NEW APPROACHES TO

RABBIT & FOX CONTROL

Viruses genetically engineered to sterilise pest animals may bring relief to a ravaged land

When the ships of the First Fleet dropped anchor in Sydney Cove 204 years ago, they carried more than the 966 sick, hungry and dispirited convicts and soldiers who had survived the long voyage from England.

As well as their reluctant human cargo and a pitifully small supply of seeds and livestock, the ships brought the advance party of an invasion force: five English rabbits.

Nothing more was heard of those first rabbits: they all appear to have died, despite their owners' care. Some two dozen successful introductions occurred in subsequent years, but the invasion proper did not begin until 1860, when 24 wild English rabbits imported by Thomas Austin and released on his property, Barwon Park (near present-day Geelong), found an environment to their liking. Austin's rabbits not only survived, they prospered beyond belief: in the first 8 months of 1887, for example, 10 million rabbits were destroyed in New South Wales alone.

Rabbits not only changed forever Australia's environment; they also changed the face of its economy. A 1942 report estimated that the damage caused by rabbits each year added up to 10 times the amount earned from the trade in rabbit skins and flesh. Today, between 200 million and 300 million rabbits cost Australia an estimated $70 million each year simply in lost agricultural production: it is impossible to calculate how much damage rabbits cause through habitat destruction or through competition with native fauna.

The story of the 'grey blanket' and of the sudden impact of myxomatosis has become an integral part of Australia's history. Less well known, perhaps, is that in early 1950 the CSIRO scientists responsible for testing the myxoma virus sent a dispirited telegram to their chairman, Dr Ian Clunies Ross, informing him that field trials of the virus on a dairy farm at Gunbower,

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Vic., had failed and that they were convinced the virus would never be successful.

Fortunately they persevered with their program of inoculating rabbits in the Albury district. The results were initially disappointing, but by December that year myxomatosis had spread along the Murray, Murrumbidgee, Lachlan and Darling rivers. Within 2 years it had reduced Australia's rabbit population from about 600 million to fewer than 100 million animals.

The success was not without its unfortunate complications, however. At the same time as myxomatosis was beginning to reduce rabbit populations by up to 99%, CSIRO was accused — in a forerunner of today's often exaggerated concerns about the release of genetically modified organisms — of having released a 'killer disease' when a simultaneous outbreak of human encephalitis in the Murray Valley caused severe illness in hundreds of adults and even led to the deaths of several children.

The accusations were silenced when it was announced that three leading Australian scientists, Sir MacFarlane Burnet, Dr Clunies Ross and Professor Frank Fenner, had courageously injected themselves with live myxoma virus to demonstrate that myxomatosis was specific to rabbits and would not affect humans.

The myxoma virus has become less effective since those exciting early days, evolving to ensure its host's — and thus its own — survival. Myxomatosis now kills between 30% and 80% of rabbits, depending on the virulence of the strain involved, levels of resistance and environmental factors such as temperature or drought. The main myxomatosis vectors, the European rabbit flea *Spilopsyllus cuniculi* and the mosquitoes *Culex annulirostris*, *C. pipiens* and *Anopheles annulipes*, have specific moisture requirements that prevent myxomatosis being a viable control method in arid areas. However, Australian researchers

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Ms Kathleen Saint isolates cloned colonies of the myxoma virus genome.

Tania Bubela

Even before they emerge from the den, fox cubs have determined the dominance hierarchy that will determine whether or not they breed.

Graeme Chapman

Dr Mark Bradley employs high-pressure liquid chromatography to separate fox sperm proteins so they can be tested as potential immunocontraceptives.

Tania Bubela

The alpine country around Charlotte Pass supports a diversity of native rodents, marsupials, rabbits... and foxes. This storm-wrecked snow gum is now home to a vixen and her cubs.

have continued the tradition set by their predecessors, searching for new ways of controlling rabbits and attempting to improve established techniques.

A three-pronged approach to rabbit control through immunosterilisation (also being researched for fox control), improved efficiency in the delivery of the myxoma virus and investigations into rabbit haemorrhagic disease (RHD) is under way, with various elements being funded by the Australian Wool Research and Development Corporation, the Australian Meat Research Corporation and the Australian and New Zealand Council of Conservation Ministers (CONCOM). The Australian National Parks and Wildlife Service's endangered species program is providing funding support for research into fox control.

