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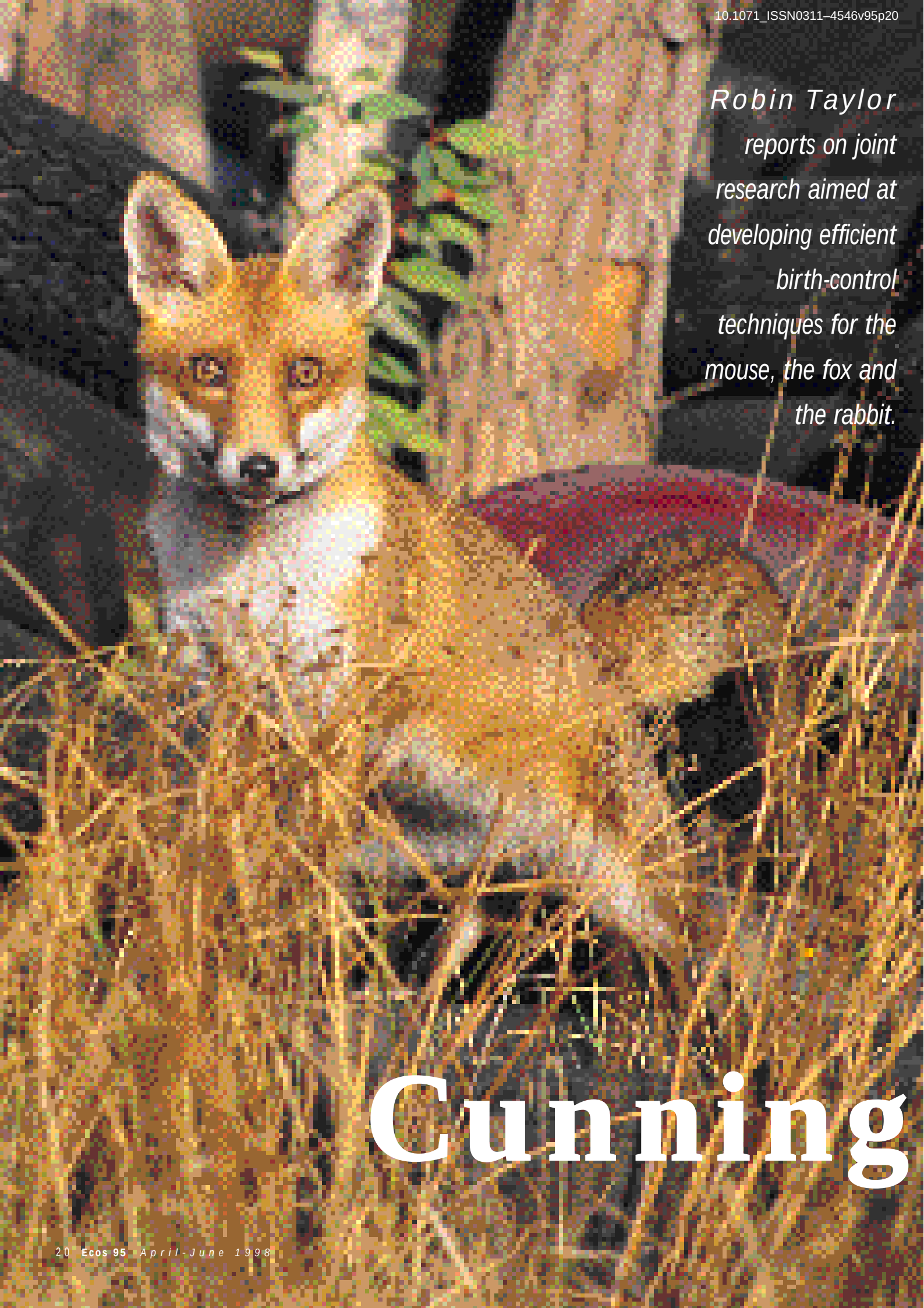


Cunning

Robin Taylor

reports on joint research aimed at developing efficient birth-control techniques for the mouse, the fox and the rabbit.

