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Comparing the refuge strategy for managing the evolution of insect resistance under different reproductive strategies

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ABSTRACT

Genetically modified (GM) crops are used extensively worldwide to control diploid agricultural insect pests that reproduce sexually. However, future GM crops will likely soon target haplodiploid and parthenogenetic insects. As rapid pest adaptation could compromise these novel crops, strategies to manage resistance in haplodiploid and parthenogenetic pests are urgently needed. Here, we developed models to characterize factors that could delay or prevent the evolution of resistance to GM crops in diploid, haplodiploid, and parthenogenetic insect pests. The standard strategy for managing resistance in diploid pests relies on refuges of non-GM host plants and GM crops that produce high toxin concentrations. Although the tenets of the standard refuge strategy apply to all pests, this strategy does not greatly delay the evolution of resistance in haplodiploid or parthenogenetic pests. Two additional factors are needed to effectively delay or prevent the evolution of resistance in such pests, large recessive or smaller non-recessive fitness costs must reduce the fitness of resistance individuals in refuges (and ideally also on GM crops), and resistant individuals must have lower fitness on GM compared to non-GM crops (incomplete resistance). Recent research indicates that the magnitude and dominance of fitness costs could be increased by using specific host–plants, natural enemies, or pathogens. Furthermore, incomplete resistance could be enhanced by engineering desirable traits into novel GM crops. Thus, the sustainability of GM crops that target haplodiploid or parthenogenetic pests will require careful consideration of the effects of reproductive mode, fitness costs, and incomplete resistance.

1. Introduction

The acreage of landscape planted to genetically modified (GM) crops that produce insecticidal toxins has increased each year since they were commercialized in 1996 (James, 2007). The use of GM crops is likely to expand, as will the number and types of insect pests targeted in the future (Bates et al., 2005; James, 2007; Malone et al., 2008). The increasingly widespread use of GM crops, and the intense selection they impose on pest populations, makes the evolution of resistance a serious threat to their continued success. Several insect species have evolved resistance to GM crops under field and laboratory conditions (Gould, 1998; Tabashnik et al., 2008a,b), demonstrating that many insect pests harbor genetic variation in resistance to GM crops.

The current theory concerning evolution of resistance to GM crops focuses on insect species that are diploid and reproduce sexually (hereafter referred to as diploid) (Gould, 1998; Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a). This is not surprising, as the majority of insecticidal GM crops are engineered to produce *Bacillus thuringiensis* (*Bt*) toxins that are toxic to some diploid coleopterans and lepidopterans (Gould, 1998; Bates et al., 2005; Showalter et al., 2009). However, recent biotechnological advances such as RNA interference (Baum et al., 2007; Mao et al., 2007) and fusion proteins (Mehlo et al., 2005), and identification of insecticidal proteins from sources other than *Bt* (Liu et al., 2003), could yield GM crops with novel modes of action. It is expected that such novel GM crops may target pests that are haplodiploid and reproduce sexually (e.g. mites, thrips, whiteflies), or diploid and parthenogenetic (e.g. aphids; hereafter referred to as parthenogenetic), in addition to diploid pests (Bates et al., 2005; Malone et al., 2008). For example, cotton engineered with the snowdrop lectin gene (*lecGNA* 2) targeting the cotton aphid, *Aphis gossypii*, is being field tested in China and India, and experimental cultivars of GM eggplant, oilseed rape, potato, rice, tobacco, and wheat have been produced for controlling aphids (*Myzus persicae*, *Macrosiphum euphorniae*, *Rhopalosiphum padi*) and thrips (*Frankliniella occidentalis*) (Jayaraman, 2004; Malone et al., 2008). As pests with any reproductive mode often harbor genetic variation in resistance to insecticides and GM crops (Gould, 1998; Whalon et al., 2006; Tabashnik et al., 2008a,b), developing strategies to delay the evolution of resistance will

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continue to be a major issue involved in commercializing new crop varieties.

Refuges of non-GM crops are used widely to delay resistance to GM crops in diploid insect pests (Gould, 1998; Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a). The theory underlying the refuge strategy was first developed in a seminal paper by Comins (1977), who showed that gene flow between refuges and insecticide treated fields could delay the evolution of resistance. Refuges promote the survival of large numbers of susceptible pests that can mate with rare resistant individuals surviving in GM fields. When resistance is inherited as a recessive trait and GM crops kill all or nearly all hybrid offspring produced by such matings, resistance can be substantially delayed (Gould, 1998; Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a). Furthermore, the refuge strategy is most likely to delay the evolution of resistance when refuges are large, the initial frequency of the resistance allele is low, resistant individuals have lower fitness in refuges than susceptible individuals (fitness costs), and resistant individuals have lower survival on GM crops than on non-GM crops (incomplete resistance) (Gould, 1998; Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a). Field outcomes of resistance evolution to GM crops in diploid pests are consistent with theory underlying the refuge strategy (Tabashnik et al., 2008a), suggesting that population genetics models are appropriate for characterizing the factors affecting resistance evolution in key pests. However, the effectiveness of the refuge strategy has not been evaluated for insect pests with different reproductive modes.

Here, we developed analytical and simulation models to expand and generalize the theory underlying the refuge strategy for managing the evolution of resistance to GM crops. We created models for diploid, haplodiploid, and parthenogenetic species to compare the effects of various factors on resistance evolution. Although some authors have suggested that a refuge strategy would likely be ineffective for managing the evolution of resistance in non-diploid pests (Denholm et al., 1998; Rausher, 2001), we show that this strategy could delay or even reverse the evolution of resistance in haplodiploid and parthenogenetic pests. In a broader sense, our results illustrate the effects of mode of reproduction on the rate of adaptation under selection.

