

representing the Safari Club International, told of the contribution that wildlife use has made to the protection of wetlands in Victoria, many of which have been conserved due to pressure from recreational shooters. In the United States, landowners' economic interest in recreational hunting of deer has encouraged the maintenance of habitat which would otherwise have been cleared for cattle grazing. The retention of wildlife habitat on privately owned land is an addition to that

preserved in the national reserve system and thus effectively expands that system.

Sustainable use of wildlife presents an opportunity to raise revenue for conservation management. To this end, it was suggested that mechanisms be formulated to "capture" revenue deriving from both consumptive and non-consumptive wildlife use by applying the "user pays" principle and that this revenue

be redirected into nature conservation programmes to reinforce government expenditure.

The proceedings of the conference will be available by the end of 1994. This volume will include the keynote addresses, presentations from symposia and many of the contributed papers. It will certainly be compelling reading and provide readers with the latest in research, opinion and strategies on this topic.

The CRC for Biological Control of Vertebrate Pest Populations: fertility control of wildlife for conservation

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IN the last four years there has been a growing awareness of fertility control as a means of reducing or eliminating pest mammals. It is the preferred option of animal welfare groups in Australia (Tyndale-Biscoe 1991) and in North America (Denver Wildlife Research Center 1993), and the expectations have accordingly been raised for its imminent use for the control of Australia's most intractable species, the rabbit, the fox and the cat. In this article I will outline the progress so far achieved in developing this approach for the fox and rabbit, the major obstacles that still remain including the perceived risks, and the long-term prospects for these and other species if fertility control is shown to be an effective means of controlling pest populations.

THEORETICAL BASIS

The size of a population of animals is determined by the birth rate, death rate, migration and quality and suitability of the habitat for the particular species. In arid Australia the major factor in the decline and extinction of native species has been alteration of the habitat (Morton 1990), although increased mortality from introduced predators is more commonly cited as the major cause. Conversely, the very changes in the habitat, which worked

against the native species, favoured several introduced species, particularly the rabbit and the fox.

To control a mammalian pest species the commonest approach has been to use mortality agents, usually poisons. However, in the case of the rabbit it has recently been shown (C. K. Williams and R. J. Moore, in prep.) that the destruction of the warrens, where successful breeding occurs, is far more effective and long lasting than any poisoning regime; when the warrens were left intact the recovery of the rabbit populations after fumigation or line poisoning was very rapid because the warrens could rapidly be reoccupied. Similarly, after the initial highly successful reduction of rabbits in 1951-54 with the myxoma virus, rabbit populations recovered in some areas because reproduction of the survivors was not curtailed and resistance to the virus evolved (Fenner and Ratcliffe 1965). Clearly, the most important factor in pest control is the rate of recovery after a treatment has been applied; if that could be curtailed, as with warren ripping, the effect of all methods would be enhanced.

Fertility control is sometimes regarded as mortality applied at an earlier stage of the life cycle but for many species it could be much more

than this. Among social species reproduction by subordinate members of the group may be inhibited by dominant members (Mykytowycz 1959), while in other species there is active competition among males for access to breeding females. In both situations sterilization of dominant members could theoretically affect realized fecundity of the population disproportionately (Caughley *et al.* 1992). In the best known example of applied population control, using sterilization, the American Screw-worm Fly, *Callitroga hominivorax*, (Spradberry 1994) the important aspect is that the females mate only once; so by greatly increasing the male population with sterile but sexually active males, the probability of a female being fertilized is greatly diminished.

For both the above examples the essential components of fertility control are that the sterilized individuals remain sexually active and retain or improve their status in the social hierarchy of the population, and that a sufficiently high proportion of the population is sterile. While these conditions have long been recognized (Knipling 1959; Davies 1961), it is only in the past five years that advances in reproductive immunology and molecular biology have made it theoretically possible to achieve this in wild mammals.

CURRENT RESEARCH BY THE CRC

The Co-operative Research Centre for Biological Control of Vertebrate Pest Populations, established by the Federal Government in 1992, is the largest and most integrated commitment of research effort to fertility control for wildlife in the world. The main idea that is driving the work of the CRC is that the genes for proteins that are critically involved in fertilization or implantation can be inserted into a virus that infects the target species. An animal infected with the recombinant virus would simultaneously raise antibodies to the virus and the reproductive protein and fertilization or implantation would be prevented, without affecting the animal's sexual activity or social status in the population. The current aims of the CRC are to answer four central questions that derive from this concept:

Question 1: What proportion of females in a wild population must be sterile in order to reduce significantly the rate of growth of the population?

