

Pied-pipers wanted: The search for super-lures of New Zealand mammal pests

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Wayne Linklater is a wildlife biologist and science writer with a penchant for 'poking his nose' into others' disciplines. His writing about ecology and politics, and odd-toed wildlife like horses and rhinoceros, can be found on New Zealand's SciBlog network, <http://sciblogs.co.nz/politecol/>, and WordPress <http://perissodactyla.wordpress.com>. He posts about these topics on Facebook (<https://www.facebook.com/RestorationEcology>, <https://www.facebook.com/wayne.linklater.9>) and can be followed on Twitter (@EcologyVictoria, @Perissodactyla). He is Director of the Centre for Biodiversity & Restoration Ecology at Victoria University of Wellington, and Research Associate at the Centre for African Conservation Ecology, Nelson Mandela Metropolitan University, South Africa. Wayne is currently Visiting Scholar in the Department of Geography at University of California-Berkeley where he is *turning on, tuning in, and dropping out* to the study of human-animal relationships where the humanities and sciences meet.

Dave Greenwood is a biochemist with a long-standing interest in the biosynthesis and metabolism of natural products. He began his career examining how insects detoxify plant-derived compounds. He has applied this experience to researching novel methods of pest and pathogen control; vertebrate and invertebrate chemosensory systems; and functional gene discovery. He developed the field of proteomics within Plant & Food Research to a point where his skills were ported to oversee the development of proteomics at the Centre for Genomics, Proteomics & Metabolomics (CGPM) at the University of Auckland (UoA) concentrating on high-end mass spectrometry using Fourier transform ion cyclotron resonance mass spectrometry. His 0.2 FTE position at University of Auckland was honorary until 2008 but is now incorporated into the Joint Graduate School in Plant & Food Sciences, where he is an associate professor and a co-director of the CGPM.



Rob Keyzers carried out his BSc(Hons) and PhD studies at Victoria University of Wellington. His thesis research, carried out under the guidance of Assoc Prof. Peter Northcote focused on spectroscopy-guided isolation of sponge metabolites. He then carried out postdoctoral research with Mike Davies-Coleman (Rhodes University, South Africa) and Raymond Andersen (University of British Columbia, Canada) before a short role as a flavour and aroma chemist with Paul Boss at CSIRO in Adelaide, Australia. He was appointed to the faculty at his alma mater in 2009 where he is currently a Senior Lecturer.

Janine Duckworth is a reproductive biologist and immunologist researching alternative methods of pest control for vertebrate species such as possums and stoats. Her current roles include researching possum and stoat sex pheromone attractants; species-specific fertility control vaccines and delivery systems; oral chemosterilants; and high-virulence RHD strains for rabbit biocontrol as humane and environmentally safe methods of pest control in New Zealand.



Introduced mammalian competitors and predators are the leading threat to New Zealand's native wildlife (Craig *et al.* 2000). The recent Pest Summit (3–4 December, 2012; Linklater 2013a) identified improving ways of detecting and killing mammals as the top three research priorities. One of these was the development of better lures to attract pest mammals to monitoring and killing devices.

We have fought conservation battles largely with existing food-based lures. Mammals have been eradicated on islands and their populations depressed on the mainland. But we are not yet winning the war. Eradication of the worst mammal pests remains improbable on the mainland because current technologies cannot operate at the required scale and intensity within probable budgets. The extensive deployment of killing devices for extermination at greater scales is juxtaposed logistically against the intensity of effort required to kill the few last, most difficult to detect, animals.

The attractiveness of a lure determines the effectiveness of killing devices like traps and poisons. Food lures do not attract all animals and have important limitations. First, food lures largely attract only animals whose range already includes the site where the poison or trap is placed – animals are not drawn in from across the landscape. Second, food lures become less attractive when other foods are plentiful. Third, after trapping and poisoning operations, the few animals remaining are more wary of killing devices and not fooled by food lures. Survivors and immigrants re-populate the landscape rapidly. Lastly, the attractiveness of food lures is often short-lived. Lures need to be frequently replenished and that means costly labour, made slower and more expensive by often difficult terrain.

