

Ecological, behavioral, and genetic factors influencing the recombinant control of invasive pests

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Abstract. Invasive species are a major threat to biodiversity, cost the world economy billions of dollars annually, and are often difficult, if not impossible, to control using current approaches. Recombinant technologies could revolutionize management of such pests but would be subject to a range of genetic, behavioral, and ecological factors that could limit their efficacy or applicability. We use a realistically parameterized combined population dynamics/genetics model to assess the potential of, and constraints on, a suite of recombinant approaches that have been suggested for pest control. We show that, of the options suggested to date, a genetic construct that distorts operational sex ratios by sterilizing, killing, or sex-changing one gender and being inherited through the other, is not only potentially the most effective means of pest control, but also one that remains effective over the widest range of ecological and behavioral conditions. All methods, however, are sensitive in particular to the degree of density dependence in the pest population and to operational issues such as maximum copy number and stocking levels, which affect introgression rates. Optimal investment strategies for an integrated pest management program that includes the nonlinear interactions of recombinant strategies and complementary management options can be assessed through the sensitivity analyses. The subtle effects of even minor variability in some parameters, such as extra mortality due to the presence of the construct, further suggest that genetic techniques be applied in an active adaptive management framework, so that strategies can be regularly optimized as the impacts of a release program are assessed.

Key words: *biological control; genetic control; integrated pest management; invasive species; population control; recombinant techniques; sex-ratio distortion; sterile male release; Trojan gene.*

INTRODUCTION

Invasive species are changing the world's natural environment and our use of it at an unprecedented rate, acting as vectors for new diseases, altering ecosystem processes, reducing biodiversity, and causing major economic losses (Vitousek et al. 1996, Mack et al. 2000). As many as half a million species have been introduced to new geographical regions as a result of human activities, causing annual costs to the United States alone of \$120 billion and costs to global agriculture of \$248 billion (Pimental et al. 2001, 2005). A few species, such as rats and cats, contribute disproportionately to these impacts (33% and 29%, respectively, U.S. data only; Pimental et al. 2001), while a few others, such as the mosquitofish, rosy wolf snail, and the brown snake, have more localized but extreme environmental impacts (data available online).² These high-impact species are prime targets for management.

If detected soon after establishing, eradication of invasive pests may be possible (Dahlsten and Garcia

1989, Culver and Kuris 2000, Bax et al. 2002), but can be difficult and needs to be considered against environmental, economic, political, and social risks (Meronek et al. 1996, Simberloff 1997, Meyers et al. 2000, Bax et al. 2001). Approaches to managing established pests range from adapting to their impacts through to a variety of options for physical removal, biocides, and biological control (Meyers et al. 2000, Wittenberg and Cock 2001, Thresher and Kuris 2004). For well established and widely distributed pests, the only realistic options have been augmentative and classical biological control (van Driesche and Bellow 1996) and sterile-male release programs (Krafsur 1998, Benedict and Robinson 2003). Despite some dramatic successes using both approaches, both also have significant constraints on their application (Whitten and Foster 1975, Simberloff and Stilling 1996, Follett and Duan 1999). As a result, most invasive pests remain uncontrolled.

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 acting through a mutant Y chromosome had apparently driven some insect populations to extinction. Practical development of such techniques lay fallow, however, until recent developments in recombinant genetics stimulated renewed interest in the field

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² www.issg.org/database/

(Krafsur 1998, Davis et al. 2000, Schliekelman and Gould 2000a, b, Gould and Schliekelman 2004, Schliekelman et al. 2005, Thresher 2008). Currently, at least four recombinant methods for pest control (repressible male sterility, virally vectored immunocontraception, female-biased sex-ratio distortion, and autocidal control) are being tested in the laboratory (Thomas et al. 2000, Hinds et al. 2002, Horn and Wimmer 2003, Gong et al. 2005, Thresher et al. 2005, Phuc et al. 2007). Another widely publicized study 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 1999, 2002, Hedrick 2001, Stokstad 2002)—a considerable threat if accidentally released, but also possibly a very powerful tool for pest control. The effect of a “Trojan gene” on a laboratory fish population is also currently being tested (A. Kapuscinski, *personal communication*).

Whether any of these methods, even if genetically feasible, would be effective in the “real world” remains unknown. Several methods have been modeled, which has highlighted the relative strengths of some techniques (e.g., female lethality, late-acting autocidal control; Schliekelman and Gould 2000b, Phuc et al. 2007) and identified sensitivities to genetic factors, such as increased per capita mortality due to the insertion of a genetic construct (Gong et al. 2005, Schliekelman et al. 2005), or the stage at which the genetic system operates (Atkinson et al. 2007, Phuc et al. 2007). When applied to the control of insect pests, the relative success of different genetic options as modeled can be informative as they can be compared with laboratory trials (Chen et al. 2007, Phuc et al. 2007), and with field data on the success (and failures) of the less powerful sterile-insect technique (e.g., Krafsur 1998). However, when applied to the broader question of the control of invasive species, theoretical analyses to date suffer from one or more limitations: first, they rarely compare under similar conditions the full range of genetic options available for pest control and hence make difficult an informed choice of approach for a particular species or situation; second, they rarely consider potential synergistic effects of sometimes simple, complementary actions that could be undertaken by managers as part of an integrated pest management (IPM) program (e.g., selective culling of noncarrier pests); third, they assume release numbers comparable to the wild population size, which would be unrealistic for many noninsect invasive pests; and fourth, perhaps most importantly, they typically either ignore or unrealistically simplify potential effects of key ecological processes, such as density dependence (Muir and Howard 1999, Schliekelman and Gould 2000a, b, Davis et al. 2001, Huang et al. 2007).

We assess the potential of, and potential constraints on, nine recombinant methods that have been suggested for pest control (Table 1) using a combined genetic and

population dynamics model with underlying dynamics common to all gonochoristic, bisexual vertebrate species. Similarities between some of our results and those for the control of insects using recombinant techniques (Schliekelman and Gould 2000a, b, Schliekelman et al. 2005) suggest applicability of our results beyond gonochoristic, bisexual vertebrates, but the importance of species’ ecology in the success of control programs cannot be overstated.

Using this model, we address three specific questions. First, how robust are the proposed methods in driving a pest population to quasi-extinction over a wide range of conditions? We chose 1% of the pre-control population as the quasi-extinction level, as populations below this level may become unstable due to stochasticity, Allee effects, inbreeding, and so forth, and model assumptions are less likely to be valid. Second, how effective are the proposed methods under a set of relatively optimistic parameters (e.g., highest practically achievable copy number and stocking rates, no leakage/gene silencing), and which factors have most influence on success? This provides an initial assessment of the risk of a pest control program failing in the light of factors, such as environmental variability and density dependence, the effects of which cannot easily be controlled and whose impact on pest population dynamics is often not well documented. Third, what are the benefits of different management options that could be used in a recombinant-based integrated pest control program (e.g., selective harvests of males or females, investment in high copy number individuals), and are there optimal integrated strategies?

We do not include in the comparison internally or externally vectored recombinant effects, such as *Wolbachia*-induced cytoplasmic incompatibility (e.g., Dobson et al. 2002, Rasgon and Scott 2004, Sinkins and Gould 2006), selfish genetic elements (e.g., Chen et al. 2007, Huang et al. 2007), virally vectored immuno-contraception (Hardy et al. 2006), or toxins produced using recombinant approaches, on the basis that their effectiveness depends only in part on pest population dynamics and genetics, and more on the baiting strategy or the dynamics of the vector.

METHODS

We use an age-structured deterministic model to simulate births, mortality, sex ratios, and gene frequencies in a freely interbreeding population. The assumptions and formulation of this model are typical of models used for assessing vertebrate populations (e.g., Punt and Hilborn 1997), except in this formulation we track the copy number of introduced genes in addition to age classes and sex.

Age-structured population model

The number of individuals (N) of age a ($a = 1$ to z with z representing all ages of z and above), sex (k), and copy number (g) is described by the following equations:

TABLE 1. Evaluation of recombinant methods for pest control.

