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GENETIC OPTIONS FOR THE CONTROL OF INVASIVE VERTEBRATE PESTS: PROSPECTS AND CONSTRAINTS

Ronald E. Thresher
Invasive Animals Cooperative Research Centre and CSIRO, Division of Marine and Atmospheric Research, Hobart, Tasmania, Australia

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RONALD E. THRESHER, Invasive Animals Cooperative Research Centre and CSIRO, Division of Marine and Atmospheric Research, Hobart, Tasmania, Australia

Abstract: Conventional methods for the control of invasive pests are generally effective only on small-space scales or short-time frames. For most well established pest populations, longer-term efforts to manage the problem have been largely abandoned. I examine the potential of using "autocidal" genetic techniques to control terrestrial vertebrate pests, based on the inheritance through males of transgenes that either sterilise females or convert them into functional males ("daughterless"). Simulation analysis of two high profile pest species, the cane toad (*Bufo marinus*) in Australia and brown rats (*Rattus norvegicus*) in an urban environment, using realistic parameters, suggests that virtual eradication could be achieved at apparently realistic stocking rates within 100 years for toads, and in less than 20 years for rats. The essential genetic requirements for autocidal technology (the ability to genetically transform the pest, genes that when blocked cause sex-specific infertility or sex change, and a means of shutting off the construct for breeding purposes) have already been demonstrated in rodents and are likely to be available in other pests, based on broad conservatism of genetic mechanisms of sex differentiation in vertebrates. Hence, there appear to be no major logistical or technical impediments to developing a genetic control program against many pest species. However, the models also indicate that a recombinant pest control program would be difficult against species whose populations are under strong density dependent regulation or are so large that absolutely high numbers of carriers need to be stocked to achieve control. More potent genetic options than those modelled could be feasible, but their use needs to be tested against public acceptability, due to the apparently higher risk they pose for non-target populations and species.

Key Words: amphibian, cane toad, genetically modified, invasive species, model, Norway rat, policy, public acceptability, recombinant genetics, risk, rodent.

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INTRODUCTION

Methods to manage invasive species range from ignoring them and hoping they will go away through to a variety of options for physical removal, biocides and biological control (Thresher and Kuris 2004). For well established and widely distributed pests, the only realistic options currently available are augmentative and classical biological control, and sterile male release programs, both of which have significant constraints on their application (Whitten and Foster 1975). As a result, most invasive pests remain uncontrolled except at small scales and for short periods.

In the 1960s, entomologists speculated that genetic techniques could be a powerful means of controlling pest populations (Hamilton 1967), based on the observation that meiotic drive (a genetic sex ratio distorter) had apparently driven some insect populations to extinction. Practical development of such techniques lied fallow, however, until recent developments in recombinant genetics stimulated renewed interest in the field (e.g., Thomas et al. 2000). Currently, at least three recombinant methods for pest control (repressible male sterility, virally vectored immuno-contraception, and female-biased sex ratio distortion) are being tested in the laboratory. Another widely publicised study has speculated that the escape of even one carrier of a "Trojan gene" (a construct that pleiotropically enhances mating advantage while otherwise reducing fitness) could cause species extinction (Muir and Howard 2002), an issue of considerable concern with regards to accidental escapement of genetically-modified (GM) organisms, but also one that offers options for pest control if properly managed. A number of recent studies have modelled the potential for pest control of methods that have been proposed, incorporating varying degrees of ecological reality (Davis et al. 2000, Schliekelman and Gould 2000a,

b, Gould and Schliekelman 2004, Schliekelman et al. 2005, N. Bax and R. Thresher, unpublished data). All conclude that pest control using genetic methods is feasible, at least under the conditions specified in the models.

In this paper, I briefly review the genetic options that have been proposed thus far, discuss their potential when applied to two very different invasive vertebrates (the cane toad [*Bufo marinus*] in Australia and urban populations of the brown rat [*Rattus norvegicus*]), and then consider possible ecological and logistical constraints on the application of the technology for the control of terrestrial vertebrate pests.

GENETIC OPTIONS FOR PEST CONTROL

Three broadly different approaches for using recombinant genetics to control pests have been investigated: genetically engineered viruses that, when incorporated into a bait, act as a species-specific toxin or sterilizer; self-disseminating engineered viral diseases; and non-disseminating (i.e., sexually transmitted) "autocidal" genes. In the last, I include as a special case chromosomal modifications designed to achieve the same outcomes as the recombinant approaches (Gutierrez and Teem 2006, Cotton and Wedekind 2007). Viruses as toxins will not be considered further here, as their dynamics and efficacy largely parallel those of a conventional baiting program (see Torres et al. 2001 for an example of using a virus to disseminate a recombinant vaccine in rabbits [*Oryctolagus cuniculus*]). Genetically engineered viral diseases are being examined principally for mammals, in which the target is a gene crucial for reproduction that can be disrupted by a suitably modified, otherwise low impact vector (usually a virus). "Immuno-contraception" has been investigated for rabbits, mice (*Mus musculus*), foxes (*Vulpes vulpes*), and cane toads in Australia (Hardy et al. 2006, Robinson et al. 2006) and brushtail possums (*Trichosurus vulpecula*) in New Zealand (Cowan 1996). Despite promising results in the laboratory, a decade long program to develop immuno-contraception against introduced mice in Australia has recently been terminated, in part because of perceived difficulties in obtaining public approval to release the virus and in part because of problems finding a suitable virus.

Gould and Scheikelman (2004) coined the term "autocidal" to refer to genetic modifications that essentially resulted in a species breeding itself to extinction. Five autocidal approaches have thus far been suggested as possible control options (Table 1). All appear to be genetically feasible. Projects to develop or assess the population impacts of four are currently in progress, principally on insects or fish: female-specific lethality (Thomas et al. 2000), "daughterless" and female-specific sterility (Thresher et al. 2005), and a pleiotropic "Trojan" gene (A. Kapuscinski, personal communication). Autocidal techniques are of interest in part because they offer possibilities for control where none now exist (e.g., most invasive pests), but also because they have several intrinsic advantages over conventional biological control: theoretically, at least, and depending on the specific method used, the genes can be constructed to be species-specific; they can target particular life-history stages or sexes, so as to maximise efficacy or minimise damage to non-target species; their effects can potentially be reversed if something goes wrong (using a genetic "off switch" built into the construct); and some approaches lend themselves to relatively quick and inexpensive modification to target other species, while retaining species-specificity for each. The relatively low cost of changing targets spreads the benefits of the high-cost genetic program required to develop an autocidal approach, and contrasts with the virtually "start-all-over-again" efforts needed to find a separate pathogen for each pest species targeted by conventional biological control.

POTENTIAL APPLICATION TO TERRESTRIAL VERTEBRATE PESTS: EXAMPLE 1 – CANE TOADS

Cane toads were introduced from Hawaii to tropical Australia in 1935, as a biological control agent for cane beetles (Lever 2001). The toad proved singularly ineffective as a biocontrol agent, but rapidly increased its range, population densities and conspicuousness in Australia, to the point where it is considered one of the countries most damaging invasive terrestrial vertebrates, and one of the worlds' 100 worst invasive alien species (Lowe et al. 2001). Currently, the toad occupies about half a million km^2 of northern Australia (primarily Queensland and the Northern Territory), but its range is expanding rapidly (Phillips et al. 2006) and it appears that most of tropical and sub-tropical Australia is vulnerable to the species. The toad has been implicated in the decline of native predatory mammals and reptiles, as well as native frogs (Lever 2001), but compelling data of long-

Table 1. Autocidal approaches suggested as possible options for controlling invasive pests.

