The new **revolution**

Science writer **Jodie Harris** visits one part of NERC's Molecular Genetics Facility to meet the researchers involved in a new wave of genetic research.

For many people, it is impossible to imagine life without the internet or text messaging – the speed at which we can obtain information has transformed our lives. So too with genetic research. New technologies are completely changing the way we understand life on Earth. Over the past decade, this technological revolution has culminated, perhaps most famously, in the mapping of the entire human genome, our genetic blueprint.

NERC's Molecular Genetics Facility has three laboratories at Edinburgh, Liverpool and Sheffield offering a complete range of cutting-edge DNA technologies. Together, they place NERC science at the forefront of genetic research in the UK.

At the invitation of the head of one of these laboratories, Terry Burke, I visited the Sheffield lab to find out more. The lab, which is part of the University of Sheffield's Department of Animal and Plant Sciences, is home to two very important resources: a state-of-the-art molecular genetics laboratory and the highly experienced staff who have trained and supported 74 young scientists in genetic technologies.

Scientists, mainly PhD students from across the UK, often stay at one of the facility's labs for one season before returning to their own university with a new set of skills. With such a constant flow of people, the facility is a melting pot of fantastically varied projects, from understanding shorebird mating systems and working out just how far fig wasps fly from tree to tree, to discovering new organisms playing a role in the nitrogen cycle.

Terry Burke explained their ethos. 'We like to support genetic research from inception to publication and the training component is very important. NERC scientists are typically working with unusual organisms and need specialist help. For example, they might be working on a really obscure plant where it's really hard to extract the DNA, or they might be working on unusual DNA sequences. Our facilities are built to deal with curious requests.'

'We're still a long way from understanding the function and role of every gene but we're climbing that curve. Liverpool and Edinburgh have, together, just launched the latest revolution in sequencing technology that is so powerful it can simultaneously sequence several bacterial genomes in an afternoon – it used to take months. This opens up entirely new approaches to scientific enquiry', added Terry.

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Eric Wanders/Foto natura/FLPA

Night-time adultery

PhD student Clemens Kuepper, who has been working at the Sheffield lab since 2005, has a professional interest in adultery. For biologists like Clemens, molecular techniques such as DNA fingerprinting have been a revolution. It allows them to infer kinship between animals without knowing all of their family history. This goes a long way to explaining cooperative behaviour: animals tend to invest time and energy in their own offspring, or those of close relatives.

Theories on the evolution of cooperative behaviour hinge on the idea that individuals cooperate to promote their own genes. But in the late 1980s, the first DNA fingerprinting uncovered wholesale adultery throughout the animal kingdom: a father cannot be too sure he is raising his own child.

The Kentish plover is a shorebird with an interesting line in parental care. In a Mediterranean population of breeding pairs, only half are monogamous, staying together to care for their young. In a few families, the male abandons the family, but in around forty percent of cases the deserter is female.

Both parents want to re-mate, but at least one of them must remain with the brood. This is what ecologists call sexual conflict over care. Clemens wants to know if a pair's relatedness plays a part in whether the couple stay together or not, and whether this influences the survival of the offspring.

He explained, 'We know that relatedness increases cooperation. Being cooperative could mean that one of the parents looks after the young, so that the other can go off to mate again. But if a breeding pair is too closely related, inbreeding results in all kinds of deleterious traits becoming expressed in the offspring. We think there must be a trade-off between the advantages of mating with a relative versus the advantages of bringing in new genetic diversity by mating with a less related partner. We want to find out if there's an optimum relatedness.'

The first DNA fingerprinting uncovered wholesale adultery throughout the animal kingdom.

The problem is that, in the field, it is extremely hard to view these extra-pair copulations, as they often happen at night. Also, several hundred plovers can breed together, making it tricky to work out the family pedigree. Instead, Clemens took the easier route of paternity and kinship testing in the comfort of the lab.

So far, paternity tests support the idea that the more related the pairs, the more likely it is that one of the pair members is unfaithful, and the chicks do not necessarily belong to both caring partners. The next question is why is this happening?

'We don't know how this behaviour works; whether it's a passive mechanism or if it could be that somehow the birds know about their relatedness,' said Clemens.

Environmental factors have a strong influence on offspring survival. Whether a single-parent family does well or not depends largely on the quality of the environment. Plover chicks are precocious. They are up and running within hours of hatching. The parent watches for predators, leads them to feeding grounds and protects them from other aggressive families. If it is a good environment, there will be plenty of food, few predators and little competition – one parent is enough to look after the chicks.