Method	Description	Definition (see Table 2)
Neutral gene	neutral gene included for comparison of fixation rate due to stocking	$U_{y,0}^{k,g} = U_{y,0}^{k,g}$
Lethal construct	construct induces embryonic death of offspring; when homozygous results in sterility and is equivalent to a sterile male/female release	$U_{y,0}^{k,g} = 0$
Sex-specific lethality construct	as above, but male- or female-specific; transmitted through male or female line	$U_{y,0}^{f,g} = 0$ or $U_{y,0}^{m,g} = 0$
Sex-specific sterility	construct causes offspring of one sex to be sterile; transmitted through male or female line	$U_{y,0}^0 = \sum_{g=1}^G U_{y,0}^{m,g}$ or $U_{y,0}^0 = \sum_{g=1}^G U_{y,0}^{f,g}$
Gender distortion (“daughterless” or “sonless”; cf. Hamilton 1967, Thresher et al. 2005)	construct causes offspring to develop as specified sex, irrespective of sexual genotype; note that $U_{y,0}^{f,0} = U_{y,0}^{f,0} + U_{y,0}^{m,0} - \sum_{a=1}^z N_{y,a}^{m,0} \Omega_a^0 \gamma^0 / N_y^m \times 2 \sum_{g=0}^{2G} Q_y^g$ $U_{y,0}^{m,0} = \sum_{a=1}^z N_{y,a}^{m,0} \Omega_a^0 \gamma^0 / N_y^m \times 2 \sum_{g=0}^{2G} Q_y^g$ for daughterless and equivalently for sonless, as transgenic carriers are genetic females or males, respectively	$U_{y,0}^{f,g} = 0$ $U_{y,0}^{m,g} = U_{y,0}^{m,g} + U_{y,0}^{f,g}$ or $U_{y,0}^{f,g} = U_{y,0}^{f,g} + U_{y,0}^{m,g}$ $U_{y,0}^{m,g} = 0$
Inducible mortality (cf. Gould and Schliekelman 2000)	construct causes death when externally triggered by, e.g., extreme environmental variability or artificial trigger; construct maintained in population by further stocking	$U_{y,0}^{k,g} = 0$ for $y = y_t$, where y_t is the time period when the lethal gene is triggered
“Trojan gene” (cf. Muir and Howard 1999, 2002)	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	adjustment of $D^{k,g}$, γ^g , A_m^g , M_a^k as required
Mutual incompatibility	construct is lethal when present in two or more copies (unless genes are identical)	$U_{y,0}^{k,g} = U_{y,0}^{k,g}$ for $g = 1$ $U_{y,0}^{k,g} = U_{y,0}^{k,g} \times (1 - 2/2^g)$ for $g > 1$
Engineered under-dominance (cf. Davis et al. 2001)	construct is lethal when only one copy present (or more than one copy, but genes are identical)	$U_{y,0}^{k,g} = 0$ for $g = 1$ $U_{y,0}^{k,g} = U_{y,0}^{k,g} / 2^{g-1}$ for $g > 1$

Note: Definitions apply to copy number (g) ≥ 0 unless otherwise specified.

$$N_{y+1,1}^{k,g} = U_{y,0}^{k,g} e^{-M_1^{k,g}} \quad a = 1 \quad (1a)$$

$$N_{y+1,a}^{k,g} = N_{y,a-1}^{k,g} e^{-M_a^{k,g} - F_{y,a}^{k,g} - gT} \quad 1 \leq a < z \quad (1b)$$

$$N_{y+1,z}^{k,g} = (N_{y,z}^{k,g} + N_{y,z-1}^{k,g}) e^{-M_a^{k,g} - F_{y,a}^{k,g} - gT} \quad a = z \quad (1c)$$

where $U_{y,0}^{k,g}$ represents births at start of time period y , $M_a^{k,g}$ is the instantaneous rate of natural mortality, $F_{y,a}^{k,g}$ is the instantaneous rate of harvest mortality or culling, and T is the instantaneous rate of mortality per copy of the genetic construct. With the exception of T , all parameters can be specified to sex. The number of new individuals born in each time period is defined as

$$U_{y,0}^k = 0.5 N_y^f V e^{\alpha[1 - (N_y^f / N_0^f)]} + \varepsilon \quad g \geq 0 \quad (2)$$

where α is the shape parameter of the second form of the Ricker (Ricker 1954) stock–recruitment relationship, corresponding to the intrinsic rate of population increase (Turchin 2003), though the effect is attenuated

as the age structure operates similarly to a time lag. N_0^f and V are the number of mature female equivalents and the replacement number of births per female in the undisturbed population at the start of the simulation, respectively, while N_y^f and $U_{y,0}^k$ are the number of mature female equivalents and the number of births produced of sex k from all mature females in time period y , respectively:

$$N_y^f = \sum_{g=0}^G \sum_{a=1}^z N_{y,a}^{f,g} \Omega_a^g \gamma^g \quad g \geq 0. \quad (3)$$

Ω_a^g is the fraction of females that are mature

$$\Omega_a^g = [1 + e^{-\ln(19)(a - A_m^g) / (S_m^g - A_m^g)}]^{-1} \quad (4)$$

and γ^g is the fraction of year-0 wild-type birth rate. A_m^g and S_m^g are the ages at which 50% and 90% of females mature when measured off a logistic cumulative frequency distribution. ε in Eq. 2 is variability in births introduced as random starts on a broadscale environ-

TABLE 2. Parameter definitions.

Symbol	Parameter
$N_{y,a}^{k,g}$	number of individuals of age a , sex k , and copy number g in time period y
$U_{y,0}^{k,g}$	number of births
$\Omega_a^g; A_m^g; S_m^g$	fraction of females that are mature; ages at which 50% are mature; ages at which 90% are mature
V	replacement births per mature female in undisturbed population
γ^g	fraction of year-0 wild-type birth rate
α, ϵ	parameters of the Ricker stock–recruitment curve
$M_a^{k,g}$	instantaneous rate of natural mortality
$F_y^{k,g}$	instantaneous rate of harvest mortality or culling
T	instantaneous per copy rate of mortality of the construct
$P_y^{k,h}$	proportion of gametes based on binomial for single-sex constructs and Eq. 9 for dual-sex constructs
$D^{k,h}$	mating disadvantage; default value represents no advantage or disadvantage
S	non-expression of construct
L	number of loci (used in Eq. 9)
Q_y^g	proportion of offspring accounting for mating (dis)advantage

mental signal (the Southern Oscillation Index, log-transformed and normalized), that provided us a mechanism to represent the randomness and serial correlation typical of many environmental signals. For the sensitivity analysis of environmental variability we used the same sequence for each scenario but adjusted the amplitude.

Inheritance of introduced genes

One copy of the introduced gene was assumed to be sufficient to cause the phenotype or action specified by the recombinant method being evaluated. Copies on multiple loci were assumed to segregate independently during meiosis. A key concept in modeling the genetics is that recombinant techniques permit the insertion of multiple copies g of a construct into released organisms, so that the expected copy number in a subsequent generation t is $g/2^t$ (assuming independent segregation). Other options for maximizing inheritance of the construct are discussed next. For a homozygous unilocus trait under Mendelian inheritance, $g/2^t$ reduces to $2/2^t$. Under these conditions, genotype frequencies for a diploid organism where the introduced gene can only be carried by one sex (e.g., female lethal, female sterile, daughterless; see Table 1 for definitions) are obtained by disassociating males and females into the proportion of gametes of each copy number and sex $P_y^{k,h}$ based on a binomial distribution

$$P_y^{k,h} = \sum_{g=0}^G \binom{g}{h} 0.5^g (1 - 0.5)^{h-g} \times \sum_{a=1}^z N_{y,a}^{k,g} \Omega_a^g \gamma^g / N_y^k \tag{5}$$

where sex of the haploid gametes h distinguishes it from the sex of the diploid parents and offspring g and G is the highest copy number in a released organism.