2. Methods

2.1. General conditions for the evolution of resistance

We used analytical models to determine conditions under which the frequency of resistance alleles in a population increases or decreases over time. We assumed resistance is controlled by a single locus with two alleles (*S* for susceptibility; *R* for resistance), a realistic scenario because mutations at single loci confer resistance to GM crops in several species (Tabashnik and Carrière, 2008). In diploid and haplodiploid species, we assumed the gene frequency before selection was the same in males and females, with random mating between genotypes (in parthenogenetic species no mating occurs).

In diploids, the change in the frequency of the *R* allele each generation is

$$\Delta q = pq(W_R - W_S)/W_M \tag{1}$$

where p is the frequency of the *S* allele, q is the frequency of the *R* allele, W_S is the marginal fitness of the *S* allele, W_R is the marginal fitness of the *R* allele, and W_M is the mean fitness of all the genotypes (Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a). The direction of change is determined by $(W_R - W_S)$, such that q decreases when $W_R < W_S$. Since $W_S = pW_{SS} + qW_{RS}$ and $W_R = qW_{RR} + pW_{RS}$, where W_{SS}, W_{RS}, and W_{RR} are the fitness of the *SS*, *RS*, and *RR* genotypes, respectively (Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a), q decreases when

$$qW_{RR} + pW_{RS} < qW_{RS} + pW_{SS} \tag{2}$$

In haplodiploids, females contribute twice as much to the gene pool as males (Hartl, 1972). In a randomly mating population with equal numbers of females and males and the same gene frequency in males and females, a typical equilibrium condition before selection (Hartl, 1972), the change in the *R* allele frequency each generation is

$$\Delta q = (2/3)\Delta q_{Fem} + (1/3)\Delta q_{Mal} \tag{3}$$

where Δq_{Fem} and Δq_{Mal} are the change in the frequency of the *R* allele in females and males, respectively (Hartl, 1972). Thus, the overall change in the *R* allele frequency each generation is

$$\Delta q = [2pq(W_{R/Fem} - W_{S/Fem})/3W_{M/Fem}] + [pq(W_{R/Mal} - W_{S/Mal})/3W_{M/Mal}] \tag{4a}$$

where $W_{S/Fem}$ and $W_{R/Fem}$ are the marginal fitness of the *S* and *R* alleles, respectively, in females, $W_{S/Mal}$ and $W_{R/Mal}$ are the fitness of the *S* and *R* genotypes in males, $W_{M/Fem}$ is the mean fitness in females and $W_{M/Mal}$ is the mean fitness in males. Eq. (4a) can also be expressed as

$$\Delta q = \{pq[2W_{M/Mal}(W_{R/Fem} - W_{S/Fem}) + W_{M/Fem}(W_{R/Mal} - W_{S/Mal})]\}/3W_{M/Fem}W_{M/Mal} \tag{4b}$$

The direction of change is determined by $[2W_{M/Mal}(W_{R/Fem} - W_{S/Fem}) + W_{M/Fem}(W_{R/Mal} - W_{S/Mal})]$, and Δq is negative when $(2W_{M/Mal}W_{R/Fem} + W_{M/Fem}W_{R/Mal}) < (2W_{M/Mal}W_{S/Fem} + W_{M/Fem}W_{S/Mal})$. When q before selection is low $W_{M/Mal} \approx W_{M/Fem}$, and q declines when

$$2W_{R/Fem} + W_{R/Mal} < 2W_{S/Fem} + W_{S/Mal} \tag{5a}$$

Eq. (5a) can also be expressed as

$$2qW_{RR} + 2pW_{RS} + W_{R/Mal} < 2qW_{RS} + 2pW_{SS} + W_{S/Mal} \tag{5b}$$

In parthenogenetic organisms, selection proceeds based on the fitness of each genotype relative to the mean fitness. Because mating does not occur, each genotype represents a distinct lineage. When resistance is recessive, the frequency of the *RR* genotype reflects the frequency of resistant individuals in the population. In contrast, when resistance is dominant, the sum of the frequency of *RS* and *RR* is the frequency of resistant individuals in the population. Thus, depending on the dominance of resistance, the change in the frequency of both the *RS* and *RR* genotypes determines the change in resistance frequency. Accordingly, the change in the *R* allele frequency each generation, which is based on frequency of both the *RS* and *RR* genotypes, is

$$\Delta q = F'_{RR} + 0.5F'_{RS} - q \tag{6}$$

where F'_{RS} and F'_{RR} are the frequency of the *RS* and *RR* genotypes, respectively, after selection and q is the frequency of the *R* allele before selection. The change in the frequency of a genotype is based on its frequency and fitness, such that $F'_{RS} = W_{RS}F_{RS}/W_M$ and $F'_{RR} = W_{RR}F_{RR}/W_M$. As $q = F_{RR} + 0.5F_{RS}$, Eq. (6) becomes

$$\Delta q = [(W_{RR}F_{RR} + 0.5W_{RS}F_{RS})/W_M] - F_{RR} - 0.5F_{RS} \tag{7a}$$

