Model organisms in genetics research

Introduction

Almost every major breakthrough in human and veterinary medicine has depended on the use of animals in the research, development or testing of new therapies. Wellcome supports the use of animals in research if researchers can show that it is legally, ethically and scientifically justified but works hard to limit this use as set out by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (https://www.nc3rs.org.uk/).

In the UK any research with animals must comply with regulations set out by the Home Office.

The zebrafish

We have an extensive knowledge of a select group of animals, plants and microorganisms that are used in labs around the globe. The results of studies in these so-called 'model organisms' are used to help us understand biological phenomena in humans and animals.

It would come as no surprise if you hadn't heard of the zebrafish – and no, it's not just a black-and-white striped seahorse. These slender fish, characterised by white and neon blue stripes running down their flanks and fins, have become crucial model organisms over the past four decades or so. Despite being small and unknown to many people, these animals are of massive significance in a vast number of genetics labs.

Where did the zebrafish come from?

Known as *Danio rerio* in Latin, the zebrafish is native to the slow streams and rice paddies of East India and Pakistan. In these waters, they rarely grow larger than 4 cm long. This once-quiet existence has now been replaced by scientific stardom. Zebrafish have a far shorter relationship with science than many other animals: they were first used in research in the 1960s. By 1976, the USSR [the Soviet Union] had launched a zebrafish into orbit on the Salyut 5 space mission.

Despite their meteoric rise in use by scientists, many people know the zebrafish as just a popular pet. Marketed by pet shop owners as 'hard to kill', they continue to inhabit the tanks of many (possibly slightly neglectful) exotic fish owners.

Who were the pioneers in zebrafish genetics?

Hailed as the father of zebrafish genetics, George Streisinger – who worked at the University of Oregon – began the fish frenzy. A fish hobbyist himself, Streisinger was the first to recognise the potential for using zebrafish in the lab in the late 1960s.

In order to develop the zebrafish as a model animal for research, Streisinger had to create new techniques to study their genetics. At the time, Aaron Novick was the director of Oregon's Institute for Molecular Biology, and Streisinger was able to take the time he needed to work on zebrafish thanks to Novick's ability to see the long-term potential of the study. Amazingly, despite receiving funding for the whole period, Streisinger didn't publish a single paper on zebrafish in the 1970s. This would be unheard of in a modern research environment, where scientists are under pressure from research funders and universities to publish their work. But Novick trusted Streisinger, and he was right to do so.

The big breakthrough came in 1981 when Streisinger and his colleagues created cloned zebrafish. The paper on this work made the front cover of 'Nature', one of the most prestigious scientific journals. As zebrafish research began to make significant gains, Streisinger sadly died from a heart attack while scuba diving.

But, like many scientific pioneers, Streisinger inspired a generation to continue his work. One such individual was Charles Kimmel, a colleague of Streisinger's, who continued to study zebrafish throughout the 1980s and remains at the forefront of zebrafish developmental genetics today.

Why are zebrafish suited to genetics research?

Given his own experiences, Streisinger knew why zebrafish were popular among fish owners: they were hardy and easy to maintain. In the wild, they live in small freshwater pools – conditions that are easy to replicate in a tank. In addition, zebrafish are vertebrates, unlike other popular genetics model organisms such as fruit flies and nematode worms. This, along with the fact that zebrafish embryos develop outside the body and are almost completely translucent, means they are ideally suited for studying vertebrate development.

The embryos can be manipulated genetically to alter the expression of certain genes as they grow, and 84 per cent of known disease-causing genes in humans have counterparts in zebrafish. As a result, zebrafish are not just useful to developmental biologists but are also useful in studying disease states.

A pair of zebrafish can produce 200-300 fertilised eggs a week. This extraordinarily high fecundity (the ability to produce lots of offspring) allows scientists to have vast numbers of samples. Within a matter of months, these eggs will have developed into adult fish. A short generation time (the time between two generations of offspring) means that scientists can complete studies quickly and that the costs of rearing zebrafish are lower than for other organisms.

Have zebrafish been used in any landmark studies?

Zebrafish genetics had firmly established itself in Oregon in the 1980s, but it was the work of scientists outside the university and outside of the field of zebrafish genetics that really brought the field to the fore.

Christiane Nüsslein-Volhard had worked on screening fruit flies for mutations that affected their development. By looking at the mutant flies, it was possible for Nüsslein-Volhard and her colleagues to identify genes that were important for development. This work, which was carried out at the end of the 1980s, later won her the 1995 Nobel Prize in Physiology or Medicine.