Combining male and female gamete proportions, assuming random mating and (for simplicity in this example), a 50:50 sex ratio gives the proportion of offspring of sex k and copy number g in time period y , $Q_y^{k,g}$,

$$Q_y^{k,g} = 0.5 \times \sum_{h=0}^g P_y^{f,h} \times P_y^{m,g-h} \tag{6}$$

which is adjusted to account for the mating (dis)advantage (or relative fertilization success) of genetically modified (GM) individuals $D^{k,h}$ and normalized as follows:

$$Q_y^{k,g} = \sum_{h=0}^g (P_y^{f,h} \times D^{f,h}) \times (P_y^{m,g-h} \times D^{m,g-h}) / \sum_{k=1}^2 \sum_{g=0}^{2G} Q_y^{k,g} \tag{7}$$

Offspring proportions are adjusted for the non-expression of the construct S and multiplied by the total number of pre-recruits $U_{y,0}^k$ to give the number of age-0 pre-recruits of sex k and copy number g in time period y :

$$U_{y,0}^{k,g} = Q_y^{k,g} \times S \times U_{y,0}^k \tag{8}$$

For introduced genes that can be carried by males and females (e.g., lethal genes, pleiotropic genes, underdominance, mutual incompatibility), the assumption of independent segregation breaks down because two genes can be carried at each locus. Schliekelman and Gould (2000a) derived an algorithm for the independent segregation of genes on multiple loci for diploid organisms accounting for homozygous loci. A simplified close approximation that tracks the distribution of genotypes rather than gametes differs only slightly in genotype distribution over the first few generations (P. Dunstan, *personal communication*). In this formulation,

genotype frequencies depend on the copy number of the adult and the number of loci (L) on which the introduced gene can be carried. For those approaches in which the construct can be carried by both sexes, we replaced the binomial expression in Eq. 5 with

$$P_y^{k,h} = \begin{cases} 0 & \text{if } h > g \\ \frac{\binom{g}{h} \binom{2L-g}{L-h}}{\binom{2L}{L}} & \text{if } h \leq g \end{cases} \times \sum_{a=1}^z N_{y,a}^{k,g} \Omega_a^g \gamma^g / N_y^k \tag{9}$$

where the denominator is the number of possible ways of generating L loci on a gamete, given that there is a total of $2L$ to choose from, and the numerator is the product of the binomial coefficient of the h transgenic alleles from a total of g transgenic alleles and the binomial coefficient of the natural alleles in the remaining $L - h$ loci.

The effects of the alternative formulations on inheritance are illustrated for an individual carrying two copies of an introduced construct. If the construct can be carried by only one sex (e.g., female lethal), then the carrier must be heterozygous at two loci, its gametes will be distributed according to the binomial distribution (Eq. 5), resulting female carriers will die, and the construct is passed on only to the next male generation: the construct remains heterozygous. If, on the other hand, the construct can be carried on both sexes, then a carrier with two copies of the construct could either be heterozygous at two loci or homozygous at one. Without knowing beforehand which loci carry the construct, gamete distribution probabilities and resulting offspring copy numbers will depend on both the number of constructs and the number of available loci (Eq. 9), and the construct can be carried at homozygous or heterozygous loci in subsequent generations.

Robustness

We started with a model specified to provide annual births of 1000 individuals at carrying capacity, a sex ratio of 1, and equivalent population parameters for both sexes (Table 2 and Appendix). Stocking rate was constrained not to exceed the number of animals produced in the wild, so declined as the wild-type population approached zero. Results would be more rapid than reported here for species with nonoverlapping generations, where wild-type adults predated the control would be rapidly removed from the population.

Introgression rate was quantified as the number of generations after initial stocking at which the number of females carrying the wild genotype was reduced by 99%. Similarly extinction rate was quantified as the number of generations required for the number of all viable females to reach 1% of initial population size. The choice of 1% is a reasonable management target, but wholly arbitrary,

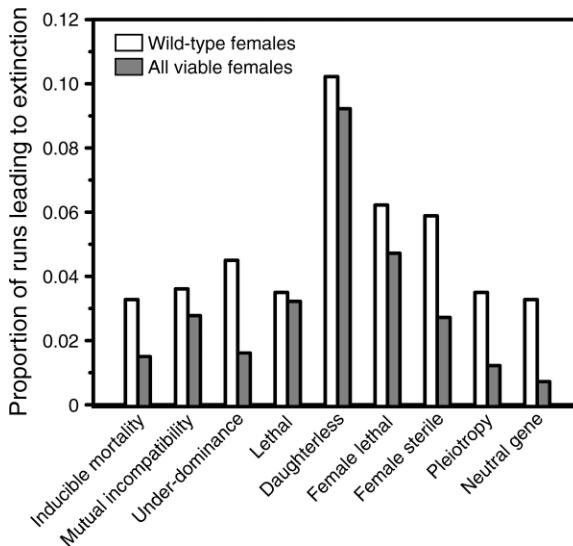


FIG. 1. Robustness of the nine recombinant pest control methods to effects of changes in genetic, operational, and environmental factors on the likelihood they will result in either fixation of the genetic modification (open bars) or pest extinction (solid bars), quantified as the proportion of runs leading to quasi-extinction of wild-type females or all viable females, respectively. See Table 1 for an explanation of recombinant methods.

noting that the dynamics of gene flow at very low population levels would be increasingly dominated by unknown, but probably species-specific, patterns of reproductive and other social behaviors. Results from our models were not qualitatively different for alternative endpoints of 5% or 0.1%. We limited the scenarios to a maximum of 25 generations, on the basis that any method that took longer than this to constrain a pest population would have less general applicability to invasive pest populations (although potentially acceptable in groups such as insects where generation times are short enough that 25 generations would fit within management time frames). Results were not qualitatively different for longer term scenarios.

We varied the nine parameters simultaneously over 10 levels, with the level for each parameter chosen independently and randomly with equal probability. There are 10^9 possible combinations of the nine parameters, and we randomly selected 20 000, which gave smooth response curves for single-parameter effects. We chose parameter ranges to be both realistic and informative over a wide range of scenarios and not centered on any one application. We restricted density dependence to values of $\alpha > 0.5$, because lower values than this led to extinction on more than half of all model runs with or without any construct, which was both unrealistic and reduced the discrimination power of the sensitivity analysis. For the ‘Trojan gene’ (pleiotropic effect) option, we used an additional (higher) level of mating success for male carriers of four times higher

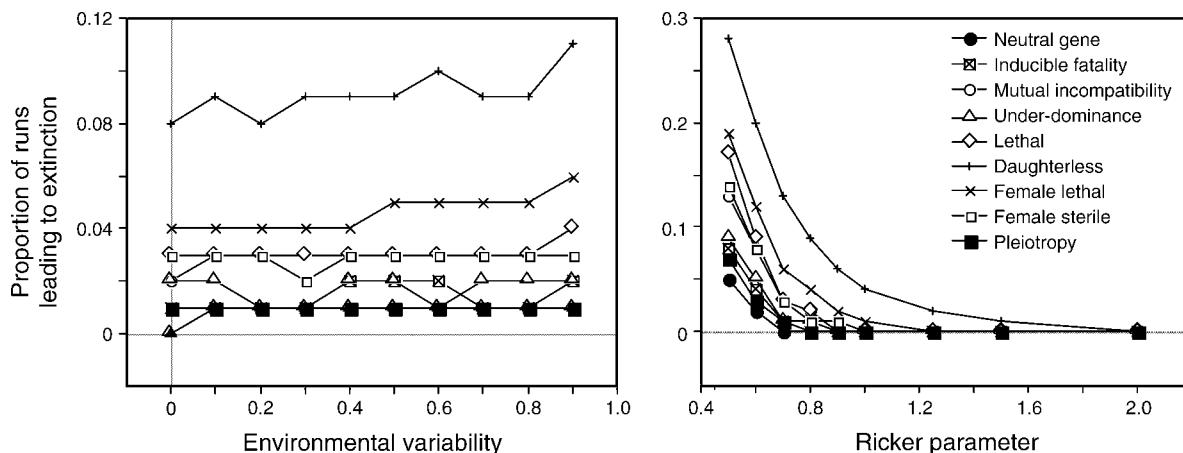


FIG. 2. Robustness: effects on introgression and extinction rates of varying density dependence in the targeted population (through adjusting the Ricker parameter, Eq. 2) and increasing the effect of environmental variability on recruitment (through multiplying mean annual recruitment by a standard series of values from the Southern Oscillation Index with increasing amplitude, Eq. 2). Each line represents 10 model scenarios with all parameters at default values, except for that specified on the abscissa. Note the difference between graphs in scale of the y-axes. Simulations were performed for a maximum 25 generations.

than the wild type. This represents an increased male size at mating resulting from an added growth hormone gene. A fourfold increase in male mating success and a juvenile survival of 0.995 relative to the wild type were identified by as most likely to lead to population extinction by Muir and Howard (2002). We also tested this level of mating success on the most effective sex-ratio distorter construct for comparison.