Eq. (7a) can also be expressed as

$$\Delta q = [2F_{RR}(W_{RR} - W_M) + F_{RS}(W_{RS} - W_M)]/2W_M \quad (7b)$$

The direction of change is determined by $[2F_{RR}(W_{RR}-W_M)+F_{RS}(W_{RS}-W_M)]$. Thus, q decreases when

$$2F_{RR}W_{RR} + F_{RS}W_{RS} < 2F_{RR}W_M + F_{RS}W_M \quad (8)$$

We used these models to explore conditions where resistance can be prevented (i.e. q decreases over time) based on the proportion of landscape planted to refuges (P_{Ref}), fitness costs associated with resistance (c; the proportion reduction in fitness of the *RR* or *R* genotypes compared to the *SS* or *S* genotypes in refuges), the initial *R* allele frequency (q_0), and reproductive mode. A recent review by Gassmann et al. (2009) defined a fitness cost as "a trade-off in which alleles conferring higher fitness in one environment (e.g., presence of *Bacillus thuringiensis* [Bt] toxins) reduce fitness in an alternative environment (e.g., absence of Bt)." It remains unclear how costs might be manifest on GM crops, because costs are often differentially expressed across environments with varying characteristics (i.e. refuges versus GM crops; Gassmann et al. 2009). Further, data to estimate costs can only be collected in the absence of toxins, where survival of resistant and susceptible individuals can be compared without the confounding effects of insecticide exposure. Thus, as it remains unclear how costs might affect fitness of resistant individuals on GM crops, we assumed that costs only affect fitness in refuges. However, we considered a special case where costs were expressed in all crops using simulation models (see Simulation models). We focused on cases where resistance is recessive, as this condition is most likely to delay or prevent the evolution of resistance in diploid pests (Gould, 1998; Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a).

2.2. Simulation models

The analytical models provide general conditions for resistance evolution, but do not characterize the evolutionary dynamics over time. We therefore used deterministic simulation models to evaluate effects of various factors on the evolution of resistance (Table 1). Diploid individuals had three possible genotypes (*SS*, *RS*, and *RR*), while haploid males had two possible genotypes (*S* and *R*). The landscape consisted of GM crops and non-GM refuges. Each generation, individuals mated randomly in the landscape (except in the parthenogenetic model where no mating occurred) and females laid eggs randomly throughout the landscape. Individuals mated once per generation. Events in the model occurred in the following order: mating, oviposition, and selection. Each generation, both the marginal and mean fitnesses were recalculated based on the gene frequencies after selection.

We used sensitivity analyses to determine the effects of reproductive mode, refuge proportion, initial allele frequency, dominance of resistance, fitness costs, incomplete resistance, and survival on GM crops on the evolution of resistance (Table 1). Survival was determined solely by fitness on GM and non-GM crops. Unless otherwise noted, we assumed that the fitness of *SS* and *S* individuals on GM crops was 0 and the fitness of *RR* and *R* individuals on GM crops was 1. The fitness of *SS* and *S* individuals in refuges was 1, and the fitness of *RR* and *R* individuals in refuges was $1-c$, where c is the fitness cost. The fitness of *RS* individuals on GM crops was 0, $W_{RR}/2$, or W_{RR} with recessive, additive, or dominant resistance, respectively. Similarly, the fitness cost for *RS* individuals in refuges was 0, $c/2$, or c with recessive, additive, or dominant costs, respectively. Although fitness costs were generally assumed to only affect survival in the absence of toxins (i.e. refuges; Gassmann et al. 2009), in some cases fitness costs could also affect survival of resistant individuals on GM crops (see model of Gould et al., 2006). Thus, we also modeled the special case where fitness costs affected survival of resistant individuals in both refuges and GM crops. In this case, the fitness of *RR* and *R* individuals in all crops types was $1-c$, and the fitness cost for *RS* individuals in both crop types was 0, $c/2$, or c with recessive, additive, or dominant costs, respectively. We modeled incomplete resistance by decreasing the fitness of the *RR* or *R* genotypes on GM crops, where the fitness of *RR* or *R* genotypes was always lower on GM crops than refuges (Table 1). Thus, incomplete resistance always reflected lower survival of resistant genotypes on GM crops compared to refuges, regardless of whether fitness costs affected survival of resistant phenotypes in refuges. Under standard conditions where the fitness of the *RR* or *R* genotypes in refuges was 1, the reduced fitness of *RR* or *R* genotypes on GM crops represents the ratio of survival on GM crops to survival in refuges.

We used a phenotypic criterion and report the time to resistance as the number of generations for the frequency of resistant individuals to increase from the initial value to 0.5. When resistance is recessive, the phenotypic criterion is met when 50% of individuals are *RR*. When resistance is dominant, the phenotypic criterion is met when 50% of individuals are *RS* or *RR*. We input the parameters from three studies (Gould, 1998; Carrière, 2003; Tabashnik et al., 2008a) to determine if predictions with the diploid and haplodiploid models matched previously published models. In all cases model output exactly matched the published results, which showed that the models were programmed accurately. We could not compare results with the parthenogenetic model to any previous studies because no published model exists to our knowledge. However, equilibrium in resistance allele frequency occurred in simulations as predicted by Eqs. (1), (3), and (6), demonstrating that the models embodied the relevant assumptions.

Table 1
Parameter values used in simulation models.

