

ORIGINAL ARTICLE

Genetic control of invasive plants species using selfish genetic elements

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cytoplasmic male sterility, genetic control, invasive species, selfish elements, spatial structure.

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Abstract

Invasive plants cause substantial environmental damage and economic loss. Here, we explore the possibility that a selfish genetic element found in plants called cytoplasmic male sterility (CMS) could be exploited for weed control. CMS is caused by mutations in the mitochondrial genome that sterilize male reproductive organs. We developed an analytical model and a spatial simulation to assess the use of CMS alleles to manage weed populations. Specifically, we examined how fertility, selfing, pollen limitation and dispersal influenced extinction rate and time until extinction in populations where CMS arises. We found that the introduction of a CMS allele can cause rapid population extinction, but only under a restricted set of conditions. Both models suggest that the CMS strategy will be appropriate for species where pollen limitation is negligible, inbreeding depression is high and the fertility advantage of females over hermaphrodites is substantial. In general, spatial structure did not have a strong influence on the simulation outcome, although low pollen dispersal and intermediate levels of seed dispersal tended to reduce population extinction rates. Given these results, the introduction of CMS alleles into a population of invasive plants probably represents an effective control method for only a select number of species.

Introduction

The economic and environmental impact of weeds and invasive plants is profound. Although no global estimates are available, introduced weed species are responsible for approximately US\$23.4 billion in annual crop losses in the United States (Myers and Bazely 2003) and AU\$3.9 billion in Australia (Sinden et al. 2004). The ecological damage caused by weeds and invasive species appears to be even more substantial. Indeed, alien weeds are spreading through US wildlife habitat at a rate of approximately 700 000 ha/year (Babbitt 1998). These invasive weeds have a negative impact on native species directly, through out-competing and hybridizing with indigenous species (Myers and Bazely 2003; Pimentel et al. 2005), and indirectly, through modifying physical features of their environment (Vitousek et al. 1987; Sala et al. 1996; Mack and D'Antonio 1998). In response to

this problem, numerous strategies have been employed to manage the global epidemic of invasive weeds.

Three major methods have been adopted to control the spread of weeds: mechanical, chemical and biological (Tu et al. 2001). Mechanical methods are particularly useful if the population is relatively small. Although this method can specifically target weed species and thereby minimize harm to other plants, mechanical removal can be labor intensive and impractical for large infestations. Chemical control has also been employed to manage weed populations, but the use of herbicides can result in damage to desirable plants and have other harmful environmental impacts. In natural populations of alien species, insect herbivores can be introduced to control the spread of their invasive hosts. Biological control has met with considerable success in some species (Myers and Bazely 2003) but poses some additional concerns, including the potential for the control agent to switch hosts from the

introduced plant to a native species (e.g. Louda et al. 1997). Consequently, because of the limitations of these major methods of control, there has been considerable incentive to develop alternative ways of managing weeds and invasive plants.

Here, we explore the use of selfish genetic elements as a means of weed control. Selfish genetic elements can invade a population despite causing a reduction in fitness to their hosts. One common type of selfish genetic element in plants is cytoplasmic male sterility (CMS), where mutations arising in the mitochondria result in a dramatic reduction in male fertility (reviewed in Frank 1989; Touzet and Budar 2004; Pelletier and Budar 2007). Because CMS alleles are inherited maternally, they can spread in populations despite causing a severe reduction in male fitness if the female fertility of male sterile individuals is higher than that of the hermaphrodites (Lewis 1941; Frank 1989). If the invasion of male sterile individuals is not countered, this process will result in population extinction through the loss of hermaphrodites. In natural populations, male fertility can be rescued through the evolution of nuclear restorers. Furthermore, as females become common, pollen limitation will result in strong selection for hermaphrodites (Charlesworth and Ganders 1979; Frank 1989). Alternatively, populations could escape extinction through the evolution of self-fertilization or asexual reproduction. Although there have been many theoretical models examining the evolution and maintenance of CMS alleles and nuclear restorers (e.g. Lewis 1941; Charlesworth and Ganders 1979; Frank 1989; Gouyon et al. 1991; McCauley and Taylor 1997; Couvet et al. 1998), few have explored the population dynamics of CMS, and none of these studies have examined effects of CMS, on the risk of extinction.

The central goal of this study is to determine if the introduction of CMS loci into an invasive population can result in extinction and act as an effective control method. Such loci are frequently uncovered in hermaphroditic species through crossing divergent populations (Barr 2004) and could also be introduced through molecular techniques from species where CMS loci have been characterized (Chase 2006). Specifically, we address three questions regarding the use of CMS genes as a method of weed control. (1) How long will it take for the population to go extinct once a CMS allele has been introduced? The more quickly the CMS allele spreads to fixation, the less opportunity populations will have to escape extinction. To address this question, we developed an analytical model to estimate the conditions under which male sterile individuals will invade a population, rise to fixation, and result in extinction. (2) What is the net effect on reproductive output of the population during the invasion of a CMS allele? For a CMS gene to spread, the female fertility of the male sterile mutant must

be enhanced (Frank 1989). This boost in female fertility could result in an increase in seed set, and potentially population size, of the invading species and have important management implications. To quantify this effect, we used our analytical model to assess the annual and total effect on seed set. (3) What is the effect of spatial structure on the probability of population extinction? To address this question, we developed a spatially explicit model and ran simulations to examine the influence of pollen and seed dispersal on the fate of the CMS allele.

Model and results

We constructed a one-locus, two-allele model, where the locus is maternally inherited through the cytoplasm. The frequency of the hermaphrodites, q , in the next generation is given by:

$$q_{t+1} = \frac{q_t(s(1-\delta) + (1-s)F(q))}{W_t} \quad (1)$$

Where s equals the selfing rate, δ represents the level of inbreeding depression in the hermaphrodite and W_t is the mean fitness of the population at time t . The function, $F(q)$, measures the fertilization rate of outcrossed ovules, a measure of pollen limitation.

Similarly, we determined the frequency of a female with a CMS mutation, p (where $q = 1 - p$), in the next generation as:

$$p_{t+1} = \frac{p_t(1+f)F(q)}{W_t}, \quad (2)$$

where f equals the increase in fertility resulting from the CMS mutation. Consequently, the mean fitness of the population is:

$$W_t = p_t(1+f)F(q) + q_t(s(1-\delta) + (1-s)F(q)) \quad (3)$$

As pollen becomes more limiting when there are more females we assume that $F(q)$ rises as a function of the frequency of hermaphrodites, q , within the population. Specifically, we assume that fertilization rate follows a hyperbolic function $F(q) = cq/(q+a)$, where c represents the asymptote and a represents the frequency of hermaphrodites that gives $1/2$ the maximum fertilization success. Given that the maximum success of outcrossed seeds is c and the maximum of inbred seeds is $1 - \delta$, the model that we develop depends only on the ratio $(1 - \delta)/c$ and not on each of these parameters individually. Thus, without loss of generality, we set c to one; other values of c can be explored simply by multiplying $1 - \delta$ by the new value of c . The parameter a influences the shape of the hyperbolic function $F(q)$ describing fertilization success. This function rises rapidly, implying little pollen limitation, when a is low. Conversely,

fertilization success approaches a linear function of hermaphrodite frequency, implying substantial pollen limitation, when a is high. The dose–response relationship between pollination and fruiting has been shown to asymptote in several species (reviewed in Mitchell 1997), and a hyperbolic function can approximate this relationship (Cane and Schifffhauer 2003). Note that, to the extent that $F(q) < 1$, selfers have greater reproductive assurance than outcrossers.