Approach	Description	References
Sex or stage-specific lethality/sterility	Construct induces death of offspring at specified stage, or kills or sterilises offspring of one sex, in which case the gene is transmitted through the other sex.	Thomas et al. 2000
Gender distortion ("daughterless" or "sonless")	Construct causes offspring to develop as specified sex irrespective of sexual genotype.	Hamilton 1967, Thresher et al. 2005
Inducible mortality	Construct causes death when externally triggered by, e.g., extreme environmental variability or artificial trigger; construct maintained in population by further stocking.	Grewe 1997, Schliekelman and Gould 2000
Pleiotropy "Trojan gene"	Construct pleiotropically has positive effect on one or more fitness components, and negative effects on others, e.g., increases mating advantage while decreasing viability of genetically modified offspring.	Muir and Howard 1999, 2000
Selfish genes	Operational construct, e.g., one that causes gender distortion or sex-specific lethality, is packaged into a genetic element that has a high probability of reproducing itself within a genome, increasing both its spread and that of the construct.	Burt 2003, Burt and Trivers 2006

term impacts on Australian ecosystems are still sparse (see papers in Molloy and Henderson 2006). The history of efforts to control the toad in Australia is reviewed by Tyler (1998) and Lever (2001). A variety of methods have been suggested to control it, ranging from topical biocides to traps, sterile male release programs and classical biological control (see papers in Molloy and Henderson 2006). To date, no safe and effective biological control agent against the species has been identified, and other approaches have proven successful only at small scales.

The possible effects of a recombinant program of pest control on cane toads have been examined by Thresher and Bax (2006), the results of which are summarised here. The assessment was made using a deterministic, age-structured population genetics model that incorporated the main features of cane toad demography, based on information from Lampo and De Leo (1998) and R. Shine (persoanl communication). The population genetics model simulates recruitment, age-specific mortality, sex ratios and gene frequencies in a freely interbreeding population; i.e., it assumes no emigration and immigration. Initial conditions for the cane toad specify an annual mean recruitment of 1,000 individuals at carrying capacity, a sex ratio at birth of 1:1, a constant post-metamorphic mortality rate, an age at maturity (95% mature) of 2 years, and a maximum age (95% mortality) of 5

years. Recruitment (number of individuals surviving metamorphosis) was related to adult population size by means of a discrete logistic (Ricker) model. Lampo and De Leo (1998) suggest strong density dependence in the egg and larval stage, but only slight density dependence among juveniles and adults. This was captured in the model by using as a baseline a Ricker parameter of 1.25, which specifies low recruitment to the juvenile stage when populations are at less than half carrying capacity (due to the small number of breeding females) but which stabilises asymptotically at larger population sizes due to competition among pre-metamorphs. The model also assumes recruitment is strongly affected by rainfall (proxied as the Southern Oscillation Index), on the basis that high water levels increases the number of available breeding ponds and resources for developing tadpoles. Extinction is arbitrarily defined as when the number of viable females falls to 1% of initial population size, and the model runs are limited to a maximum of 100 years, as any method that took longer than this to control cane toads would probably not be attractive to managers or the public.

The effects of physical removal on the modelled toad population are shown in Figure 1, for removal levels of up to 20% of the breeding population each year. Physically removing toads reduces immediate population density, obviously, but at low removal rates this is largely over-ridden by large inter-annual differences in breeding success and by density dependence. To achieve a significant long-term effect on population size, about 20% of the adults need to be removed each year; long-term extinction requires annual removal in excess of 40% – a huge task.

Figure 2 compares the effect of four recombinant options on cane toad numbers: a gene construct that biases offspring sex ratios towards males ("daughterless"), one that sterilises females, but not males ("female sterile"), one that causes pre-maturational female-specific mortality ("female lethal"), and a Trojan gene. For the last, the mating success of male carriers was set 4 times higher than that of wild type males, but they also had 0.5% lower first-year survival of their offspring, a combination suggested by Muir and Howard (2002) as most likely to lead to population extinction. For the comparison, stocking rate was set at 5% of mean annual pre-stocking recruitment per year, and carriers were assumed to have eight

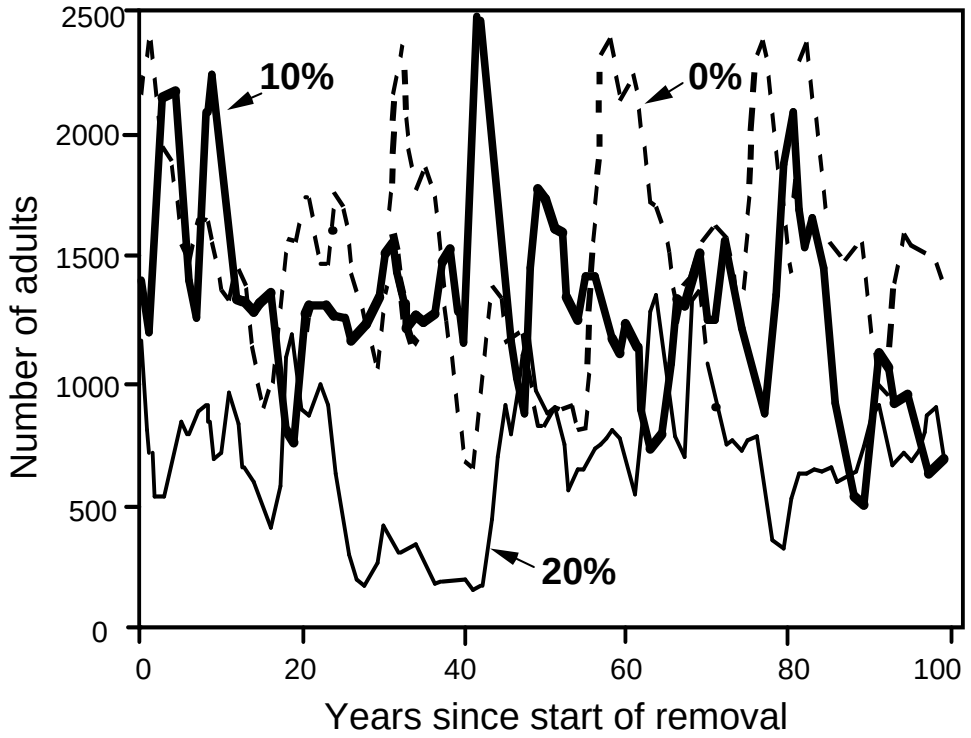

Figure 1. Effects of physical removal on modelled number of cane toads, at annual removal rates of 0, 10 and 20% of the post-metamorph population.