Parameter	Values used
Proportion of landscape planted to refuge (P_{Ref})	0.1, 0.3, 0.5
Initial *R* allele frequency (q)	0.001, 0.01
Fitness cost (c) for *RR* and *R* individuals (proportion reduction in survival in refuges)	0, 0.1, 0.3, 0.5
Fitness of *RR* and *R* individuals on GM crops (incomplete resistance)	0.1, 0.5, 1

3. Results

3.1. General conditions for the evolution of resistance

Eqs. (2), (5b), and (8) demonstrate that for any type of pest, reducing the fitness of the *RR* and *R* genotypes, or increasing the fitness of the *SS* and *S* genotypes, produces conditions favorable for preventing resistance. Additionally, because p is typically greater than q before selection, reducing the fitness of the *RS* genotype delays the evolution of resistance.

Based on the analytical equations, in species with any reproductive mode, when resistance and fitness costs are recessive (i.e. $W_{SS} = W_{RS}$), q decreases when (SI Methods)

$$1/(1+c) < P_{Ref} \quad (9)$$

Thus, the direction of resistance evolution is solely based on refuge size and fitness costs, but not on allele frequency or reproductive mode. As fitness costs increase, smaller refuges are

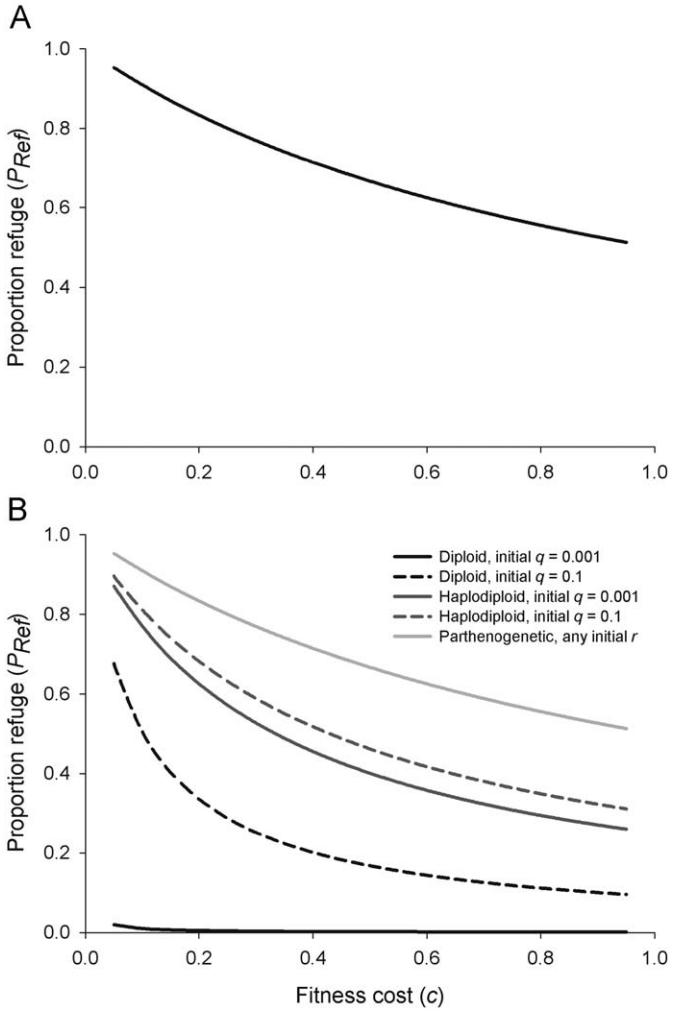

Fig. 1. Proportion of landscape planted to refuge (P_{Ref}) for which the R allele frequency (q) decreased over time for diploid and haplodiploid species with recessive resistance and (A) recessive fitness costs (c) and (B) dominant fitness costs. For these simulations, fitness costs only affected fitness in refuges. In (A) the line represents species with any reproductive mode and any initial R allele frequency (q_0). In (B) lines are shown for species with different reproductive modes and initial R allele frequency. For each line, combinations of P_{Ref} and c above the line will result in a decrease in q over time, while values below the line will result in an increase in q over time. Values intersecting the line represent equilibrium conditions where q does not change.

needed to prevent the evolution of resistance (Fig. 1a). However, even with large costs, refuges occupying over 50% of the landscape are needed to prevent the evolution of resistance.

In another scenario, when resistance is recessive and fitness costs are dominant (i.e. W_{RS} in refuges $= W_{RR}$ in refuges), reproductive mode and allele frequency affect the evolution of resistance. Under these conditions, in a diploid species q decreases when (SI Methods)

$$q/(q+c-qc) < P_{Ref} \quad (10)$$

In a haplodiploid species under the same conditions, q decreases when (SI Methods)

$$(2q+1)/[2q+1+c(3-2q)] < P_{Ref} \quad (11)$$

In a parthenogenetic species, q decreases under the same conditions when costs are dominant compared to when costs are recessive (SI Methods, Figs. 1a,b). Thus, because q is typically small before selection, even small dominant fitness costs can prevent the evolution of resistance in diploid pests. However, dominant fitness costs have less of an effect on the evolution of resistance in haplodiploid and parthenogenetic species compared to diploid pests (Fig. 1b). Additionally, q before selection has the largest effects for diploid species (Fig. 1b).