Australia has suffered massively since European settlers colonised the continent two centuries ago. Among the scores of introduced mammals that have gone wild, three in particular have caused enormous environmental and economic damage. Rabbits, cats and foxes are a dire threat to many native species: today 57 native mammals are classified as endangered, and 54 others are under threat.

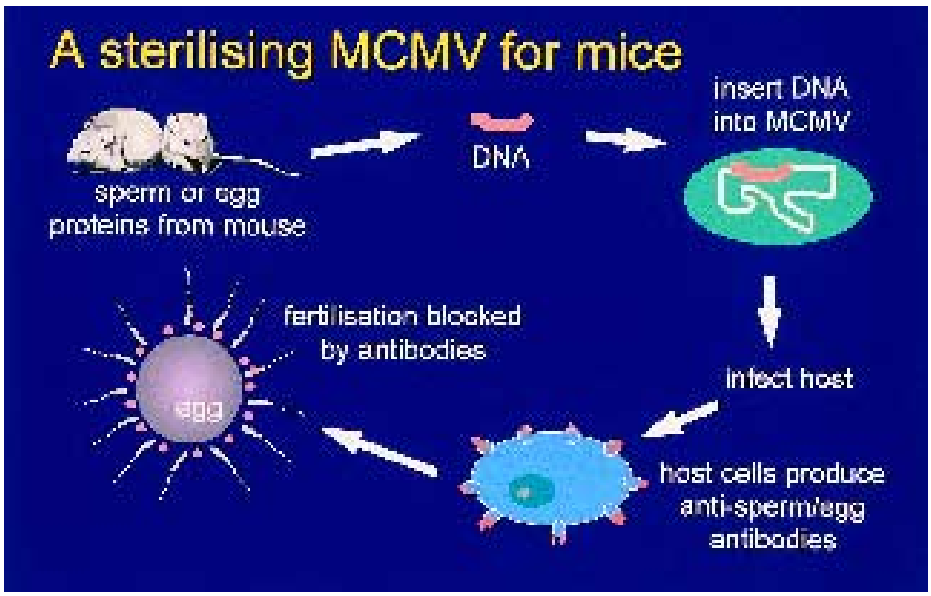
In the rangelands that span some 75% of the continent's dry interior, the European rabbit, the fox and the cat have contributed to the extinction of almost the entire suite of the mammals weighing between 35 grams and 5.5 kilograms. By itself, the rabbit costs Australian agriculture close to 600 million dollars annually in lost production.

Traditional control measures for feral mammals – poison baits, trapping, shooting and warren-ripping – have relied on killing their target. But all have failed to contain their numbers. The one notable success against Australia's worst pest, the European rabbit, came in 1950 when CSIRO released the myxoma virus, which causes the lethal disease myxomatosis. In 1969, the European rabbit flea was introduced to enhance the virus's spread in drier regions.

The original Brazilian strain of myxoma virus killed more than 99% of rabbits when it was released into the field. Myxoma virus remains an important control agent for rabbits, but co-evolution between microbe and host has resulted in reduced mortality. Some strains kill only 10% of rabbits; the survivors become immune to reinfection by more virulent strains.

The latest weapon against the rabbit, rabbit calicivirus, has been spectacularly successful in some areas, but not in others (see story on page 25). A campaign in Western Australia to control foxes has seen the return of endangered animals, but its success relies on continued lethal baiting. As for feral cats, the other main introduced predator of Australian wildlife, and mice, which periodically erupt in Australia's grain-growing areas, effective controls simply don't exist.

At the Vertebrate Biocontrol CRC, researchers are taking a different approach to feral pest control. Instead of trying to kill them, they are developing ways of limiting their birth rates, using a process known as immuno-contraception. The pioneering research involves designing vaccines that 'trick' the animal's immune system into treating certain proteins found on sperm and egg cells as foreign, that is, as antigens. The immune system of the vaccinated animal then produces antibodies that bind to these proteins in its own testis or ovary, preventing fertilisation (see diagram).



Strains of cytomegalovirus (MCMV), a benign virus common in wild mice populations, are being genetically engineered to carry an infertility agent among mice populations.