By the early 1990s, fruit-fly genetics was far more advanced than that of vertebrates. To establish the significance of her work in flies, Nüsslein-Volhard turned her hand to zebrafish. The resulting project – looking for developmental mutations in zebrafish – was led by Nüsslein-Volhard at the Max Planck Institute in Germany and an ex-student of hers, Wolfgang Driever, in Massachusetts, USA. It became known as ‘The Big Screen’ and culminated in the publication of 37 papers in a 1996 volume of a publication called ‘Development’, in which 4,264 mutations were identified.

How are zebrafish being used today?

The Zebrafish Mutation Project at the Wellcome Trust Sanger Institute, Cambridge, aims to generate ‘knock-out’ mutants for every one of the protein-coding genes of the zebrafish genome – meaning fish in which the genes don’t work. This, in turn, could reveal details of the function of over three quarters of all human genes, since we share so many with fish. Currently, the project has generated nearly 4,000 different alleles (genetic variants), in over 60% of the fish’s protein-coding genes.

All the data are made publicly available and researchers can buy the fish carrying the mutant alleles. Researchers can treat the mutant strains with potential drugs to develop new therapies for genetic diseases. As the project continues, subsequent work may reveal novel treatments for a variety of diseases.

QUESTIONS FOR DISCUSSION

- List at least three advantages and three disadvantages of using zebrafish instead of mice in research. Think about the facilities needed to raise each animal and how similar the animals are to humans.
- Why do you think it’s important for scientists to make their research findings publicly available? Think about who might be funding the research and how other scientists might benefit. Who or what might be blocking the release of such information?

The mouse

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Man and mouse have an ancient love–hate relationship, and our fascination with this creature stretches far beyond cartoons such as Tom and Jerry and Mickey Mouse. Once considered an agricultural pest, the mouse, or *Mus musculus* in Latin, has been used to make huge advances in human health.

Where did the lab mouse come from?

Records of research using mice date back to the 17th century when William Harvey (an English doctor), gave a detailed description of blood circulation, but the lab mice we know today originated in 18th-century Asia. The Japanese took to collecting ‘fancy’ mice and, much like the trading of any item, the rare and unusual ones became highly sought after. Such was the popularity of the hobby that it soon spread to Europe and the US, and it held particular appeal for the Victorians in Britain.

At the same time, Abbie Lathrop, a failed poultry farmer, began breeding and selling mice from her barn at home in Illinois, initially for mouse fanciers. She picked up a new client in the geneticist William Earnest Castle at Harvard University and established herself as a long-term supplier.

Modern lab mice are believed to have descended directly from some of the original stocks. The high level of inbreeding during the fancy mouse trade has resulted in a remarkable level of genetic similarity between these modern mice, making them ideal for genetics research.

The Augustinian monk Gregor Mendel was also taken by the ‘mouse craze’. Mendel is known for his experiments on pea plants in the mid-19th century, but if it weren’t for the objections of Mendel’s bishop, mice may well have been the subjects of his studies of inheritance. Mendel was forbidden from breeding mice within the monastery, so he took to gardening as a less racy alternative.

Who were the pioneers in mouse genetics?

It wasn’t until 40 years later that Mendelian inheritance was observed in mammals. Mendel had shown that mating of two heterozygous parents – who carry one dominant and one recessive allele of a particular gene – produced offspring with a 3:1 ratio of dominant phenotype to recessive phenotype. A phenotype, put simply, is the way an organism looks, or their ‘observable traits’. Mendel found that the green gene in peas was recessive and the yellow gene was dominant: when the offspring had one green gene and one yellow gene, its phenotype would be yellow.

In 1902, French biologist Lucien Cuénot used coat colours to demonstrate the same ratios of inheritance in mice. Cuénot’s work is arguably the first example of a mouse-based genetic study.

The same year Castle placed his first order with Lathrop for some of her fancy mice. From 1900 to the publication of his final paper in 1961, Castle would devote his entire academic career to studying genetics in mammals.

Castle’s dedication and scrupulous method left a lasting impression on many of his students, including Clarence Little. Together with Little, Castle produced a series of seminal papers on coat-colour genetics in mice. Little went on to develop strains of inbred mice, descendants of which are still used in modern laboratories today. He recognised the need for genetic uniformity to be able to compare new variations and, in 1909, developed the first inbred ‘lab mouse’, DBA (dilute brown non-agouti). His inbreeding experiments led to stable strains for studying cancer. One of Little’s strains became the first mouse genome to be sequenced in 2002.