Table 2
Effects of reproductive mode, initial R allele frequency (q_0), refuge size, and dominance of resistance on the number of generations for the frequency of resistant individuals to reach 0.5.

q_0	Dominance of resistance	Reproductive mode		
		Diploid	Haplodiploid	Parthenogenetic
Refuge = 10%				
0.001	Recessive	118	7	6
	Additive	5	4	6
	Dominant	3	3	3
0.01	Recessive	16	5	4
	Additive	4	3	4
	Dominant	2	2	2
Refuge = 30%				
0.001	Recessive	438	14	12
	Additive	10	8	12
	Dominant	6	6	6
0.01	Recessive	49	9	8
	Additive	7	6	8
	Dominant	4	4	4
Refuge = 50%				
0.001	Recessive	>1000	25	20
	Additive	18	14	20
	Dominant	10	10	9
0.01	Recessive	109	16	14
	Additive	12	10	14
	Dominant	6	7	6

Fitness of the *RR* and *R* genotypes on GM crops was 1; fitness of the *SS* and *S* genotypes on GM crops was 0; fitness of all genotypes in refuges was 1 (i.e. no fitness costs).

3.2. Effects of reproductive mode, allele frequency, refuge size, and dominance

Resistance evolved faster in simulations with increases in initial R allele frequency or dominance of resistance and smaller refuges (Table 2). Resistance evolved slower in diploid compared to haplodiploid and parthenogenetic species when resistance was recessive. However, with additive or dominant resistance, there were only small differences in the rate of resistance evolution in species with different reproductive modes (Table 2).

3.3. Effects of fitness costs and incomplete resistance

Unless otherwise noted, all results shown represent the standard case where fitness costs only affected fitness in refuges (not GM crops). When fitness costs affected survival in both refuges and GM crops, the time for resistance to evolve increased with any mode of reproduction compared to when fitness costs affected survival only in refuges, especially as the magnitude of costs increased (Fig. 2). Thus, our standard assumption that costs only affected fitness in refuges may underestimate the effects of fitness costs if costs are expressed in both refuges and GM crops in the field.

For diploid pests, resistance evolution was delayed or prevented when fitness costs were associated with resistance (particularly with non-recessive costs), and when resistance was incomplete (Tables S1 and S2). Similar results have been shown in

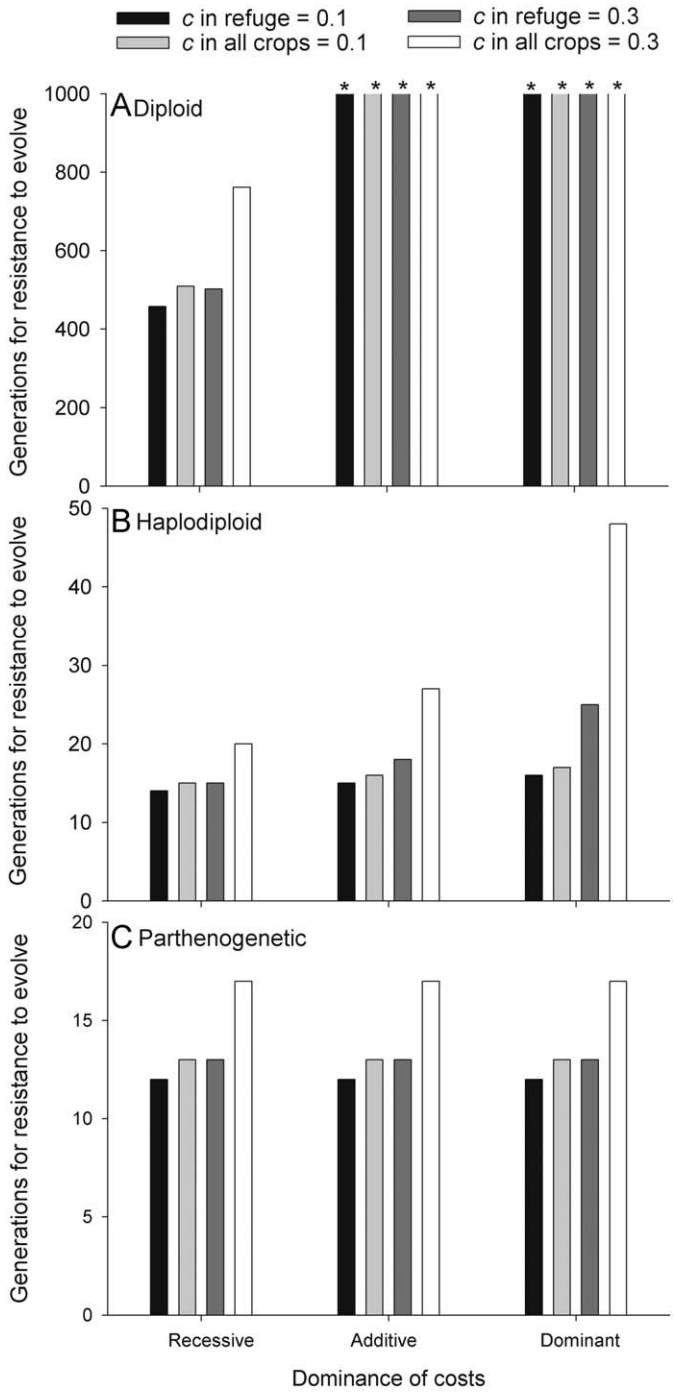

Fig. 2. Effects of fitness costs (c), magnitude of costs, and dominance of costs on the number of generations for the frequency of resistant individuals to reach 0.5 in (A) a diploid, (B) a haplodiploid, and (C) a parthenogenetic species. Costs were assumed to affect fitness in only the refuge, or on both refuges and GM crops. For all simulations, fitness of the *SS* and *RS* genotypes on GM crops was 0 (i.e. recessive resistance) and fitness of the *RR* and *SS* genotypes in the refuge were $1-c$, and 1, respectively. The initial *R* allele frequency was 0.001, and the proportion of the landscape planted to refuge was 0.3. For any other set of initial conditions, resistance was delayed in simulations where fitness costs affected fitness of resistant individuals on both crops compared to refuges alone. *: Resistance never evolved (i.e. the resistance allele frequency declined over time).

other diploid models (Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a).