Our model is similar to the classic model for gynodioecy derived by Frank (1989) except we used a different pollen limitation function. The hyperbolic pollen limitation function allowed us to model a decelerating relationship between seed set and hermaphrodite frequency while still remaining analytically tractable. In particular, we were able to derive a general solution to this model and use this solution to determine the time until population extinction unlike previous analyses that incorporated pollen limitation.

We found equilibria when $p_1^* = 0$ and when:

$$p_2^* = \frac{-s(1+a)(1-\delta) + c(f+s)}{-s(1-\delta) + c(f+s)}$$

The first equilibrium represents a situation where male sterile individuals are absent from the population. The second equilibrium represents a polymorphism for females and hermaphrodites (gynodioecy). This second equilibrium can approach the fixation of male sterile individuals under certain conditions, such as when inbreeding depression and the fertility advantage of females is high, while a is small. Conversely, when a is large, pollen limitation will have a strong influence on the fitness of females, and this can result in a polymorphism with a low frequency of females (Fig. 1). Setting $c = 1$ and $a = 0$ (i.e. removing pollen limitation from the equation) we recover the same result as Charlesworth (1979) where the second equilibrium then becomes $p_2 = 1$, implying that females will become fixed if the male sterile cytoplasm is maintained in the population.

We then determined the stability of the equilibria. The slope with respect to p of eqn (2) at the first equilibrium ($p_1^* = 0$) is:

$$\lambda_1 = \frac{c(1+f)}{c - cs + s(1+a)(1-\delta)},$$

which must be less than one for the equilibrium to be stable. When maximum seed set is set to 1 (i.e. $c = 1$), p_1^* will be stable when:

$$f < a(s - \delta s) - \delta s,$$

where f , δ , s and a are constrained to be >0 . Consequently, male sterile individuals cannot invade the

population when the female fertility advantage is slight, the product of the inbreeding depression and selfing rate is low, and a is large (pollen limitation is strong).

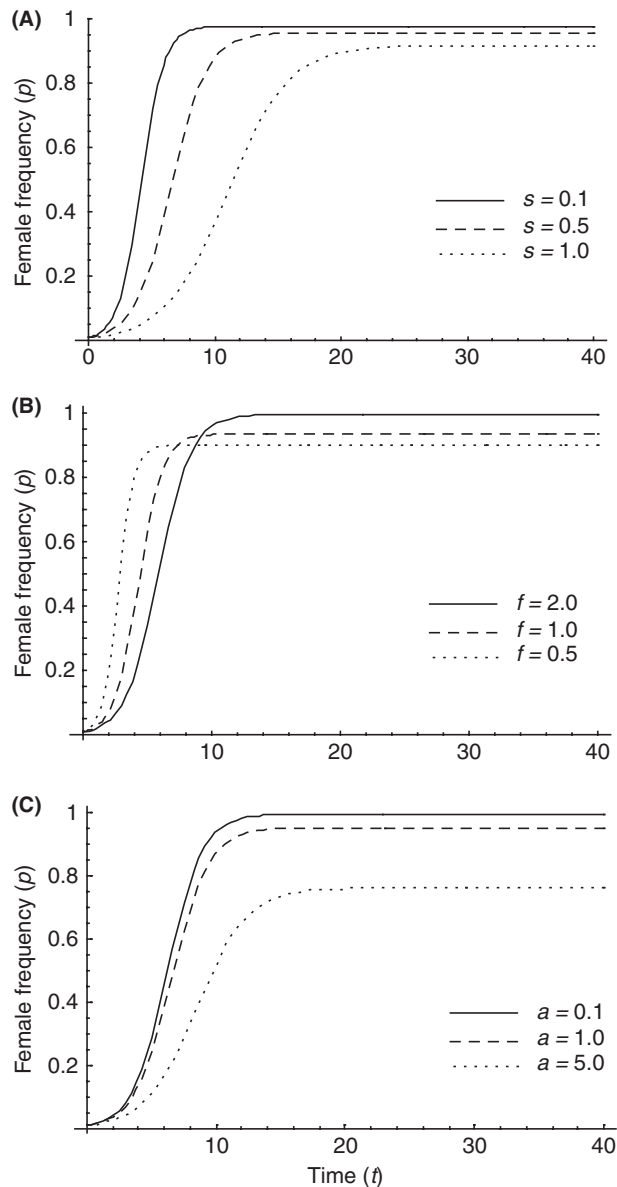


Figure 1 The frequency of male-sterile (female) individuals through time in populations with different parameter values. (A) Differences in the level of self-fertilization, s . Solid lines $s = 0.1$, dashed lines $s = 0.5$ and dotted lines $s = 1$ (other parameters are $p_0 = 0.01$, $\delta = 0.5$, $f = 1$, $a = 0.9$ and $c = 1$). (B) Differences in the female fertility advantage, f . Solid lines $f = 2$, dashed lines $f = 1$ and dotted lines $f = 0.5$ (other parameters are $p_0 = 0.01$, $s = 0.1$, $\delta = 0.5$, $a = 0.9$ and $c = 1$). (C) Changes in the shape of the pollen limitation function, a . Solid curves $a = 0.1$, dashed curves $a = 1$ and dotted curves $a = 5$ (other parameters are $p_0 = 0.01$, $s = 0.1$, $\delta = 0.5$, $f = 1$ and $c = 1$).

The second equilibrium is biologically valid only when:

$$s > -\frac{cf}{c - (1 - \delta) - a(1 - \delta)},$$

which occurs if and only if p_1^* is unstable, allowing the CMS allele to invade. The second equilibrium is stable when:

$$\lambda_2 = \frac{c - cs + (1 + a)(1 - \delta)}{c(1 + f)} = \frac{1}{\lambda_1} < 1,$$

which means the polymorphism will be stable when it exists and when $p_1^* = 0$ is unstable. Therefore, when the asymptote is set to 1 (i.e. $c = 1$), the polymorphic equilibrium will be stable when $f > a(s - \delta s) - \delta s$. Under these conditions, the fertility advantage of females offsets the reduction in seed set due to pollen limitation, allowing the invasion of the CMS allele.

The general solution for p_t , where p_0 represents the frequency of females at time 0 is:

$$p_t = \frac{p_0 \lambda_1^t s(1 + a)(\delta - 1) + c(f + s)}{c(f + s)(1 + p_0(\lambda_1^t - 1)) + s(\delta - 1)(1 + a + p_0(\lambda_1^t - 1))} \quad (4)$$

From the general solution, we were able to calculate the time until extinction (τ) by assuming the population will reach extinction when the number of hermaphrodites falls below a single individual and solving for t . This assumed that the initial number of male sterile individuals was one (i.e. $p_0 = 1/n$) and that the population size (n) remained constant until extinction. That is, we assume that density dependent regulation occurs in a manner that does not depend on female and hermaphrodite frequencies until only one hermaphrodite remained. This extinction time was:

$$\tau = \frac{\ln\left(\frac{(n-1)((\delta-1)(n+an-1)s+c(n-1)(f+s))}{s(\delta-1)(1+an)+c(f+s)}\right)}{\ln(\lambda_1)} \quad (5)$$