Meanwhile, Lathrop was carrying out her own studies on cancer in some of her mice and co-authored 10 papers on the subject with Leo Loeb at the University of Pennsylvania. It is not clear how much Little learned from Lathrop, but he seems to have sidelined her research.

Little established the Jackson Laboratory in Maine, USA, in 1929. To this day, the centre remains a haven for mouse genetics and more than 3 million 'Jax' mice of almost 10,000 varieties are shipped to laboratories all over the world every year.

Why are mice suited to genetics research?

We have more in common with mice than we may like to think. Beyond our mutual enjoyment of cheese, almost every gene in the human genome has a counterpart in the mouse.

In a short film that was shown to visitors to the Jackson Laboratory, Little described the mouse as "a miniature human being". This has since held true; from their metabolism to the structure of their internal organs, mice are very similar to humans.

In addition, our strong affiliation with mice in the past has allowed us to develop an extensive knowledge of how they work. We are now able to manipulate, insert and delete genes with relative ease. This, paired with the physiological resemblance, has been crucial in developing how we understand disease.

Have mice been used in any landmark studies?

Since the beginning of the 20th century, studies using mice have accounted for dozens of Nobel Prizes, and they continue to win. 13 of the last 20 awards (up to 2018) in Physiology or Medicine have all been for work involving mouse models. Mice have been involved in all kinds of research, from the discovery of penicillin to understanding the brain.

Of particular note is the work that won George D Snell the 1980 Nobel Prize in Physiology or Medicine. Snell, who was also a student of Castle, used mice to uncover the genes that make up the major histocompatibility complex, a group of genes central to the immune system. Snell also created the first congenic mouse strain, animals that differ from other strains by a single gene.

More recently, Mario Capecchi, Martin Evans and Oliver Smithies shared the 2007 Nobel Prize in Physiology or Medicine for developing ways of introducing specific gene modifications into mice using embryonic stem cells. They were able to generate offspring that expressed specific gene mutations that were carried (but not expressed) by the parent.

What research in mice is being done today, and where is it going?

Given our long history, mice will no doubt play a major part in research that tackles future challenges to human health. Arguably the greatest challenges today include understanding brain disorders, and mice are already being used in some very promising research in this area.

Optogenetics is a revolutionary technique that enables scientists to target individual neurons in the brain with remarkable precision. First, researchers insert genes that encode light-activated molecules called opsins into specific mouse neurons. Then, they fire a laser onto the brain, which induces an action potential and mimics a particular disease. The field of optogenetics is already helping us to understand the neuro-circuitry involved in Parkinson's and addictive behaviours.

In the UK alone, 2.6 million mice were used in scientific procedures in 2018. This represents almost three quarters of all scientific procedures on animals. Total numbers of animals used in scientific research are falling in the UK, where scientists now operate under the principles of the "3Rs" – replacement of animal studies with other methods, reduction of the numbers of animals used and refinement of the techniques used on animals to improve welfare. In the future, advances in technology may allow computers to more accurately simulate disease states. Lab-grown tissues could also help reduce the need for animals. Currently, no methods provide a completely suitable alternative, but scientists continue to aim to reduce, refine and – hopefully, one day – replace the use of animals in research.

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- Annual statistics of scientific procedures on living animals Great Britain 2018
- Making sense of optogenetics
- George D. Snell, the father of immunogenetics and the MHC
- Nobel Prizes in Medicine

QUESTIONS FOR DISCUSSION

- Thinking about independent and dependent variables and the scientific method, why is it important to have genetically similar mice for experiments?
- The discoveries made by Capecchi, Evans and Smithies allowed scientists to introduce specific mutations into a mouse's DNA. Considering what we know about the effect of some DNA mutations, how can these techniques be used to study disease? There might be some useful information on the Nobel site (see the References).
- Populations across the world are ageing. Why is a better understanding of diseases such as Parkinson's becoming more important?

The nematode worm

We have an extensive knowledge of a select group of animals, plants and microorganisms that are used in labs around the globe. The results of studies of these so-called 'model organisms' are used to help us understand biological phenomena in humans as well as animals.

At approximately 1 mm in length and transparent, the nematode worm known as *Caenorhabditis elegans* (*C. elegans*) might seem an unusual choice of animal to study in such detail. But this peculiar soil-dwelling roundworm has proved itself to be a vital research tool. In fact, it's arguably the single most described animal in scientific literature.