In haplodiploid species, as predicted by the analytical equations, recessive fitness costs generally had small effects on the evolution of resistance except with large refuges (Table 3).

Table 3
Effects of fitness costs (c), refuge size, and dominance of costs on the number of generations for the frequency of resistant individuals to reach 0.5 in a haplodiploid species.

Fitness Cost (c)	Refuge (%)	Dominance of Cost		
		Recessive	Additive	Dominant
0.1	10	7	7	7
	30	14	15	16
	50	27	31	35
0.3	10	7	7	8
	30	15	18	25
	50	34	59	∞
0.5	10	7	8	10
	30	16	25	∞
	50	44	∞	∞

Fitness of the *RR* (and *R*), *RS*, and *SS* (and *S*) genotypes on GM crops were 1, 0, and 0, respectively (recessive resistance). Fitness of the *RR* (and *R*) and *SS* (and *S*) genotypes in the refuge were $1-c$, and 1, respectively (i.e. costs only affected fitness in refuges). The initial *R* allele frequency was 0.001.

Table 4
Effects of recessive fitness costs (c), refuge size, and incomplete resistance on the number of generations for the frequency of resistant individuals to reach 0.5 in a haplodiploid species with recessive resistance.

Fitness of *R* on GM crop	Fitness of *RR* on GM crop	Refuge (%)	Fitness cost (c)		
			0	0.1	0.3
Fitness of R = Fitness of RR					
0.1	0.1	10	27	30	38
		30	87	147	∞
		50	194	Stable	∞
0.5	0.5	10	10	10	10
		30	23	24	28
		50	44	54	100
Fitness of R<Fitness of RR					
0.1	1.0	10	19	21	24
		30	58	85	∞
		50	128	>1000	∞
0.5	1.0	10	9	9	9
		30	21	22	25
		50	40	48	83
Fitness of R > Fitness of RR					
1.0	0.1	10	8	8	8
		30	16	17	18
		50	29	33	45
1.0	0.5	10	7	7	7
		30	15	15	16
		50	27	30	37

Fitness of the *SS* and *S* genotypes on GM crops was 0. Fitness of the *RR* (and *R*) and *SS* (and *S*) genotypes in the refuge were $1-c$, and 1, respectively (i.e. costs only affected fitness in refuges). The initial *R* allele frequency was 0.001.

Fitness costs also had small effects on resistance evolution when resistance was additive or dominant (Table S3). Large, non-recessive costs were necessary to prevent the evolution of resistance (Table 3). With fitness costs and incomplete resistance, resistance evolution was delayed or prevented, particularly with large refuges (Tables 4, S4 and S5). In most cases, incomplete resistance in males delayed resistance more than incomplete resistance in females (Tables 4, S4 and S5). However, large delays in the evolution of resistance were less likely when resistance was additive or dominant compared to recessive (Tables 3, S3–S5).

In parthenogenetic species, fitness costs associated with resistance had small effects on delaying the evolution of resistance (Table S6). Over the range of values simulated,

Table 5
Effects of recessive fitness costs (c), refuge size, dominance of resistance, and incomplete resistance on the number of generations for the frequency of resistant individuals to reach 0.5 in a parthenogenetic species.

Fitness of *RR* on GM crop	Refuge (%)	Fitness cost (c)		
		0	0.1	0.3
Recessive resistance				
0.1	10	22	24	30
	30	66	111	∞
	50	145	Stable	∞
0.5	10	9	9	9
	30	18	20	23
	50	35	42	76
Dominant resistance				
0.1	10	7	7	7
	30	19	19	19
	50	41	41	41
0.5	10	3	3	3
	30	6	6	6
	50	10	10	10

Fitness of the *SS* genotype on GM crops was 0 and fitness of the *RR* and *SS* genotypes in the refuge were $1-c$, and 1, respectively (i.e. costs only affected fitness in refuges). The initial *R* allele frequency was 0.001.

incomplete resistance delayed resistance evolution more than fitness costs, and in some cases resistance was prevented with incomplete resistance and fitness costs (Table 5). Preventing the evolution of resistance was possible only when resistance was recessive, as fitness costs and incomplete resistance had little effect with dominant resistance (Tables 5, S6). Results were similar with recessive or additive resistance, although in no case with additive resistance did the frequency of resistant individuals decline over time.

4. Discussion

The effects of sex and reproduction on the rate of adaptation under selection have long been an issue of great interest and debate among biologists and evolutionary theorists (Hartl, 1972; Bull, 1979; Kondrashov and Crow, 1991; Orr and Otto, 1994). Models suggest that under most conditions, asexual haploid populations evolve more rapidly than sexual diploid populations (Orr and Otto, 1994). Models have also suggested that resistance generally evolves slower in diploid compared to haplodiploid insect pests because resistance alleles are exposed from the outset to selection in haploid males (Caprio and Hoy, 1995; Denholm et al., 1998; Carrière, 2003; Crowder et al., 2006). However, the effects of sex and reproduction on the evolution of insecticide resistance remain largely unknown, and generalizing the theory of resistance evolution for insect pests with different reproductive modes has been lacking.