For inducible mortality, where the lethal construct remains dormant until externally triggered, we assumed the lethal gene was 100% effective and was triggered every sixth generation, based on Davis et al. (2000).

Effectiveness

Robustness testing assessed method performance over the entire parameter space and included many situations where genetic control techniques would be unsuccessful. In order to assess the potential effectiveness of the different methods under a more realistic control scenario (i.e., assuming that prescreening had occurred), we restricted the model to a particular scenario and explored the sensitivity to parameters around that scenario. Each potential method was tested under the same set of relatively optimistic parameters (i.e., copy number of eight and 5% stocking rates, no leakage/gene silencing), varying one parameter at a time. A copy number of eight may be close to the limit of what is achievable before the effects of silencing (e.g., Schubert et al. 2004), though the equivalent of higher copy numbers may be possible through use of selfish genetic elements or simultaneous use of different constructs, all of which have a similar phenotypic effect, e.g., causing female sterility (Thresher 2008). The metric used to compare performance was the number of generations until extinction, i.e., method efficiency. Model details

and outputs are provided in the Appendix. Results are summarized here.

Interaction of recombinant control and harvesting

We based the analyses of the combined effects of harvesting and genetic control on the same scenario used to test the effectiveness of the different methods (Appendix). In this section we examined the interaction of female sterility with a variety of selective and nonselective harvesting options to determine the value of developing an integrated pest management strategy and its effectiveness.

RESULTS

When all methods are implemented under the same set of ecological, genetic, and behavioral conditions, there are marked differences between the nine recombinant options in their impacts on the targeted populations (Appendix: Figs. A1 and A2). These differences continue when testing for robustness. The robustness of each method is summarized over the wide range of values used in this analysis (Fig. 1). Robustness is considered in two ways: in the context of how frequently the genetically modified (GM) females displace wild-type females (introgression) and how frequently all viable females are eliminated (pest extinction). With regard to introgression, for every method there are at least 500 scenarios (out of 20 000) in which wild-type females go extinct within 25 generations. Among the options examined, the most robust method for introgressing a novel construct is daughterless (a construct that converts genetic females into functional males; 10.2% of simulations) and the least robust is the pleiotropic Trojan gene (3.5%), when constrained to the maximum male-mating advantage used in the robustness analysis, 140%. The

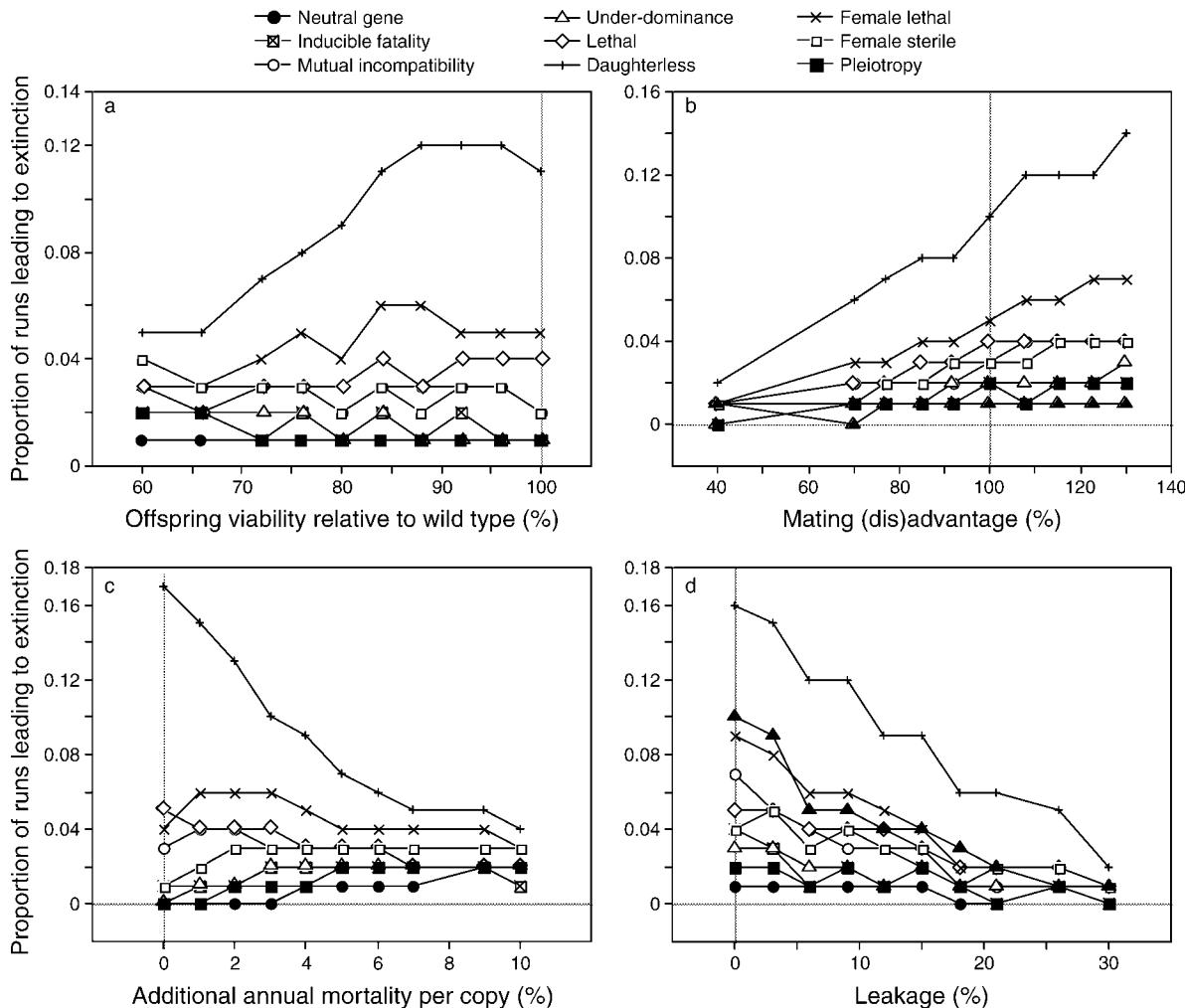


FIG. 3. Robustness: effects of variation in four genetically based factors on the proportion of runs leading to pest extinction using the nine recombinant methods compared: (a) genetically modified (GM) offspring viability relative to wild type, (b) proportional mating disadvantage (<100%) or advantage (>100%) of GM individuals, measured as relative fertilization success, (c) additional annual mortality per copy due to the presence of the construct, and (d) rate of gene leakage/silencing per generation. Simulations were performed for a maximum of 25 generations.

efficacy of the pleiotropic construct is similar to that of both stocking a neutral gene and one causing non-sex-specific lethality, where there is no mechanism for introgression, as all heterozygote offspring die. If harvesting is excluded from the analyses, the percentage of scenarios for the neutral gene that lead to extinction of wild-type females falls to 0.9% in this broad parameter space. Of the methods we examined, only under-dominance and the three sex-ratio-distorting methods (daughterless, female sterility, and female lethality) performed significantly better than the base level. Introgression frequencies increase markedly, to 12.1%, if the mating advantage of a male carrier of a pleiotropic Trojan gene is increased to four times the wild type, an outcome consistent with Muir and Howard (2002), who modeled the impact of inserting an additional growth hormone construct. The same four-

fold mating advantage applied to male daughterless carriers increases the method's introgression rate to 38.5%.