In southwestern Australia and in New South Wales two large experiments on wild rabbits were begun in 1993 to test the effect of sterilizing various proportions of the female population, on rate of increase and survival of young. In each experiment 12 warren systems, each carrying 50–100 rabbits, have been isolated by fences and buffer strips and each was allocated randomly to one of four treatments. In each warren all the rabbits were caught and marked and 80 per cent of the adult females were subjected to surgery, either laparotomy or ligation of the oviducts, the proportion sterilized being 0 per cent, 40 per cent, 60 per cent or 80 per cent. In addition, the impact of these levels of sterility on the European Flea, *Spilopsyllus cuniculus*, and the incidence of infection with myxoma virus are being investigated. This is because the life cycle of the flea is intimately tied to the reproductive cycle of the rabbit, particularly late pregnancy and new born kittens. With a high proportion of the females sterile, will the fleas remain in sufficient numbers to transmit myxoma virus? These experiments are planned to run for three years and the results of the first year are currently being

analysed and the second season of treatment is in progress now.

Logistics preclude us from conducting a fully replicated, orthogonal experiment of this sort on foxes. Also with current methods it is impossible to catch more than 80 per cent of any fox population. Nevertheless, we have begun a study in New South Wales on fox populations depleted by 75 per cent and comparing the effects of leaving the residue intact or sterilizing 75 per cent of the residual females, on rate of population recovery and the response of key prey species. Similar experiments, but without imposed sterility, have also commenced in Western Australia.

Question 2: Can gamete specific proteins be presented to the animal in such a way as to provoke an effective and long lasting immune response that interferes with fertilization or fetal development?

Effort is presently concentrated on interfering with fertilization by identifying proteins present on the surface of the sperm and the ovum which are involved in the processes leading to fusion of the male and female nuclei. These processes are the locomotion of the sperm, which brings it into the vicinity of the ovum; the first contact between the sperm head and the outer coat of the egg, called the zona pellucida, which induces the acrosome reaction of the sperm; the release from the acrosome of enzymes that breakdown the zona and allow the sperm to pass through and lie against the plasma membrane of the ovum; and fusion of the sperm and egg plasma membranes involving proteins on the equator of the sperm head, so that the sperm nucleus can enter the egg and fuse with its nucleus.

These several proteins can be identified and isolated by the use of monoclonal antibodies targetted to each of them. The antibodies can then be used to test the importance of the proteins in fertilization by culturing eggs and sperm in the presence of each antibody and observing whether fertilization is prevented. To date in the rabbit and fox about 40 monoclonal antibodies have been prepared against the sperm of each species and some of them have been shown to block sperm-egg binding *in vitro*. In the rabbit sperm monoclonal antibodies have also been shown to prevent fertilization *in vitro*.

In both species the genes encoding for some of these proteins have been sequenced and comparisons with the sequences from other species have begun. In addition, the first *in vivo* trials of the purified proteins have begun. In the fox two experiments have been conducted to test the effect of immunizing vixens with specific sperm proteins. The proteins provoked strong immune responses in the serum and vaginal secretions prior to the breeding season. While fertilization was not prevented, most of the treated animals failed to sustain pregnancy.

Question 3: Can recombinant viruses or bacteria that express the genes encoding the gamete proteins be constructed, and can they act as vectors to immunosterilize the proportion of the wild population of the target species identified in the first question?

This question concerns the means of delivery of the antigens identified in the previous question and the proportion of the population that must be exposed. The choice of delivery systems ranges from direct presentation of the selected protein in a bait to the development of infectious recombinant viruses. The former is the safest but the most costly, the latter the cheapest but poses the highest risk; all options are being considered.

