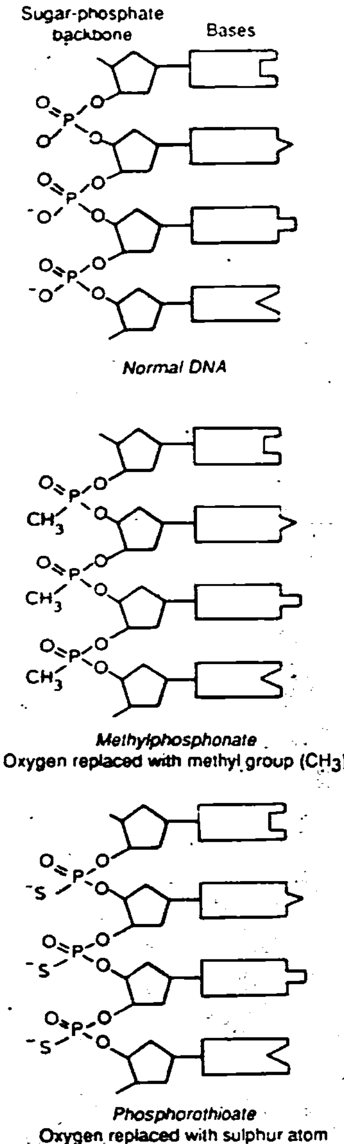
How to go wild

Zoos rightly gain kudos by providing animals for reintroduction to the wild. But setting captive-bred animals free involves far more than simply opening the cage door

Mark Stanley Price and Iain Gordon

THROUGHOUT the world, we are driving species towards extinction at an ever increasing rate. In the early 1960s, zoos began to emphasise their role as arks that might save such endangered species. By breeding animals in captivity and then releasing them in protected reserves, we might rescue some species. Yet well-meaning conservationists often give too little thought to how the animals will fare once they have gone "back to nature". Ecologists now realise, for instance, how much harder it is to survive as an orang-utan than an oryx. Reintroductions of some species, it seems, are fraught with difficulty and may even be doomed to failure.

In the past, many animals have been reintroduced to the wild in an unplanned and haphazard manner. In the 1970s, for example, conservationists returned the nene, the Hawaiian goose, to its native islands after it bred successfully at reserves run by the Wildfowl Trust in Britain. But the release of 1244 birds on Hawaii and 391 on Maui Island over 16 years has failed: the nene has not established a self-sustaining population anywhere in the Hawaiian archipelago. The reintroductions failed for several reasons. The nene spends much time on the ground, and the adults moult when leading their young, and so cannot fly, making both the adults and young



Chemical analogues that mimic antisense DNA may prolong its life in the cell. Researchers have synthesised these two variations by replacing oxygen atoms

inoculations by altering them so that they produce more antisense RNA (see "Brave new botany", *New Scientist*, 3 June 1989).

No one knows why the antisense RNA protects plants from the virus. Unlike most viruses, CMV uses RNA rather than DNA as its genetic material. The virus does not need to produce mRNA to make its coat protein because it can trick the ribosomes in the plant cells into using the RNA already carried by the virus. The antisense RNA engineered into the plants binds to the RNA that codes for the coat protein and so it might prevent ribosomes from using this RNA to synthesise proteins. But there is another possible explanation. The sequences that the virus needs to replicate its RNA are near the gene for the coat protein. The antisense RNA might also bind to these sequences and so perhaps it protects plants by preventing the virus from replicating rather than by reducing the supply of coat protein. Support for this idea comes from work by Keith O'Connell at Monsanto in St Louis, Missouri. Researchers have produced tobacco plants that make antisense RNA from the coat protein gene that does not bind to the nearby replication sequences. These plants are not protected from the virus.

Experiments to investigate the implications of antisense technology for medicine are still at an early stage. Both cancer and viral diseases might one day be treated by injecting short lengths of antisense DNA, synthesised artificially. This would remove any need to alter the cells of the patient by "gene therapy".

The obstacle to all cancer therapies is the need to kill or inhibit cancerous cells without harming healthy cells. The pairing of complementary sequences of bases in DNA or RNA is one of the most accurate systems of recognition found in nature; harnessing it with antisense DNA may be the key to the treatment of some cancers.