We have shown that reducing the fitness of individuals harboring *R* alleles delays the evolution of resistance in any pest. In diploids before selection, most *R* alleles are expected to be in heterozygous *RS* individuals. The fitness of *RS* individuals decreases when resistance is recessive and/or non-recessive fitness costs are associated with resistance. Under these conditions, resistance evolves slower or is prevented (Gould, 1998; Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a).

In haplodiploid species, females contribute twice as much to the gene pool as males (Hartl, 1972). Despite this, results show that the evolution of resistance is often driven by selection for resistant males. In populations at genetic equilibrium, which is a typical condition before selection (Hartl, 1972), the initial frequency of resistant males is equal to the *R* allele frequency (q), while the frequency of homozygous resistant females is q^2. Thus, because resistant males are much more common than resistant females, decreasing the fitness of resistant males can have the largest effect on delaying or preventing the evolution of resistance. As in diploids, the majority of *R* alleles in females of a haplodiploid species are present in *RS* individuals, but all of the *R* alleles in males are found in *R* individuals. With recessive fitness costs, the fitness of the *RR* and *R* genotypes is reduced in refuges. However, since the majority of *R* alleles in females is carried by *RS* rather than *RR* individuals, the cost in females has smaller effects on resistance evolution than the cost in males. Similarly, on GM crops, decreasing the fitness of *R* males delays resistance more than decreased fitness of *RR* females. In contrast, when fitness costs are additive or dominant, the fitness of *RS* females is reduced, and females have more influence on the evolution of resistance.

In parthenogenetic species, the genotype with the highest fitness becomes most common, and resistance evolves unless the *SS* genotype has the highest fitness. When resistance is recessive and the *RS* genotype does not have the greatest fitness, resistance can be prevented when the *SS* genotype has higher fitness than the *RR* genotype, which can occur with large refuges, fitness costs, and incomplete resistance. When resistance is additive or dominant, resistance can be prevented when the *SS* genotype has higher fitness than both the *RS* and *RR* genotypes, which can occur with large refuges, non-recessive fitness costs, and incomplete resistance. Resistance stabilizes at levels that may or may not lead to control problems when the *RS* genotype has the highest fitness. These results represent species that reproduce via obligate parthenogenesis. However, many species are cyclically parthogenetic, and mate occasionally. In such species, resistance evolves slower as the number of generations between mating decreases, as the reproductive mode becomes more similar to diploid pests that reproduce sexually.

Confirming results of previous studies (Gould, 1998; Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a), large refuges of non-GM crops, low initial frequency of resistance alleles, and recessive inheritance of resistance delayed the evolution of resistance in diploid pests. Several of these factors also delayed resistance evolution in haplodiploid and parthenogenetic pests, suggesting that some tenets of the refuge strategy are broadly applicable for resistance management. In both haplodiploid and parthenogenetic pests, larger refuges and lower initial resistant allele frequencies delayed the evolution of resistance. However, while recessive resistance along with refuges greatly delays resistance evolution in diploids, such delays are not possible in haplodiploid or parthenogenetic pests without additional factors such as fitness costs and incomplete resistance.

So, can resistance evolution to GM crops be delayed or prevented in haplodiploid and parthenogenetic pests with a refuge strategy? A low frequency of resistant alleles before selection is clearly important, an expected condition when fitness costs are associated with resistance (Carrière et al., 1994). Low frequencies of resistance alleles before selection ($r<0.01$) are commonly observed in diploid pests targeted with GM crops (Tabashnik et al., 2008a). Thus, it is unlikely that the frequency of resistance alleles would be high in many populations of haplodiploid or parthenogenetic pests before the introduction of GM crops. Although the initial resistance allele frequency may be difficult to control, dominance of resistance could be modified by properties of the GM crops to delay the evolution of resistance (Carrière and Tabashnik, 2001; Tabashnik et al., 2005, 2008a). High toxin concentrations that kill all heterozygous individuals and make resistance functionally recessive should be implemented in GM crops targeting haplodiploid and parthenogenetic pests, as resistance evolved faster with non-recessive resistance.

Additionally, fitness costs and incomplete resistance had smaller effects with non-recessive resistance.

One option to delay or prevent the evolution of resistance in haplodiploid and parthenogenetic pests would be to plant refuge crops that induce large, non-recessive fitness costs. Fitness costs are often associated with resistance to *Bt* crops, and are not recessive in about 25% of cases (Gassmann et al., 2009). For insects feeding on plants (as opposed to artificial diet), costs affecting survival associated with resistance to *Bt* crops or sprays averaged 0.23 (range = −0.20 to 0.63) (Gassmann et al., 2009), indicating that values used in our simulations are realistic. The magnitude and dominance of costs can vary greatly based on host plants, environmental conditions, and interactions with natural enemies (Gassmann et al., 2009). For generalist herbivores, planting refuges of specific crops that increase the magnitude and dominance of fitness costs without reducing the survival of susceptible individuals could delay resistance evolution (Janmaat and Myers, 2005; Bird and Akhurst, 2007). For specialists, choosing specific crop cultivars could affect fitness costs (Carrière et al., 2005). Furthermore, natural enemies or pathogens that increase the magnitude or dominance of costs could enhance resistant management (Raymond et al., 2007; Gassmann et al., 2008). Additionally, if costs are expressed on GM crops in addition to refuges, resistance will be further delayed.