Very few bird species have such a diverse mating system as the Kentish plover. Many other species of plover have a similar lifestyle and habitat but most of them are monogamous, suggesting a genetic influence to the Kentish plovers' behaviour. Clemens said, 'Another aim of this research is to look at whether the different regional populations have different genetic profiles that could explain this. We hope that one day, we'll be able to identify the genes that predetermine whether a female stays or deserts her family.'

A Kentish plover from Mexico. Contrary to its name, the Kentish Plover is a cosmopolitan species, found on almost all continents.

The pros and cons of cohabitation

PhD student Paula Senior found that bat paternity turns out to be no less interesting than the plovers'. In summer in Yorkshire's Wharfedale, female Daubenton's bats group together to rear their young in colonies along the lower stretches of the valley. But not everyone joins the party; just ten percent of the males roost here with the females, the rest live in the higher parts of the dale. Paula, from the University of Leeds, conducted paternity tests to reveal the consequences of living in different habitats for male mating success.

An explanation for the separation of males and females is that females need a constant supply of food during pregnancy and lactation, whereas males can survive with a more interrupted food supply by entering a sleepy state called torpor which helps them conserve energy. High up in the dale, the food source sometimes becomes patchy, making foraging difficult for females.

But why is there segregation amongst the males? Is the lower dale the better habitat for all of the bats: so good that the majority of the males are excluded and must make the best of the harsher conditions upstream? Alternatively, are the males higher in the dale simply avoiding the intense competition for food in the lower dale… and thriving?

These two explanations have very different consequences for the two groups of males. If the males living in the maternity roosts with the females are the fittest and strongest and are pushing their weaker colleagues up the dale, they have not only secured the best food and the best roost in town – they probably also get to father most of the offspring too.

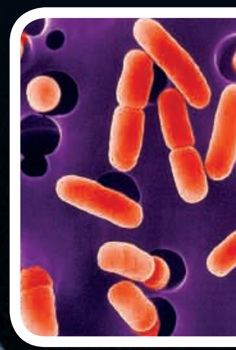
With the help of the facility to work out the genetic profiles of the adults and juveniles, Paula's team did indeed find that the males living alongside the females father proportionally more of the offspring than the other males.

'Females become sexually receptive at the time they leave the roost in late summer to go to a swarming site. This is where bats mix and mate with other populations, which maintains genetic diversity. The males roosting in the maternity sites could get the early advantage by mating with the females before they set off,' she said.

Bats are unusual mammals in that mating happens a long time before the female ovulates in the spring, and the sperm is stored throughout the winter in the lining of the oviduct. Early mating could allow sperm to obtain the key positions within the oviduct wall.

There might also be a difference in male bats' ability to mate during swarming. One of Paula's supervisors, John Altringham, explained, 'Our guess is that during the summer the males at the maternity sites may be excluding the other males. They may also out-compete them during swarming. Swarming involves a lot of chasing, but it's difficult to see exactly who is chasing who. Also, it's possible that when the males from the upper dales arrive at the swarming sites, they are less able to spend time here because they have fewer reserves.'

Sexual segregation is very common in mammals, birds and fish. It has been studied mostly in ungulates and there is a lot of speculation as to whether it is down to differential use of resources, for example, where the sexes can cope with different quality environments, or whether some individuals are actually excluded from prime areas by others. Because this study revealed that the Daubenton's bats higher up the hillside are less reproductively successful, it suggests that they have been actively excluded. This study could have parallels in wider studies in the animal kingdom.



Daubenton's bat.

Arco Images / Alamy

The males roosting in the maternity sites could get the early advantage.

The bacteria hunters

Searching for the unknown is tricky and requires a bit of lateral thinking. Scientists from a NERC consortium studying marine microorganisms are using the Sheffield and Edinburgh labs to determine exactly what thousands, if not millions, of unknown organisms are doing.

Classically, people have grown microorganisms in cultures and then worked out their function. But most microorganisms won't grow in the lab. So, in the last ten years, scientists have developed new molecular techniques that can analyse all the genetic material in a particular environment, such as a bucket of water, or a handful of soil or sediment. This new field is called metagenomics. Instead of trying to pinpoint particular organisms within the sample, they identify the genes for specific functions such as nitrogen cycling or carbon fixation.