The nine methods differ substantially in the proportion of runs leading to total extinction of all viable females, i.e., pest eradication (Fig. 1). Over the parameter space examined, three methods do only slightly better than a neutral gene (0.7% of simulations, 0% without the effects of harvesting): inducible fatality, under-dominance, and the pleiotropic Trojan gene operating to a maximum mating advantage of 140%. The method most robust over the explored parameter space is daughterless, which results in population extinction in 9.2% of simulations. Again, performance of the pleiotropic Trojan genes increases significantly when applied with a large advantage in male mating success (4.4% extinction rate at a 400% male mating

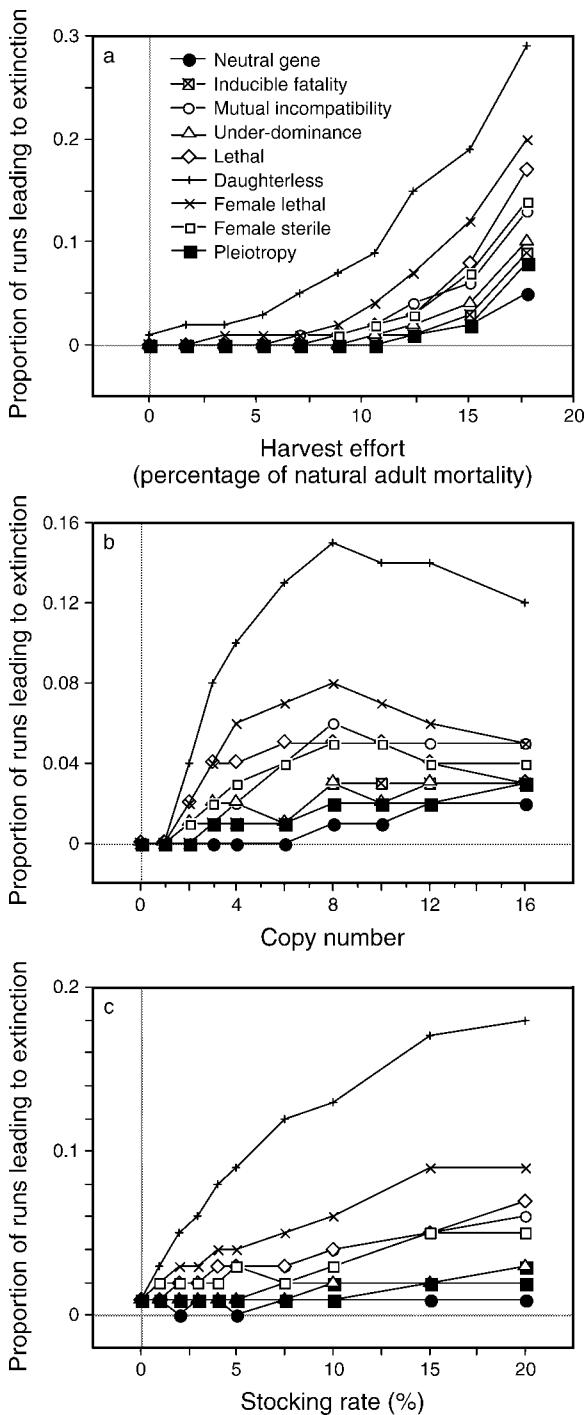


FIG. 4. Robustness: effects of variation in three factors amenable to management manipulation on the proportion of runs leading to pest extinction using the nine recombinant methods compared: (a) annual harvest/removal effort, quantified as the percentage of natural annual mean adult mortality at carrying capacity, (b) number of copies of the construct in each stocked carrier, (c) stocking rate (as percentage of natural annual mean recruitment at carrying capacity). Simulations were performed for a maximum of 25 generations.

advantage), but this is dwarfed by a 37.4% eradication rate when daughterless males also are provided a fourfold mating advantage.

The effects of individual parameters on the frequency of pest extinction are broadly similar for all control methods (Figs. 2–4); however, the relationship between increasing parameter values and extinction frequency differs widely among parameters. The factor that affects control methods least is environmental variability, for which large increases in variability only slightly increase the proportion of populations driven to extinction. We speculate that this slight increase is the result of prolonged periods of poor natural recruitment that magnify the effect of relatively constant stocking rates. The parameters to which control methods are most responsive are the degree of density dependence in the pest population, stocking rates, and copy number. Increasing all three parameters initially results in a rapid decline (density dependence) or increase (stocking rate and copy number) in the robustness of control methods, though the rate of change declines at higher parameter values. Increasing copy number becomes counterproductive at higher levels, due to the effects of additional mortality per transgene copy.

In the highly diverse parameter sets of the robustness analysis, few scenarios for any genetic method achieve pest eradication within 25 generations at a copy number ≤ 2 or an annual stocking rate $\leq 2\%$ of mean recruitment at carrying capacity (Fig. 4). To assess the effectiveness of the different methods in a control scenario where they were more likely to be applied, we re-ran the analyses with parameters at relatively optimistic levels. Under these conditions, all three sex-ratio manipulating options (daughterless, female lethal, and female sterile) have similar efficiencies and are affected similarly by changes in parameters (Figs. 5–7). Effects of changes in parameters were broadly similar for all control methods examined (other than offspring viability, for which the pleiotropic Trojan gene method requires low viability of GM offspring to be effective). The parameters that have the greatest effect on effectiveness are any additional mortality induced by the construct, the degree of density dependence in the pest population, leakage, mating (dis)advantage, and up to a point, stocking rates and copy number. Virtually any additional mortality to carriers caused by the presence of the construct renders every option examined nonviable within the 25-generation limit. Mating (dis)advantage also has a large, but two-edged effect. Up to a point, any advantage that accrues to carriers as a result of the construct markedly increases the efficiency of the control program, whereas any reduction in mating advantage severely hampers control efforts. The effect has to be severe to have a major impact; relatively slight changes in mating advantage (approximately $\pm 20\%$) as a result of the construct do not markedly change the outcomes of a control program, although the number of generations to extinction increases from 15 to 25 at a 20% mating



PLATE 1. (Left) Carp (*Cyprinus carpio*) were introduced to Australia in 1859 but did not spread widely until the 1960s. They are now abundant throughout southeastern Australia and the subject of a major integrated control program. (Right) Netting enhanced with telemetry and olfactory attractants is eradicating this species from two Tasmanian lakes. Photo credits: (left) Paul Brown DPI Victoria; (right) Inland Fisheries Service, Tasmania.

disadvantage and decreases to 12 generations at a 20% mating advantage. The parameter that has the least effect on the effectiveness of control programs is environmental variability. With regard to options amenable to management action, extinction occurs within 25 generations for the three sex-ratio-manipulating methods and inducible fatality, at any level of harvesting. The same four methods lead to extinction for copy numbers greater than four (female sterile) or six (the other three) and at stocking rates >2% (the three sex-ratio-manipulation options) and >4% (inducible fatality). The other approaches do not generally lead to extinction, although mutual incompatibility and lethality can contribute to extinction under high harvest effort.

The interacting effects of different management options were explored using female sterility (Fig. 8).

As expected from the single-parameter analyses, the interaction of copy number and stocking rate is nonlinear (Fig. 8). Without complementary pest removal (harvesting), achieving extinction in ≤ 25 generations when using carriers with a copy number ≤ 4 requires stocking rates $\geq 6\%$ (Fig. 8). Harvesting or otherwise removing pests as part of an integrated pest management (IPM) program substantially reduces the time required to achieve eradication, or alternatively, allows for meeting this objective within the specified 25-generation limit using carriers with lower copy numbers or lower stocking rates (Fig. 8; see Plate 1). Conversely, physical removal alone does not result in pest extinction except under conditions of low density dependence, but its effect is greatly enhanced when combined with a genetic control method (Fig. 9).

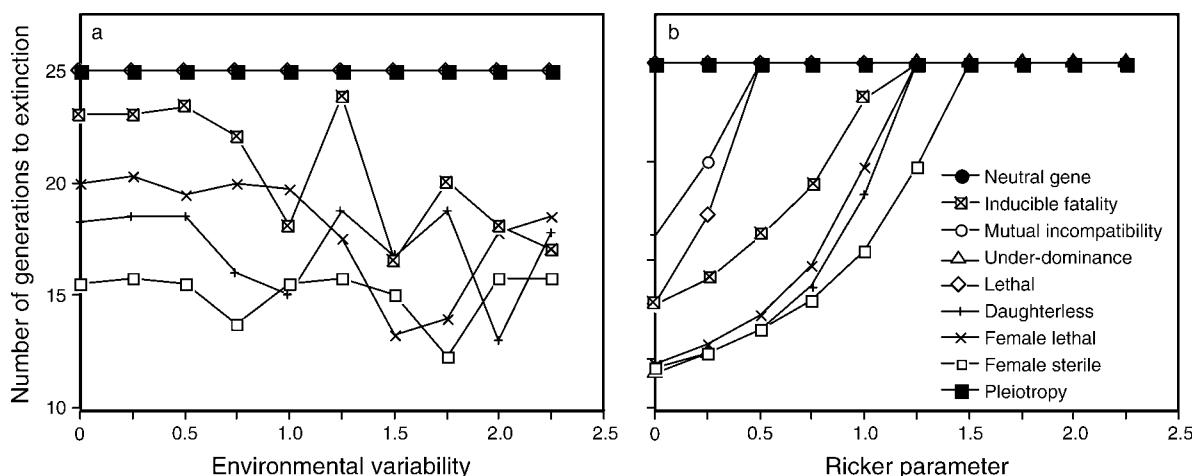


FIG. 5. Effectiveness: (a) effects of the magnitude of effects of broadscale environmental variability and (b) the variation in degree of density dependence in the targeted population on recruitment on the number of generations required to achieve pest extinction using the nine recombinant methods compared. Values for parameters not being varied were: leakage, additional mortality per copy, harvest effort, and environmental effects = 0; offspring viability relative to wild type, mating advantage, and Ricker parameter = 1; copy number = 8; and stocking rate = 5%.