Population extinction can occur quite rapidly when $p_2^* > 1 - 1/n$. If the selfing rate and inbreeding depression are high, if the fertility advantage of the females is substantial and if pollen limitation is mild unless hermaphrodites are very rare, population extinction can occur in a few generations (Fig. 2). For example, when $s = 0.95$, $\delta = 0.95$, $a = 0.01$ and $f = 1$ the population will go extinct in ~ 5.67 generations. This is because the fertility of hermaphrodites is greatly reduced when both the selfing rate and inbreeding depression are high. If hermaphrodites are largely outcrossing (e.g. $s = 0.05$), the time to extinction rises, but not by much, to $\tau = 18.65$ generations. Population size also influences extinction time although the relationship is strongly dependent on

the level of pollen limitation (Fig. 3). Extinction was much more likely to occur and to be rapid in smaller populations. In very large panmictic populations, extinction did not tend to occur, because pollen limitation prevented the spread of females to sufficiently high frequencies ($> 1 - 1/n$). Reducing pollen limitation increased the size of the population at which extinction was still observed. Therefore, under certain scenarios (i.e. very high inbreeding depression, a large fertility advantage, reduced pollen limitation, and small to moderate population sizes), extinction would likely occur prior to the introduction of restorer genes through mutation, particularly if multiple mutations that cause CMS were introduced into the same individual, as this might require several restorers to arise.

Assuming a constant population size until extinction, we obtained the total increase in seed production as follows:

$$\frac{\int_0^\tau W_t dt}{\tau(s(1 - \delta) + (1 - s)c/(1 + a))}, \quad (6)$$

where W_t (eqn 3) represents the combined seed production per individual for females and hermaphrodites in each generation and $(s(1 - \delta) + (1 - s)c/(1 + a))$ represents the relative seed output that would have been expected if females were absent from the population. The total excess seed production is then obtained by integrating the above equations from time 0 to the extinction time, τ . We can also obtain the maximum excess seed production by solving for the time at which:

$$\frac{d(W_t/(s(1 - \delta) + (1 - s)c/(1 + a)))}{dt} = 0 \quad (7)$$

and examining the seed output in that generation.

We found that seed production initially increases as females become more common because we assumed that they have higher seed production due to the reallocation of resources from male function to female function. However, as females become even more frequent, this increase in seed production is countered by pollen limitation, and excess seed production declines (Fig. 4A). For outcrossing species with high inbreeding depression, the total excess seed production is greatest when the fertility of females is large. When the female fertility advantage is small there may actually be a reduction in total seed production compared to a purely hermaphroditic population, as pollen limitation will reduce the seed set of both hermaphrodites and females when hermaphrodites are rare (Fig. 4B). However, even when total seed production is not greatly affected, maximum seed production can be substantially increased when the fertility of females is large (Fig. 4C).

Simulation methods

To explore the dynamics of a CMS allele when pollen and seed dispersal is spatially structured, we ran simulations using a program developed in R (see Supporting Information). In the model, we assumed equal viability, seed dispersal and pollinator visitation for females and her-

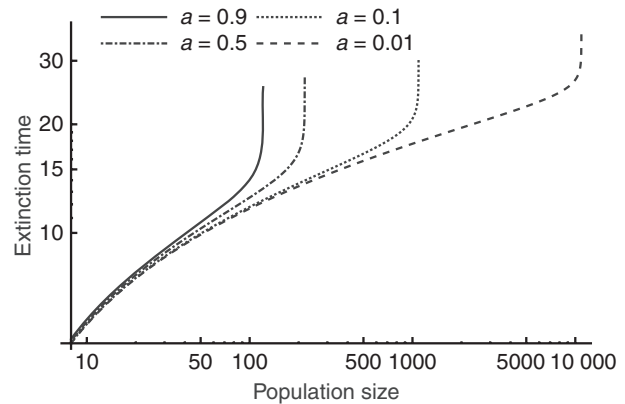
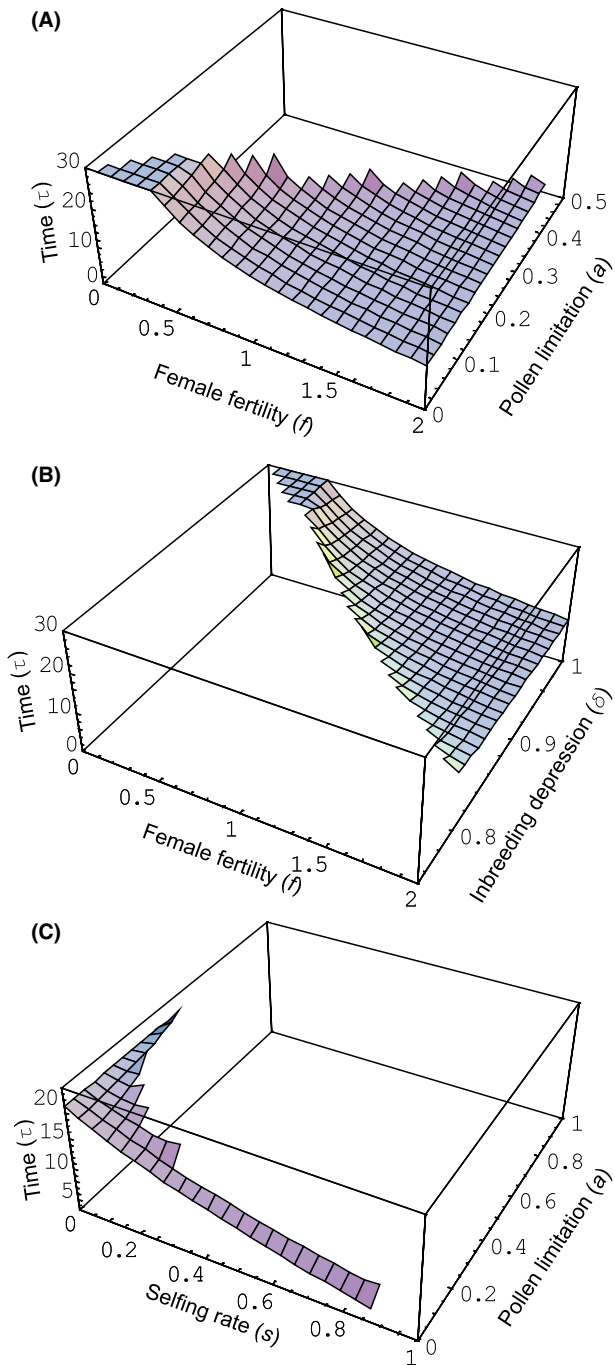


Figure 3 The extinction time for populations of varying size when male sterile individuals invade a population of hermaphrodites. Populations were considered to be extinct when the frequency of hermaphrodites reached $1/n$ and population size was assumed to be constant until extinction (other parameters are $f = 1$, $\delta = 0.95$ and $c = 1$). Different levels of pollen limitation were investigated ($a = 0.01$ dashed line, $a = 0.1$ dotted line, $a = 0.5$ dashed and dotted line, and $a = 0.9$ solid line). Both axes are plotted on a log scale.

maphrodites. Pollen discounting was not incorporated into our model and self-fertilization in the hermaphrodites occurred prior to outcrossing.