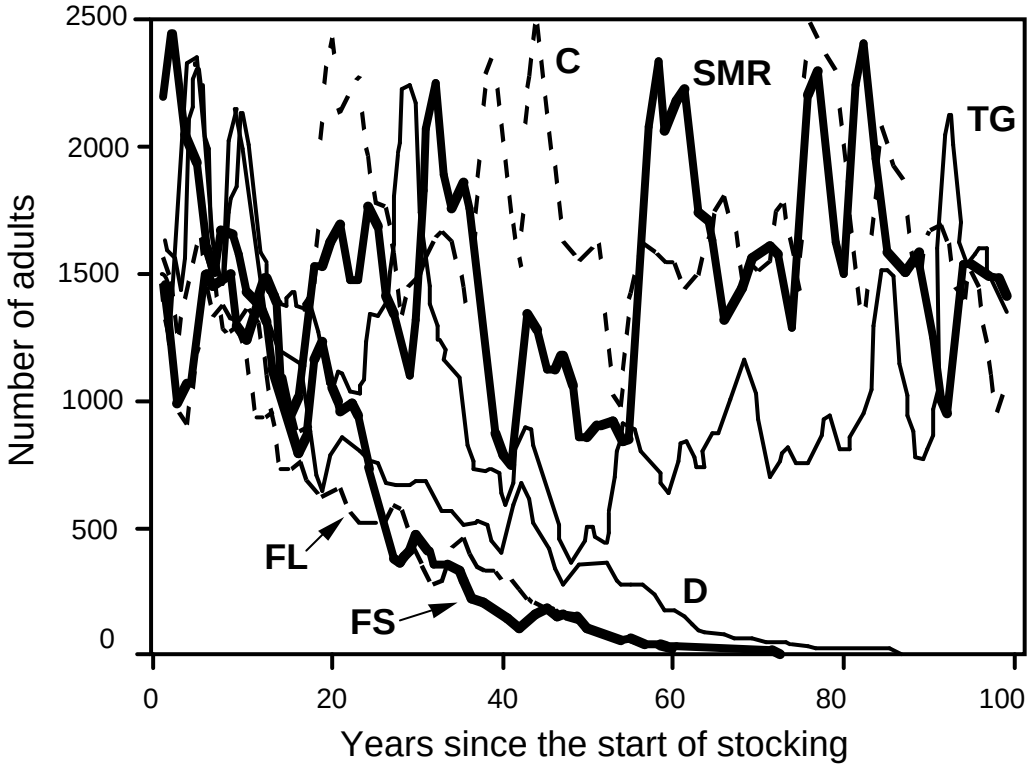

Figure 2. The effect of six genetic control methods on numbers of adult cane toads. C = control (stocking of neutral gene); SMR = Sterile male release; TG = Trojan gene; FL = Female-specific lethality; FS = female-specific sterility; D = daughterless.

independently segregating copies of the relevant construct. The model also assumes that one copy of the construct was sufficient to cause the desired phenotype (sex change, sterility or death), that there was no leakage due to gene silencing, and that the stocked animals had a fitness equal to that of the wild-types. The effects of varying these parameters on the efficacy of a recombinant program are explored by N. Bax and R. Thresher (unpublished data). For completeness, the model also assesses the effects on the cane toad of stocking a neutral gene (a stocking control) and the release of sterile males (also at 5%) as a possible alternative management option.

At the 5% per annum stocking rate, neither releasing sterile males nor carriers of a Trojan gene had a significant long-term effect on toad numbers. The model suggests that achieving extinction using a sterile male release program requires annual releases in excess of 50% of the number of wild type males and even then, takes about 90 years of annual stocking. The Trojan gene method results in a rapid replacement of the wild genotype (N. Bax and R. Thresher, unpublished data), but does not lead to population extinction even at stocking rates as high as 70% of mean pre-stocking recruitment. In contrast, 5% annual stockings of carriers of female-lethal, female-sterile or daughterless constructs cause the toad population to go extinct in less than 80 years. The minimum annual mean stocking rates required to achieve extinction within 100 years are similar for the female-lethal and female-sterile constructs (at about 2.5% of natural recruitment levels) and is slightly higher for a daughterless construct (3%).

POTENTIAL APPLICATION TO TERRESTRIAL VERTEBRATE PESTS: EXAMPLE 2 – THE NORWAY RAT

Urban rodents are among the world's most damaging invasive species as disease vectors and threats to public health, contaminators and consumers of food products, and destroyers of infrastructure (Pimental et al. 2000, Sullivan 2004). Despite this, and despite an almost universal dislike of urban rats, in particular, most urban governments have effectively abandoned efforts to control mice and rats at anything other than local scales and over short periods, using poisons, traps, barriers and

acoustic devices to lure, repel or, most frequently, kill rats and mice. The long-term effects of these control efforts are negligible (Davis 1987, Easterbrook et al. 2005), which has led to calls for better means of managing the pests (Colvin and Jackson 1999).

To assess the potential of a recombinant approach to control invasive rodents, I use as a test case an urban population of the Norway or brown rat. The model structure and key assumptions are similar to that described above for the cane toad. Basic parameters for the Norway rat model are based on Davis (1953): sex ratios are equal at birth, average age to maturity is 3.5 months, 99.9% of the newborn die before or at weaning (annualised mortality rate), the subsequent annual mortality rate is 91%, and recruitment is density dependent and strongly affected by environmental variability. As for cane toads, I assume as a first approximation that the rat population is closed; this assumption seems reasonable based on field data showing that Norway rats are relatively non-migratory in urban environments (Davis 1953).

The effect of introducing multiple copies of a female-sterilising construct, inherited through males, on a Norway rat population the size of Baltimore's (approximately 50,000 rats) (Easterbrook et al. 2005) is shown in Figure 3, with and without complementary rat control. Alone, releasing adult carriers equivalent to 5% of wild-type (WT) recruitment at carrying capacity (approximately 1,750 rats per month, of which half are male carriers and the rest sterile females) reduces the rat population to virtual extinction in less than 20 years. Combining the 5% monthly stocking with a control program that removes genetically modification and WT rats indiscriminately at a rate that doubles the natural mortality of the adults reduces the modelled rat population by more than 99.9% in about 10 years. In contrast, just increasing the rat mortality without the genetic modification technology only depresses rat numbers without eradicating them (Figure 3), and the population quickly rebounds if the killing stops (Davis 1987). Producing about 2,000 rats per month to release requires a significant effort, but