Super-lures are long-lived lures that target the wary and attract them from substantially greater distances – often from outside their familiar range. Thus, super-lures are proposed as a tool for biosecurity and animal eradication to be used after traditional approaches have done the easy killing. Improving lures is a sound research investment because a super-lure could likely be retrofitted to existing technologies such as traps and poisons. The logistics of implementing advances in lures are, therefore, apparently small but the gains potentially large.

The Department of Conservation proposed workshops on each of the research priorities supported at the Pest Summit to develop their detail and form credible consortia of researchers and stakeholders across multiple agencies to bid for substantial research funding. Workshops would provide background and facilitate the formation of collaborative teams to write and deliver on grants from, for example, the Ministry for Business, Innovation and Employment or from environmental and research philanthropies.

In April, Victoria University of Wellington's Centre for Biodiversity and Restoration Ecology hosted the first of these workshops (Linklater 2013b). Thirty-two experts and stakeholders from businesses, Crown research institutes, universities, and the Department of Conservation attended. The group included an enormous range of experience from research with invertebrates, like bees, to mice and elephants; it illustrated that many groups are already engaged in the search, whether it be for lures for rats, possums, or stoats. It was clear from the outset that there is a substantial interest in the topic and an existing breadth of experience in New Zealand to draw from.



Peter Banks is an Associate Professor in Conservation Biology at the University of Sydney, Australia. He has been working on the ecology and impacts of invasive mammals for more than 15 years, studying foxes, dogs, cats and rats in Australia and American mink in Finland. His research interests focus on the behavioural aspect of interactions between alien and native species and aims to manipulate such behaviour to achieve novel solutions to difficult conservation problems.

Jamie MacKay has been working on invasive mammals since arriving in New Zealand in 2005. A MSc thesis project on rat island invasions led to a PhD (completed in 2011) with Professor Mick Clout at the University of Auckland on improving mouse eradication success. During his PhD he developed a particular interest in the way animal behaviour changes as population density decreases and the implications this has for pest control. He is currently following this research theme in possums, looking at their movement behaviour and their interactions with control devices at different population densities



Christine Stockum has a background in biochemistry and proteomics, and a PhD in molecular biology. A passion for applied science has recently led her to join Victoria University of Wellington's mammalian pheromone research team as a postdoctoral fellow. Her role is protein identification and production to aid the search for a super-lure for possums and rats. Christine maintains the research programme's colonies of wild caught possums and rats, which are used as a source of potentially attractive secretions, the components of which could be used as super-lures.

Lured to aspire

The Department of Conservation wants a super-lure because they are thinking big – eradication of mammalian predators in areas over 10,000 hectares (Bell *et al.* 2013). They are proposing a *Future of Predator Control* programme that will target three to five such large sites on mainland New Zealand as catalysts for the development of new technologies or novel applications of existing technologies that will extend the current technical, logistical and financial limits to pest eradication. Large-scale mammal pest eradication using existing trapping technologies would require about one trap per four hectares, so 50,000 ha would require 12,500 traps – a logistic task beyond probable human and financial resources. A super-lure would reduce the trap density required by drawing animals to them from further away and increase animal interaction rates with fewer traps. It might even work for more than one species.

A ‘back of an envelope’ calculation illustrates, albeit crudely, the benefits of small increases in the effectiveness of a lure for reducing trap density. For example, a 20% increase in a lure’s attraction distance results in a 44% increase in the effective area trapped or an approximately 31% decrease in the required trap density (Figure 1). Even larger cumulative gains might be achieved if super-lures attracted non-resident animals because the requirement for trapping grids to provide a nearly continuous cover of the landscape would be further relaxed. These improvements should translate into substantial reductions in the costs of animal control programmes or a commensurate increase in the area over which control can occur. Thus, small improvements in trap effective area quickly make large gains in control programme efficiency.