We began each simulation with a 50×50 lattice array (Pannell 1997; Heilbut et al. 2001). The lattice was initially filled with hermaphrodites and following 100 generations we introduced the CMS allele into a single randomly chosen cell. We modeled seed and pollen

Figure 2 The extinction time for populations when male sterile individuals invade a population of hermaphrodites. Populations were considered to be extinct when the frequency of hermaphrodites reached $1/n$ and population size was assumed to be constant until extinction. (A) The extinction time for populations with varying levels of the female fertility advantage, f (range 0–2) and differences in the shape of the pollen limitation function, a (range 0–0.5) (other parameters are $n = 1000$, $s = 0.1$, $\delta = 0.95$ and $c = 1$). When both the level of pollen limitation is high and the fertility advantage is low, extinction does not occur (upper corner of the graph). (B) The extinction time for populations with differences in the level of inbreeding depression, δ (range 0.75–1) and varying levels of the female fertility advantage, f (range 0–2) (other parameters are $n = 1000$, $s = 0.1$, $a = 0.1$ and $c = 1$). Throughout most of the parameter space, extinction does not occur, but when the level of inbreeding depression is very high ($\delta > 0.8$) and the fertility advantage is substantial ($f > 0.7$), extinction can occur in <20 generations. (C) The extinction time for populations with varying amounts of self-fertilization, s (range 0–1) and differences in the shape of the pollen limitation function, a (range 0–1) (other parameters are $n = 1000$, $f = 1$, $\delta = 0.95$ and $c = 1$). For most of the parameter space, extinction does not occur, but when the level of pollen limitation is low and the outcrossing rates are high (i.e. a and s are small), extinction can occur in <20 generations.

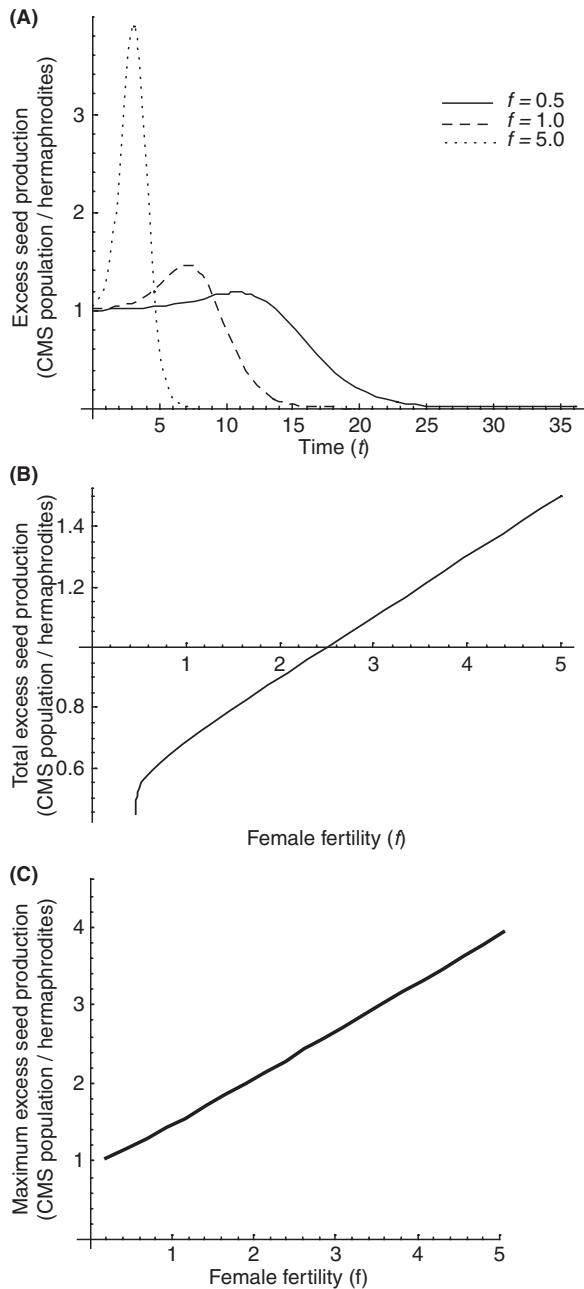


Figure 4 Excess seed production in a largely outcrossing population with high inbreeding depression as a CMS allele is invading. (A) The relative seed production of a population with females compared to a population of only hermaphrodites through time. Solid curves $f = 0.5$, dashed curves, $f = 1$, dotted curves $f = 5$. (B) The influence of female fertility (f) on total excess seed production in a largely outcrossing population with high inbreeding depression where a CMS allele causes population extinction compared to an identical population of hermaphrodites. (C) The influence of female fertility (f) on maximum seed production in a largely outcrossing population with high inbreeding depression where a CMS allele causes population extinction compared to an identical population of hermaphrodites. For all panels, other parameter values are $n = 1000$, $s = 0.05$, $\delta = 0.9$, $a = 0.1$ and $c = 1$.

dispersal with and without edge effects, so seeds or pollen were either lost from the population when they landed outside of the lattice or they landed on cells located on the opposite side of the lattice respectively. We modeled both wind and animal pollination, as the mode of pollen dispersal may influence the level of pollen limitation and the success of the CMS allele. For wind pollination, the number of pollen grains for each hermaphrodite was randomly drawn from a normal distribution (base parameter set as mean = 1000 and SD = ± 100) and assigned to each individual in the lattice. A proportion (h) of pollen was randomly distributed to all cells in the lattice while the remainder ($1 - h$) was randomly dispersed to the neighboring eight cells. Similarly, for animal pollination, we simulated the total number of pollinator trips from each hermaphrodite to the other cells in the lattice. A proportion (h) of the pollinator trips were randomly distributed to all cells in the lattice while the remainder ($1 - h$) were randomly assigned to the neighboring eight cells. We modeled pollinator trips as a visit from a pollen source to a stigma, followed by the pollinator exiting the patch (no pollen carryover). The number of pollinator trips leaving each plant was set to 10, unless otherwise stated.

We modeled seed production in the following way. The expected number of ovules for each hermaphrodite, o , was set to 50 for all simulations. For each hermaphrodite, the total number of seeds produced through self-fertilization was randomly sampled from a Poisson distribution with mean $(1 - \delta)so$, where s is the selfing rate, δ is the level of inbreeding depression and o is the average number of ovules for hermaphrodites. The total number of ovules available for outcrossing in hermaphrodites was randomly determined by sampling from a Poisson distribution with a mean $(1 - s)o$. Similarly, for females, seed set was determined by randomly sampling from a Poisson distribution with mean $(1 + f)o$, where f represents the fertility advantage of females over hermaphrodites due to reallocation of resources from male function. For wind pollination, when the number of pollen grains was less than the number of ovules available for outcrossing, the number of pollen grains landing in each cell determined the number of outcrossed progeny for each individual. For animal pollination, we assumed that pollen carryover did not occur and that a single visit by a pollinator was necessary and sufficient to fertilize all ovules.

We modeled seed dispersal in a manner similar to pollen dispersal. A certain proportion (d) of seeds produced in each cell were randomly dispersed to all the cells in the lattice while the remaining seeds ($1 - d$) were dispersed to the neighboring eight cells as well as the cell of origin. To create the next generation, a seed was randomly drawn from the pool of seeds landing in the cell and if there were no seeds landing in the cell the cell remained empty.