Several cancers are known to be associated with mutations in a particular gene. For example, 40 per cent of cancers of the colon are associated with mutations in a small section of the gene known as c-Ki-*ras*. David Tidd from the University of Liverpool is exploring the feasibility of using antisense DNA in the treatment of these cancers. He predicts that antisense DNA may be able to distinguish between normal and mutated genes. This would mean that it could be used as the basis of a therapy that inhibited only the cancer cells bearing the mutated genes, perhaps even converting them back into normal cells.

This idea might also work in the treatment of viral diseases. So far research has concentrated on inhibiting HIV, the virus that causes AIDS, which, like CMV, uses RNA as its genetic material. John Goodchild from the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, has attempted to use short sequences of antisense DNA to stop HIV from replicating in cultured cells. He tested 20 different antisense DNA sequences, all of which were complementary to regions of the viral RNA. They all inhibited the virus to some extent, and the best of them compared favourably with drugs now in use. Although antisense DNA can inhibit the virus in tissue culture, it will be a long time before researchers can do clinical trials because there are many problems in administering it safely.

Mimicking DNA makes sense

One of the problems of using antisense DNA is that it is rapidly broken down by enzymes inside cells. So researchers must dose cells with too much antisense DNA so that enough survives to bind to mRNA. For this approach to be successful, we must find a way to protect antisense DNA from these enzymes. In view of this, many researchers are looking at the effects of using chemical analogues that mimic antisense DNA.

Chemists can make such analogues by replacing one of the oxygen atoms in the phosphate groups of the sugar-phosphate backbone of DNA with either a methyl group (CH_3) or a sulphur atom (see Figure). These DNA analogues are not recognised by the enzymes that would otherwise destroy them. Unfortunately, however, the analogues do not seem to be much better than normal DNA at blocking genes. This is because the effectiveness of antisense DNA partly relies on enzymes that destroy the mRNA strand when it is bound to a strand of DNA. If the enzymes do not destroy this bound mRNA, it is eventually freed from the DNA and so able to direct the synthesis of proteins. The difficulty with the analogues is that the enzymes are less able to destroy mRNA bound to them. So although the analogues survive for longer than normal DNA, in the end they lead to the destruction of a similar amount of mRNA.

Tidd created DNA analogues in which he replaced the oxygen in the bases at the ends of the strands with a methyl group. These analogues appear to be promising because mRNA attached to them was destroyed while the analogues were still protected from the enzymes that destroy normal single strands of DNA.

Another way of improving the effectiveness of antisense DNA is to bind chemicals to the DNA strand that help to stabilise the hybrid double helix made of strands of mRNA and DNA. The idea is to prevent the mRNA from escaping before it is destroyed by enzymes. Claude Helene from INSERM in Paris has pursued this approach with encouraging results.

Charles Jennings and his colleagues at Harvard University are working to combine antisense technology with the recently discovered ribozymes. Ribozymes are enzymes made from RNA that are able to cut through other RNA strands. By attaching antisense RNA to ribozymes, they have been able to make them bind to and cut specific mRNAs found in eggs of the frog *Xenopus laevis*. So far they have achieved this only in the test tube, but they hope to be able to extend the technique to cells in living organisms. If they succeed, antisense RNA-ribozymes hybrids might make antisense technology even better at switching off genes.

Even if these improvements fail, antisense technology is already established as a powerful technique in both pure and applied research. Once a gene has been isolated, introducing an antisense version can switch it off in almost any organism. By switching off selected genes, scientists will be able to analyse how genes control complex biological processes such as growth and development—the knowledge we need for further advances in biotechnology and medicine. □

