Can science save endangered species?

It's generally agreed that humans have a responsibility to preserve the planet's other species. This may involve protection and conservation, but in some cases we can give plants, insects and animals a more direct helping hand.

By **Tim Fryer**

The Guam Kingfisher is now extinct in the wild following the introduction of brown tree snakes

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ACCORDING TO THE International Union for the Conservation of Nature (IUCN) 67,222 species of animals, of which 5,674 are mammals, are on its 'red list' of endangered species – fish and birds are the most heavily hit. Of these, 1,337 (109 mammals) are already either extinct, possibly extinct or extinct in the wild, and a further 13,267 (1,204 mammals) are critically endangered, endangered or vulnerable.

Mammals are highlighted because it includes many of the world's most iconic species such as polar bears, tigers, blue whales, snow leopards and rhinos, but ornithologists might argue that various eagles, ospreys and, of course, the dodo are equally charismatic.

Perhaps the most important question is why extinction of plants, insects and animals actually matters all that much. Certainly we are all aware of the demise of the dodo and if we had our time again we would do our best to ensure its survival, but even with the knowledge we now have it has done little to help the northern white rhino, the last male of which died this year. Then there are species such as Australia's Bramble Cay mosaic-tailed rat; who noticed when that became extinct two years ago? Has it made any material difference to the ecosystem it left behind?

"Once you start removing large predators from the food chain, then you start destabilising the ecosystem, which could cause a massive ecosystem collapse all of its own," says David Williams-Mitchell, director of communications at EAZA – the European Association of Zoos and Aquaria. It's a case, then, of all flora and fauna, great and small, having their roles to play.

"The bottom line is that pretty much all life on Earth, with the exception of the deep

The Least Water Lily, now only found in one place in England, is the subject of a conservation project involving Kew's Millennium Seed Bank

Propagation experiments as part of the Least Water Lily conservation programme

sea and hydrothermal vents, depends on plants," concurs Keith Manger, manager of the Millennium Seed Bank Partnership, the Kew-led project to preserve plant species from around the world. "We have a duty of care to the plants – to creatures on the planet that depend on them so that they can also survive – but also these plants have a huge potential use to man."

Mass extinction

Arguably we are now experiencing the sixth mass extinction event to affect planet Earth. A mass extinction event means a large number of species are wiped out over a short period of geological time. Estimates of how many species we have changes all the time – generally considered to be between two and five million but some estimates go up to 30 million – so to determine exactly when we reach the threshold is virtually impossible. However, with 67,000 species on the IUCN Red List it is clear there is a problem and the List provides a focal point.

Williams-Mitchell adds: "Given we're in a conservation crisis, we have to use the tools which are available to us, and the best way to go about doing it is not through emotion. It's really through scientific practice.

"Of course there's an emotional element there as well. We need to educate people to not change their phone every three months, to not just throw their plastic in the sea, all this kind of stuff. That's the educational side of zoos – they can help with that. But for the really hard-science, down-to-earth conservation work that's being done, if the techniques exist, then we'd be stupid not to use them."

So what can be done? There is no silver bullet – there are too many causes ranging from climate change to habitat destruction, >

Hogar is an Amur Leopard, the world's rarest cat and the subject of an EAZA breeding programme

< and from pesticides to poaching. The article by Helena Pozniak in this issue ('The tech battle we must not lose') looks at how technology is being deployed to combat the poachers, but very different technologies and initiatives are emerging that could be a lifeline.

> 'We have to work on human behaviour change first, and also solid conservation work, in order to save species and habitats.'
> **David Williams-Mitchell** EAZA

Breeding programmes

Time to meet the rarest cat in the world – the Amur leopard. Hogar is an inhabitant of the Big Cat Sanctuary in Kent, and one of only 200 Amur leopards distributed in zoos around the world. In the wild there are only about 80, all surviving in a dedicated conservation area in Russia. It sounds precarious, and it is, but a decade ago there were only 30, so the programmes put in place are working, albeit slowly.