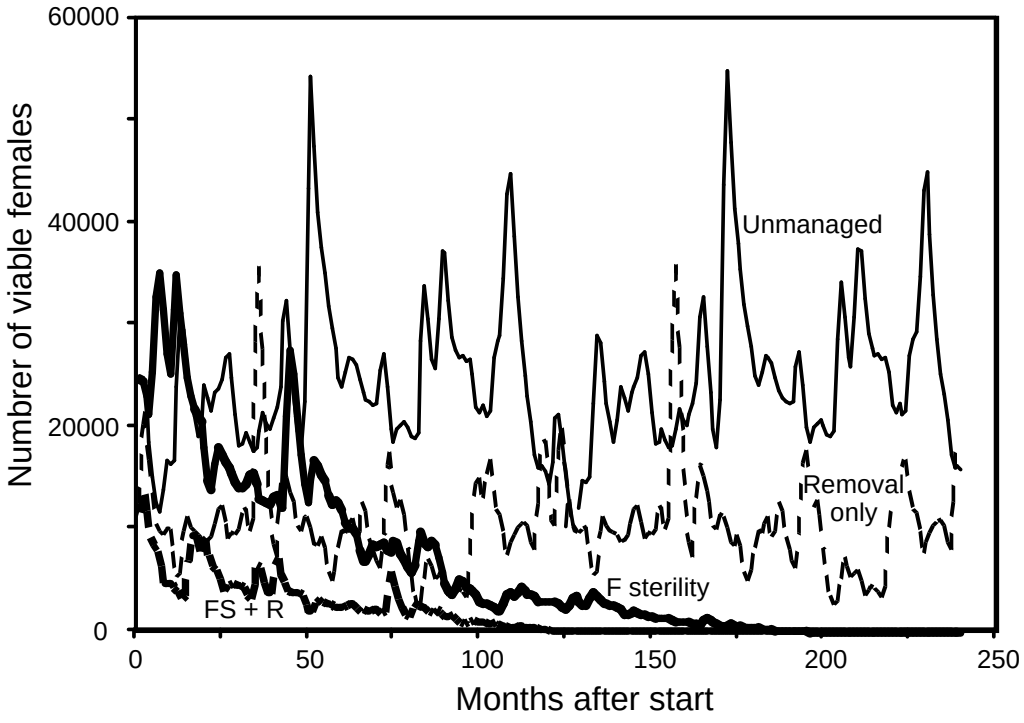

Figure 3. Modelled trajectories for an urban rat population under four management conditions: unmanaged, sustained annual removals/killings that double the rate of adult natural mortality (removal only), a female-sterility construct stocked at 5% of natural pre-treatment recruitment (F sterility), and a combination of both the recombinant method and non-discriminate removal of rats (FS + R), as above.

appears to be feasible given the scale of the commercial rat breeding industry. The ease with which rats can be bred suggests options for community involvement in a "breed-and-release" program, which could also build local interest in and support for the program.

GENETIC FEASIBILITY OF RECOMBINANT CONTROL METHODS

The model analyses indicate that (1) physical removal (killing, trapping, etc.) alone is insufficient to control either well-established invasive vertebrate – cane toads or rats – at anything approaching realistic effort levels, (2) recombinant methods can achieve control, and possibly eradication, within what are likely to be acceptable time frames and at effort levels that are likely to be costly, but achievable, and (3) combining the recombinant method with physical removal reduces the time required to control the pest. The limited impact of physical removal as a pest control option with some species is well established. Nonetheless, the analyses indicate that it can play a significant role in a pest management program based on recombinant methods. In an idealised scenario, recombinant approaches can be considered "silver bullets" for pests, but they work best when combined with other pest control options in an integrated pest management framework. The modelled control programs are based on a construct inherited through one sex (males) that sterilises, kills or changes the sex of the other (females). As such, they require the ability to genetically transform the target species, genes that when knocked-out or mis-expressed cause the phenotype of interest in females, and a method for repressing the effect of the construct(s) in order to maintain breeding lines (conditional gene expression). The extent to which these requirements are satisfied at present varies widely across taxa, depending on the amount of genetic interest in them for other purposes. Hence, existing technologies appear to satisfy all three requirements in rodents: transgenic rats and mice have been routinely produced for medical research since the 1990s (Tesson et al. 2005), numerous genes that when disrupted cause sex-specific infertility in rats and mice have been described (e.g., Al-Shawi et al. 1992, Sharpe et al. 1995), and conditional gene expression, using the Tet-off system (Gossen et al. 1995), has been demonstrated in mice (Kistner et al. 1996). In contrast, considerably less background work has been done on amphibians, in general, and toads in particular. There are no reports in the literature of genetic modification of toads, but there is a large literature on the use of sperm-mediated gene transfer to produce transgenic frogs (Ny et al. 2006). The African clawed frog (*Xenopus laevis*) is a model species for studying the genetics of development in lower vertebrates (e.g., Heasman 2006), and hence techniques for manipulating gene expression in the species have been well worked out. It is highly likely that the methods used to produce transgenic tadpoles and adult *X. laevis* would be broadly applicable to cane toads. With regard to suitable genes, a general program to develop at daughterless construct in cane toads is described by Koopman (2006). More specifically, development of a daughterless construct in fish has focused on disrupting the expression of the enzyme aromatase, which is crucial in female sexual differentiation (Thresher et al. 2005, in preparation). Chemical inhibition of aromatase modifies phenotypic sex in amphibians (Chardard and Dournon 1999, Mackenzie et al. 2003), as it does in fish, suggesting that an approach similar to that being developed for fish would be effective in the toads.

More broadly, patterns of steroid and hormone expression and activity are similar across a range of vertebrate lineages (Devlin and Nagahama 2002, Eggert 2004), as are many elements of the underpinning genetics (Devlin and Nagahama 2002, Koopman 2006, Wilhelm and Koopman 2006). This conservatism should facilitate identification of suitable target genes in novel species. Again, aromatase provides a possibly useful example. The enzyme serves a similar function in most lower vertebrate lineages, has been sequenced from taxa as diverse as fish, reptiles and mammals (including humans), and when blocked chemically, sex reverses genetic females into functional males in fish, amphibians, reptiles and birds (Elbrecht and Smith 1991, Piferrer et al. 1994, Wibbels and Crews 1994, Charnaud and Dournon 1999). The ubiquity of the enzyme and its key role in sex determination across lower vertebrates suggest it to be a good candidate gene on which to base sex-specific pest control options. Similarly, methods for genetic modification appear to have wide applicability, with a range of commonly used techniques, such as micro-injection, sperm- or virally-vectored transfection, demonstrated in fish, a frog, a diverse range of mammals (including rabbits, cows [*Bus taurus*], pigs [*Sus scrofa*], cats [*Felis catus*], sheep [*Ovis aries*], and goats [*Capra hircus*]) (Houdebine 2005) and, recently, a bird

(Koo et al. 2006). The Tet-off/Tet-on system of conditional gene expression also appears to be widely applicable across vertebrate lineages, having been demonstrated in animals ranging from fish to small mammals. Hence, although there will be non-trivial challenges to implementing a recombinant program in each new target species, generic approaches and information suggest the principal constraints are likely to be logistical, rather than conceptual. A possible exception are those species that lay eggs, as conventional applications of methods to transform species are greatly facilitated by access to the developing egg and embryo. Genetic transformation has recently been reported in the chicken (Koo et al. 2006) using a retroviral vector, but whether this approach can be safely applied to other egg-laying vertebrates, such as invasive Burmese pythons (*Python* spp.), is not clear.