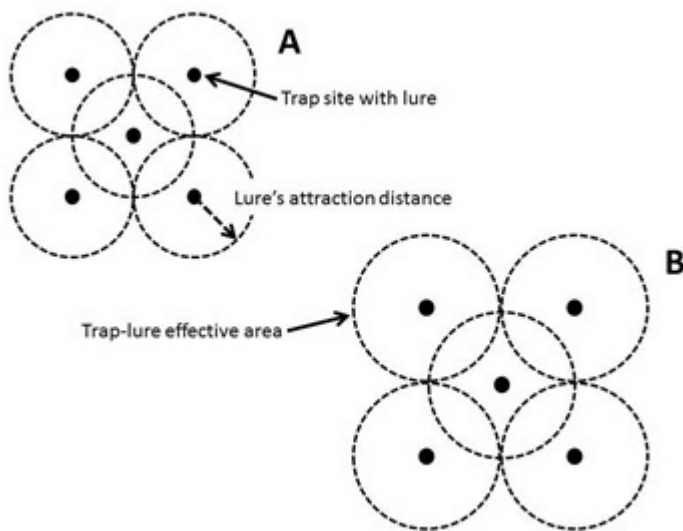


Figure 1: The influence of improvements in lure attraction distance on a trap's effective area making substantial reductions in trap density possible or increases in the area trapped for the same number of traps. A 20% increase in a lure's attraction distance (illustrated from A to B) results in a 44% increase in the area trapped or an approximately 31% decrease in required trap density.

It is simple, but complicated

Lures have long been a fundamental part of human culture. Early hunters placed decoys resembling ducks on ponds and anglers suspended decoys resembling invertebrate prey from

lines thousands of years ago. Real-estate agents brew coffee and bake bread in open homes – the result, you stay longer. Sophisticated super-lures have been routine for invertebrate trapping for decades (Karlson & Luscher 1959; Wyatt 2009; Witzgall *et al.* 2010). New Zealand has been a major player in research for invertebrate lures, especially Plant & Food Research (Suckling *et al.* 2013) where the world's largest database on the topic resides (El-Sayed 2013). The application of super-lures for the control of invasive invertebrate species, therefore, is not new (El-Sayed *et al.* 2009).

Lures for invertebrates have largely involved pheromones responsible for sexual attraction because they stimulate a specific and invariant response. Insects are renowned for consistent ‘stupidity’ by being attracted to the pheromone-laced traps. They simply cannot resist these semiochemicals,¹ behaviour-modifying chemicals, that mimic the signal of a mate. But super-lures have so far eluded us for mammals. Prof. Jane Hurst explained why in her plenary presentation, which began the day (Hurst & Beynon 2013).

Mammals are too smart, too adaptable, and therefore too complicated. Mammals are less likely to communicate with pheromones, at least in the way it is understood for invertebrates, where highly specific responses occur to relatively small and volatile molecules. For mammals, experience and learning modify responses and lead to the rapid, perhaps periodically runaway, evolution of complex signals and interpretation. Mammalian pheromones, where they exist, are likely to be more complex cocktails of large as well as small molecules.

It is the complexity of signal production and interpretation in multi-dimensional networks (communities) of animals, cautioned Associate Prof. Peter Banks during his plenary presentation, which began the afternoon session, that makes the ecology of new lures and their application even more challenging than their chemistry and production (Banks *et al.* 2013). A lure in one context might be ignored, or worse, avoided, in another. The scent of a rodent, for example, might be interpreted by conspecifics both as a place to be or a place to avoid, depending on the presence of predators that might also be attracted to rodent scent. The interpretation and, therefore, utility of scent signals as lures becomes substantially more complex when one begins to consider the olfactory landscape of predators, parasites, mutualists, competitors, and conspecific kin and mates, amongst many other relationships, that must occur.

Sweet smell of success

If all this complexity is discouraging, one need only look to recent advances in mammal pheromone work to, nevertheless, inspire the search. Remarkably, researchers have found insect alarm pheromones identified decades ago, in particular those of bees, also generate avoidance responses in Asian elephants (Greenwood 2013). Crop raiding by elephants is a serious threat to family livelihoods and can result in injury and death

¹ semiochemical. A chemical that affects the behaviour of an organism. Such chemicals include pheromones, which are used for communication between members of the same species, and allelochemicals, which act as chemical signals between members of different species. Editor. Retrieved May 22, 2013 from Encyclopedia.com: <http://www.encyclopedia.com/doc/1O6-semiochemical.html>

to people and elephant alike. Work to develop scents that could be used to reduce human–elephant conflict, because elephants avoid them, led researchers to their discovery (Rasmussen & Riddle 2004). The researchers found that a simple bee alarm pheromone component, sprayed onto traditional crop-field barriers, deterred elephants from crossing into crops. If mega-animals like elephants can be manipulated with scent – and insect ones at that – then why can't we similarly manipulate rats? The experience with elephants raises the possibility that some of the thousands of semiochemicals already identified and developed from insects may be usefully applied to modifying pest mammal behaviour.