An immuno-contraceptive vaccine aimed at preventing fertilisation can be targeted at two main sites: antigens important for fertilisation on the surface of sperm, or those involved in sperm attachment to the outer coat of the egg (the zona pellucida).

The mouse

In 1997, researchers from the Vertebrate Biocontrol CRC, working at CSIRO's Division of Wildlife and Ecology, Canberra, and the John Curtin School of Medical Research at the Australian National University, achieved a major breakthrough. They showed in laboratory enclosures that a virus could be used to carry an infertility agent that induced temporary infertility in mice.

Dr Ron Jackson, Dr Deborah Maguire, Dr Lyn Hinds and Professor Ian Ramshaw infected laboratory mice with an ectromelia (mouse pox) virus equipped with a gene coding for zona pellucida proteins.

Stimulated by the virus, the mouse's immune system formed a barrier of antibodies that stopped the mouse sperm attaching to the surface of the egg, preventing fertilisation. The researchers showed that, following infection by the engineered virus, laboratory mice were immunised against reproduction for periods up to 28 weeks: potentially long enough to prevent mouse populations reaching plague levels.

These experiments offered proof that fertility control can be achieved by using viruses as vectors for immuno-contraceptive vaccines. It's a breakthrough that has direct implications for the control of mouse plagues experienced by farmers and country towns in eastern Australia.

The mouse pox virus will not be used to carry the infertility agent into populations of the wild house mouse field, because it is not present in Australia (outside the controlled conditions of the John Curtin Medical School). Performing the role as 'taxi' is mouse cytomegalovirus (MCMV) – a benign virus common in wild populations.

Dr Mal Lawson and Professor Geoff Shallam at the University of Western Australia (a collaborator in the Vertebrate Biocontrol CRC) are genetically engineering MCMV to include the zona

Scientists are investigating the potential impact of birth control on mice in the wild.

Above: Dr Lyn Hinds surgically sterilising a mouse. Trials have established that sterilising 60-65% of the population appears sufficient to control mouse numbers.

Above left: Enclosures at Walpeup in the Victorian Mallee, used for mouse experiments.

Far left: Mice are fitted with radio collars, enabling their movements to be monitored over a 12-month period.

pellucida proteins. They have already made a marker strain of the virus which is being used to infect mice and to compare how well different strains compete.

This year, leader of the mouse species group, Dr Grant Singleton, and his colleague, Lisa Chambers, will conduct trials in enclosures at Walpeup in the Victorian Mallee. A virus-free strain of mice will be infected with MCMV to study its rate of spread in the population and whether it persists in the enclosure after the mice are removed.

Because the virus needs close contact to spread, either by sexual transmission or in saliva, the researchers need to know a lot about mouse social behaviour in the wild. Using mice fitted with radio collars, Chambers has been following their movements over a 12-month period, collecting information about their home range, movements and habitat use. Her results will provide information about the likely rate of spread of the virus through the wild population.

Chambers is also investigating how populations in enclosures respond when a proportion of females are sterilised. In an operation that could almost be described as micro-surgery, Dr Lyn Hinds carried out tubal ligations on 60-70% of female mice; they were still hormonally competent, but not fertile. In a second group, the ovaries were also removed.

The researchers wondered whether allowing females to remain hormonally competent and keep their status in the population would prevent younger mice from breeding. But they found no difference between the two groups.

In the treated populations, some compensation occurred through bigger litter sizes and mice becoming pregnant more often, but Chambers says that sterilising 60-65% of the population appears to be sufficient to maintain mouse numbers at an acceptable level.

Unless the infertility is maintained in each successive generation, the population quickly rebuilds. This indicates that a bait-delivery system would not be appropriate for mice, because baiting would need to be carried out virtually every four weeks.

The fox

Working closely with the CRC's mouse team are researchers whose target is the fox. In contrast to the mouse-control system, the fox contraceptive vaccine will be bait-delivered. This is because a virus suitable for spreading an infertility agent into fox populations has not yet been identified in Australia.