ECOLOGICAL CONSTRAINTS ON RECOMBINANT APPROACHES

The model analyses also indicate several features of autocidal control programs that constrain their utility. The first is that like any control program, they perform poorly when the targeted pest population is under strong density dependent regulation (N. Bax and R. Thresher, unpublished data) (Figure 4A). If population regulation is density independent, then any control strategy that consistently reduces the number of breeding adults will have a cumulative, negative effect on population size. For most species, an assumption of density independent population regulation is not easily justified. Estimation of the degree of density dependence in natural populations is difficult (Sibly et al. 2005), but recent meta-analyses (Brook and Bradshaw 2006) suggest

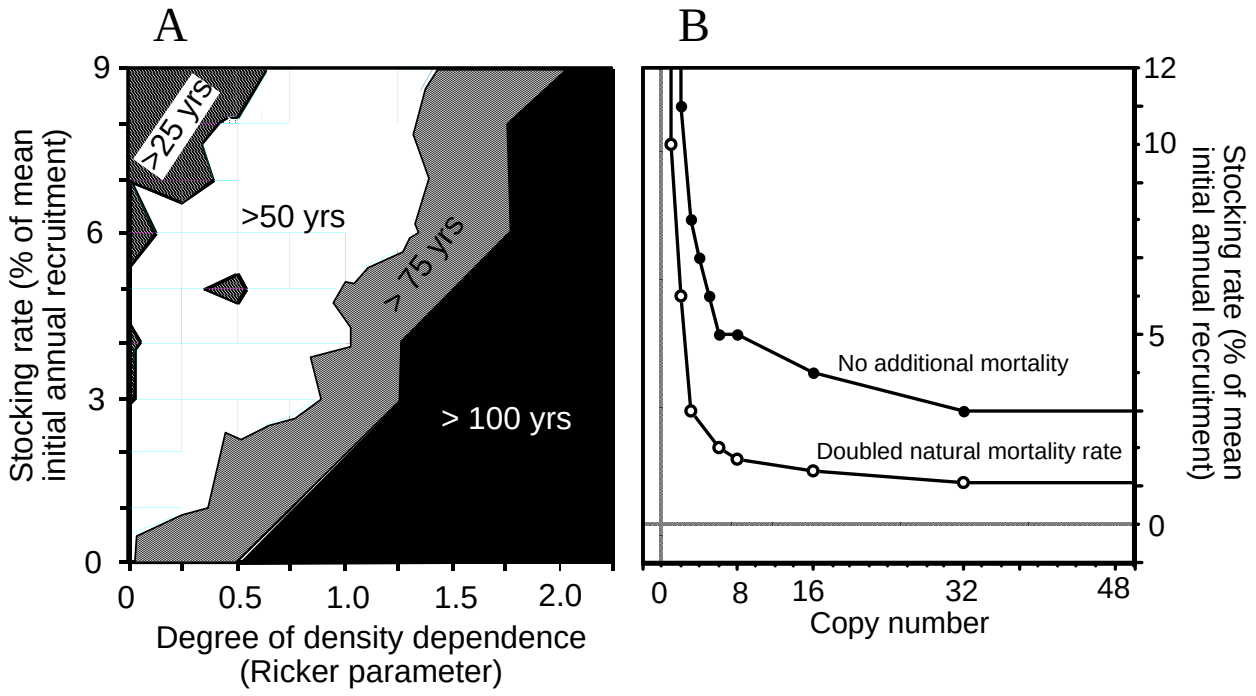

Figure 4 (A.) The effect of different degrees of density dependence on the stocking rate required to reduce a cane toad population to <1% of virgin biomass within 100 years, based on the model depicted in Figs. 1 and 2. (**B.)** The relationship between the stocking rate and copy number required to drive a 50,000 rat population to <50 rats within 20 years, based on the model depicted in Figure 3, with and without complementary rat management activities. The slight irregularities in the relationships reflect stochastic effects of environmental variability.

widespread evidence of density dependence across almost all taxonomic groups for which long time-series of population data were available. Density dependence slows and potentially stops the fall in pest numbers as control efforts are maintained, an effect that can be countered only with increasing difficulty by increasing control efforts (Figure 4A).

The second conspicuous feature of autocidal control programs is that they are inherently slow acting. Even under optimal conditions, effective population control (e.g., populations reduced to < 1% of virgin biomass) typically requires more than 10 generations, and can take much longer. On the positive side, this slow impact allows an affected ecosystem time to adjust to the absence of the pest, there are no mass mortalities of pests, and if a problem develops, there is adequate time to launch counter-measures, such as a second gene construct that effectively shuts off the first. On the negative side, managers and the public are likely to be unimpressed with, and may not be prepared to invest in, such a slow response to the problem. For urgent problems, such as when a native species is at risk, a genetic approach may be too slow to be useful. More fundamentally, the slow action of a recombinant pest program could allow time for the evolution of strongly counter-selected mechanisms to, for example, re-balance operational sex ratios. Logically, as sex ratios in a daughterless program become increasingly skewed towards males, those animals able to continue to produce female progeny would have a very high fitness and be selected for. Such rapid genetic responses to distorted sex ratios have been reported in wild populations (Charlat et al. 2007). Whether or not it can occur, or would occur frequently, in vertebrate pest populations can only be determined empirically. If it is a problem, countering it is likely to require simultaneous application of multiple approaches to pest control (integrated pest management) and possibly a suite of autocidal constructs, that could be sequentially released into a target population to over-come counter-selective pressures.

The amount of time required to control the pest problem depends in part on management objectives (complete eradication or a sustained reduction in pest numbers/impacts) and effort expended. For the modelled approaches to work effectively, minimum stocking rates equivalent to 3-5% of annual mean natural recruitment are required, sustained for at least 10-20 generations, to achieve virtual pest extinction; lower stocking rates can depress pest numbers under most conditions, but do not result in extinction. Achieving a stocking rate equivalent to 5% of natural recruitment is not a trivial task for any highly successful invasive species. The scale of the effort depends in part on the biology of the pest, and particularly the ease with which it can be bred, in part on the size of the target population (and hence the absolute number of carriers required), and in part on the extent to which other management factors can be manipulated to optimise effects of stocking (N. Bax and R. Thresher, unpublished data). The efficacy of a stocking program is determined by three factors amenable to management action: stocking rate itself, copy number, and the amount and specificity of complementary efforts to remove the pests.

The interaction of these factors is illustrated for urban rats in Figure 4B. Copy number is the number of independently segregating copies of the construct carried by each stocked animal. The higher the copy number, the larger the number of generations over which offspring of the carriers will express the desired phenotype (Gould and Schliekelman 2004). In principal, at an infinite copy number the release of a single carrier will drive a pest species to extinction, as the proportion of carriers in the population increases geometrically with each subsequent generation. In practice, (1) genetic mechanisms silence redundant copies of genes, in plants at least (Schubert et al. 2004), and (2) the interaction between copy number and stocking rate is non-linear (N. Bax and R. Thresher, unpublished data) (Figure 4B). Hence, with no complementary management activity, a rat population the size of Baltimore's could be virtually eradicated in less than 20 years by releasing 42 rats (21 carrier males and 21 sterile females) per month, 1.2% of natural recruitment, if each had an infinite copy number, 4% per month for a copy number of 16, and 5% at a copy number of 8. If physiological and genetic silencing mechanisms constrain the carriers to be homozygous at a single locus for the construct (copy number = 2), eradication within 20 years is still possible, but requires a stocking rate of 11% per month. Adding non-selective removal (killing or trapping) of rats as part of an IPM program, however, substantially reduces the stocking rate required to achieve eradication. Doubling the rate of natural mortality of post-weaning juvenile and adult rats reduces the required monthly stocking rate of copy number 2 carriers to only 4%, results in virtual extinction within 20 years even with single copy carriers, at a stocking rate of 10%, and