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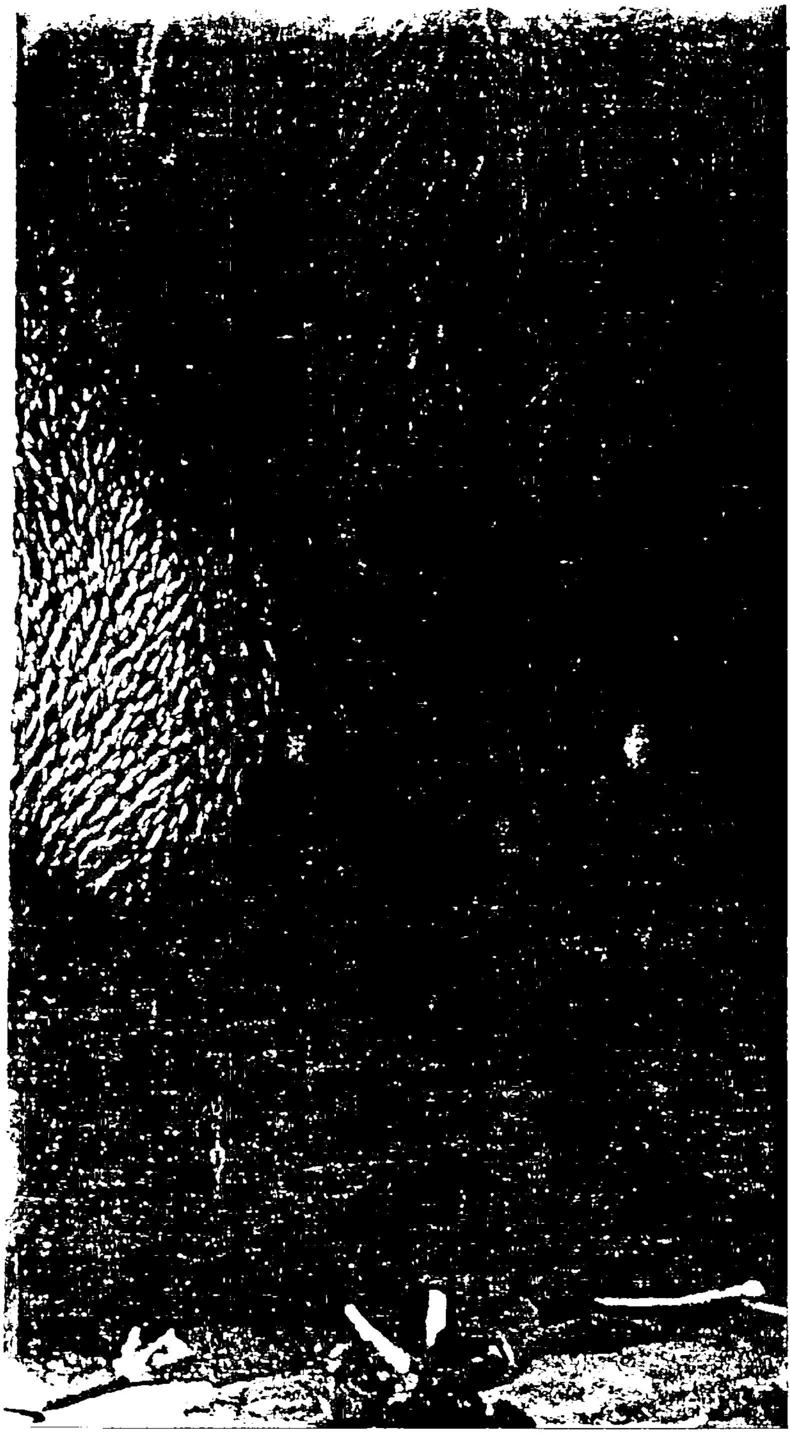


vulnerable to hunters, and to predators that people have introduced. Another factor which may have reduced their breeding success was that biologists released most of the geese in the mountains, which the birds originally used for only a short period each year.

Conservationists now realise the importance of a scientific approach to managing reintroduction. The Oman project to reintroduce the Arabian oryx is a shining example of this new attitude. Hunters exterminated the last wild herds of the Arabian oryx in Oman in 1972. Biologists in the US began to try to establish a captive herd of this antelope in 1963, and by the late 1970s the American herd was thriving. Between January 1982 and 1984, a team headed by Mark Stanley-Price released a total of 21 oryx as two herds into the Jiddat-al-Harasis, a stony desert plateau in central Oman. Seventeen of the founder animals came from the American herd, two from the Gladys Porter Zoo in Brownesville, Texas, and the remainder from the San Diego Zoological Society. One male oryx originated from the Jordanian national herd in 1984. Several calves were born in a large enclosure erected in an area of natural vegetation.