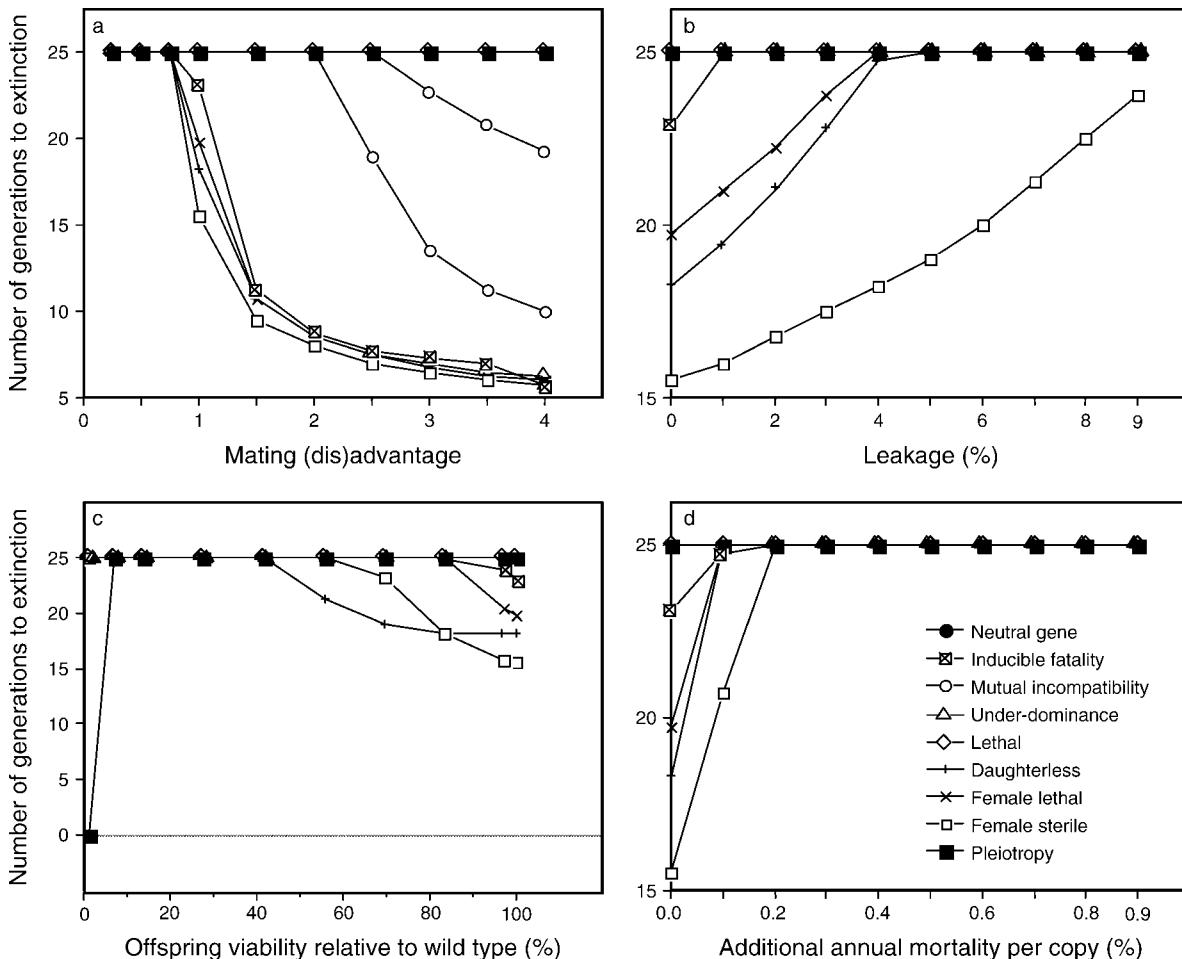


FIG. 6. Effectiveness: effects of variation in four genetically based factors on the number of generations required to achieve pest extinction: (a) proportional mating disadvantage <1 or advantage >1, (b) rate of gene leakage/silencing per generation, (c) GM offspring viability relative to wild type, and (d) additional annual mortality per copy due to the presence of the construct. Values of other parameters are as in Fig. 5.

The effect of harvesting is further enhanced if the pest can be removed selectively. We examined three options: non-selective pest removal, removal of only females (GM and wild type indiscriminately), and removal of wild type, but not GM individuals (both sexes; Appendix: Fig. A3A). Complementary nonselective or female-specific harvesting of the pest population at 50% of the annual mean natural mortality rate at carrying capacity reduces the pest population after 25 generations to about half of what it would have been if only the female-sterility method had been used. However, the same level of harvest mortality selectively targeting wild-type individuals drives the pests to extinction in <10 generations. A similar outcome can be achieved (extinction in 15–17 generations) by nonselective removal of juveniles, but only when harvest activities effectively double the natural rate of juvenile mortality (Appendix: Fig. A3B).

DISCUSSION

There are two main risks of the recombinant approaches: that they will have unexpected, undesirable outcomes (the gene constructs will jump to and affect other species) or they will not work as effectively as planned. With regard to the former, risk assessment for genetically modified (GM) methods of pest control is very preliminary and is likely to differ significantly among the recombinant methods considered, at least in part due to probable differences in species specificity (Thresher 2008). An appropriate set of risk assessment frameworks can probably be built based on those developed for containment of GM crops and animals (Hayes et al. 2007; see also Muir and Howard 2004, Burgman 2005), but there will be wide uncertainties in the assessments pending experimental studies and field trials. We can, however, better estimate the second risk, that of program failure, based on fundamental genetics and population ecology. In that regard, our results

broadly confirm previous theoretical studies indicating that pest control, and even eradication, in reasonable time frames is possible using recombinant techniques (Hamilton, 1967, Davis et al. 2000, Schliekelman and Gould 2000a, b, Schliekelman et al. 2005, Atkinson et al. 2007, Phuc et al. 2007). Importantly, the results also show that control can be achieved by adding the construct at a small enough percentage of wild-type recruitment (<5%) that the approach may be logistically feasible for a variety of pest species. However, our analyses also show that the efficacy of the methods proposed to date vary widely, in part as an inherent function of the genetics and in part due to differing sensitivities to ecological and genetic factors, only some of which are likely to be controllable.

Approaches that distorted population sex ratios are by far the most robust and effective of the methods that we examined. A construct that converts genetic females into functional males (daughterless) and is inherited through (phenotypic) males is robust over the widest range of conditions explored. Constructs that kill or sterilize only female offspring were also widely successful, but in our simulations were less robust than daughterless in the face of wide parameter variation and in most situations took longer to reduce the female population to below 1% of initial population size. This result is similar to that reported by Schliekelman et al. (2005), though their analysis was based on populations subject only to low levels of density dependence. The relative power of sex-ratio distorters also varies with the specifics of the approach (construct released on only males, only females, etc.), the effects of which may be complex (e.g., Schliekelman et al. 2005) and depend on reproductive behavior, sperm competition, and non-assortative mating. Any application of these approaches will need to be preceded by modeling to test effectiveness under the particular circumstances to which it is to be applied.