Where did the nematode worm come from?

In the lab, the nematode worm lives on nutrient-enriched agar jelly in a Petri dish. But this clinical environment is worlds apart from its natural, and significantly dirtier, habitat. Although researchers had a wealth of knowledge about the genetics and physiology of the worm, its natural history has remained largely a mystery.

The wild-type (naturally occurring) strain of nematode worm that was used in the pioneering work of the mid-20th century was first located in British mushroom compost. These types of rotting environments are the Park Lane and Mayfair of the nematode Monopoly board, as they are rich in bacteria for the worms to feed on. By 1956, small-scale research on the nematode worm had begun in Berkeley, California.

Who were the pioneers in nematode worm genetics?

During the 1950s and early 1960s, researchers made a number of crucial discoveries in molecular biology. Suddenly the mysteries of biology seemed eminently solvable.

While working in Oxford, the South African researcher Sydney Brenner was one of the first people to see the structure of DNA, discovered by the Cambridge scientists Watson and Crick. Clearly impressed, Brenner soon moved to Cambridge and began working with Crick on bacterial genetics.

By the 1960s, Brenner was looking for more complex biological problems, such as those in development and the nervous system. He wanted to apply the skills gained from studying bacterial genetics to multicellular life.

Drawing on the literature that had begun to emerge on the nematode worm, he concluded that it ticked all the boxes for his research (see below). His decision to focus on *C. elegans* ultimately led to the research landscape we see today – within which the genome of the nematode has been completely sequenced and characterised.

Brenner remarked, after winning the 2002 Nobel Prize in Physiology or Medicine, that choosing the right organism is "as important as finding the right problems to works on".

Why are nematode worms suited to genetics research?

The nematode worm offers simplicity to a researcher, as it has fewer than 1,000 body cells when fully grown. Despite lacking bones, a heart and a circulatory system, it has muscle, nerve and gut cells. It is also transparent, which means that its cellular activity can be easily observed.

The sheer scale of the work on nematode worms in the 1960s and 1970s (particularly that coming out of Cambridge) was one of the driving factors behind making the nematode worm a great model organism. The relationship between worm and scientist was relatively new, and this translated into a group of people excited about breaking new ground in the field of genetics.

Have nematode worms been used in any landmark studies?

Geneticists are working in a 'post-genomic era', in which the genome sequences of a significant number of organisms are known. The question now is: how can we find out what each of these genes does? As the first organism to have its whole genome sequenced, the nematode worm is the perfect candidate for research that tackles this question.

Two scientists, Craig Mello and Andrew Fire, developed a technique in nematode worms that is now used for understanding the function of specific genes as well as in the development of new medicines based on switching off rogue genes. Using their technique, referred to as RNA interference, or RNAi, scientists can silence a specific gene, to give them an indication of what the function of that gene may be. The first RNAi drug, Onpattro, was approved in 2018 for treatment of a rare genetic condition that causes proteins to build up in the nervous system.

The beauty of the technique, which was originally published in the journal *Nature* in 1998, is that it's relatively easy to do in nematode worms. By simply injecting the worms with specific double-stranded RNA sequences, genes can be targeted for silencing. This technique has revolutionised how we investigate the function of genes and ultimately can give an indication of which genes are affected by certain diseases. For this work, Mello and Fire shared the 2006 Nobel Prize in Physiology or Medicine.

What research in nematode worms is being done today and where is it going?

The nematode worm continues to be an important model organism. While it is especially challenging to unravel the mysteries of ageing in complex organisms such as humans, scientists are now looking at nematode worms in an attempt to understand, at a much more simple level, how living things age. Interest in *C. elegans* for ageing studies began in the 1980s, when mutant worms with altered lifespans were discovered. As ageing research continued into the 1990s, the gene FOXO was identified as having an important influence on lifespan in both nematodes and fruitflies. Later, it emerged that gene variants of the human FOXO gene are associated with longer life. Overall, hundreds of genes involved in controlling lifespan have been identified. So if life-extending therapies ever become available, chances are they'll have come courtesy of a tiny transparent worm.

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- The Nobel Prize in Physiology or Medicine 2006
- Using *C. elegans* for aging research

QUESTIONS FOR DISCUSSION

- The discovery of RNAi by Mello and Fire allows us to investigate genetic disease. Discuss how the ability to silence specific genes can allow us to investigate the causes of genetic diseases.
- Discuss what Brenner meant when he said that choosing the right organism can be just as important as asking the right questions in scientific research.