The first step towards any successful reintroduction is a feasibility study. We must know why the species became extinct in the wild, whether these conditions persist and whether suitable habitat remains to support a population. One such study, to examine the possibility of reinforcing the last remnants of a unique population of skinks, a type of lizard, living on Round Island, off Mauritius, concluded that reintroduction was likely to fail. The researchers found that the island was still ridden with introduced rats which had caused the original population to decline.

In the case of the Arabian oryx, little was known about the original populations, and so conservationists working to reintroduce this species relied on information about a close relative, the fringe-eared oryx in Kenya. Studies showed that oryx live in herds made up of males and females in roughly equal numbers. Bachelor herds do not occur, and single territorial males are rare. Herds establish a straightforward hierarchy that involves all females and males above the age of about seven months. In Oman the project workers tried to establish a herd of not less than 10 animals, with a roughly equal number of males and females and a range of years. The animals lived together in the enclosure long enough to develop a stable social group. When the oryx first arrived in Oman, Price and his colleagues kept them in groups in small pens for a few days before releasing them into the enclosure. Covering an area of 100 hectares, the enclosure was large enough for the animals to graze as a unit, and contained a variety of natural types of vegetation. As there was no artificial shade or shelter provided, the animals had to learn to exploit their environment in the full face of the desert climate.

Ready for freedom

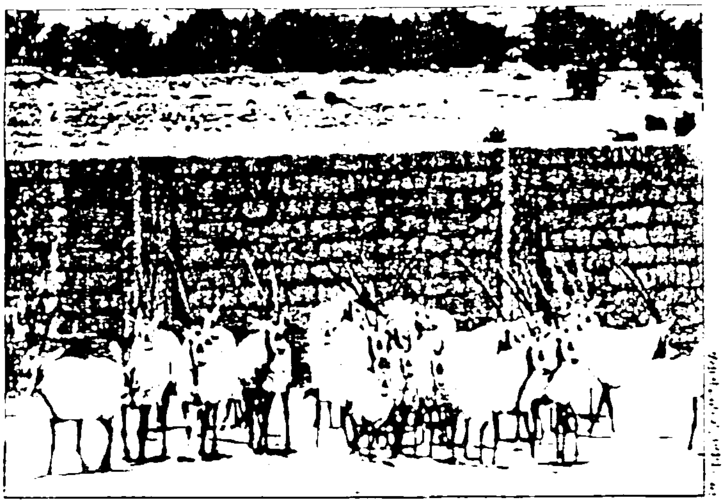
The herd had to meet two main criteria before we released it. First, the oryx had to have developed a stable and unambiguous hierarchy, with male A, say, always dominating male B in social encounters. Secondly, they had to exhibit the full range of social and sexual behaviours that are normal in a wild herd. For instance, dominant male oryx defecate in conspicuous places in a squatting position. In the first herd assembled in Oman, a male in the enclosure assumed dominance at the age of 24 months. But it was another 18 months, following the release of two older males into adjacent pens, before he started to squat-defecate in the enclosure. This indicated his social maturity and increased the likelihood that he would keep his herd together in the desert.

Monitoring the animals after release is also crucial. In the short term, the way released animals disperse is one measure of their response to the new environment. Knowing why any of the animals die also enables us to improve methods of managing them immediately, or at least before any more are released. In the reintroduction of another oryx species, the scimitar-horned oryx, to the Bou-Hedma National Park in Tunisia, the dominant male killed a young oryx calf, and several members of a herd of a related species of addax antelopes reintroduced to the same area. The project workers removed this aggressive animal from the main herd and kept a close watch on the interactions of the oryx and addax herds.

This emphasises the importance of being able to manipulate and manage the released animals in their native environment. The monitoring phase is often neglected once the released animals appear to be surviving. Because no one monitored the fate of the Hawaiian geese after their release, we still do not really know why only four of the 1600 released over 16 years managed to survive.

Not all species are equally amenable to reintroduction. We can draw up general rules to determine whether a species might be successfully re-established. Two contrasting reintroductions, the Arabian oryx and the orang-utans, illustrate the importance of trying to do this.

Many people have tried to reintroduce orang-utans to their native habitat. But it is not easy for many reasons. These



Captive-bred oryx adapt well to the wild, if the social relations within a herd are well established before the animals are released

animals need to learn much about their exceptionally complex environment and how to relate socially to other orang-utans. Conservationists soon found that reintroduced orangs were more likely than native animals to be killed by predators, probably because many of the newcomers often move about on the forest floor whereas the wild animals spend all their time in the trees.