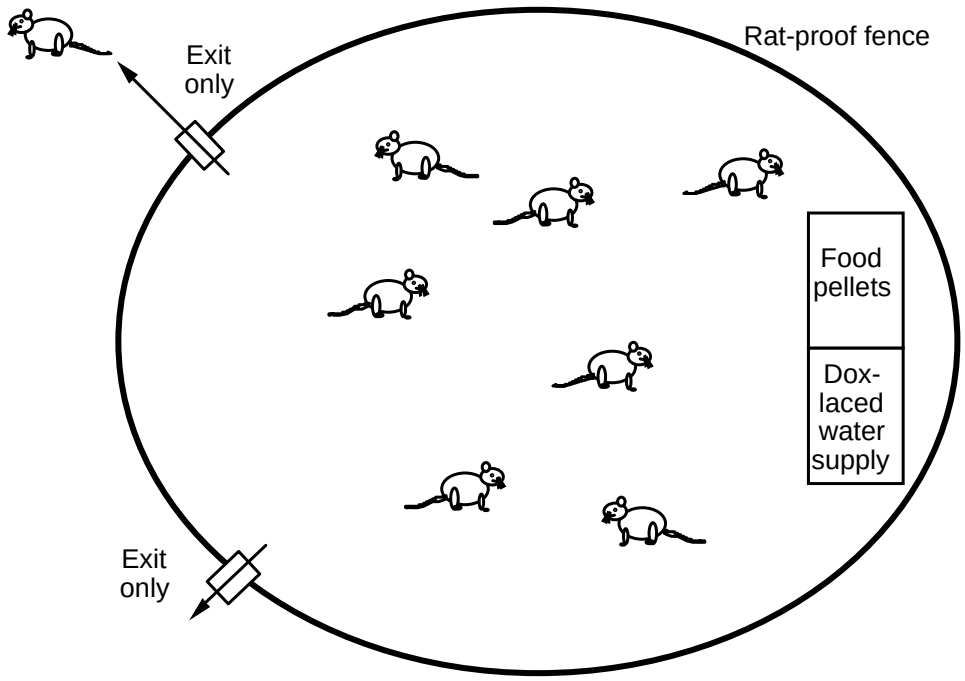

Figure 5. Diagram of a self-sustaining seed population of rats, that could be deployed to control island populations. The enclosure is seeded initially with a pair of high copy number rats, with closed exits, a food supply and a water supply dosed with doxyclycline, which represses the female sterility construct and hence allows the pair to breed and numbers in the enclosure to build. At intervals, the exits are opened, allowing some juvenile carriers to escape and spread the autocidal gene to the target population. The system, as diagrammed, would be largely self-contained, requiring only occasional visits to add food and doxycycline supplies.

makes possible eradication at stocking rates of <2% for copy numbers ≥ 6.

The interactions between stocking rate, copy number and complementary management actions on the efficacy of a control program have three implications.

First, complementary management activity enhances a pest control program based on recombinant methods. Selective removal of wild type animals, while leaving the genetically modified carriers to breed, is the most effective approach (N. Bax and R. Thresher, unpublished data), but even non-selective removal can significantly reduce the time to extinction or the stocking rate required. Recombinant methods add value to physical removal programs that otherwise would have little long-term impact.

Second, the interactions imply that recombinant approaches, as they are outlined above, are probably applicable only to pest animals that are present in relatively small numbers (10s to 100s of thousands), that inhabit discrete habitat patches each of which is treated independently, or that can be efficiently and inexpensively bred and stocked out in large numbers. Hence, urban rats rather than free-ranging nutria (*Myocastor coypus*). As many pest species are highly fecund, large-scale breeding programs could often be feasible. Combining the autocidal construct with a conditional regulatory element; i.e., a sequence that turns a gene on or off in response to an environmental stimulus, offers potential for the development of breeding colonies of high copy number individuals, that can seed surrounding areas. Again using rodents as an example, a Tet-off regulatory sequence shuts down gene expression when carriers are provided drinking water dosed with tetracycline or one of its derivatives (Kistner et al. 1996). Hence, a seed population of high copy number rats could consist of a semi- enclosed colony that is provided food and a tetracycline-dosed water supply, from which most, but not all juveniles are allowed to escape (Figure 5). When the escaped carriers breed with wild type rats, the autocidal gene expresses in their

offspring, leading to its spread and a long-term population decline. On small scales, self-sustaining breeding enclosures like this could control rats on islands where biocidal approaches are not feasible or risk damage to high value non-target species. On larger scales, aggressive stocking so as to genetically fix the construct in source regions of a meta-population, which are then sustained by conditional repression as long as is required, could constitute an inexpensive, large-scale source of carriers that either disperse naturally or can be stocked into other areas. In such cases, continuous conditional repression of the construct may not be required for species in which the original breeding adults are long-lived, e.g., pythons.

The third implication is that for pests that are very abundant or in which production of carriers is costly, more aggressive genetic approaches may be required to affect control. Three options have been suggested. First, if silencing prevents production of high copy number individuals, the same effect could be achieved by the simultaneous use of multiple autocidal constructs. In rodents, the diversity of genes known to affect fertility appears to provide considerable scope for multiple constructs, each based on disrupting or mis-expressing a different gene and each subject to its own constraints on copy number. The combination of 4 autocidal constructs, even if each is individually constrained, could result in an effective high copy number, minimising required stocking rates. Second, a single autocidal construct could be packaged in a "selfish" gene element, so that it replicates within the genome and thereby spreads as if present at an infinite copy number (Burt 2003). Options for achieving a self-replicating autocidal gene are discussed by Burt and Trivers (2006), but thus far it remains only a theoretical possibility. Finally, as noted above, it has been suggested that the release of a single carrier of a pleiotropic Trojan gene, i.e., one that increases male reproductive attractiveness while decreasing the viability of its offspring, could lead inevitably to population extinction (Muir and Howard 1999, 2002). More recent analyses suggest extinction is very unlikely under most ecologically realistic scenarios (N. Bax and R. Thresher, unpublished data), but the broader concerns about aggressive genetic approaches raised by Muir & Howard (1999, 2002) remain valid. Whereas a construct that can cause population collapse following the release of a handful, or even one carrier is superficially attractive, it can also constitute a serious threat to non-target species and populations. The use of low copy number carriers necessitates a substantial stocking program to affect the target population, which while logistically costly, makes it unlikely that the accidental release of a few carriers in areas where they are not wanted, or that the spread of a single autocidal construct across a species barrier, would cause long-term damage.

CONCLUSION

There appear to be no major logistical or technical impediments to use recombinant technology as a core element of a program to control at least some vertebrate pests, such as rats in urban environments, though the required constructs need to be assembled and tested for functionality and fitness effects. Such programs could have major impacts on environmental amenity, biodiversity conservation, health, and even sanitation, as well as significant economic benefits. However, the viability of such a program also depends critically on whether the public accepts the approach (Gaskell et al. 1999) and on a policy and legislative framework that effectively regulates environmental applications of GM technology (Kapuscinski and Patronski 2005). Australian studies suggest that the extent of public acceptability will depend on whether or not the public is consulted during the development of the technology, the transparency of the decision-making process leading to its potential use, and the risk of the construct jumping to non-target species (Thresher and Kuris 2004, Fisher and Crib 2005). Genetic threats to other species need to be assessed on a case-by-case basis, as it is likely to depend on the particulars of the autocidal construct involved and whether or not the target species co-occurs with related species. Currently, in most jurisdictions, autocidal programs will also have to cope with policy and legislative frameworks that provide few clear mechanisms for evaluating and regulating GM technology for purposes of environmental remediation. In the United States, for example, numerous overlapping international, federal and state legislations could affect program viability and it is very ambiguous even as to which regulatory agencies have primary carriage of the issue (Kapuscinski and Patronski 2005). At the other end of the spectrum, Australia has established a national Office of the Gene Technology Regulator, which in principle provides a single point of contact for developing, evaluating and potentially approving environmental release of an autocidal construct. As

the release of the GM carriers constitutes a form of biological control, regulatory frameworks based on those in place for biological control may often be appropriate, suitably modified to encompass expert input into the risk elements specific to recombinant genetics. Unfortunately, regulatory frameworks for biological control in some jurisdictions also appear to be confusing and inconsistently applied (Strong and Pemberton 2000).