ALTA (The Amur Leopard and Tiger Alliance) was set up to protect both species in the conservation area. The Amur tiger, also called the Siberian tiger, now has a wild population of nearly 600, but none in Siberia. Actions from ALTA include setting up anti-poaching teams, compensation schemes for farmers who lose livestock, forest-fire-fighting measures and monitoring of populations.

Hogar was born in captivity in the Czech Republic in 2007 and sired two cubs in 2012 at the Big Cat Sanctuary. These have now joined the breeding programme at other zoos. In fact all animals that are part of EAZA's EEP (EAZA Ex-situ Programme) are owned by the programme, they cannot be bought or sold. There are around 400 of these programmes, each focusing on a separate species.

"What these breeding programmes do," explains Williams-Mitchell, "is lots of demographic and genetic analysis of the population that we have and the population that's available beyond our network as well. And then we try and find the best matches between animals so that we can continue to maintain a high level of genetic diversity and demographic diversity as well.

"If you're a zoo and you receive a recommendation to breed, then you don't have much of a choice in that."

EAZA has around 300 zoos and aquaria as its members and works with similar organisations around the world. All affiliates feed into a database called the Zoological Information Management System, or ZIMS for short, which is run by a non-profit organisation called Species 360. Information includes studbook data, medical data, feeding data, sperm counts, menstrual cycles – all information that zoologists, population biologists, geneticists and others can use to either do research or to make recommendations for breeding.

While the database is a great use of IT, there is some serious science behind its content as well. It goes way beyond putting two animals together that are both feeling frisky at the same time. They have to be the right animals and their mating must aim to produce the right offspring. Neither is it just a numbers game, although objectives vary from programme to programme.

Dr Graham Etherington is senior computational biologist in the Di Palma Group at the Earlham Institute in Norwich, whose work includes genetics research. He says: "We use DNA sequencing to sequence the genomes of multiple individuals from an endangered species to see how much

CASE STUDY

MAURITIUS PINK PIGEON

The Mauritius Pink Pigeon was in dire straits in the 1970s. Famous zoologist Gerald Durrell found only 20 of them existing on their native island and distributed some to zoos in an attempt to protect the species. The Pink Pigeon gained some fame as a conservation project and now, after a number of measures including habitat recreation and predator removal in Mauritius, the global population has increased to a still perilous but nonetheless more healthy 400.

"Unfortunately they are quite aggressive and stupid," concedes research student Camilla Ryan, who is working at the Earlham Institute on a project with the Mauritius Wildlife Foundation to look at the genetics of the pigeon. She adds: "At the moment our work here is academic research but we hope it will directly benefit the pigeon. So far the zoo population has been kept separate from those in the wild, so we are hoping that we can use captive birds to introduce further genetic variation."

variation there is in those populations. It gives us an idea to the amount of inbreeding in the population and also if there are any specific gene families, like immune genes or development genes, that are particularly affected by the declining population."

In other words if a species is to adapt and survive it needs a degree of diversity. When populations of a species get very low then there is increased danger this diversity can be lost. Earlham Institute's head of genomics pipelines, Dr Karim Gharbi, takes up the story: "Breeding is important, but breeding in a genetically smart way. It's misguided sometimes to try and breed species that are endangered – accelerating expansion but resulting in generating organisms that are not very diverse. So it's important when we are breeding to preserve species that this is done in a way that will maximise the retention of the genetic diversity.

"If it becomes so genetically non-diverse then anything, any change in the environment, might wipe them out because there is no individual in the population that has the ability to adapt to the change."

Genetic editing

Genetically guided selective breeding is one thing. More controversial is genetic modification. Dr Gharbi continues: "The CRISPR technique is what people are really excited about at the moment, possibly to target particular genetic defects in the genomes to try and remove disease alleles [gene variants]. It's not GMO – genetically modified organism – as we've known it in the last few decades where you introduce a piece of foreign DNA coming from a different species into another species. This technology is different, it's acting directly on the genome of a particular species, a particular individual, and editing it."

With tools of such potential it may be that species that are functionally extinct – where animals are alive but there seems no chance of it making a successful comeback – could still have a future.