Social skills are also very important, and depend in part on each animal's previous history: for example, animals that have spent a period in the wild in early life find it much easier to lead an independent life when reintroduced. Orang-utans also fare better if they have spent time with wild or more experienced individuals before their release.

In the wild, however, adult male orangs and adolescents live mostly alone, associating with females only to mate. Females live with dependent offspring. So orang-utans rarely interact with complete strangers, making the reception of the reintroduced orangs that much more fraught. Reintroduced animals generally move only about half a kilometre from where they are released because of the aggressive behaviour of wild orangs. This reluctance to move greatly reduces their chances of self-sufficiency because wild orangs need to move over wide areas of forest, following the fruiting pattern of the trees.

By contrast, Arabian oryx have a single social unit, the mixed male-female herd with long-term bonds between individuals that can be cemented before release. The herd then moves freely as a self-contained unit: the first herd of Arabian oryx established a home range of 1700 square kilometres two years after its release.

The desert environment also made life easier for the reintroduced oryx. This habitat lacks diversity: the Oman desert has only three species of low trees and between 30 and 40 common grasses, herbaceous plants and shrubs. Once released, the oryx ate almost every species available, and both after rains and in the dry season they ate staple grasses supplemented by a few ephemeral plants. In contrast, the rainforest that the orang-utans inhabit is a complex, mixed forest made up of a small-scale mosaic of habitats, with some trees as tall as 60 metres. Each orang-utan in the Sumatran forest may have to range over 2 to 3 square kilometres to find enough food, and individuals of the species live far apart. Unlike the oryx the orang-utans have a diverse diet. They eat fruits, leaves, bark, shoots and sometimes fungi and birds' eggs. These foods are highly dispersed: of the 28 chief species of fruit they eat in Borneo, 18 had densities of less than two trees per hectare. Each species of fruit is also available for only a very short period of time. So a rehabilitated orang-utan has to develop a varied diet through experience in a forest with complex and irregular fruiting patterns.

The reproductive biology of the orang-utans also makes it more difficult for them to establish a self-sustaining population. Despite the similar weights of Arabian oryx (55 to 75 kilograms) and orangs (30 to 80 kilograms) and gestation periods of 266 and 245 days, oryx become sexually mature much earlier and reproduce thereafter much more frequently than orang-utans. A wild female oryx has her first calf before she is three years old and can then calve every 9 to 12 months. In Oman the number of animals in the herds increased by 22 per cent each year. By comparison, wild orangs conceive for the first time when they are between 13 and 15 years old and produce offspring only once every six to seven years thereafter. So their low rate of increase hinders the establishment of a viable population unless vast numbers are released.

The reintroduction of Arabian oryx into Oman also showed that success partially depends upon the ability to monitor the population's performance. Monitoring in the desert and in the orang-utan's forest requires very different techniques. Visibility in the oryx's desert environment is good and the terrain is easy for a vehicle to move through. Because oryx live in herds and remain in a relatively circumscribed area for weeks or months, they are easy to track down daily. The forest is the opposite in every respect: visibility is low, and locating the orang-utans on consecutive days is almost impossible because the dispersed nature of their food supply requires them to be constantly on the move. All of this makes the reintroduction of orang-utans labour-intensive and costly compared to oryx, particularly as each orang-utan needs intensive rehabilitation before it is released.

All these factors permit the orang to be firmly placed into a class of animals whose biology and ecology makes them difficult and expensive to reintroduce. It is a highly specialised species, with a low reproductive rate, living in a hazardous and competitive environment, which allows researchers to observe released animals only sporadically. But the Arabian oryx project is Oman shows how successful reintroductions can be. Large ungulates, hoofed mammals, appear to be good candidates for reintroduction. Most successful efforts show how important it is not merely to have good scientific data on the species, but also a deeper understanding of how naive animals bred in captivity will perceive and respond to their native, but novel, habitat. Our success at reintroducing wild ungulate species may be helped by our long history of managing and domesticating their relatives, cattle, sheep and goats. But we are a long way from being able to "save" any given species by reintroducing zoo-bred animals. □

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