The New Zealand government and the New Zealand Agricultural and Marketing Research and Development Trust are providing $NZ100 000 a year for the 3-year RHD research project in the hope that the virus will provide a means of controlling rabbits in the South Island. New Zealand is also considering supporting research into immunosterilisation with a view to using this method, should it prove suitable, to control possums, which have caused immense damage to native forests in that country.

The Division of Wildlife and Ecology has recognised the importance of this new approach and has, over the past two years, redirected substantial resources to it. As well, the CSIRO Board has accepted a recommendation from Chief Executive Dr John Stocker to allocate additional funds to advance the immunosterilisation aspects of the program. Dr Stocker (himself an immunologist) supports the program because, he says, environmental degradation, especially of Australia's fragile rangelands, and the accompanying extinction of native animals are matters of immediate concern to every Australian.

Vertebrate pests such as rabbits and foxes are major contributors to that process, and the application of modern science to their control carries with it the promise of benefiting native animals as well as the environments on which they depend. Dr Stocker adds that the research program meets the criteria the CSIRO Board has set for high-priority research, relating to its potential benefits to Australia, its feasibility and the capacity of Australian scientists to investigate the problem and develop solutions appropriate to this country.

He also stresses that he would not have supported the program had he not been convinced of its inherent safety and specificity to target species. Public concerns about the release of genetically manipulated organisms or viruses cannot be dismissed, he says, but must be addressed honestly, with the benefits and difficulties discussed openly.

Dr Hugh Tyndale-Biscoe, leader of the CSIRO Division of Wildlife and Ecology team looking for new ways of bringing rabbits under control, concedes that complete eradication of these pests will not be possible. However, he points out that a significant long-term reduction in rabbit numbers, especially in fragile environments such as arid and semi-arid areas, will relieve much of the pressure on native plants and animals as well as reducing the economic cost of rabbits' competition for food with sheep and cattle.

Even more important than competition is the fact that rabbits have changed the rules of the ecological game: in arid regions a density of no more than one or two rabbits per hectare — an apparently low population — is enough to destroy all acacia seedlings; in some areas, populations of native plants now consist entirely of senescent, century-old trees. Some idea of the extent of this damage can be gleaned from the 400-ha T.G.B Osborne Vegetation Reserve, in north-eastern South Australia, where an annual rabbit-control program since the 1970s has led to a dramatic increase in the numbers of native plants.

Dr Tyndale-Biscoe also stresses the importance of controlling foxes, which depend on newborn rabbits (called kittens) for up to 80% of their food supply: a long-term reduction in rabbit numbers, if not accompanied by a reduction in fox populations or breeding capacity, could see the predators turn to other prey, such as native birds and mammals. The removal of competition from rabbits may seem to be an immediate blessing for native animals, but if it means increased predation by foxes it could spell complete extinction for some endangered species or, as has happened in arid regions, the local extinction of even common animals such as brush-tailed possums.

As Division of Wildlife and Ecology ecologist Dr Kent Williams points out, the central difficulty in controlling both rabbits and foxes lies in their biology. In the case of rabbits, when food and water are plentiful and rabbit numbers

are relatively low a doe can give birth to four or five kittens every month for five months a year — in exceptional circumstances, her daughters from her first and second litters can themselves be reproducing before the breeding season ends. Except in arid regions, few does breed for a second year, since the energy costs of lactation render them susceptible to a number of diseases. Lactation also takes such a toll on their bodies that they are easier prey for foxes and other predators.

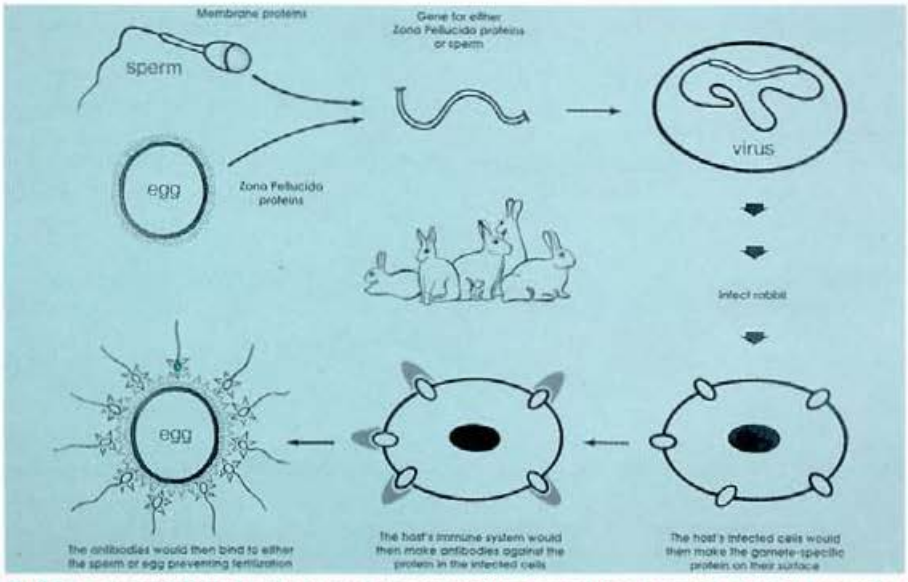
And while rabbits' community structure is hierarchical, with a dominant buck and one or two dominant does, subordinate does can and do mate with both dominant and subordinate males whenever possible. Together, these factors promote a maximum level of reproduction that is limited only by food and territory.