Williams-Mitchell says of the possibilities of genetic editing: "I don't think it will happen for the northern white rhino, although I may be wrong. But in the future, if we get down to such a bottleneck as that, it may be possible to use genes from those animals to artificially inseminate a similar subspecies or something like that. There are many techniques which are coming up that could be useful."

Cold storage

When those techniques exist it will be essential to have the DNA of the original species. Frozen Ark is a project that intends to achieve just that. This project was actually inspired by a biological control project that went wrong (see Rebecca Northfield's article 'Top 10 invasive species' in this issue), resulting in the extinction of native Partula snails in French Polynesia. As a last-gasp effort to save the species, tissue samples were taken and frozen to preserve the DNA. This enabled the continuation of the speciation study and an international captive breeding programme was established at the Zoological Society of London (ZSL), with the aim of eventually returning the small land snails to their natural habitat.

The project developed and not at a snail's pace – there are now 48,000 samples from 5,000 species held across the Frozen Ark consortium. There are 27 consortium partners based in five continents which include zoos, universities and museums.

"In theory, we would like to have a representation of all the endangered species within our consortium collection," says Mafalda Costa, research assistant at the Frozen Ark Project, Cardiff University, "but this in itself is an ambitious goal to achieve. Firstly, the list of endangered species is continuously changing, secondly many species that are already potentially endangered will go extinct without being discovered."

Different types of samples are useful in different contexts. A conservation (population) genetics or a pedigree study will probably only need access to tissue/DNA or non-invasive samples; a comparative genomics study requires high-quality tissue/DNA samples, while a breeding programme of an endangered species will rely on the use of cryopreserved gametes [reproductive cells], especially if the individuals are no longer able to reproduce without assisted reproductive technology >

< or have died since. Samples held include the scimitar-horned oryx, which is now extinct in the wild, and Hogar's Amur leopard relatives.

Preserving DNA

Knowing how to store the samples is difficult when future technologies for making best use of these samples is not yet known.

"Our main research focus is to understand the best methods for collection, transport, storage and curation of different types of biological samples from a large variety of animal species," says Costa. While it is acknowledged in many fields of biology that appropriate preservation of tissue samples is vital to isolate good-quality DNA, there is a surprising lack of comprehensive and systematic studies testing available methods for specific tissue storage of wild animal species and how continued re-use of frozen samples impacts DNA stability.

Costa continues: "To address some of these questions, we are currently running a series of experiments on DNA degradation. We are specifically testing how temperature and humidity and increased freeze-thaw cycles impact DNA quality."

Lower temperatures are best, is the general thinking, with liquid nitrogen (at -196°C) considered the gold standard for sample preservation. Although expensive, it is used for long-term storage of DNA, RNA, protein samples, and also for reproductive cells. This reduces the likelihood of damage from ice crystal formation and virtually all biological and chemical processes are halted at this temperature.

In university laboratories, DNA is most commonly stored at 4°C, -20°C or -80°C, either dry or in a buffer fluid or ethanol, which avoids chemical and enzymatic degradation. Costa says: "Ethanol is easy to use, but it is relatively expensive, flammable and evaporates quickly.

"There are not many long-term studies looking at DNA degradation over time under different temperatures, but it is acknowledged that samples maintained in pure ethanol at -80°C remain suitable for use for years, while samples stored in liquid nitrogen are useable for decades. Ultimately, the final intended use for the sample determines whether that sample is still valuable or not."

Biodiversity means the preservation of all species of flora as well as fauna, so the challenges of storing and collecting animal tissue are shared by Keith Manger and his team at the Millennium Seed Bank (MSB) at Wakehurst in Sussex.

Manger says: "When you've dried the seed, cleaned it, hermetically sealed it and banked it at -20°C, theoretically most seeds will live for hundreds or thousands of years. Within that there are some species that are a bit shorter-lived which we also put in liquid nitrogen – we do cryogenics here as well. About 10 per cent of our seeds are triplicated into liquid nitrogen."

Seeds are 'triplicated' because the MSB works with partners, so all individual collections are stored in two places to ensure preservation. The cryogenics therefore forms a third option. In total, MSB has seeds from 189 countries, with in excess of 80,000 seed collections and 34,000 species.

