Cane toad chemical ecology: getting to know your enemy

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Abstract

Surprisingly little is known about the way chemical secretions modulate behaviour in amphibians, and this is particularly so with respect to cane toads. For example, anecdotal observations and some preliminary experimental data suggest that cane toads may deploy sex, alarm or aggregation pheromones. If true, the existence of such ecologically significant cane toad chemicals would support our proposition that "...knowledge of cane toad chemical ecology will reveal potential control strategies." To test this proposition we have recently assembled a multidisciplinary research team based at the University of Queensland, with a view to answering the following questions. (1) Can we establish methodologies capable of the qualitative and quantitative chemical analysis of ecologically significant cane toad chemicals? (2) Can we isolate, characterize and identify these chemicals? (3) Does cane toad chemistry vary between individuals, with life cycle, male vs. female, sexual maturity, season, geographic location etc.? (4) Can we establish cane toad behavioural assays, and do any cane toad chemicals display activity in these assays? (5) Can we use knowledge of cane toad chemicals to disrupt cane toad survival?

Introduction

Most animals (and indeed many plants) use chemicals (pheromones, toxins, venoms...) to improve their individual survival, and in doing so contribute to the success of their species. That is, those species that can produce or acquire key chemicals that significantly enhance survival will prosper and proliferate within an ecosystem, at the expense of competing species that are less chemically adept. The chemicals can provide advantage in the form of sex, alarm and trail pheromones, which facilitate intra-species communication for the purpose of enhancing reproduction, defence and feeding. They can also take the form of venoms or defensive secretions which can be used to improve inter-species interactions - for example - a venom that rapidly kills or immobilizes prey, or an unpleasant tasting secretion that deters (or even kills) would be predators - each in their own way facilitates the desire to feed well, and not be fed upon! The study of this diverse array of chemicals and their biological roles is encapsulated in the science of chemical ecology. Species that co-evolve within an ecosystem eventually establish a stable balance, an accord between competing chemical ecologies where prey and predators acquire, lose, and reacquire immunity to successive generations of venoms and defensive secretions - and as individual species trial and refine new pheromonal solutions. While this balance will ebb and flow as species and indeed ecosystems evolve, the peace can be shattered by the enforced relocation of an invasive species into an unfamiliar ecosystem - unaccustomed or prepared for the chemical onslaught launched by the invader. The appearance of the cane toad in Australian ecosystems may be characterised as just such an onslaught. An understanding of the chemical ecology of cane toads could offer a way to control them. As new participants in the quest to control cane toads in Australia, we bring a molecular skill set, quite different from but complementary to that which has been historically applied to this problem.
Our ability to explore and challenge the cane toad at the molecular level allows us to address our primary proposition that:

Knowledge of cane toad chemical ecology will reveal potential control strategies.

As Anuran amphibians (frogs and toads) have a pronounced calling behaviour, it has long been assumed that chemicals played little or no role in their behaviour and ecology (e.g. Houck and Sever 1993). Recent results have changed this view (Brizzi, et al. 2002, Lee and Waldman 2002, Pearl, et al. 2000, Wabnitz, et al. 1999, Waldman and Bishop 2004), and it is now clear that many, if not all, Anurans have a robust chemical ecology and use chemical signals to modulate their behaviour.

In reviewing the scientific literature on cane toad chemistry we were surprised to note that it was largely decades old, was superficial and incomplete by modern standards, and was bereft of ecological context. Furthermore, these studies were limited to narrowly defined investigations into the organic soluble defensive secretions from cane toad parotoid glands, and the appearance of related chemistry in toad egg masses – and were overwhelmingly focused on the bufadienolide class of metabolites – which are broadly represented across many animal and plant species and are known to be cardioactive toxins. For example, marinobufagin is representative of the ten bufadienolides that have been reported from adult cane toads and egg masses (Chen and Osuch 1969, Matsukawa, et al. 1997). The skin secretions of the toad also contain alkaloids and bioactive peptides and proteins (Heatwole 1994). The incomplete nature of this chemical knowledge begs more questions than it answers, and prompted us to formulate and seek to address a series of questions.

Question 1

Can we establish methodologies capable of the qualitative and quantitative chemical analysis of ecologically significant cane toad chemicals?

We make no assumptions on the molecular nature of cane toad chemical ecology, and require that methodologies we develop are capable of analysing small (i.e. bufadienolides) and large (i.e. proteins and peptides) molecules, across all biosynthetic structure classes, whether they are water or organic soluble, volatile or non-volatile, stable or non-stable, and whether they are major or very minor components. Our analytical technologies of choice are automated HPLC/DAD/ELSD/ESIMS and GC/MS, and build on our extensive experience in the analysis of molecules across all biosynthetic structure classes. Preliminary studies on parotoid gland secretions have already revealed a far more complex molecular picture than previously recognized – and hint at issues of chemical stability and interconversion post-secretion.

Question 2

Can we isolate, characterise and identify these chemicals?

Knowledge of molecular structures is a key step in understanding origin, relationship and function. Pure standards can be used to calibrate analytical procedures, to more accurately monitor variations (see question 3), and to screen for ecologically relevant properties (see question 4). Our technologies of choice are HPLC/DAD/NMR, and advanced chromatographic and spectroscopic methods.
Question 3

Does cane toad chemistry vary between individuals, with life cycle, male vs. female, sexual maturity, season, geographic location etc.?...