Conventional methods of rabbit control are relatively effective when they are applied in concert and over successive seasons. Government authorities and landholders spread poison baits, fumigate warrens with toxic gases and destroy warrens through deep ripping and ploughing. Where they are a problem, foxes are primarily controlled through shooting by landholders or recreational hunters, and through poison baits or trapping.

Myxomatosis remains an integral element in the suite of control methods for rabbits, although, as noted, it is significantly less effective today than it was during the early 1950s. All strains of myxoma released so far are derived from a few localities in Brazil, which means rabbits have been able to develop resistance to the virus as it has evolved: myxoma is thus limited in how well it is transmitted from one individual to another or from one population to a potentially more resistant population, and how lethal it is. Suitable insect vectors are generally absent from the semi-arid rangelands; mosquito vectors occur there only during infrequent wet seasons, and the European rabbit flea can only persist in climatic regions where it can reproduce frequently (with breeding seasons separated by fewer than 6 months).

Little can be done to improve the efficiency of warren-ripping — it is already highly effective — and fumigating warrens will also remain a useful method of controlling discrete and easily accessible populations. Spreading vegetable baits laced with 1080 poison will always be of limited utility, since there is a constant danger that non-target species (especially those native animals already under pressure from competition by rabbits) will be destroyed.

Dr Brian Cooke of the South Australian Animal and Plant Control Commission has been looking at improving the delivery of myxomatosis through the introduction of *Xenopsylla cunicularis*, a Spanish species of flea that can survive and prosper in arid and semi-arid areas. He is currently breeding the flea under quarantine to determine its potential impact on non-target animals, and has found that it does not parasitise marsupials. It has some affinity for two species of native rodents — the southern Victorian subspecies of the southern bush rat, *Rattus fuscipes*, and the stick-nest rat *Leporillus conditor* — but neither is likely to come in contact with it. The Spanish flea persists in dry areas (with less than 600 mm annual rainfall) while *Rattus fuscipes* lives in wetter country, where myxomatosis is spread by the European rabbit flea, and *Leporillus conditor* is extinct on the mainland. Neither species is susceptible to myxomatosis, so Dr Cooke is confident that field releases of *X. cunicularis*, planned for this year, will not affect native animals.



Building on established techniques for constructing recombinant viruses, CSIRO researchers hope to 'engineer' a myxoma virus strain, carrying rabbit genes, that will make infected rabbits infertile.

Dr Cooke is also involved in assessing the impact of RHD in Europe. This viral disease, which attacks the liver and spleen of rabbits, killing them in 2–3 days, first appeared in China in 1984 and has since spread to Europe (where it killed some 64 million farmed rabbits in Italy) and Mexico.

It appears to be specific to rabbits (a similar disease that also affects the liver and spleen of hares is spread by a different virus), and has considerable potential as a rabbit-control agent in Australia. It is spread by direct contact, so it may persist in arid areas even when rabbit densities are relatively low: it therefore offers some hope of keeping rabbits' ecological impacts at a minimum in these extremely sensitive ecosystems.

Dr Harvey Westbury and colleagues at the high-security Australian Animal Health Laboratory (AAHL) at Geelong, Vic., will spend the next 2–3 years determining: whether RHD affects Australian and New Zealand rabbits in a similar manner to those in Europe, Asia and Mexico; whether it is a safe, effective and species-specific biological control agent; and whether it has any deleterious effects on the ability of myxomatosis to keep rabbit numbers low.