Developing GM crops that can induce incomplete resistance is another feasible option to delay the evolution of resistance in any type of pest. Resistant populations of pest species often have lower survival on single-toxin *Bt* crops than on non-*Bt* crops with the same genetic background (Fig. 3, Table S7). For seven pests where survival on both *Bt* and non-*Bt* crops were measured, the ratio of fitness on *Bt* crops relative to non-*Bt* crops averaged 0.63 (range = 0.11–1.5) (Fig. 3, Table S7), indicating that values used in our simulations are realistic. Furthermore, one strategy to increase incomplete resistance could be to incorporate more than one toxin (pyramided toxins) into GM crops. Pyramided toxins are increasingly used to control diploid pests, and are effective because insects resistant to one toxin may be killed by the other toxin (Bates et al., 2005). If multi-toxin crops contribute in lowering fitness of resistant individuals more than single-toxin crops, multi-toxin crops could be a valuable alternative for delaying resistance evolution in haplodiploid or parthenogenetic pests (Fig. 3).

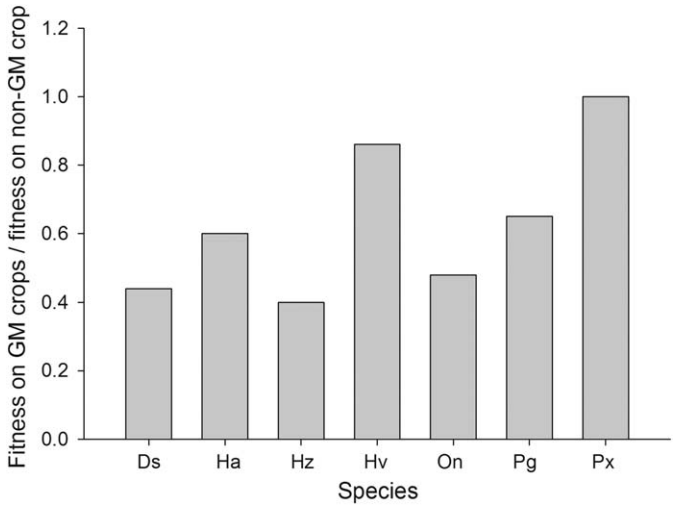

Fig. 3. Ratio of the fitness of resistant individuals on GM crops compared to non-GM crops for seven pest species based on data from laboratory and greenhouse studies (see Table S7 for full dataset). For species where data was collected on multiple crops or with multiple toxins, the average value is shown. Species abbreviations: Ds: *Diatraea saccharalis*; Ha: *Helicoverpa armigera*; Hz: *Helicoverpa zea*; Hv: *Heliothis virescens*; On: *Ostrinia nubilalis*; Pg: *Pectinophora gossypiella*; and Px: *Plutella xylostella*.

The use of modified toxins designed to specifically "bypass" known resistant mechanisms and kill resistant insects could perhaps also induce incomplete resistance (Soberon et al., 2007). A similar approach could be to use toxins that promote negative cross-resistance (NCR), which are toxins that can significantly delay the evolution of resistance to GM crops by causing hyper-susceptibility in resistant insects (Pittendrigh et al., 2008). As screening methods to identify and synthesize modified or NCR toxins improve, such toxins could be incorporated into pyramided cultivars or sprayed on GM crop fields to counteract the evolution of resistance. Furthermore, in haplodiploid organisms, decreased fitness of resistant males often has the greatest effect on delaying the evolution of resistance. Thus, if individuals of only one sex are affected by pyramided, modified, or NCR toxins, reducing the survival of males is the most effective strategy. Many genes in insects are differentially expressed in males and females, and the use of bacteria or conditional lethal genes have been commonly used for sex-specific control or sterile-insect release programs (Heinrich and Scott, 2000; Veneti et al., 2005). Similar methods could be used to induce incomplete resistance in males of targeted haplodiploid pests.

Our results suggest that the standard refuge strategy alone is insufficient to considerably delay or prevent the evolution of resistance in haplodiploid or parthenogenetic pests. Similarly, Denholm et al. (1998) and Rausher (2001) suggested that the refuge strategy would be ineffective for managing the evolution of resistance in non-diploid pests. However, unlike these studies, we show that the standard refuge strategy can be effective when resistance is incomplete and associated with fitness costs. Thus, resistance management strategies will be most effective if refuges not only promote survival of susceptible insects, but also increase the magnitude and dominance of fitness costs. Additionally, resistance management will be most effective if novel GM crops induce incomplete resistance, or are used in concert with externally applied toxins to lower the fitness of resistant individuals. However, it remains to be seen if such strategies would be feasible from economic, environmental, and practical perspectives. GM crops for control of haplodiploid and parthenogenetic species could be a major boon to agriculture in many countries. Our results show that with proper considerations, the evolution of resistance in such pests can be delayed or prevented as in diploid pests. Although these results are based on several simplifying assumptions, such as no survival of susceptible insects on GM crops and random mating between genotypes, they provide a framework for exploring the effects of mode of reproduction on the evolution of resistance and for studying alternatives to the standard refuge strategy. Future models should build upon this framework to further explore the role of sex and reproduction on the evolution of resistance and to design strategies to delay or prevent resistance in pests with different reproductive modes.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtbi.2009.08.017.

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