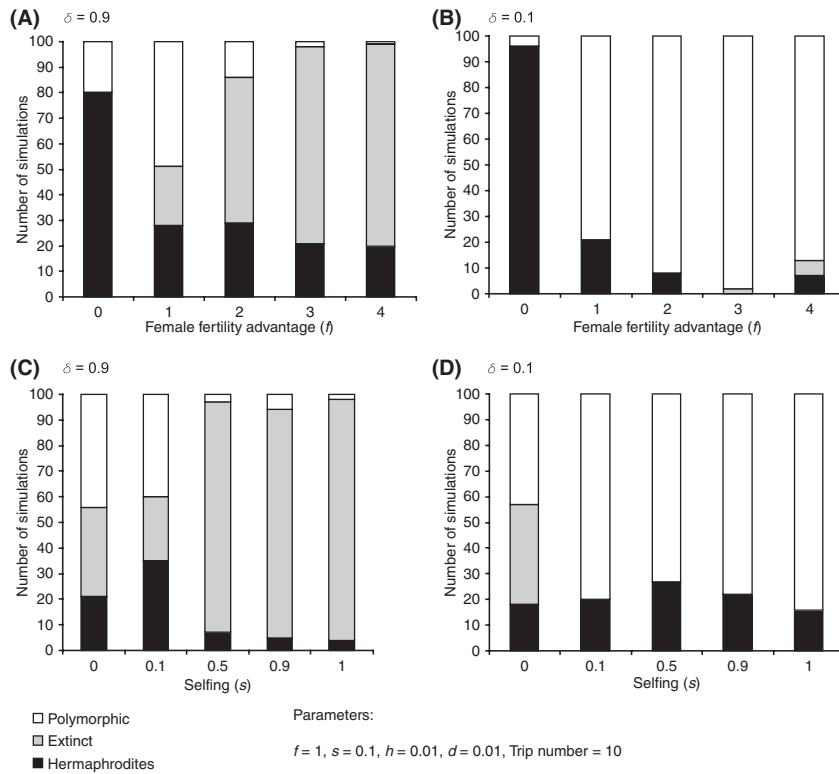


Figure 5 The results of a spatially explicit simulation examining the influence of the female fertility advantage (f) and selfing rate (s) on the fate of an introduced CMS allele. An animal pollinated species with edge effects and high inbreeding depression ($\delta = 0.9$) and low inbreeding depression ($\delta = 0.1$) was modeled in a 50×50 lattice. The number of hermaphroditic, polymorphic and extinct populations after 100 generations following the introduction of a CMS allele into a hermaphroditic population without restorers is shown. All parameters unless otherwise stated are: $o = 50, f = 1, s = 0.1, h = 0.01, d = 0.01$. The number of pollinator trips was 10. (A) The effect of increased female fertility advantage (f) when inbreeding depression is high ($\delta = 0.9$). (B) The effect of increased female fertility advantage (f) when inbreeding depression is low ($\delta = 0.1$). (C) The effect of increased self-fertilization (s) when inbreeding depression is high ($\delta = 0.9$). (D) The effect of increased self-fertilization (s) when inbreeding depression is low ($\delta = 0.1$).

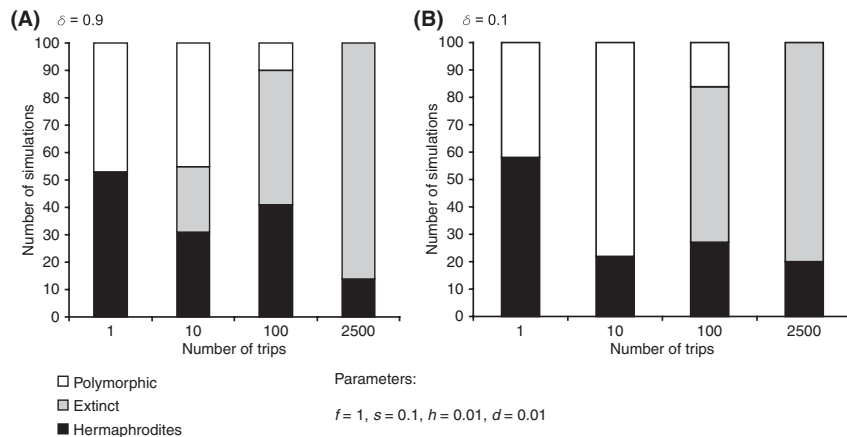


Figure 6 The results of a spatially explicit simulation examining the influence of pollinator visitation rate on the fate of an introduced CMS allele. The number of hermaphroditic, polymorphic and extinct populations after 100 generations following the introduction of a CMS allele into a hermaphroditic population without restorers is shown. All parameters unless otherwise stated are: $o = 50, f = 1, s = 0.1, h = 0.01, d = 0.01$. (A) The effect of increasing the number of pollinator trips from each plant in the lattice when inbreeding depression is high ($\delta = 0.9$). (B) The effect of increasing the number of pollinator trips from each plant in the lattice when inbreeding depression is low ($\delta = 0.1$).

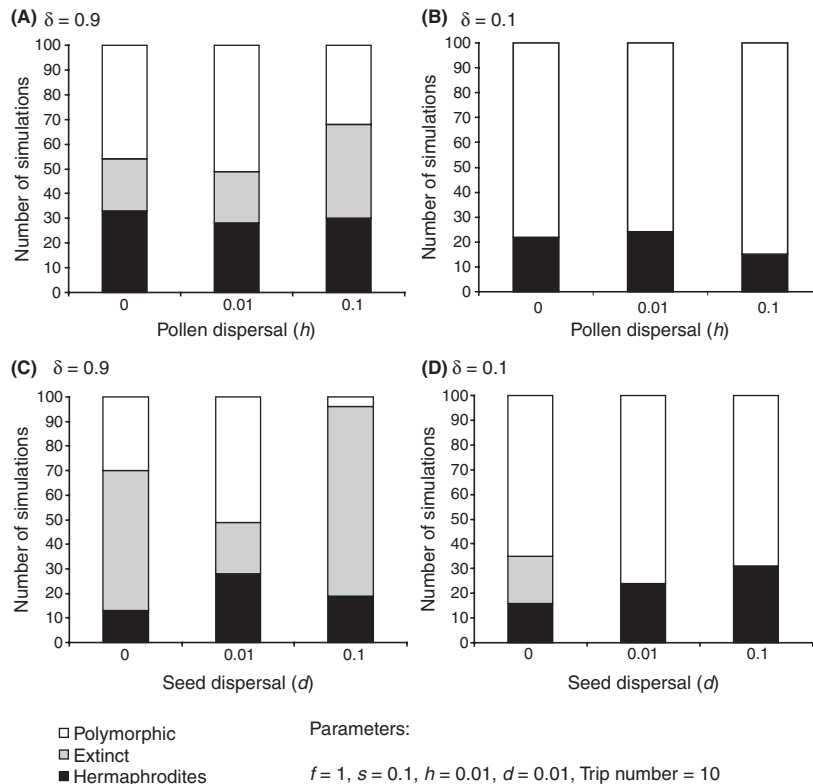


Figure 7 The results of a spatially explicit simulation examining the influence of long-distance pollen (h) and seed dispersal (d) on the fate of an introduced CMS allele. The number of hermaphroditic, polymorphic and extinct populations after 100 generations following the introduction of a CMS allele into a hermaphroditic population without restorers is shown. All parameters unless otherwise stated are: $\sigma = 50, f = 1, s = 0.1, h = 0.01, d = 0.01$. The number of pollinator trips was set to 10. (A) The effect of increased long-distance pollen dispersal (the proportion of pollen going to random cells in the lattice, h), when inbreeding depression is high ($\delta = 0.9$). (B) The effect of increased h when inbreeding depression is low ($\delta = 0.1$). (C) The effect of increased long-distance seed dispersal (the proportion of seeds going to random cells in the lattice, d), when inbreeding depression is high ($\delta = 0.9$). (D) The effect of increased d when inbreeding depression is low ($\delta = 0.1$).