If RHD proves suitable in Australia and New Zealand, its release will be carried out only after all relevant State, federal and New Zealand bodies have given their approval, after environmental and animal welfare issues are considered and after a full public debate.

The AAHL research program also involves considerable basic research into the virology and epidemiology of RHD: the disease first appeared so recently that very little is known about any aspect of its 'biology', and much of what Dr Westbury and his colleagues

find will be ground-breaking work that has implications far beyond the control of rabbits.

Myxomatosis has so far been employed solely to kill rabbits, but it also has potential as a delivery system for other control methods. Dr Mike Holland co-ordinates the Division of Wildlife and Ecology team that is examining the immunology and reproductive biology of the rabbit, with a view to engineering the myxoma virus so that a new strain — carrying with it rabbit genes — can be released. As well as producing antibodies to the myxoma virus, rabbits could produce an immmunological response to the inserted genes: if those genes carry the code for components of rabbit sperm and egg that immunological response should result in infertility.

The myxoma virus replicates rapidly inside rabbits' bodies, which means an engineered virus should quickly stimulate the production of antibodies that could, as Dr Tyndale-Biscoe describes it, act to 'interrupt the intimate conversation between sperm and egg'.

This exciting project takes as its starting point the immunological complexities of conception. In very basic terms, if fertilisation of an ovum is to occur, sperm must overcome a number of barriers in the vagina, uterus, fallopian tubes and, finally, the coating of the ovum, convincing each layer of protection of their friendly intentions.

To accomplish this, the sperm contain proteins that enable an ovum to 'recognise' them. By applying extensive knowledge of the vaccinia virus (a pox virus) to the closely related myxoma virus, and building on research by Professor Helen Garnett, head of Department of Biology at Wollongong University, Dr Holland and Dr Ron Jackson are extracting from the surface of rabbit sperm and the zona pellucida (the outer covering of the ovum) those proteins that enable sperm and ovum to 'recognise' each other.

They will then clone the genes that express those protein antigens and add them to the myxoma virus, turning it into a species-specific recombinant virus that will stimulate the production of antibodies against sperm and neatly shut off the recognition system, rendering female rabbits unable to conceive.

As well as engineering genes so they can accomplish that complex task, virologist Dr Peter Kerr, a colleague of Dr Holland, is seeking a strain of myxoma that will survive for long periods, providing more opportunities for transmission. This in turn requires investigating the mechanisms that control how well particular strains of the myxoma virus are transmitted in order to determine whether the engineered virus would be spread more efficiently by mosquitoes, which are attracted to both male and female rabbits of all ages and irrespective of their reproductive activity, or by rabbit fleas, which are attracted solely to pregnant rabbits.

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Dr Scott Crerar and Mr Andrew Cleary hold one of the Division's foxes, which will play a part in research into the development of antibodies to sperm proteins.

The signal advantage of immunosterilisation is that it strikes at the heart of the reason for the rabbit's success — its high reproductive capacity. A genetically altered myxoma virus of a strain that is virulent enough to be spread widely and quickly — but which does not kill its victims before they have many opportunities to transmit the disease to others — will sterilise those rabbits that survive.

Importantly, immunosterilisation is not expected to cause a female rabbit to lose her libido, which would lead to her losing her place in the dominance hierarchy. Were this to happen, the sexually inactive female would immediately be replaced by a subordinate and reproduction would continue uninterrupted. Experiments by Dr Lyn Hinds employing tubal ligation (which, like immunosterilisation, induces sterility while leaving intact the hormonal system that controls sexual behaviour and dominance) of captive female rabbits have shown that dominant females continue to mate and to build nests; indeed, their dominance is enhanced rather than diminished because they do not have to meet the energy costs of pregnancy and lactation.

Mathematical models of rabbit reproduction suggest that sterilising 55–60% of a population will reduce the recruitment rate to below replacement levels; if more than 75% of rabbits are sterilised, the population will drop to very low levels and remain there, although 90% sterilisation rate may be needed to accomplish the same result in arid and semi-arid areas.

The accuracy of these models will have to be tested under field conditions, in which levels of sterilisation could be lower than in captive situations. Immunity conferred by previous exposure to myxomatosis could adversely affect susceptibility to the recombinant virus, as could a lack of suitable vectors.