For the fox there is no species specific virus available and the current approach is to deliver the agent by bait. However, in order for it to provoke an immune response, it must reach the small intestine unaltered and enter the Peyer's patches, where lymphocytes will become programmed to synthesize antibodies to the protein. To achieve this the protein can be packaged in microspheres, which will pass through the stomach without being digested. Initial trials with microspheres containing fox sperm antigens fed to rats provoked good antibody response. While this approach may be useful for laboratory trials, the cost of production may make it prohibitive for biological control in the field. An alternative approach is to make a recombinant bacterium or virus that expresses the sperm antigen and deliver this to foxes in bait. These would be microorganisms that can replicate in the mouth or small intestine of the animal that eats the bait but would not be transmitted to other animals thereafter.

This approach has been used very successfully in Europe to immunize wild foxes against rabies. Part of the DNA from the rabies virus was inserted into the vaccinia virus and, when eaten in a bait, replicated in the mouth and provoked a strong antibody reaction to the inserted rabies protein. This technique has proved to be so successful that fox populations have now increased in Europe. For our purposes the advantage of this system is that a variety of viruses can be considered and the selected virus could also be incapacitated to ensure that it cannot spread.

For the rabbit any system that relies on oral bait delivery would be impractical and too expensive and our aim is to develop a recombinant form of the myxoma virus; it would kill some of the rabbits that were infected with it and would sterilize those that recovered and developed antibodies. To date recombinant myxoma viruses have been constructed in which foreign genes encoding for harmless or neutral proteins have been inserted. Rabbits infected with these recombinant viruses developed mild symptoms of myxomatosis and produced high and sustained antibody levels to both the myxoma virus and to the foreign protein. This has shown that the foreign gene was being fully expressed in infected rabbit cells and provoking the rabbit's immune system. The next step will be to make a myxoma recombinant virus containing the gene that encodes for a sperm antigen.

Question 4: Can this be achieved in a way that does not put at risk other species?

National concerns about the eventual outcome of this work are the possible consequences to human health, to domestic stock and companion animals, and to native fauna. These have in common the concern of the specificity of the contraceptive agent to the target species and the species specificity of the viral vector. Both matters are regularly scrutinized by the CRC Advisory Committee and by the Genetic Manipulation Advisory Committee of the Federal Government prior to each stage of the research. For instance, at the present time permission is being sought from GMAC for the construction of myxoma virus encoding genes for reproductive antigens for trial in rabbits and for a vaccinia virus recombinant expressing

the gene for a fox sperm antigen. In addition the CRC has commissioned an assessment of its procedures which will take place in September.

International concerns are directed at the risk to target species in countries where the species is indigenous and well regarded; the concern is the risk of accidental or malicious export of the agent from Australia. Such doubts could adversely affect Australian trade if the risk was considered unacceptable by the foreign country. These concerns can be addressed in three ways: assessment of the possibility of escape from Australia and means to prevent it; assessment of the probability that the agent could become established in a country where the target species is endemic; and the development of contingency plans to counter an outbreak, should one occur. Since the work on the fox is currently directed at oral delivery of non-disseminating vectors this poses no risk internationally; the only presently perceived risk relates to the work on developing a recombinant myxoma virus that could affect rabbits in other countries, where they are not considered in the same light as in Australia.

CONCLUSION

It must be evident that the concept, if shown to be effective and safe in one species, could be developed or adapted for other species. At present the concept is being considered in New Zealand for the eventual control of brush-tail possums and the government is investing substantial sums in the research needed for this. The difficulties for the possum cannot be underrated, since there is little knowledge about possum gametes and fertilization, no knowledge of the immune system of the possum and, at this stage, no known vector. In Australia there is growing interest in developing the concept for the control of wild house mice in the wheat belt. For this species the prospects are much better because so much is known about the reproduction, genetics and immune system of the mouse and there are at least two mouse specific viruses that could be used as vectors. If the mouse work is successful the major pest of rice crops in South East Asia, *Rattus argentiventer*, could be targeted. The great advantage of immunocontraception for the rice rat would be that the bait would not be

harmful to the human population that depends on the crop, as are the poisons currently in use, and (a not insignificant factor) the affected rats would still be edible. Other candidate species for the future in Australia are feral cats and feral pigs but to tackle each of these species would still require substantial resources. In our view it is not prudent to commit funds to these species until we have positive answers to the four questions posed at the beginning in at least one species — the fox, rabbit or mouse.

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For further information on the work of the CRC for Biological Control of Vertebrate Pest Populations refer to the 1992–93 Annual Report, available from the Executive Officer, P.O. Box 84, Lyneham, ACT 2602.