We ran each simulation for a maximum of 100 generations, unless the population went extinct or the CMS allele was lost. We ran each set of parameters 100 times and recorded each of three possible outcomes: (i) population extinction; (ii) polymorphic populations composed of females and hermaphrodites; and (iii) populations composed entirely of hermaphrodites (the CMS allele was lost). The effects of all parameters were examined. Unless otherwise stated, we used a base set of parameters of $f = 1, s = 0.1, h = 0.01$ and $d = 0.01$. We examined the parameter sets with high inbreeding depression ($\delta = 0.9$) and low inbreeding depression ($\delta = 0.1$). We also examined perennality. In these sets of simulations, individuals in each cell would die and be replaced by a seed with probability P in each generation. Significance was compared for different parameter combinations using chi-squared tests. When the expected number of populations for some categories was low, we combined or eliminated categories to test for significance. All analyses were conducted in R.

Simulation results

Edge effects had a relatively minor influence on the probability of population extinction. Therefore, we have chosen to present only the results of simulations that included edge effects. Moreover, the mode of pollination did not qualitatively influence the results so we report the outcome of simulations where animal pollination was modeled.

In agreement with the analytical results, we found that increased seed set of the females relative to that of the hermaphrodites (i.e. female compensation; Delph et al. 2007) through either increased allocation of resources to ovules in females or strong inbreeding depression in selfing hermaphrodites increased the probability of population extinction. We found a significant difference in the fate of a population as the fertility advantage of females (f) increased. This was true for high levels ($\delta = 0.9$: Fig. 5A; $\chi^2 = 261.46, df = 8, P < 0.001$) and low levels of inbreeding depression ($\delta = 0.1$: Fig. 5B; $\chi^2 = 275.48,$

df = 6, $P < 0.001$; extinct and polymorphic data were combined). When inbreeding depression was strong, higher rates of self-fertilization increased the probability of extinction ($\delta = 0.9$: Fig. 5C; $\chi^2 = 211.63$, df = 8, $P < 0.001$ and $\delta = 0.1$). The exception to this pattern was the slightly higher level of population extinction when the selfing rate was zero compared to the extinction rate when the selfing rate was low ($s = 0.1$). This difference was marginally significant ($\chi^2 = 5.36$, df = 2, $P = 0.069$) and was likely a function of the reproductive assurance secured by selfing hermaphrodites when mates became limiting. In contrast, when inbreeding depression was weak (Fig. 5D; $\delta = 0.1$), the degree of self-fertilization did not influence the outcome of the simulations in those populations where some selfing occurred ($\chi^2 = 3.75$, df = 3, $P = 0.30$). In purely outcrossing populations, extinction occurred 39% of the time, while when hermaphrodites were able to self-fertilize none of the populations went extinct, although the frequency of non-CMS populations was similar in outcrossing compared to selfing populations ($\chi^2 = 0.50$, df = 1, $P = 0.48$). The higher extinction rate in outcrossing populations was likely due to the fact that outcrossing hermaphrodites had no opportunity to escape extinction through self-fertilization when they became rare in populations, allowing females to rise to fixation.

Also in accordance with the results of the analytical model, those parameter sets that reduced the degree of pollen limitation generally increased the probability of population extinction. We found a significant difference in the number of populations going extinct, remaining polymorphic or becoming fixed for hermaphrodites as pollinator visitation increased ($\delta = 0.9$: Fig. 6A; $\chi^2 = 193.42$, df = 6, $P < 0.001$ and $\delta = 0.1$: Fig. 6B; $\chi^2 = 294.37$, df = 6, $P < 0.001$). In particular, high pollen export through frequent pollinator visits reduced the amount of pollen limitation, favoring females and increasing the risk of extinction. Pollen limitation negatively affects females because, unlike hermaphrodites, females cannot self-fertilize when mates or pollinators become limiting.

Using these spatially explicit simulations, we were also able to test the influence of pollen and seed dispersal on population extinction. When there was strong inbreeding depression, we found a significant difference in the number of populations going extinct, remaining polymorphic or becoming fixed for hermaphrodites when we varied the level of pollen dispersal (Fig. 7A; $\chi^2 = 43.65$, df = 4, $P < 0.001$). Specifically, the number of populations going extinct increased when more pollen landed in random cells. Enhanced pollen dispersal favored females, thereby increasing the probability of population extinction, as pollen dispersal likely reduced pollen limitation

when hermaphrodites became rare locally. However, when inbreeding depression was low, population extinction did not occur and the number of hermaphroditic populations was the lowest when pollen dispersal was greatest ($h = 0.1$), although the difference was not significant (Fig. 7B; $\chi^2 = 2.76$, df = 2, $P = 0.25$). This was likely because the effect of pollen dispersal on female fertility was weak relative to the benefits of selfing to hermaphrodites experiencing low inbreeding depression when pollen became limiting.

We also found a significant difference in the number of populations going extinct, remaining polymorphic or becoming fixed for hermaphrodites when seed dispersal distances changed. This was true both when inbreeding depression was strong ($\delta = 0.9$: Fig. 7C; $\chi^2 = 76.00$, df = 4, $P < 0.001$) and when it was weak ($\delta = 0.1$: Fig. 7D; $\chi^2 = 6.24$, df = 2, $P < 0.05$, extinct and polymorphic populations were combined). When inbreeding depression was strong, high levels of seed dispersal resulted in the largest probability of extinction, and when 10% of the seeds landed in random cells 77% of the populations went extinct. This is likely because pollen limitation of female plants decreased when females were able to establish in different areas of the population, reducing the formation of female clusters and lessening pollen limitation, allowing females to spread until the entire population went extinct. However, for these same parameters, intermediate levels of seed dispersal resulted in the lowest probability of population extinction (Fig. 7C). Seed dispersal solely to near neighbors resulted in population extinction in 57% of the populations while when 1% of the seeds went to random cells in the lattice only 21% of the populations went extinct. This difference was significant ($\chi^2 = 25.75$, df = 1, $P < 0.001$). When inbreeding depression was low, 19% of populations went extinct when all the seeds went to near neighbors while none of the populations went extinct when some of the seeds went to random cells.

Thus, both with high inbreeding depression and low, we find that females are more likely to spread when seed dispersal is restricted to neighboring cells compared to when there is a small level of random dispersal. When seed dispersal is local, female clusters are likely to form. Females in the center of the clusters may become pollen limited and consequently local extinction can occur in the center of the cluster, while those females on the edge next to hermaphrodites will not suffer to the same degree from pollen limitation. However, any seeds that land in the central cells will be female as limited seed dispersal prevents the hermaphrodites from establishing within female clusters. Thus, the cluster of females can expand and restricted dispersal prevents re-colonization within the cluster by hermaphrodites. However, if intermediate levels

of random seed dispersal occur, hermaphrodite seeds are able to re-colonize the cells within the center of the clusters and hermaphrodites will have an advantage under these circumstances when mates become limiting as they can self-fertilize.