Co-leader of the fox species team, Dr Peter Bird, says the approach is based on the success of a vaccine delivered in baits for control of rabies in Europe. (In fact, the program against rabies has been so successful that researchers in Europe are now looking with interest at the Australian work to control fox numbers.)

Bird and his colleagues are assessing a number of different ways of delivering an immuno-contraceptive vaccine in a bait form.

Molecular biologists have developed a strain of *Salmonella* that cannot survive in the natural environment. The researchers hope to induce infertility by adding selected genes for fox gamete (sperm or egg) antigens to this mutant bacterial strain which is then given to foxes in baits. Another possible delivery system is a vaccinia virus, similar to that used to immunise foxes against rabies, which has been genetically engineered to express fox gamete antigens.

The researchers have already engineered *Salmonella* bacterium and vaccinia virus to include gamete genes and showed they can produce an immune response. This indicates that the system works. The next step is to see whether infertility follows.

A major problem with conducting fertility trials in foxes is that the breeding season is limited to one cycle per year, each winter. But the researchers are collaborating with two groups in the Northern Hemisphere, in France and Finland, enabling them to conduct trials year-round.

A key question for ecologist Dr Alan Newsome is how far must fox populations be reduced to ensure survival of prey populations. Since 1994, Newsome and his colleagues at the Vertebrate Biocontrol CRC have studied the relationship between foxes, cats, and their prey on about 60 square kilometres at Lake Burrendong in the New South Wales central tablelands.

Eastern Shield?

A SUCCESSFUL campaign in Western Australia to reduce fox numbers and reintroduce endangered native species has prompted the idea for a similar campaign in the east.

WA has a natural advantage when it comes to controlling introduced predators: a group of plants known as poison peas. These native species contain the poison sodium fluoroacetate, which is manufactured synthetically as 1080. The native animals that evolved alongside these plants have a natural resistance to the poison; but to rabbits and foxes, even minute amounts are lethal. Meat baits carrying small amounts of 1080 will kill foxes, but won't harm native animals.

Western Shield is a campaign being run by the WA Department of Conservation and Land Management, (one of the partners in the Vertebrate Biocontrol CRC). The campaign covers an area of nearly five million hectares and has an annual budget of $1 million.

The program involves increasing fox baiting to a scale never before attempted, substantially increasing research into feral cat control; and, as predators are controlled in target areas, returning native animals to their former habitats.

This baiting has led to spectacular recoveries of populations of woylies (a small, scarce wallaby species), numbats, chuditch (marsupial cats), possums and carpet pythons. In fact, the fox baiting program has allowed woylies to increase in number to the point where they are no longer considered endangered in WA.

In the long term, it is hoped that biological control methods will stop foxes reproducing, but baiting with 1080 will probably be the key weapon against them for another decade or so.

Director of the Vertebrate Biocontrol CRC, Dr Bob Seamark, says three million dollars was spent on fox baiting in New South Wales in 1996-97. Based on the WA experiences, that should be sufficient to control foxes on about 100 000 square kilometres, or one-sixth of NSW.

Seamark is talking to government and non-government agencies about the possibility of implementing a program similar to Western Shield in eastern Australia. To be effective, it would have to control foxes on farmland to protect lambs, as well as in reserved lands to benefit wildlife.

'Could we use the catchment-management approach to make the annual fox baiting program more effective?' This is the question he has asked the Central West Catchment Committee which manages an area of 92 000 square kilometres in NSW (10% of the Murray-Darling Basin).

Seamark hopes the CRC's large fox-removal experiment within that catchment area – on the foreshores of Lake Burrendong on the Macquarie River – can be used as a core example.

The site is divided into three areas: the first has been heavily baited to try to eliminate foxes, the second has been baited to kill between half and two-thirds of the fox population, and the third area has been left unbaited. Spotlight counts reveal that on the fully-baited site, fox numbers have declined to almost zero; on the intermediate site, numbers have stabilised at about half their former level; and on the untreated site, fox numbers have doubled.

The impact of the fox reduction is being followed by measuring survival rates of rabbits and brush-tailed possums (an alternative prey of foxes and feral cats). Radio-mortality transmitters are fitted to captured animals which are released back into the wild. About 60 rabbits and 30 possums are maintained with these collars across all treated sites. When an animal is killed, the pulse rate of the collar increases. The collar is located and the predator responsible identified from tooth marks.