"When you enter the main dry room downstairs and the cold room complex, which comes off the dry room, you're actually entering the most biodiverse place on planet Earth, probably the universe," says Manger. "It's a very special place and it has very high security as a result, partly because the collections are priceless and they're living and they're there for a very long time."

The vault, which is below ground, has been designed to last 500 years irrespective of what happens at ground level.

Collections vary enormously as seeds are not standard, nor are the environments they are taken from. Seeds can be as small as 100µm for orchids and as big as a fist for some palms, but 1-2mm is typical for wild plants. Collecting, cleaning, drying and storing are all technical disciplines in their own right and MSB has been instrumental in creating global standards and protocols for them.

Humidity is one of the key factors in storing seeds successfully. Manger observes: "Equilibration relative humidity [ERH] measurement is something that we brought to the table globally and that pretty much originated for seeds in the Millennium Seed Bank. We want to be environmentally responsible as well – our cold rooms are possibly some of the lowest-energy cold rooms you'll find: each of the 150-cubic-metre cold rooms running at -20°C are actually consuming just 1kW of electricity, which is ridiculously low."

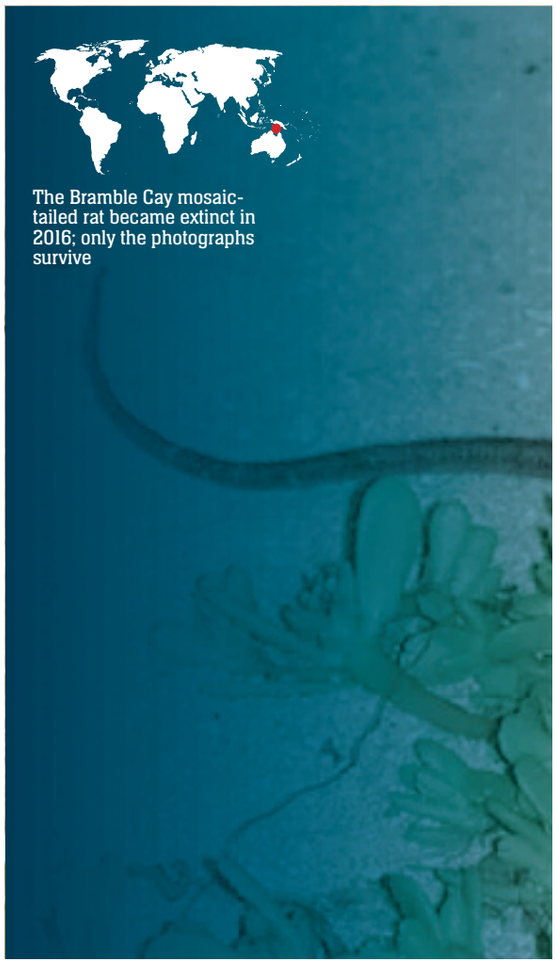
There are also extensive research facilities at MSB, even living quarters for visiting scientists, as investigation into the species, how to store, germinate and propagate, is a large part of the objective.

One recently announced project aims to conserve England's last population of the IUCN Red-listed *Nuphar pumila* – the Least Water Lily, to give it its common name – which can be found at Colemere, Shropshire, and the MSB is involved in research behind its genetics and propagation. A fuller understanding of both is critical if it is to survive in not only England, but also Scotland and other countries where this rare lily currently struggles for survival.

Making changes

Most people who mix scientific and conservation interests don't believe that a solution lies in the laboratory. Williams-Mitchell sums up the sentiment: "We have to work on human behaviour change first, and also solid conservation work, in order to save species and habitats. We can't expect engineering of the weather, for example, to be something that will save us from climate change. It's too easy, and it doesn't really address the main issue, which is that we live unsustainably.

"If we have technology to continue to allow us to continue living unsustainably, that doesn't really help anything." •



The Bramble Cay mosaic-tailed rat became extinct in 2016; only the photographs survive

Genetics is guiding breeding practice and could play a role in preventing extinctions

Inside the cold room at Kew's Millennium Seed Bank, where seeds are stored at -20°C