Although the plenary speakers set the scene by describing complexity, both researchers have been a part of significant advances in the application of olfactory signals to change mammal behaviour. Saturating habitat with the scent of native prey has been shown to reduce the ability of introduced predators to find the same prey (Price & Banks 2012). This power to manipulate predator success by changing the olfactory landscape is an exciting development for New Zealand conservationists – particularly useful for protecting remnant populations of our most endangered species.

Rodents secrete protein in their urine and these proteins are known to contribute to complex olfactory signals. Recently, a protein secreted by male mice was shown to be attractive to female mice (Roberts *et al.* 2010). The researchers called the protein darcin – after the character Darcy in Jane Austin's *Pride and Prejudice*. Darcin appears to induce rapid associative learning in female mice such that the same attraction is subsequently shown towards the pheromone's remembered location and towards other odour cues associated with darcin. The effect of this learning is to provide strong reinforcement of attraction by females to specific males. The advance that darcin's discovery makes is that it demonstrates that a protein which rodents secrete in their urine might be, in combination with small volatile compounds, a true mammalian pheromone. The prospect, therefore, is for cocktails of proteins and volatile molecules to be useful lures of mice and perhaps other mammals that also secrete protein signals. The work to reach darcin, however, is also a glimpse of the large amount of detailed research required to make progress in the search for super-lures. In Britain, such work has been motivated by the greater food security afforded by better rodent control, but it clearly also has applications in New Zealand for biodiversity and biosecurity.

Odeur en Nouvelle-Zélande

It was the work identifying the pheromonal properties of protein-volatile complexes in mouse urine (Roberts *et al.* 2010) that inspired the search for similarly attractive pheromones in rats and Australian brushtail possum (Paske *et al.* 2013a). Researchers at Victoria University recently confirmed the presence of proteins belonging to the same family as darcin in the urine of Norway rat (*Rattus norvegicus*) and ship rat (*Rattus rattus*). It is, however, yet to be shown if one of these proteins functions as part of a pheromone in the same way as darcin does in mice.

Work at Landcare Research is also under way to identify lures for Australian brushtail possum. These largely solitary-

living species are successful at finding mates even at low densities. Researchers, therefore, regard sexual secretions to be the most likely source of super-lures. They have shown that objects scented with the oestrous secretions collected from female possums are more investigated and manipulated by captive male and female possums (Duckworth *et al.* 2013). Using advances in radio frequency identification tags on wild possum and motion activated cameras, which record possum movements and their behaviour at traps, the researchers have begun field trials of oestrous secretions as lures of possum.

Trials of olfactory, visual and audio lures and new lure delivery systems for Australian brushtail possum are also under way at the Centre for Wildlife Management and Conservation, incorporating researchers from Lincoln and Auckland Universities, and Connovation Ltd² (MacKay *et al.* 2013). Aerosol canisters to improve lure longevity and dispersal have been developed and appear to perform better than food lures in field trials.

The search for rat and stoat lures also occupies researchers from Lincoln University's Department of Ecology (Murphy *et al.* 2013). Urine and droppings from both male and oestrous female stoats, the urine and droppings from ship rats, and rabbit muscle, pelt and fat are being tested for their ability to attract stoats and rats in outdoor enclosures. Evidence for attraction will help refine the search for the critical semiochemicals involved.

With several research groups and multi-institutional collaborative projects under way, it is clear that New Zealand researchers are highly motivated by the potential of olfactory lures and not discouraged by the complexity and size of the task.

Even a whiff of success...