Though it performed poorly in our robustness simulations, inducible mortality is a potentially powerful technique depending upon the specifics of the stocking and triggering frequencies employed (Davis et al. 2000), but because it requires a two-stage approach and multiple, large-scale triggering events, it is logistically complex. We did not model the Trojan Y chromosome approach proposed by Gutierrez and Teem (2006), but this should be comparable to the daughterless approach with a copy number of two. Of the other methods we examined, two proved to be particularly poor at causing pest extinction within 25 generations: under-dominance and the pleiotropic Trojan gene (Table 1). In robustness testing, under-dominance was slightly more effective than control scenarios at causing the introduced construct to disseminate through the targeted population and was consistently more effective than control scenarios under the restricted parameter set. This design feature of under-dominance has potential importance in combat-

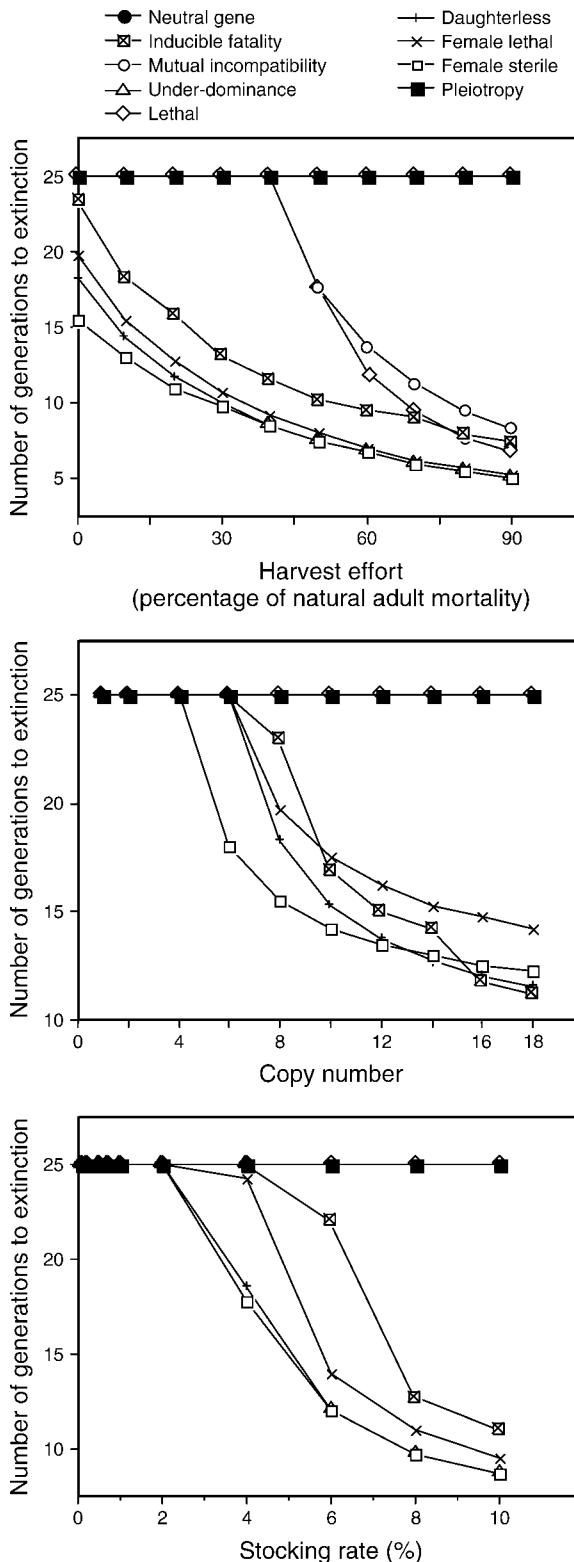


FIG. 7. Effectiveness: effects of different levels of harvest effort, copy number, and stocking rate on the number of generations required to achieve pest extinction. Values of other parameters are as in Fig. 5.

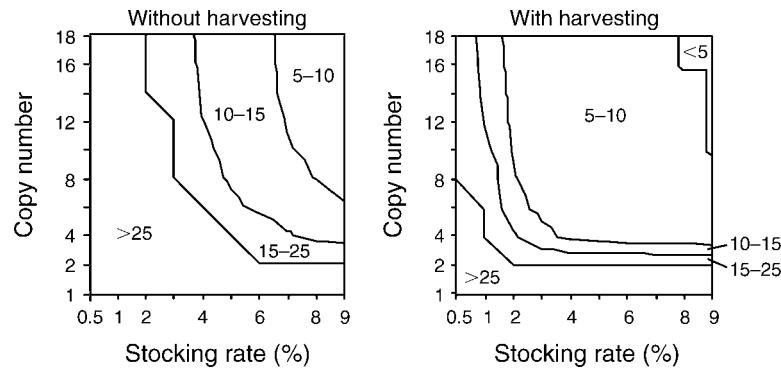


FIG. 8. Interactive effects of copy number and stocking rate on the number of generations required to achieve pest extinction for the female-sterile option with and without complementary pest harvesting (nonselective pest removal at levels equal to annual mean rate of natural mortality at carrying capacity). Values of other parameters are as in Fig. 5. Regions defined by lines identify parameter combinations that lead to extinction over a similar number of generations.

ing insect disease vectors (Davis et al. 2001, Magori and Gould 2006) but only in replacing the wild-type individuals with GM ones—it has only minor effects on population viability under most conditions. The poor effectiveness of a Trojan gene as a control approach, even when used at parameter settings that should have maximized its impact, suggests that the risks of extinction due to the accidental release of a Trojan gene carrier (Muir and Howard 1999) have been overestimated. Even if we relax the 25-generation limit for pest extinction, the impacts of a Trojan gene construct are constrained by density dependence in the affected population. While it may be risk averse to assume no increase in population fecundity as numbers decline in a species threatened by unwelcome extinction (Muir and Howard 1999), a similarly risk-averse strategy for controlling an unwanted species needs to assume compensatory increases in population growth rates at low population numbers (Krafsur 1998).

All varied parameters affected the performance of all methods but to differing degrees (Fig. 10). The most

robust methods were affected the most as they had the largest response to the control methods, but the relative effect of parameters was similar across all methods. Of the nine parameters varied, two groups, broadly classified as environmental factors and genetic factors, may be difficult to manipulate, whereas a third set (“management”) is broadly amenable to management investment decisions.

With regard to environmental factors, the two we examined differed markedly in their effects on pest control. The degree of density dependence in the targeted population had a large influence on the robustness of the control program, to the extent that in the robustness analysis, only daughterless achieved the 25-generation objective when Ricker’s $\alpha > 1.25$, and few were successful when $\alpha \geq 1.0$, whereas most methods (including just physical removal without a genetic component) led to pest extinction when population regulation was low (Fig. 2). Results were similar when we analyzed the effectiveness of the different control methods. Recombinant control methods are not

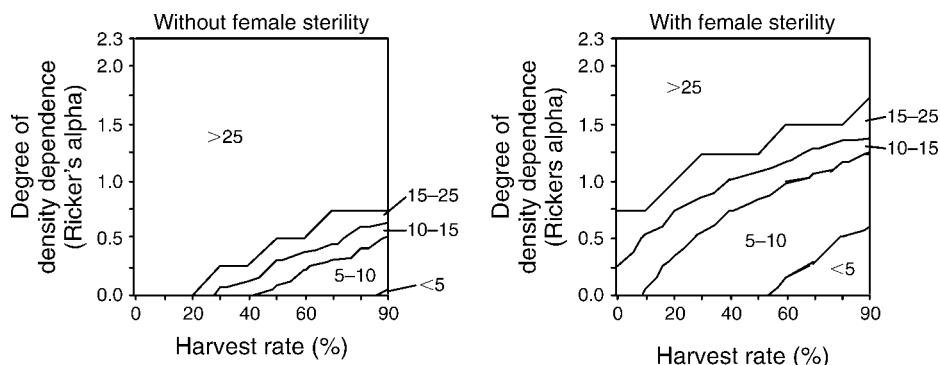
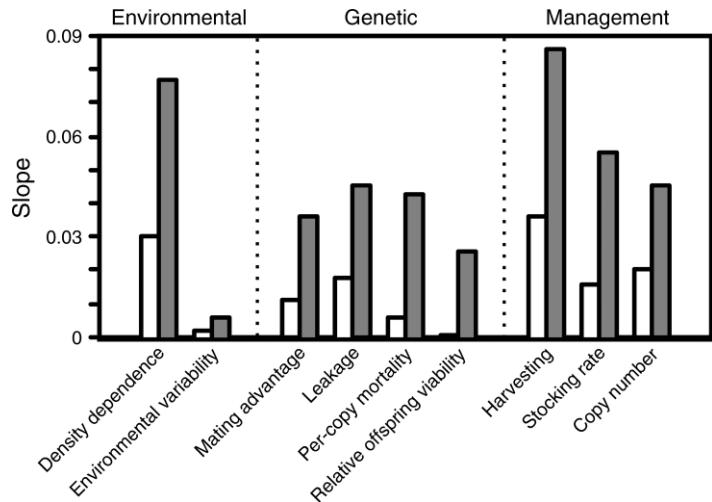


FIG. 9. Interactive effects of degree of density dependence in the targeted population and nonselective harvest/removal rate (as percentage of annual mean rate of natural mortality at carrying capacity), with and without a sterile-female pest control program, on the number of generations required to achieve pest extinction. Values of other parameters are as in Fig. 5. Regions defined by lines identify parameter combinations that lead to extinction over a similar number of generations.