The study is showing that cats are major predators of possums, with both foxes and cats taking rabbits as their main prey, but with foxes turning to grasshoppers in summer. Eagles or goannas kill possums and rabbits. Myxomatosis is still killing rabbits, but the 1996 outbreak of rabbit calicivirus disease killed over 90% of the rabbit population. Since then, foxes and cats have suppressed rabbit populations at these low levels, but their take of possums has increased.

A novel experiment by a student from the University of Sydney working with Newsome has shown that possum behaviour can indicate whether a high enough proportion of foxes has been removed for native prey species to benefit. Shaan Gresser monitored possum behaviour by attaching a bobbin of cotton to their collars and determining their movements, Hansel and Gretel like, by following the trail of cotton caught on vegetation.

The cotton trails revealed that, in the area where foxes had been removed, possums used all habitats available, foraged more widely, and spent more time foraging on the ground and in open areas. Where foxes were abundant, the possums restricted their range to dense undergrowth and trees.

In another experiment, Gresser found that the smell of fox urine (fresh or stale) was sufficient to discourage possums' foraging activities. Another experiment with possums is being conducted by Karolyn Pickett, a student from Toronto University, Canada. Pickett uses evenly-spaced food sources to see how much food is taken at varying distances away from the animals' nest trees. This is also showing the sensitivity of possums to foxes, and is another indication that foxes cause behavioural changes in possums.

Newsome believes such changes in behaviour will provide the necessary indicators of the effectiveness of fox control induced by immuno-contraception.

The rabbit

The concept of immuno-contraception began with the rabbit, when Dr Hugh Tyndale-Biscoe and Dr Steve Robbins proposed using the myxoma virus as a

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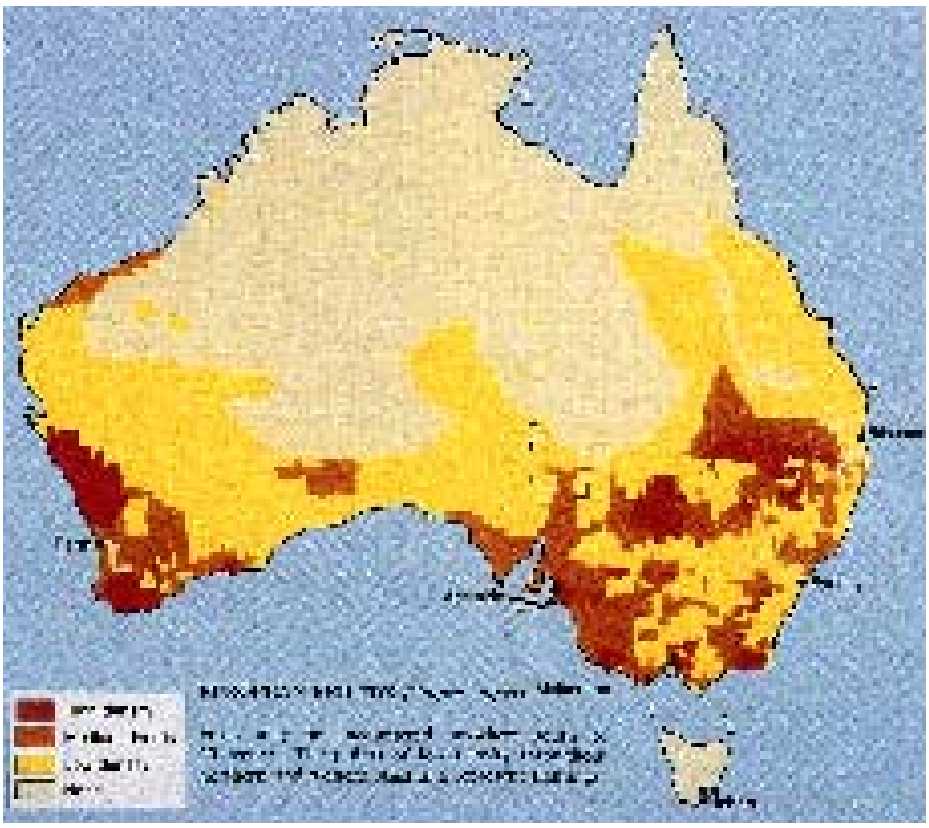

vector for synthetic vaccines that would sterilise infected rabbits.