In the Colne Estuary, Essex, scientists from the Universities of Sheffield, Cardiff and Essex, want to know which microorganisms are responsible for cycling nutrients. In particular the groups at Sheffield and Essex are keen to see how these organisms deal with inorganic nitrogen from agricultural fertilisers and sewage treatment plants when it reaches the estuary. High levels of nitrates can result in eutrophication – where runaway growth depletes oxygen levels suffocating other aquatic life.

Organisms alter levels of nitrate by a process called nitrate reduction. Historically, our knowledge about nitrate reduction has been based on bacteria such as *Escherichia coli (E. coli)* and *Pseudomonas* species that researchers can grow in culture. However, these don't actually appear at very high levels in the environment and are not responsible for most of the nitrate reduction. What scientists lack is an understanding of which organisms are present and what genetic systems are responsible for these processes. They have assumed that the processes are the same as those in well-studied organisms such as *E. coli*, but this might not necessarily be the case.

The team at Sheffield have sequenced DNA from a mud sample using the Edinburgh lab and compared the extracted DNA with a global database of the genomes (complete genetic profiles) of more than 600 bacteria.

So far, the scientists think that they have characterised one of the dominant groups of genes in bacteria responsible for nitrate reduction found within the sediments at the high-nitrate end of the estuary. Mark Osborn, who leads the Sheffield group, said, 'It turns out that the genomes most closely related to the DNA found in the samples are from delta-proteobacteria. What is interesting is that these sequences look like they are most closely related to organisms which have hitherto been known for their role in sulfate reduction – not nitrate reduction.' If understanding is based on organisms, such as *E. coli* and *Pseudomonas*, that are historically isolated from soils or medical environments, have researchers been studying the wrong organisms? And are they the appropriate models for understanding the fate and turnover of nitrates in estuaries? The team hope the facility will provide some of the answers.

The future of genetic research

Terry Burke explained that the facility is evolving from the analysis of anonymous genetic markers towards understanding the functions of specific genes, keeping the facility at the cutting-edge of environmental research. He predicts that in the foreseeable future the facility will be able to compare whole genomes instead of just small sections.

'We'll be able to answer questions that we can't even think about now – such as the origins of species, species relationships, adaptations to the environment and much more,' he added.

Further information about the research

'The pros and cons of cohabitation': scientists working on this project were Paula Senior and John Altringham both from the University of Leeds, and Roger Butlin from the University of Sheffield. 'Sex and segregation in temperate bats', *Proceedings of the Royal Society* B, 272, 2467-2473 (2005).

'Night-time adultery': Clemens Kuepper is based at the University of Bath and can be contacted by email: c.kupper@bath.ac.uk.
For more information: www.bath.ac.uk/bio-sci/biodiversity-lab

'The bacteria hunters': scientists working on this project were Mark Osborn (A.M.Osborn@sheffield.ac.uk), Ashley Houlden and Cindy Smith with collaboration from David Nedwell, University of Essex, and Andy Weightman, University of Cardiff. This project is part of the NERC consortium Aquatic Microbial Metagenomic and Biochemical Cycling headed by Ian Joint at the Plymouth Marine Laboratory.
For more information: www.genomics.ceh.ac.uk/mm

More information on genetic research: www.darwin.rcuk.ac.uk

NERC has three laboratories within its Molecular Genetics Facility. The facility offers many different technologies and services to the NERC science community, and each laboratory has strengths in particular areas.

Molecular Genetics Facility – Edinburgh offers custom DNA sequencing and sequence analysis services using both conventional and the latest high-throughput technologies. The strength of the lab is in its ability to help researchers integrate and analyse the huge amount of data they generate. For example, researchers can get support to make their results available on the web. Academic Manager: Mark Blaxter, email: mark.blaxter@ed.ac.uk

Molecular Genetics Facility – Liverpool specialises in microarray technology that can measure the activity of thousands of genes simultaneously. This gives scientists a uniquely detailed, genome-wide, overview of biological responses from microbes, plants and animals. Scientists also have access to high-throughput sequencing technologies and receive support right from design and planning, to getting results and analysis.
Director: Andrew Cossins, email cossins@liv.ac.uk

Molecular Genetics Facility – Sheffield specialises in developing and applying genetic markers to answer questions in population genetics and behavioural ecology. Head of Laboratory: Terry Burke,
email: T.A.Burke@Sheffield.ac.uk

To find out more about the facility's technical equipment and support visit: www.nerc-molgen.org