CSIRO Cane Toad Research

The cane toad (*Bufo marinus*)

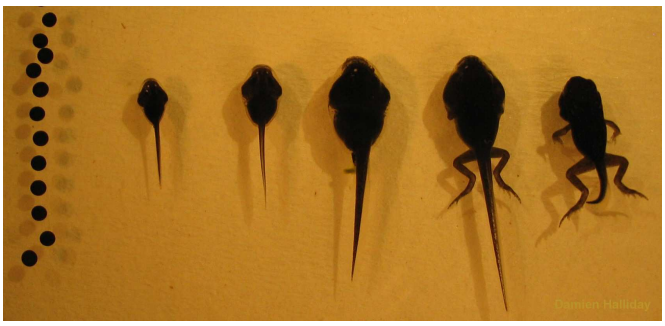
Background

From 1991 to 1997, CSIRO, with co-investment from the Australian Government, undertook major research on cane toads with a view to discovering biological methods for their control. Viruses and other infectious agents were sought in cane toads in their natural range in Venezuela. Although some agents were found, they proved to be infectious for Australian native species. The conclusion was that there were no readily available naturally occurring infectious agents of cane toads that could be used directly as biocontrol agents in the way that myxoma virus and rabbit calicvirus had been used as successful biocontrol agents of rabbits[1]. In the absence of such agents, cane toad control is restricted to small-scale control using traps, habitat manipulation, and protection of vulnerable native species over small areas.

A new approach

In the absence of any naturally occurring biocontol agent for cane toads, CSIRO, in partnership with the Australian Government's Department of Environment and Heritage, is exploring a long-term strategy for biocontrol which involves the genetic manipulation of a virus to target one of the life stages of the cane toad. This approach is one of the long-term research strategies recommended in the Natural Resource Management Ministerial Council's Vertebrate Pest Committee Cane Toad Task Force report released in June 2005[2].

In this approach the first life stage being targeted is metamorphosis. The rationale for this work is based on research that was done some years ago in the US on bullfrogs[3], where inoculation of tadpoles with adult bullfrog haemoglobin altered the type of haemoglobin produced and many of the experimental animals died as they went through metamorphosis. They concluded that the mechanism of interference was mediated by antibody to the adult haemoglobin acting on the emerging adult red blood cells. When adult haemoglobin is introduced into the tadpole, before the tadpole has started making its own adult haemoglobin, the tadpole sees the protein as foreign and initiates an immune response against it.



Cane toad metamorphosis
Photo by: Damien Halliday, CSIRO

The first objective of the CSIRO research is to see if this effect can be reproduced in cane toads. If it can, then the next step is to see what effect that has on the toad's survival. As well as testing adult haemoglobin it is

planned to identify other genes critical to toad development with an initial focus on genes critical to metamorphosis. It is now known that there are many genes first expressed at metamorphosis in amphibians and the products of these genes are all potential targets for this biocontrol approach. When this phase of the work is completed it is hoped that we will have identified a means of interfering with toad metamorphosis and prevented the transition from tadpole to adult toad. The work is also being expanded to look for targets other than metamorphosis and a number of candidate genes have been identified. Obviously, to be useful, the gene or at least regions of the gene must be cane toad specific and species-specificity testing is an important aspect of the work.

Virus "taxis"

A second objective is to develop a means of delivering the effect of such genes throughout the toad population. The strategy is to use a virus that can act as a 'taxi' in delivering the gene that interferes with metamorphosis or some other life stage. This is a challenging step as there is limited information available on cane toad viruses. We are starting the work with a ranavirus that has been isolated in Australia and has been shown to be capable of infecting cane toad tadpoles. We are using this as a model viral system to test the hypothesis that any effects seen after inoculating tadpoles with adult proteins can also be seen after delivering the protein using a virus. The first stage of the work is to weaken (attenuate) the ranavirus as a delivery mechanism in a research context. Although the ranavirus is a useful research tool, it is a virus that can infect a wide range of amphibians and fish and therefore cannot be used in the field unless there is some way of making it species-specific. In parallel, a search is under way to discover viruses of cane toads that are species–specific and which can act as a "taxi".

