal investigator.

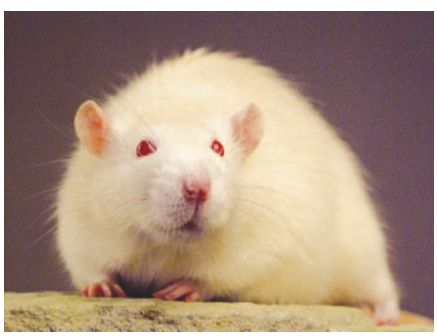
That is great news for physiologists who have flirted with the mouse in the past few years but who ultimately were left dissatisfied. Many had miniaturized equipment for measuring heart rate and blood pressure, or for imaging of blood circulation, but were disappointed with the results. "Size matters when it comes to physiological measurements," says Tim Aitman, who heads the Physiological Genomics and Medicine group at the UK Medical Research Council's Clinical Sciences Centre in London. Apart from the difficulty of working with miniaturized equipment, says Aitman, analysing the smaller quantities of blood that can be drawn from mice gives bigger margins of error.

Meeting of minds

Michael Spedding, head of research at the drug company Servier in Neuilly-sur-Seine, France, points to the superiority of rats for modelling human psychiatric stress. In stressed rats, the areas of the brain that change size are the same as those thought to be affected by stress in people. Mouse brains are just too small for researchers to measure such changes accurately.

What's more, even the most skilled experimentalists cannot get around the fact that, physiologically, rats are more similar to us than mice. For example, the mouse heart flutters away at about 600 beats per minute, whereas the rat heart beats at less than two-thirds that rate, closer to the average human resting rate of 70 beats per minute.

Behavioural scientists also prefer the larger and less manic rat. Mice are difficult to work with because they scurry around so much; they are also slow and inflexible learners. A classic experimental set-up for researchers interested in addiction, for instance, involves teaching rats to press a lever to get a reward such as heroin. "Rats are smart; they know what they are doing," says Rainer Spanagel, who studies addiction at the Central Institute of Mental Health in Mannheim, Germany. "Mice just don't have a plan." They rush about pressing the lever randomly, he complains. "You need twice as many mice as rats to get statistically sound data from an experiment." And neurosurgery, which can be used to investigate the brain structures involved in behaviour, is much more demanding in mice.



Ralph, the first cloned rat, was born last year.

As anyone who has kept rodents as pets will tell you, rats are also less aggressive than mice — which is a boon for the technicians who have to handle the animals. This is due to the species' social structure in the wild: mice live in groups where one highly aggressive alpha male monopolizes the females. Rats, by comparison, conform to the 1960s ideal of blissed-out free love, with widespread promiscuity and low levels of aggression.

The rat's natural advantages as an experimental animal, combined with a wealth of new genetic information, should lead to a surge of interest among biomedical researchers. A measure of this was seen at the biennial Cold Spring Harbor Laboratory Meeting on Rat Genomics and Models in New York state last December, which attracted 25% more abstracts and a third more attendees than the previous meeting. GenOway, meanwhile, is now trying to put together a Rat Consortium to bring together animal breeders, academics and experts from the pharmaceutical industry to discuss their priorities in the generation of genetically modified rat models. The Rat Pack, it seems, is coming of age. ■

Alison Abbott is *Nature*'s senior European correspondent.

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Rat genome
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www.nrrrc.missouri.edu

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