Discussion

Current control methods for weeds and invasive species, such as biological control, have not been effective for many unwanted plant species and can have harmful environmental side effects (Myers and Bazely 2003; Thomas and Reid 2007). Genetic control represents an alternative to these conventional methods and has been applied successfully in some insect taxa (reviewed in Gould 2008), but the utility of such methods have not been assessed in plants. CMS elements are common in plant populations and represent one potential method of genetic control in plant populations, as they can spread in populations despite causing a severe reduction in fitness to their hosts. Using an analytical model and spatially explicit simulations, we investigated the efficacy of CMS genes as a method of genetic control for weedy and invasive plant populations. We found that:

- A low effective selfing rate (i.e. the selfing rate after accounting for the effects of inbreeding depression), a large female fertility advantage, and weak pollen limitation are required for rapid fixation of the CMS allele and population extinction.
- Pollen limitation can be reduced through abundant pollinators and long distance pollen dispersal.
- Seed dispersal can have complex effects on population extinction and both severely restricted seed dispersal and long distance seed dispersal can enhance extinction rates.

Below, we further discuss our results and explore the feasibility of CMS as a method for weed management.

Compensation and population extinction

Compensation is defined as any difference in fitness between hermaphrodites and females (Delph et al. 2007) and can be due to a number of factors such as reallocation of resources from male to female function, inbreeding depression (Darwin 1877) or male biased herbivory (Ashman 2002). Lewis (1941) first noted that if male-sterile individuals have higher fitness through ovules than hermaphrodites CMS will spread through populations even if the advantage is slight, and later models also supported this finding (e.g. Lloyd 1975; Charlesworth and Ganders 1979; Frank 1989). Similarly, our analytical model and simulations reveal that compensation through

a large ovule advantage in females promotes the rapid fixation of CMS and can cause population extinction in <10 generations in some cases (Figs 2 and 5A). This result suggests that for the CMS allele to spread rapidly and act as an effective control strategy, a substantial level of compensation must be achieved in the target species.

Female compensation can also result from inbreeding depression in hermaphrodites, and our results show that considerable inbreeding depression promotes the rapid extinction of populations at all selfing rates, but particularly when selfing rates are high (e.g. Fig. 2B,C). Although theory predicts that inbreeding depression in many species is likely to be purged through substantial selfing (Lande and Schemske 1985; Charlesworth et al. 1990), several species practicing mixed mating or even high degrees of selfing are known to maintain significant levels of inbreeding depression (examples in Husband and Schemske 1996; Routley et al. 1999; Goodwillie et al. 2005). Consequently, in addition to self-incompatible species those invaders with a mixed mating system could be potentially controlled with CMS alleles, depending on the degree of inbreeding depression in the invasive range. Still, the combination of very high inbreeding depression (e.g. 0.95) and selfing rates (e.g. 0.8) is rarely found in nature (but see Herlihy and Eckert 2002; Michalski and Durka 2007). However, increased self-fertilization is predicted with colonization (Baker 1967), and it is possible that some invasive species with a recent history of outcrossing in the native range may have quite substantial inbreeding depression despite high levels of self-fertilization, which is a combination particularly conducive to the use of CMS as a control strategy.

Examination of gynodioecous and crop species, where CMS has been most studied, has shown that the fertility of females is generally greater than that of hermaphrodites (Couvet et al. 1998). In many cases, the fertility of the female is more than twice that of the hermaphrodites, a necessary condition for invasion of nuclear feminizing factors (Gouyon et al. 1991), although such measurements can be difficult to obtain because female fecundity can be obscured by pollen limitation (Ashman 2000) or seed set plasticity among hermaphrodites (Delph 2003). In addition, comparisons of offspring from females and hermaphrodites reveal that the sexual morphs can differ in a number of ways such as seed germination ability, seedling survival, plant size, flower production and survival (Shykoff et al. 2003). These factors were not explicitly incorporated into our model but they can contribute to female fertility, f , if they increase juvenile survival for the offspring of females relative to hermaphrodites. However, the strength of the female advantage and degree of inbreeding depression is species specific, and experimental work comparing the fecundity of females

and hermaphrodites as well as other fitness components later in the life cycle of the candidate species would be required to evaluate the likely effectiveness of CMS as a control strategy.

Mating system, pollen limitation and population extinction

Self-fertilization can also play an important role in CMS evolution through the reproductive assurance that it provides to hermaphrodites. In our models, self-compatible hermaphrodites can have an advantage over females, depending on the degree of inbreeding depression, when they are rare because they can set some selfed seed, while females are reliant on uncommon hermaphrodites for outcrossed pollen (Figs 1A and 2D). Therefore, CMS would only be an effective control agent in a species with a low effective selfing rate. For both the analytical model and the simulations, we included prior self-fertilization (Lloyd and Schoen 1992), as a specific level of selfing occurred in each plant regardless of the level of outcrossed pollen that reached stigmas. However, many plants have delayed selfing strategies (Lloyd and Schoen 1992; Sakai 1995; Kalisz et al. 1999) where hermaphrodites will experience the benefits of reproductive assurance when pollen is rare without the cost of inbreeding depression when pollen is not limiting. Therefore, delayed selfing would likely make the CMS-mediated extinction more difficult. In addition, although we did not model asexuality directly, for CMS to spread and cause population extinction, sexual reproduction is required. Therefore, understanding the reproductive mode of the target species is essential to predicting success of CMS as a control method.

Invasive species typically experience recurring cycles of colonization and low density, which can favor uniparental reproduction through assured reproduction when mates or pollinators are rare or absent (Baker 1967; Price and Jain 1981; Pannell and Barrett 1998). Following this prediction, many weeds and invaders tend to be self-compatible or propagate clonally (Baker 1967; Rambuda and Johnson 2004; Eckert et al. 2006; Barrett et al. 2008). Yet contrary to Baker's rule, there are several invasive and weedy species, such as common ragweed, yellow star thistle and spotted knapweed, that rely on cross-pollination for reproduction (Sutherland 2004; van Kleunen and Johnson 2007). For example, in a study of North American exotic plants, nonindigenous species with no autogamous seed production or vegetative reproduction made up 64 out of 396 of the exotic species sampled (van Kleunen and Johnson 2007). Finally, there is some evidence that outbreeding species may be less susceptible to biological control than asexual species (Burdon and Marshall 1981; but see Chaboudez and Sheppard 1995) or

selfing species because of the increased possibility of host resistance evolving in genetically variable populations. Forms of genetic control, such as CMS, represent a possible alternative to biological control in outcrossing plants.

Both our analytical model and our simulations revealed that pollen limitation decreased the probability of population extinction, due to the reproductive assurance experienced by selfing hermaphrodites. Our analytical results showed that as pollen became less limiting the time until population extinction declined as well (Figs 1C and 2A,C). Similarly, the simulations revealed that as pollinator visitation increased, the extinction rate also increased (Fig. 6). These results are consistent with previous CMS models that have shown that CMS factors should spread in the population until seed production of females becomes severely pollen limited (Charlesworth and Ganders 1979; Frank 1989). Although there is little empirical support for a global difference in pollen limitation between females and hermaphrodites in gynodioecious populations (Shykoff et al. 2003), several studies have shown greater pollen limitation of females when hermaphrodites are locally rare (Widen and Widen 1990; McCauley and Brock 1998; Graff 1999) providing evidence for the frequency-dependent interactions that can maintain both females and hermaphrodites (gynodioecy). However, many outbreeding weeds and invaders have generalist pollinators, or are wind pollinated, which should reduce the likelihood of pollen limitation. Indeed, there is little evidence that invasiveness is generally limited by the availability of suitable pollinators (Sutherland 2004; van Kleunen and Johnson 2007). Therefore, the generalist pollination system possessed by many weedy outbreeders may facilitate a CMS control strategy by ameliorating pollen limitation even when hermaphrodites are rare.