Mosquitoes and rabbit fleas have spread the myxoma virus widely in Australian ecosystems, especially in arid areas; and the CRC researchers believe that a myxoma virus modified to contain a fertility control agent, as long as it creates a normal infection, will be similarly spread.

Following the mouse experiment, Dr Mike Holland and Dr Tony Robinson have shown that they can induce infertility in about 75-80% of rabbits by injecting them with a zona pellucida antigen. While they do not know how long the effect will last, because the vaccine is still being tested, the researchers have demonstrated that it may affect at least two matings.

When the gene for the zona pellucida protein was engineered into a myxoma virus and injected into a rabbit, however, fertility was reduced by only 25%. So there is still a deal of work to do.

Dr Peter Kerr, a virologist with the group, says the exciting thing is the existence of an antigen that works and can be delivered to rabbits. The next step is tying the elements together.

Given that survivors of myxomatosis become immune to further infection, success will depend on the recombinant myxoma strain infecting the majority of young rabbits before other strains. This will mean timing the introduction of the virus to September or October, when about 50-80% of rabbits in the population are susceptible to myxoma virus.

In field experiments, the researchers have released myxoma virus containing a natural genetic marker and measured its rate of spread across four large sites. Laboratory analysis showed that, on two out of four sites, the virus spread successfully. The results are encouraging as they suggest that, if the timing is right, the virus can be established.

To determine what proportion of the population needed to be infertile to cause the reproductive rate to fall below replacement levels, Dr Kent Williams and Dr Laurie Twigg carried out full-scale field experiments in NSW and WA respectively, with wild rabbits living naturally in warren systems.

In what are some of the largest manipulative ecological field experiments ever done in Australia, they surgically sterilised 40, 60, or 80% of female rabbits in separate groups of warrens, repeating the treatment for those proportions each year on new rabbits at each site. Each level of sterility was replicated three times.

Intensive monitoring and comparison of reproduction on the sites indicated that, to reduce the number of kittens surviving on the site, between 60 and 80% of females need to be sterilised.

This is a significant challenge for the Vertebrate Biocontrol CRC scientists; but recent discoveries in the centre's laboratories and elsewhere have indicated many ways of improving the performance of the recombinant virus, both by manipulating the antigen gene itself and by manipulating the virus to boost the immune response, so increasing the prospects of success.

The cat

The Vertebrate Biocontrol CRC has accorded a lower priority to developing an immuno-contraceptive for feral cats, mainly because of limited resources. Should it eventually do so, it would have to design a contra-vaccine to protect domestic cats against immuno-sterilisation: although some cat owners might welcome a free sterilisation service. The situation may be reviewed if a successful fox control campaign results in increased predation by cats on native mammals, birds and reptiles.

Further information on the Vertebrate Biocontrol CRC is available on the Internet at http://www.dwe.csiro.au/vbc/

More about feral pest control

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abstract

Researchers at the Vertebrate Biocontrol CRC are developing immuno-contraception techniques for controlling rabbits, mice and foxes. These can target antigens on the surface of sperm, or those involved in sperm attachment to the outer coat of the egg (the zona pellucida). Laboratory research with mice has shown viruses are possible vectors for immuno-contraceptive vaccines and effective delivery systems are being tested for mice and sought for rabbits. A strain of salmonella bacterium, and a vaccinia virus genetically engineered to express fox gamete antigens, are being investigated for delivering an immuno-contraceptive vaccine in a bait form. Field trials are investigating delivery strategies that ensure the survival of prey populations.

Keywords: feral animals; birth control; biological pest control; immuno-contraception; rabbit calicivirus disease; myxoma virus; disease transmission; mice; foxes; rabbits.