Cane toad showing typical pale belly with irregular spots

Results to date

A cane toad breeding colony has been established to provide all the life stages necessary for the work. Using animals from this colony, several cane toad genes have been identified that are first expressed during metamorphosis and are being tested for their ability to interfere with the metamorphic process. These genes are also being compared to their counterparts present in other amphibians to assess their relatedness and their feasibility as candidate target genes.

The first gene to be tested has been cane toad adult β-globin (a component of adult haemoglobin) and it has been shown that introducing the protein encoded by this gene into cane toad tadpoles by injection suppresses its expression during metamorphosis and prolongs the expression of tadpole β-globin. This has provided support to the approach and confirms the conclusions reached with the earlier work on bullfrogs. It demonstrates the principle that exposure of tadpoles to these proteins first expressed at metamorphosis can affect the outcome of metamorphosis. The remaining genes identified are being tested for their affect on the viability of metamorphosing tadpoles.

To test the ability of a virus to deliver genes to a toad, the model ranavirus, in ground-breaking research, has been engineered to contain and express foreign genes. This is the first time this group of viruses has been shown capable of manipulation in this way. The ranavirus has also been attenuated (weakened) and the first recombinant constructed. This recombinant contains the adult cane toad β-globin gene. Testing is underway in cane toad tadpoles to see if it has the same effect as injection of adult β-globin on expression of tadpole and adult β-globin.

Future steps

Over the next three years the researchers will be testing the current set of cane toad genes for their effect on metamorphosis and other life stages. In addition they will be searching for other viruses of cane toads that could be used as viral “taxis”. If at the end of this time genes have been identified that affect the viability of tadpoles entering metamorphosis, or that some later life stage is affected, and a suitable viral taxi has been found, then the product development phase will commence.

Risk assessment

As the research is still at the discovery stage there are no plans for field trials at this time and all current recombinant virus research is conducted within a microbiologically secure facility. Before field trials are considered many processes must be undertaken including testing for non-target species to ensure other animals are not affected by the proposed biocontrol product. When a candidate product has been developed the next stage is an application fdor release to the Office of the Gene Technology Regulator (OGTR) and other relevant government agencies. Extensive public consultation is undertaken at this stage before approval for release is considered.

Researcher holding one of the cane toads bred in captivity

Further reading

1. Hyatt, A. D.; Parkes, H, and Zupanovic, Z. 1998. Identification, characterisation and assessment of Venezuelan viruses for potential use as biological control agents against the cane toad (*Bufo marinus*) in Australia: a report from the Australian Animal Health Laboratory, CSIRO, Geelong, Australia. May 1998. Geelong, Vic.: Australian Animal Health Laboratory.

2. A review of the impact and control of cane toads in Australia with recommendations for future research and management approaches. A report to the Vertebrate Pests Committee from the National Cane Toad Task Force. R. Taylor and G. Edwards (eds.) July 2005. http://www.feral.org.au/ref_docs_images/CaneToadReport2.pdf

3. Maniatis, G.M., L.A. Steiner and V.M. Ingram. 1969. Tadpole antibodies against frog hemoglobin and their effect on development. Science 165:67-69.

4. CSIRO Workshop report July 2004 - http://www.deh.gov.au/biodiversity/invasive/publications/cane-toad-2004/pubs/cane-toad-2004.pdf

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Links

CSIRO Cane Toad Factsheet:
http://www.ento.csiro.au/research/pdf/ctgeneral.pdf

Review of the Project: The Development of a Cane Toad Biological Control (February 2003)
http://www.deh.gov.au/biodiversity/invasive/publications/cane-toad-2003/index.html

Office of the Gene Technology Regulator:
http://www.ogtr.gov.au/