Foxes have more variable social lives than rabbits, depending to some extent on the environment in which they live. They are adept at surviving in urban areas. The size of the food supply determines whether they are solitary, with individual animals claiming hunting territories and interacting with other foxes only during a very brief breeding season (fertilisation can only take place over a few days each year), or live in groups. In bushland or farming areas with a diverse food supply, they usually live

Tania Bubela

Ms Tania Bubela is studying the effects of surgical sterilisation on the social behaviour of foxes in the Snowy Mountains, using radio transmitters attached to collars to track the movements of adult foxes.

in small family groups comprising a dog fox, a vixen, their cubs and a few subordinate animals.

Dr Alan Newsome of the CSIRO Division of Wildlife and Ecology is studying foxes' social systems, and has found that in Australia, as in Europe, fox society is strictly hierarchical, with competition for reproduction the determining factor in breeding. Only the dominant vixen breeds successfully, and the brevity of the breeding season further limits opportunities for reproduction: as Dr Tyndale-Biscoe points out, that brevity could work to the advantage of a control program, since sterility only needs to be conferred for a brief period each year in order to have a long-term effect.

It has only recently been revealed that foxes establish their group hierarchy in the den, during the first month of life: the cubs literally work out which will be top dog — or, for that matter, top vixen — before they are weaned.

While 50% of foxes die in their first 2 years, dominant vixens give birth to three or four cubs in their first breeding season and up to eight in subsequent seasons, so every surviving fox has ample opportunity to replace itself at least twice over. Indeed, foxes are such successful breeders and survivors (although there are no accurate figures on total numbers in Australia) that a public appeal in the Southern Tablelands of New South Wales for fox cubs inspired, among other offers, the promise of 30 cubs from a single property, taking CSIRO researchers aback.

Perhaps because rabbits, like other herbivores associated (deliberately or otherwise) with humans, are agriculturally and commercially important, we know much more about their biology than about the biology of their predators, in particular foxes. The life history of the fox has been studied extensively in Europe, but it was not until the 1970s that CSIRO scientists revealed how much Australia's foxes depend on rabbits for survival.

Similarly, much less is known about the reproductive physiology of foxes, which means that Dr Mark Bradley is at the preliminary stages of his investigations into the virology and immunology of their control in this country.

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Dr Kent Williams and Mr Bob Moore fumigate a warren. When it is used in conjunction with ripping and poisoning, fumigation is still one of the most effective rabbit-control methods.

Although he is confident that immunosterilisation of foxes will be a viable method, Dr Bradley does not have an established, well-known vector such as the myxoma virus to which he can add engineered genes to produce sterility. Virologist Dr Scott Crerar is concentrating his search on herpes viruses that affect only foxes, and a group of viruses called adenoviruses. Adenoviruses have already been used as vectors for other vaccines.

If the fox herpes can be transmitted sexually, the recombinant virus could be delivered precisely where it is needed. Adenoviruses have a better survival capacity in the environment and can be transmitted more widely throughout a population. They can be transmitted orally by the aerosol route (by adult foxes sneezing and by young foxes coming into contact with adults' mucous membranes during play, feeding or grooming) and may induce the production of antibodies that give growing foxes a relatively long-lasting immunity to fertilisation. That immunity could possibly be 'topped up' later, during copulation, by a herpes-transmitted recombinant virus.

Two proteins have so far been identified as likely targets for producing antibodies in the vixen's genital tract. Antibodies to one protein, from the head of the sperm, prevent the sperm interacting with the egg but may not be sufficiently species-specific to be useful. Antibodies to the second protein, from the tail of the sperm, cause sperm to agglutinate or clump together, inactivating them and thus greatly reducing their chances of reaching, much less penetrating, the ovum. This protein is also found, albeit to a lesser extent, in cats and dogs, so it is planned to determine which parts of the protein are specific to foxes so that only those portions could be used in an immunovaccine.

Dr Bradley envisages a recombinant virus carrying a 'soup' of elements from a number of proteins that affect both sperm and ovum, and is working to identify and clone proteins from both elements of the reproductive system to provide the best possible opportunity of conferring immunity to fertilisation. As well as delivery by virus, he suggests that a similar mixture of reproductive proteins and immune-

How to make rabbits 'allergic' to one another

Two strains of myxoma virus have been introduced into Australia: the Standard Laboratory Strain and the Lausanne Strain. Both were introduced in the 1950s and are highly virulent, but a number of attenuated isolates or 'field strains' have evolved from them, accounting for the reduced effectiveness of myxomatosis.