These observations suggest that the development of a program to control urban rats, cane toads or any other vertebrate pest using recombinant genetics should involve three initiatives: (1) a program of public consultation to determine the likely acceptability of the approach and the conditions under which it would be acceptable, (2) a review and rationalisation of relevant legislation, possibly in the context of broader issues associated with regulation of biological control in general, and (3) development of the technology itself. The last should include assembly of the relevant constructs and production of integrated lines, evaluation of effects on male fitness in particular, and the development of predictive models that incorporate detailed information on behavior, ecology, demography and inter-specific interactions. If results of these initiatives are positive, it may be practical to field test the genetics and models on invasive populations at physically isolated locations, such as rodents on isolated islands, a major conservation challenge in its own right, at minimum risk and under conditions in which the GM carriers could be eradicated using conventional methods, if need be (Veitch and Clout 2002). Successful tests on small isolated populations could be an important step in developing community and management support for wider application of the technology.

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LITERATURE CITED

AL-SHAWI, R., J. BURKE, J. J. MULLINS, R. M. SHARPE, R. LATHE, AND L. MULLINS. 1992. Transgenesis and infertility. Pages 195-206 *in* S. G. Hillier, editor. Gonadal development and function. Sereno Symposium. Raven. New York, USA.

BROOK, B. W., AND C. J. A. BRADSHAW. 2006. Strength of evidence for density dependence in abundance time series of 1198 species. Ecology 87:1445-1451.

BURT, A. 2003. Site-specific selfish genes as tools for the control and genetic engineering of natural populations. Proceedings of the Biological Society of London 270:921-928.

BURT, A., AND R. TRIVERS. 2006. Genes in conflict. Harvard University, Cambridge, Massachusetts, USA.

CHARLAT, S., E. A. HORNETT, J. H. FULLARD, N. DAVIES, G. K.RODERICK, N. WEDELL, AND G. D .D. HURST. 2007. Extraordinary flux in sex ratio. Science 317:214.

CHARNARD, D., AND C. DOURNON. 1999. Sex reversal by aromatase inhibitor treatment in the newt *Pleurodeles waltl*. Journal of Experimental Zoology 283:43-50.

COLVIN, B. A., AND W. B. JACKSON. 1999. Urban rodent control programs for the 21st century. Pages 243-257 *in* G. R. Singleton, L. A. Hinds, H. Leirs and Z. Zhang, editors. ACIAR Monograph No. 59.

COTTON, S., AND C. WEDEKIND. 2007. Control of introduced species using Trojan sex genes. Trends in Ecology and Evolution 22:441-443.

COWAN, P. E. 1996. Possum biocontrol: prospects for fertility regulation. Reproductive Fertility and Development 8:655-660.

DAVIS, D. E. 1953. The characteristics of rat populations. Quarterly Review of Biology 28:373-401.

DAVIS, D. E. 1987. Early behavioural research on populations. American Zoologist 27:825-837.

DAVIS, S. A., E. A. CATCHPOLE, AND G. R. FULFORD. 2000. Periodic triggering of an inducible gene for control of a wild population. Theoretical Population Biology 58:95-106.

DEVLIN, R. H., AND Y. NAGAHAMA. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological and environmental influences. Aquaculture 208:191-364.

EASTERBROOK, J. D., T. SHIELDS, S. L. KLEIN, AND G. E. GLASS. 2005. Norway rat population in Baltimore, Maryland, 2004. Vector-borne Zoonotic Diseases 5:296-299.

EGGERT, C. 2004. Sex determination: the amphibian models. Reproduction and Nutritional Development 44:539-549.

ELBRECHT, A., AND R. G. SMITH. 1991. Aromatase enzyme activity and sex determination in chickens. Science **255**:467-470.

FISHER, N., AND J. CRIBB. 2005. Monitoring community attitudes to using gene technology methods (daughterless carp) for managing common carp. Report prepared for the Cooperative Research Centre for Pest Animal Control, Valuemetrics Australia.

GASKELL, G., M. W. BAUER, J. DURRANT, AND N. C. ALLUM. 1999. Worlds apart? The reception of genetically modified foods in Europe and the U.S. Science 285:384-387.

GOSSEN, M., S. FREUNDLIEB, G. BENDER, G. MULLER, W. HILLEN, AND H. BUJARD. 1995. Transcriptional activation by tetracycline in mammalian cells. Science 268:1766–1769.

GOULD, F., AND P. SCHLIEKELMAN. 2004. Population genetics of autocidal control and strain replacement. Annual Review of Entomology 49**:**193-217.

GREWE, P. 1997. Potential of molecular approaches for the environmentally benign management of carp. Pages 119-127 *in* J. Roberts, and R. Tilzey, editors. Controlling carp: exploring the options for Australia. Murray-Darling Basin Comm., Canberra, Austraila.

GUTIERREZ, J. B., AND J. L. TEEM. 2006. A model describing the effect of sex-reversed YY fish in an established wild population: the use of a Trojan Y chromosome to cause extinction of an introduced exotic species. Journal of Theoretical Biology 241:333-341.

HAMILTON, W. D. 1967. Extraordinary sex ratios. Science 156:477-488.

HARDY, C. M., L. A. HINDS, P. J. KERR, M. L. LLOYD, A. J. REDWOOD, G. R. SHELAM, AND T. STRIVE. 2006. Biological control of vertebrate pests using virally vectored immunocontraception. Journal of Reproductive Immunology 71:102-111.

HEASMAN, J. 2006. Patterning the early *Xenopus* embryo. Development 133:1205-1217.

HOUDEBINE, L. M. 2005. Use of transgenic animals to improve human health and animal production. Reproduction in Domestic Animals 40:269-281.

KAPUSCINSKI, A. R., AND T. J. PATRONSKI. 2005. Genetic methods for biological control of non-native fish in the Gila River system. Contract report to the U.S. Fish and Wildlife Service. Minnesota Sea Grant Publication F20. University of Minnesota, Institute of Social, Economic and Ecological Sustainability, St. Paul, Minnesota, USA.

KISTNER, A., M. GOSSEN, F. ZIMMERMAN, J. JERECIC, C. ULLMER, H. LUBBERT, AND H. BUJARD. 1996. Doxycycline-mediated quantitative and tissue-specific control of gene expression in transgenic mice. Proceedings of the National Academy of Sciences 93:10933-10938.

KOO, B. C., M. S. KWON, B. R. CHOI, J. H. KIM, S. K. CHO, S. H. SOHN, E. J. CHO, H. T. LEE, W. CHANG, I. JEON, J. K. PARK, J. B. PARK, AND T. KIM. 2006. Production of germline transgenic chickens expressing enhanced green fluorescent protein using a MoMLV-based retrovirus vector. FASEB Journal 20:2251-2260.