The search for super-lures is as much about the journey as finding the ultimate *Pied-piper*. Although the better lures for mammals are likely to be sexual signals – breeding is a biological imperative – lures might also mimic signals involved in competition or even inter-specific signals, called kairomones, like the signals of prey, predators or other species competitors (Sbarbati & Osculati 2006). A lure does also not need to be a mammalian pheromone to be super. Given the enormous number of potential signals that might have lure properties, the development of a super-lure, while potentially many decades away, will probably result in incremental improvements in existing lures. Clearly there are an enormous number of semiochemicals, many already described and commercially available, that might improve on existing lures (Greenwood 2013; Suckling *et al.* 2013). The recent possum trials by Landcare Research, Lincoln and Auckland Universities, and Connovation Ltd. illustrate that some rudimentary scents offer improvements to existing lures or improve the action of traditional lures when they are combined. Moreover, the complexity described (Banks *et al.* 2013) also raises the possibility of multi-species lures – complexity can have advantages. A competitive or sexual signal for rodents, for example, might double as a kairomone lure for their predators, such as feral cats.

² <http://www.connovation.co.nz/>

A scents of the future

Advances in super-lures for insects was made possible by technical advances which joined gas chromatography to rapid and inexpensive biological assays – enabling the olfactory-active chemicals in biological material to be identified quickly from the enormous number of chemicals present (Suckling *et al.* 2013). The same will be true in the search for the volatile constituents of mammal signals whether it is from urine, saliva or glandular secretions. The combination of gas-chromatography and mass-spectrometry is routine, comparatively inexpensive, and has been applied to understand the composition of rat urine. (Pasko *et al.* 2013b). Its combination with bio- or behavioural assays, however, is not. Our potential, therefore, to discern super-lures from the complexity of scents from biological materials is currently limited by the laborious intensity demanded by bio- and behavioural assays.

The importance of a rapid and inexpensive bioassay was emphasised at the symposium, especially by those with experience in the search for invertebrate super-lures and elephant pheromones and deterrents. Work in New Zealand towards remarkably sensitive, rapid and inexpensive bio-assays is advanced – like the use of restrained honeybees to detect the odours of human or bovine tuberculosis. The advice from experts at Plant & Food Research (Drs Dave Greenwood and Max Suckling) was to develop bioassays like these to speed the identification of mammalian semiochemicals. The incredible number of volatile chemicals released by animals makes a rapid, cheap bioassay critical to rapidly establishing the most likely candidates for closer investigation.

Current bioassays for mammals are largely rudimentary behavioural tests that are time-consuming and expensive because they require the maintenance of colonies of wild animals. Behaviour with respect to semiochemicals is also comparatively variable and complicated such that outcomes are often ambiguous and require large amounts of replication within careful experimental designs. Instead, is it possible that we might culture olfactory tissue from the olfactory bulb or vomeronasal organ of mammals as better first-step bioassays of mammalian semiochemicals as Dr Bill Jordan suggested. Some innovation in developing a bioassay for mammalian semiochemicals is required.

Like bioassays, field tests of candidate lures are similarly demanding and outcomes sometimes ambiguous. Field tests for lures of the Australian brushtail possum are under way by two research collaborations between the pest control technology firm Connovation Ltd and Lincoln and Auckland Universities, and between Landcare Research Ltd. and Lincoln University. The early results from those field trials were perhaps indicative but not yet strong. Nevertheless, they were very useful illustrations of the challenges of testing lures in the field, where the responses of animals are influenced by so many environmental characters. The complexity described by the plenary speakers – first of the signal and then of its interpretation in multi-species olfactory landscapes – might explain why clear results with captive animals are difficult to reproduce in the wild. Like the need for advances in bioassays, field trials of semiochemicals will also require the development of robust experimental designs that take advantage of advances in remote animal monitoring, like

proximity radio-telemetry, GPS, and trap- or animal-mounted cameras.

Super-lure discovery, requiring the identification of lure candidates, developing and implementing rapid bioassays, and field experiment design and interpretation, is a technical, chemical, physiological and ecological challenge. The search will be long and complex, requiring multi-disciplinary, collaborative approaches. A strong start in the search for super-lures has been made in New Zealand by multiple research groups that have approached the problem in different ways. This diversity will ensure a robust search, especially if the different groups come together periodically, such as at this symposium, to learn from each other.

We have begun.

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