Understanding the epidemiology of those isolates will allow CSIRO scientists to develop ways of releasing attenuated strains carrying recombinant viruses (or, if preferred, new and highly virulent strains).

To accomplish those aims, Dr Peter Kerr of the Division of Wildlife and Ecology is undertaking a complex and painstaking program of 'mapping' isolates so that variations in the virulence of myxomatosis throughout Australia can be identified. Myxomatosis was originally classified on a scale of one to five according to virulence; but this system provided no way of assessing whether two samples with the same apparent virulence — from, say, arid Central Australia and Cairns — belonged to the same strain or displayed equal virulence because of geographical or environmental influences.

Dr Kerr is using two techniques to identify and differentiate field isolates of myxoma. First, he is building up a portrait of myxoma DNA and identifying where restriction enzymes cleave the molecule (cleavage sites are where DNA strands divide and join other strands to exchange genetic information). Viruses with differing DNA sequences at one or more cleavage sites will have distinctive differences in the sizes of their DNA fragments. The work is often tedious: myxomatosis is a slow-growing and slow-acting virus, so Dr Kerr must wait 4 weeks before each sample is ready for electrophoresis and can be added to his 'DNA library'.

Second, the research team is employing monoclonal antibodies — sensitive reagents that 'recognise' only single, specific parts of other molecules — to study myxoma DNA. The presence or absence of a monoclonal antibody indicates whether any changes have occurred in the molecule, because it simply will not recognise an altered molecule.

When they have identified and catalogued the main myxoma isolates, the researchers will need to develop serum-scanning techniques to study rabbits' immune responses to myxomatosis so they can select suitable viruses.

Dr Ron Jackson plans to use the knowledge gained from viral differentiation and immunological studies to construct a recombinant myxoma virus that will carry genes for sperm or egg proteins... and which will stimulate the host's immune system to make it 'immune' to fertilisation.

To do this, he will use restriction enzymes to cut myxoma DNA into fragments, then build a 'transfer plasmid' that consists of myxoma DNA on either side of a foreign DNA insert that acts as a marker gene, enabling him to track the transfer plasmid.

Dr Jackson will then add transfer plasmids to a culture of rabbit cells infected with myxoma virus. Experiments with viruses that infect mice have shown that, once such plasmids have entered the cytoplasm of the cells, a crossover recombination takes place between the viral DNA sequences inside the plasmid and matching DNA sequences in the genes of the 'wild-type' (or naturally occurring) virus.

This recombination results in the integration of the whole of the plasmid into the genome of the 'wild' virus (so that the plasmid is flanked by direct repeats of 'wild' viral DNA sequences) and the production of an intermediate recombinant virus.

Intermediate recombinant viruses are then grown in a tissue culture under conditions that promote the reproduction of viruses containing the marker gene. When those conditions are removed a second crossover occurs between the directly repeated viral DNA sequences, to form either wild-type viruses or recombinant viruses at a 50–50 ratio. The recombinant viruses can then be isolated.

Already, monoclonal antibodies to rabbit sperm and zona pellucida antigens have been manufactured and their ability to inhibit sperm–egg binding or fertilisation is currently being assessed in vitro. Similar work on zona pellucida antigens is in progress, but is slower because of the difficulties of purifying enough of the zona's individual protein components.

Rabbit ovary, epididymis and testis 'DNA libraries' have also been created, and will be screened with these monoclonal antibodies to isolate the genes encoding the proteins against which the antibodies are directed.

The next step will be to make recombinant viruses that contain DNA sequences for the most effective antigens. These can then be tested in the laboratory and in strictly controlled field trials; if they are successful, a strategy for field releases will be prepared only after the immunosterilisation program has been sanctioned by government and community regulatory bodies.

system stimulants could be incorporated into baits for use against localised infestations — for example, on islands or in arid areas where there is no possibility of non-target species being affected.

Dr Tyndale-Biscoe, Dr Bradley, Dr Holland and their colleagues are at pains to point out that immunosterilisation — while it is an exciting and highly promising field of research, with potential for control of other vertebrate pests such as feral cats and pigs (in which cases domestic animals could be vaccinated against the antigens used to produce sterility) — is new and as yet barely explored.

It brings together disciplines such as ecology, anatomy, physiology, immunology and virology, provoking as many questions as it answers, but nevertheless offering a substantial potential addition to the control methods that must be used against rabbits and foxes if Australia is to conserve its native animals, plants and ecosystems.

Carson Creagh

More about the topic

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