KOOPMAN, P. 2006. Daughterless cane toads. Pages 111-116 *in* K. Molloy, and W. Henderson, editors. Science of cane toad invasion and control. Proceedings of the Invasive Animals CRC/CSIRO/Qld NRM&W Cane Toad Workshop, June 2006. Brisbane, Australia. Invasive Animals Cooperative Research Centre, Canberra, Australia.

LAMPO, M., AND G. A. DE LEO. 1998. The invasion ecology of the toad *Bufo marinus* from South America to Australia. Ecological Applications 8:388-396.

LEVER, C. 2001. The cane toad: the history of a successful coloniser. Westbury Academic and Scientific, Otley, West Yorkshire, United Kingdom.

LOWE, S., M. BROWNE, S. BOUDJELAS, AND M. DEPOORTER. 2001. 100 of the world's worst invasive alien species, a selection from the Global Invasive Species Database. IUCN-ISSG, Aukland, New Zealand.

MACKENZIE, C. A., M. BERRILL, C. METCALFE, AND B. D. PAULI. 2003. Gonadal differentiation in frogs exposed to estrogenic and antiestrogenic compounds. Environmental Toxicology and Chemistry 22:2466-2475.

MOLLOY, K., AND W. HENDERSON. 2006. Science of cane toad invasion and control. Proceedings of the Invasive Animals CRC/CSIRO/Qld NRM&W Cane Toad Workshop, June 2006. Brisbane. Australia. Invasive Animals Cooperative Research Centre, Canberra, Australia.

MUIR, W. M., AND R. D. HOWARD. 1999. Possible ecological risks of transgenic organism release when transgenes affect mating success: sexual selection and the Trojan gene hypothesis. Proceedings of the National Academy of Sciences 96:13853-13856.

MUIR, W. M., AND R. D. HOWARD. 2002. Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms. Transgenic Research 11:101-114.

NY, A., M. AUTIERO, AND P. CARMELIET. 2006. Zebrafish and *Xenopus* tadpoles: small animal models to study angiogenesis and lympangiogenesis. Experimental Cell Research 312:684-693.

PHILLIPS, B. L., G. P. BROWN, J. WEBB, AND R. SHINE. 2006. Runaway toads: an invasive species evolves speed and thus spreads more rapidly through Australia. Nature 439:803.

PIFERRER, F., S. ZANUY, M. CARRILLO, I. SOLAR, R. H. DEVLIN, AND E. DONALDSON. 1994. Brief treatment with an aromatase inhibitor during sex differentiation causes chromasomally female salmon to develop as normal, functional males. Journal of Experimental Zoology 270:255--262.

PIMENTAL, D., L. LACH, R. ZUNIGA, AND D. MORRISON. 2000. Environmental and economic costs of non-indigenous species in the United States. Bioscience 50:53-65.

ROBINSON, T., N. SIDDON, S. TARMO, D. HALLIDAY, T. SHANMUGANATHAN AND D. VENABLES. 2006. CSIRO biocontrol project: concept and progress. Pages 86-88 *in* K. L. Mollay, and W.R. Henderson, editors. Science of cane toad invasion and control. Invasive Animals CRC, Canberra, Australia.

SCHLIEKELMAN, P., AND F. GOULD. 2000a. Pest control by the introduction of a conditional lethal trait on multiple loci: potential, limitations, and optimal strategies. Journal of Economic Entomology 93:1543-1565.

SCHLIEKELMAN, P., AND F. GOULD. 2000b. Pest control by the release of insects carrying a female-killing allele on multiple loci. Journal of Economic Entomology 93:1566-1579.

SCHLIEKELMAN, P., S. ELLNER, AND F. GOULD. 2005. Pest control by genetic manipulation of sex ratio. Journal of Economic Entomology 98:18-34.

SCHUBERT, D., B. LECHTENBERG, A. FORSBACH, M. GILS, S. BAHADUR AND R. SCHMIDT. 2004. Silencing in *Arabidopsis* T-DNA transformants: the predominant role of a gene-specific RNA sensing mechanism versus position effects. Plant Cell 16:2561-2572.

SHARPE, R. M., S. M. MAGUIRE, P. T. K. SAUNDERS, M. R. RUSSELL, D. GANTEN, S. BACHMANN, L. MULLINS, AND J. J. MULLINS. 1995. Infertility in a transgenic rat due to impairment of cytoplasmic elimination and sperm release from the Sertoli cells. Biology of Reproduction 53:214-226.

SIBLEY, R. M., D. BARKER, M. C. DENHAM, J. HONE, AND M. PAGEL. 2005. On the regulation of populations of mammals, birds, fish and insects. Science 309:607-610.

STRONG, D. R., AND R. W. PEMBERTON. 2000. Biological control of invading species - risk and reform. Science 288:1969-1970.

SULLIVAN, R. 2004. *Rats*. Bloomsbury, New York, USA.

TESSON, L., J. COZZI, S. MENORET, S. REMY, C. USAL, A. FRAICHARD, AND I. ANEGON. 2005. Transgenic modifications of the rat genome. Transgenic Research 14:531-546.

THOMAS, D. D., C. A. DONNELLY, R. J. WOOD, AND L. S. ALPHEY. 2000. Insect population control using a dominant, repressible, lethal genetic system. Science 287:2474-2476.

THRESHER, R. E., AND N. BAX. 2006. Comparative analysis of genetic options for controlling invasive populations of the cane toad, *Bufo marinus*. Pages 117-122 *in* K. Molloy, and W. Henderson, editors. Science of cane toad invasion and control. Proceedings of the Invasive Animals CRC/CSIRO/Qld NRM&W Cane Toad Workshop, June 2006. Brisbane, Australia. Invasive Animals Cooperative Research Centre, Canberra, Australia.

THRESHER, R. E., AND A. KURIS. 2004. Options for managing invasive marine species. Biological Invasions 6:295-300.

THRESHER, R. E., P. GREWE, J. PATIL, AND L. HINDS. 2005. Genetic control of sex ratio in animal populations. Australian Patent Number 2001291520, Commonwealth Scientific and Industrial Research Organisation.

TORRES, J. M., C. SANCHEZ, M.A. RAMIEREZ, M. MORALES, J. BARCENA, J. FERRER, E. ESPUNA, A. PAGES-MANTE AND J. M. SANCHEZ-VIZCAINO. 2001. First field trial of a transmissible recominbinant vaccine against myxomatosis and rabbit hemorrhagic disease. Vaccine 19:4536-4543.

TYLER, M. J. 1998. Australian frogs. A natural history. Cornell University, Ithaca, New York, USA.

VEITCH, C. R., AND M. N. CLOUT. 2002. Turning the tide: the eradication of island invasive species. Auckland, Invasive Species Specialist Group of the The World Conservation Union (IUCN).

WHITTEN, M. J., AND G. G. FOSTER. 1975. Genetical methods of pest control. Annual Review of Entomology 20:461-476.

WIBBELS, T., AND D. CREWS. 1994. Putative aromatase inhibitor induces male sex determination in a female unisexual lizard and in a turtle with temperature-dependent sex determination. General Comparative Endocrinology 141:295-299.

WILHELM, D., AND P. KOOPMAN. 2006. The makings of maleness: towards an integrated view of male sexual development. Nature Reviews Genetics 7:620-631.