Spatial structure, CMS and population extinction

Maternally inherited traits are likely to show spatial structure, as seed dispersal in many plant species is local, and in natural gynodioecious species the frequency of females can vary considerable over small spatial scales (van Damme 1986; Frank 1989, 1997; Couvet et al. 1990). Moreover, as pollen dispersal is often restricted in plants (reviewed in Ouborg et al. 1999), and the fitness of females and hermaphrodites is thought to be frequency-dependent, local sex ratio should have considerable importance to the evolution of CMS. Few theoretical studies have explicitly examined the evolution of CMS in a spatial context (but see McCauley and Taylor 1997; Couvet et al. 1998). McCauley and Taylor (1997) found that with increased variance in sex ratio among demes, females tended to be harmed by pollen limitation more than hermaphrodites reducing the global frequency of females. Similarly, high levels of seed dispersal in our

simulations resulted in the highest rate of population extinction as greater dispersal caused lower levels of pollen limitation for females (Fig. 7C). However, when seed dispersal was severely restricted, high rates of extinction also occurred (Fig. 7C) because the seeds of the hermaphrodites could not disperse into female clusters (see Simulation Results). This finding has not been described in previous models of gynodioecy. Our model also found a weak effect of pollen dispersal on population extinction, which was due to its effect on pollen limitation of females. When long-distance pollen dispersal (to random cells) increased, extinction rates increased, because females experienced less local pollen limitation allowing them to rise in frequency (Fig. 7A,B). Overall, we found that greater pollen and seed dispersal aids in the spread of CMS, although the development of significant spatial structure through limited pollen and seed dispersal does not strongly hamper the spread and fixation of females.

Risk assessment of the CMS control strategy and future directions for genetic control in plants

For the CMS allele to spread, male sterile individuals must have higher female fertility than hermaphrodites. This creates a significant management concern if CMS is used as a control method, as seed production of the unwanted species should spike as females become common. For outcrossing species with high inbreeding depression, the total excess seed production is greatest when the fertility of females is large (Fig. 3). When the female fertility advantage is small there may actually be a reduction in total seed production compared to a purely hermaphroditic population over the same time period (Fig. 3B). However, even when total seed production is not greatly affected, maximum seed production can be substantially increased when the fertility of females is large (Fig. 3C). The likelihood that a new population will successfully establish as a result of this spike in seed production may be low because females alone cannot found new populations. However, this glut in seed production could enhance seed dispersal if dispersal rate is greater when seed production is large, as may be the case if animals disperse seeds. Thus, the risk that temporarily increased seed set might allow colonization of new sites must be considered when contemplating a CMS-mediated control program.

Clearly, a major obstacle for the success of CMS control is the evolution of nuclear restorers. The probability of successful restoration of male function is difficult to gauge as it depends on a variety of factors, particularly, the mutation rate of restorers, the number of CMS bearing individuals each generation, the time until extinction, and the probability of fixation of the nuclear restorer. The

probability of fixation of a restorer changes over time, rising from near zero when CMS is rare to near one when CMS is prevalent; accurate predictions about the probability of successful restoration thus require determining how the fixation probability changes as a function of the dynamics of CMS, the effective population size, and the effects of the restorer. To be conservative, we can consider the case where a single restorer allele, if it arises in time, is able to fix with 100% probability and prevent extinction. In this case, extinction would be averted as long as at least one restorer arises, which occurs with probability $1 - \exp(-2\mu n\tau)$, where 2μ is the diploid mutation rate giving rise to restorers. For extinction times on the order of 30 generations, as commonly observed in our model, the probability of restoration would be <5% as long as $\mu < 0.001/n$, which is plausible except in very large populations. On the other hand, if restorers are already segregating in the population the CMS control strategy is likely to fail. However, if multiple *de novo* mutations are required for restoration, within the brief window of opportunity that the population exists, CMS control has a much greater likelihood of success.

Understanding the natural distribution of restorer alleles as well as the restorer mutation rate is thus key to predicting the success of CMS control. Although restorer alleles in gynodioecious species are often geographically widespread due to balancing selection, no studies have examined the distribution of restorers in hermaphroditic plant populations. It has been shown, however, that a CMS allele is highly spatially localized in the hermaphroditic *Mimulus guttatus* (Case and Willis 2008), suggesting that the corresponding restorer allele might also be, particularly if there is a cost to restoration, which has been found in many gynodioecious species (de Haan et al. 1997; Bailey 2002; Delph et al. 2007). If strong geographic structure of CMS loci and their restorers is a general feature of hermaphrodites then unique CMS alleles will be more easily identified and introduced into invasive populations lacking the matching nuclear restorer.

Life history characteristics as well as genetic and demographic factors could hamper the successful application of the CMS weed management strategy. We re-ran our simulations incorporating perenniality, and as expected this reduced the number of populations going extinct within 100 time steps (results not presented). Similarly, seed banks would allow populations to be resurrected following population extinction. Moreover, it is possible that nuclear restorers of male function could enter populations through mutation or gene flow, preventing population extinction. Large population sizes not only increase extinction time (potentially indefinitely, Fig. 3), but they also increase the likelihood that restorers will

enter the population through mutation. In addition, extensive dispersal via pollen or seed would promote the spread of restorers from other populations, while movement of CMS seeds back to the native range or hybridization with indigenous plants could harm native populations.

Our model reveals that CMS elements are a potential method of genetic control for species with certain life history and reproductive characteristics. Empirical testing is now required in such species to fully assess the utility of the method for managing such weeds. The first hurdle would be to identify and then insert CMS elements into the candidate species. The CMS elements could be inserted in a variety of ways. First, crosses between divergent populations or closely related species can uncover CMS alleles, as such crosses disrupt the association of the CMS allele with their nuclear restorers (Barr 2004). Introgression of the divergent mitochondria into the candidate species could allow for the successful introduction of the CMS locus. However, caution should be taken that other genes that could enhance invasiveness are also not inadvertently introduced, particularly given the potential of introducing nuclear restorers as well. Second, genetic transformation of the maternally inherited plastid genome is routine in some crop species (reviewed in Chase 2006). Such technology could allow the introduction of CMS loci from more distantly related species into the candidate weed. Mitochondrial haplotypes from more distant species are also more likely to carry multiple CMS mutations. Multiple CMS elements could then be inserted into individuals and introduced into invasive populations. This would make the restoration of male function more difficult to evolve, as multiple restorers might be required.

Genetic control strategies represent an intriguing new method for weed management. The CMS strategy investigated here could be enhanced in a variety of ways. First, in our models we only introduced a single CMS individual. Insertion of more than one individual in multiple regions of the population would likely allow the CMS allele to spread efficiently and result in a faster time until extinction. Other factors should also be considered that may improve the effectiveness of the CMS control method, such as the simultaneous introduction of biological controls, the mechanical removal of hermaphrodites, and the application of herbicide, or additional genetic control methods. Finally, research into other existing selfish genetic elements, such as meiotic drive mechanisms or the homing endonuclease gene (HEG), could also lead to alternative methods of genetic control in plants. For example, Gould (2008) proposed the introduction of a female sterility mutation linked to an HEG element to control the invasive outcrosser scotch broom.

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Supporting Information

Additional Supporting Information may be found at the following location:

http://www3.botany.ubc.ca/rieseberglab/data/R_code_animal_pollination_edge.txt

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