FIG. 10. Sensitivity of a robust (daughterless; gray bars) and a less robust (mutual incompatibility; open bars) genetic control program to a set of environmental, genetic, and management-amenable factors, quantified as the slope of the linear regression between standardized values for each parameter and 10 different levels for each (see Figs. 2–4). The range of values for each was chosen based on the literature and personal observations as likely to be reasonable or reasonably achievable.



unique in their sensitivity to density dependence; all control methods struggle when recruitment increases as pest numbers are driven down (e.g., Krawfur 1998). Control success is improved when mortality due to the recombinant method occurs after population density dependence occurs (Phuc et al. 2007), underscoring the need to understand the target species population dynamics and ecology if control options are to be targeted effectively. Estimating density dependence in animal populations is difficult (Shima and Osenberg 2003, Sibley et al. 2005) and may be scale dependent (Murdoch 1994). However, recent meta-analyses (Turchin 1990, Sibley et al. 2005, Brook and Bradshaw 2006) suggest widespread evidence of density dependence across almost all taxonomic groups for which long time series of population data are available. Consequently, estimating the degree of density dependence in a targeted pest population is critical to predicting the efficacy of any recombinant pest control program, even though obtaining the data to make the estimation is likely to be time consuming and logistically difficult. In contrast, the magnitude of environmental effects on recruitment is not a major determinant of the efficacy of a control program, although it clearly leads to fluctuations in pest numbers which would increase the difficulty in detecting a significant effect of the control program on pest abundance and impacts.

All four genetic factors examined had moderately large effects on robustness (Fig. 3). Effects on control efficiency were much greater as the latter analyses were restricted to scenarios where control had the potential to be effective and were therefore sensitive to modifying factors. At one extreme, and similarly to the model results of Phuc et al. (2007), silencing of the construct (or leakage) had a relatively modest effect on gender distortion methods with female sterility still leading to extinction of wild-type females in <25 generations at the maximum levels we examined (10% per generation). At the other extreme, the effect of the construct on

mortality rates and the mating success of the carriers had very large impacts on program efficacy of all constructs. Not surprisingly, any factor that reduces the competitiveness and/or slows the rate of introgression of the construct into the target population has a major impact on the success of all genetic methods (see also Schliekelman and Gould 2000a, b, Schliekelman et al. 2005, Marrelli et al. 2006, Huang et al. 2007). Even so, the sensitivity of the genetics to even very small levels of construct-induced mortality of the carriers was surprising; an increase in the annual mortality rate, per copy, of as little as 0.2% dramatically reduced the efficacy of even the best options examined. The extent to which these genetic factors would affect recombinant programs is unclear, and probably differs between factors. High rates of mortality of transgenic animals during the integration process are common and are typically attributed to physiological disruptions caused by insertion of the gene construct into critical parts of the chromosome (Mackay et al. 1992). Mortality of surviving lines may be increased due to ectopic expression of a nearby gene or insertional effects (Gong et al. 2005, Marrelli et al. 2006; though see Chen et al. 2007). Severely deleterious insertional effects can be screened out when selecting brood stock (Catteruccia et al. 2003). Targeting gene constructs at specific chromosomal locations may also reduce insertional effects (Nimmo et al. 2006). We have assumed that constructs that cause mortality >10% over baseline are screened out in producing brood stock, but even so, our model suggests that even subtle effects due to additional mortality and leakage, at levels difficult to measure experimentally, could significantly reduce the efficacy of a pest control program. Resolving this issue constitutes a major challenge to a genetic approach to managing pest species (Marrelli et al. 2006).

The impact of construct-induced reductions in mating success (e.g., Catteruccia et al. 2003; though see Gong et al. 2005) and offspring viability relative to the wild-type

animal on program viability are less draconian. Mating disadvantages arise through the effects of mass rearing, individual strain variability, or through loss of fitness resulting from (multiple) genetic insertions (Schliekelman and Gould 2000a). An increase or decrease in mating success of less than ~20% has a relatively small effect on a control program, and hence is not likely to be a major determinant of program viability. Changes in mating success >20% do affect program viability, but should be discernible from experimental studies and screened out in the breeding program to create the carriers to be released. Phenotypic effects on mating competitiveness, due to captive rearing, are common in sterile male release programs (Whitten and Foster 1975, Krafsur 1998, Benedict and Robinson 2003) and obviously to be avoided in a recombinant program, but may not be significant overall because of the multi-generational effects of the integrated constructs. Conversely, differences between GM and wild-type individuals in mating advantage could be manipulated to enhance the control program. In many fish, for example, body size correlates highly with female fecundity and male competitiveness for mating (Ryan and Keddy-Hector 1992, Andersson 1994). Hence, releasing adult carriers that are larger than their wild-type competitors, as a result of better diets or growth conditions, could be used to, albeit transiently, increase introgression rates and the efficacy of stocking programs. Over the longer term, genetically linking the control construct tightly to one that, for example, increases growth rates (e.g., Caelers et al. 2005) could simultaneously reduce rates of juvenile mortality in GM individuals relative to the wild types and provide a multigenerational mating advantage, providing there were no pleiotropic effects. Adverse pleiotropic effects would be less likely in species only recently introduced to new areas and that have yet to evolve to maximize their local fitness, but careful monitoring for a loss of other components of fitness would be advisable.

More broadly, the analyses suggest options for maximizing cost-benefits of a control program, and hence can guide investment decisions. Benefits of manipulating all three parameters open to management manipulation or selection (copy number, stocking rate, and rate of pest removal/harvesting) are significant. Bigger (higher copy numbers, more stocking, and more removal) is better in all cases, although for copy number and stocking rate, only up to a point (see also Schliekelman and Gould 2000a, b, Magori and Gould 2006). Nonlinear relationships between increasing parameter values and rates of pest extinction can provide large benefits at relatively low levels (e.g., increasing copy number from two to six), but much smaller marginal gains at higher levels (increasing copy number from eight to 12) that may not be warranted as compared to other complementary activities such as harvesting, especially selective harvesting of wild types (Appendix: Fig. A3).

The prospects of increasing copy numbers and stocking rates, as well as the potential for a complementary (selective) pest removal program, will be situation specific. In practice, maximum stocking and removal rates are likely to be constrained by logistic factors, and copy number by genetic ones (Schubert et al. 2004). Nonetheless, the inflection points in the relationships between management parameters and time to extinction (e.g., Fig. 9) suggest optimal combinations of stocking, copy number, and removal rates that provide useful targets for research and management investment, as well as minimum values for each parameter below which programs may not be viable, and hence, not worth investing in. More specifically, our models consistently show considerable benefit for a means of selectively removing wild-type individuals from the pest population, while leaving carriers to reproduce and spread (Appendix: Fig. A3). Such a program requires not only a practical option for selectively removing some individuals, such as live trapping, but also an inherited phenotype genetically linked to the construct. The practicality of this linkage has not been tested, but in at least some pest species, e.g., carp and rodents, genes responsible for morphological features, such as color and coat length (e.g., Castle and King 1947), have been identified and could be useful in this context.

Finally, the importance of accounting in planning for differences in the ecology of the wild-type targets and GM carriers in a natural population cannot be overstated. Pest control based on recombinant genetics is a long-term intervention in a population and targeted intervention in an ecosystem, and an ideal candidate for active adaptive management (Walters and Holling 1990, Atkinson et al. 2007). Periodic monitoring of the integration success of different constructs or constructs inserted at different positions in the genome would provide critical feedback to focus subsequent rearing and release efforts. Like all such management interventions, assessment of the performance of the program requires ongoing monitoring and a management strategy that can be assessed against clearly defined long term goals.

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APPENDIX

Model description and output from individual model runs (*Ecological Archives* A019-036-A1).