Armed with effective chemical analysis methodologies we can address the issue of cane toad chemical plasticity. Chemicals that cycle in abundance are obvious candidates for consideration as pheromones, which need to be regulated on vs. off in order to control ecological outcomes. Similarly, we can assess whether the character of the defensive chemicals in adults vs. eggs vs. tadpoles vs metamorphs reveal a common biosynthetic origin (i.e. deposited by the adult vs. de novo biosynthesis), and whether this knowledge highlights vulnerability in the life cycle that would allow us to disrupt critical intra-species communication, and/or enhance susceptibility to predators and/or infection. Our technology of choice will be HPLC/DAD/ELSD and HPLC/MS supported by databases keyed to the chromatographic (HPLC) and spectroscopic profiles of individual samples and pure standards.

Question 4

Can we establish cane toad behavioural assays, and do any cane toad chemicals display activity in these assays?

Discussions with colleagues suggest that cane toads may deploy sex (as in Brizzi, et al. 2002, Pearl, et al. 2000, Wabnitz, et al. 1999), alarm (Alford 1994, Hearnden 1991) and aggregation pheromones. Also, there is anecdotal evidence that female toads select locations for egg laying that are devoid of other egg masses/tadpoles. Our preferred approach to addressing these questions is collaboration, although we are establishing a toad colony that may be suitable to support selected ecological and behavioural studies.

How exactly does cane toad venom (ie defensive secretion – see below) exert a toxic effect, and are individual chemicals more or less toxic? Can knowledge of the mode-of-action of these toxins suggest likely approaches to developing cane toad specific toxins? Are egg and tadpole chemicals more toxic than adult chemicals (Cohen and Alford 1993, Hearnden 1991)? Can we identify a sex pheromone (Brizzi, et al. 2002, Pearl, et al. 2000, Wabnitz, et al. 1999)? Can we identify an aggregation pheromone? Can we identify an alarm pheromone (Kraft, et al. 2005, Summey and Mathis 1998, Wilson, et al. 2005)? Can female cane toads detect chemical cues that influence choice of location for the laying of eggs, and if so can we identify the chemical(s) responsible for this effect?

Question 5

Can we use knowledge of cane toad chemicals to disrupt cane toad survival?

Clearly we will be better positioned to answer this question once we know about cane toad chemical ecology. However, as a basic principle we take the view that such knowledge will reveal potential solutions for cane toad control. Some possible outcomes include, but are not limited to:

- a synthetic sex pheromone that can be used to either disrupt mating over a wide area, and/or as a species-sex selective lure to assist trapping programs.
- a synthetic alarm pheromone that may disrupt female cane toad egg laying behaviour by chemically tagging suitable locations as “used”, or that may disrupt tadpole/metamorph behaviour, leading to reduced survival.
- a synthetic aggregation pheromone that may disrupt the natural aggregation of cane toad tadpoles, leading to reduced survival.
• a synthetic chemical that may disrupt the production and/or distribution of cane toad toxins, rendering the cane toad vulnerable to predators and/or infection, leading to reduced survival.
• a synthetic chemical that is a toad selective toxin that might be used to enhance trapping and baiting programs.

Research team and progress

Our approach to studying cane toad chemical ecology has been to assemble a specialist team with a broad chemical skill base, supported by experts in ecology and biology. The team is built around four University of Queensland research groups, these being Prof Rob Capon (Project Leader, chemistry – small molecules), Prof Paul Alewood (chemistry - peptides), Prof Richard Lewis (biology – bioassays) and Prof Gordon Grigg (zoology – toad colony). Dedicated project appointments include Dr Andrew Hayes (chemical ecology), Dr Jie Zhang (peptide chemistry) and Alexis Barrett (toad ecology). The project received 2 yrs funding from the Qld Government, via a contract research agreement with the Invasive Animals CRC, and commenced operations on Feb 1st 2006. While very early days, the players are in place, and the dynamics of the research program are taking shape. The next 12 months promise to be a significant period, as we come to terms with cane toad chemistry.

As a postscript ... to know your enemy is to respect your enemy

As described above, the existing knowledge base on cane toad chemistry is modest at best, and is inadequate to provide a scientific basis to support many of the truisms that seem to pervade the public psyche when it comes to cane toads. With a certainty verging almost on hysteria, some attribute cane toads with almost legendary powers to poison all in their path, including waterways. That such hyperbole infuses the public and legislative debate is unhelpful. For example, even the language used when discussing cane toads can be unnecessarily emotive. Use of the term cane toad venom conjures an image of a belligerent aggressor, poised and willing to attack, to inject, whereas in reality cane toad poisonings are merely the unfortunate demise of a would be predator encountering cane toad parotoid secretions. Even the ecological role of these secretions is unclear. While they undoubtedly contain toxic components, such as the bufadelonolides, it is not certain that this is the primary role of these secretions. It may be that the toxicity of these chemicals is a by-product of secretions developed for a different purpose, such as communication, or (more likely) that these components serve a dual role. Until the molecular knowledge is gained, and combined with accurate ecological information, the most sensible terminology to use is skin secretions. We do not then presuppose a function, but allow a more measured assessment.

That we identify more closely with the demise of the native predator (quoll, lizard, snake etc...) is not surprising, but under different circumstances one might reflect on the cane toad as victim not villain – not simply because it kills native animals only in defence, and can die in the process, but because it finds itself an unwilling transportee to Australia, an invasive pest, the enemy, through no fault of its own. We look forward to contributing to the debate on, and to developing technologies that may contribute to, the control of cane toads in Australia – and to do so in a humane and respectful manner.
Literature cited:


