Reversing insect adaptation to transgenic insecticidal plants

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The refuge–high-dose strategy for delaying insect adaptation to transgenic plants produces non-transgenic plants that enable survival of susceptible individuals. Previous theoretical work has suggested three requirements for success of the refuge–high-dose strategy: a low initial frequency of the resistance allele, extensive mating between resistant and susceptible adults and recessive inheritance of resistance. In order to understand an observed decrease in resistance frequency and improve the potential for managing resistance better, we used analytical and simulation models for exploring the conditions that prevent or reverse the evolution of resistance, even when resistance is not rare initially. Assuming random mating and recessive or nearly recessive inheritance of resistance, the factors favouring reversal of resistance are non-recessive costs of resistance, low initial resistance allele frequency, large refuges, incomplete resistance and density-independent population growth in refuges.

Keywords: *Bacillus thuringiensis*; fitness costs; pesticide resistance; refuge–high-dose strategy; transgenic plants

1. INTRODUCTION

Rapid adaptation of insect pests could cut short the success of crops that are genetically modified to produce toxins from *Bacillus thuringiensis* (Bt) (Tabashnik 1994; Frutos *et al*. 1999). Theory and limited experimental evidence have suggested that, under some conditions, refuges from exposure to toxin can substantially delay the evolution of pest resistance to Bt crops (Liu & Tabashnik 1997; Gould 1998; Lenormand & Raymond 1998; Shelton *et al*. 2000). Based primarily on single-locus models, previous work has identified three key assumptions of the refuge–high-dose strategy (but see Lenormand & Raymond 1998): a low initial frequency of the resistance allele, extensive mating between resistant and susceptible adults and functionally recessive inheritance. If the resistance allele is rare and mating is random, nearly all homozygous resistant adults will mate with homozygous susceptible adults emerging from refuges. If inheritance of resistance is functionally recessive, heterozygous progeny produced by such matings will be killed by Bt plants, thereby greatly slowing resistance evolution.

Results from models have led to the conclusion that the refuge–high-dose strategy requires initial allele frequencies below 0.001 (Roush 1994). Initial empirical estimates from three major lepidopteran pests targeted by Bt crops showed that this assumption was reasonable (Gould *et al*. 1997; Andow *et al*. 2000; Bentur *et al*. 2000). However, in Arizona field populations of the pink bollworm *Pectinophora gossypiella*, the estimated mean frequency of a major allele conferring resistance to the CrylAc toxin produced by Bt cotton exceeded the theoretical threshold by *ca.* 100-fold in 1997 (Tabashnik *et al*. 2000). Despite widespread use of Bt cotton and contrary to expectations from existing theory, laboratory bioassays of field-derived strains showed that the frequency of resistance declined to undetectable levels in 1999 (Tabashnik *et al*. 2000). Here, we explore the conditions under which the frequency of

resistance can decrease, even when the initial frequency of the resistance allele exceeds 0.001. Although the example that motivated this theoretical analysis involved insect resistance to Bt crops, the underlying principles may have broad applicability for understanding and managing the evolution of resistance to xenobiotics.

We first consider a single-locus analytical model with alleles for susceptibility (S) or resistance (R) to explore the conditions under which evolution of resistance can be prevented or reversed, with the following assumptions.

(i) The initial frequency of the R allele is 0.1 and 50% of the fields are planted to refuges.
(ii) Mating among individuals from transgenic plants and refuges is random.
(iii) Resistance is recessive, with the proportion surviving on transgenics being 1.0 for an RR genotype and 0.0 for SS and RS genotypes.
(iv) The proportion surviving in refuges is 0.7 for the RR genotype, 0.85 for the RS genotype and 1.0 for the SS genotype. Thus, the fitnesses of the RR, RS and SS genotypes are 0.85 $((0.7 \times 0.5) + (1 \times 0.5))$, 0.425 and 0.5, respectively.

The direction of change in the frequency of the R allele is given by

$$\Delta R = \frac{rs(W_R - W_S)}{\overline{W}}, \tag{1}$$

where r is the frequency of the R allele, s is the frequency of the S allele, W_R is the fitness of the R allele, W_S is the fitness of the S allele and $\overline{W}$ is the average fitness of all the genotypes (Crow & Kimura 1970). The direction of change in r is given by the value of $W_R - W_S$. A proportion r of the R allele occurs in the RR genotype ($r^2/r^2 + rs$) and a proportion s of the R allele occurs in the RS genotype ($rs/r^2 + rs$). Similarly, proportions s and r of the S allele occur in the SS and RS genotypes, respectively. Thus, W_R is 0.4675 (0.1 (0.85) + 0.9 (0.425)) and W_S is 0.4925. Because $W_R - W_S$ is negative, r will decrease from

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its initial value of 0.1 until equilibrium is achieved between mutation and selection (Crow & Kimura 1970).

Explicitly expressing the fitness of genotypes in the expression $W_R - W_S$, the general condition under which the frequency of a recessive R allele decreases is (see Appendix A, §a)

$$rW_{RR} < \text{ref}\,[sW_{SS} - (s-r)W_{RS}], \qquad (2)$$

where ref is the proportion of refuges and where both habitats contribute to W_{RR}, but only refuges contribute to W_{SS} and W_{RS} (the survival of the SS and RS genotypes on transgenics is 0.0). For a recessive cost of resistance, $W_{SS} = W_{RS}$. Substituting W_{SS} for W_{RS} in equation (2) and expressing the fitness of the genotypes as a function of their habitat (see Appendix A, §b), the condition reversing the evolution of resistance is

$$(1 - \text{ref})W_{RR/\text{tra}} < \text{ref}\,(\text{ref}\,W_{SS/\text{ref}} - W_{RR/\text{ref}}), \qquad (3)$$

where $(1 - \text{ref})$ is the proportion of transgenic fields, $W_{RR/\text{tra}}$ and $W_{RR/\text{ref}}$ are the the fitnesses of the RR genotype on transgenic and refuge plants, respectively and $W_{SS/\text{ref}}$ is the fitness of the SS genotype on refuge plants. Therefore, the evolution of resistance is reversed with large refuges, low survival of the RR genotype on transgenics ($W_{RR/\text{tra}}$) and a large cost of resistance ($W_{RR/\text{ref}} \ll W_{SS/\text{ref}}$). Moreover, the value of the right-hand side of equation (2) increases as the costs become less recessive (i.e. W_{SS} and W_{RS} become less similar). Therefore, an increase in the dominance of costs also hinders the evolution of resistance.

The above equations address the direction but not the dynamics of the evolution of resistance. We thus used simulation models to investigate the effects of the dominance and magnitude of resistance costs, refuge size, initial frequency of the resistance allele, non-recessive resistance, incomplete resistance and mode of population regulation on the direction and rate of the evolution of resistance.

2. SIMULATION MODEL

In this deterministic model we assume that the fitness-reducing effects of transgenics and fitness costs in the absence of toxins only affect survival. Resistance is conferred by a single locus with two alleles (R and S). Events in the model occur in the following order: migration, mating, oviposition and selection. Mating is random among individuals from the transgenic fields and refuges. Individuals mate only once and females lay eggs at random between the two habitats. Hence, we specifically consider cases where distances between refuges and transgenic fields are small relative to the dispersal of adults. Based on data for the pink bollworm and other lepidopteran pests, we assume that the inheritance of resistance to transgenics is either completely recessive or nearly recessive (Frutos *et al.* 1999). With the high levels of mortality imposed by transgenics and nearly recessive resistance, the combination of random mating and random oviposition generally provides some of the best conditions for delaying the evolution of resistance (Caprio 2001).

We first assume that 'hard selection' (Wallace 1968) affects the survival of larvae in both habitats. Thus, the

Table 1. Effects of the dominance and magnitude of resistance costs, refuge size and initial frequency of resistance allele on the number of generations required for resistance evolution.

(The survival of RR individuals on transgenics was 1.0 and the resistance was recessive. The survival of SS individuals on non-transgenics was 1.0. The time to evolve resistance was the number of generations required to reach an R allele frequency of greater than 0.5. ∞ represents a decrease in the frequency of the R allele.)

cost (%)	initial R allele frequency	dominance of cost	refuge (%) 10	refuge (%) 30	refuge (%) 50
30	0.01	recessive	16	34	37
30	0.01	additive	∞	∞	∞
30	0.01	dominant	∞	∞	∞
30	0.1	recessive	3	7	17
30	0.1	additive	3	10	∞
30	0.1	dominant	4	∞	∞
30	0.3	recessive	1	2	4
30	0.3	additive	2	2	5
30	0.3	dominant	2	2	6
60	0.1	recessive	3	8	18
60	0.1	additive	3	∞	∞
60	0.1	dominant	∞	∞	∞
60	0.3	recessive	2	3	7
60	0.3	additive	2	2	stable
60	0.3	dominant	1	3	∞

contribution of genotypes to transgenic fields and refuges is independent of population density in the habitat where the genotypes develop. We also model 'soft selection' (Wallace 1968) in which density-dependent factors separately determine genotype success within the different habitats.

When tested with the parameters from Gould (1998), the output from our simulations matched the output of that author. Moreover, equilibria in resistance frequency occurred in the simulations as predicted by equation (1), which suggests that our model embodied the relevant assumptions.

3. RESULTS

(a) *Dominance and magnitude of resistance costs and initial frequency of the resistance allele*

As predicted from equation (2), reversal of resistance was more likely when the resistance cost was additive or dominant (table 1). With a fitness cost of 30 or 60% (the fitnesses in the refuge were 1.0 for the SS genotype and 0.7 or 0.4 for the RR genotype, respectively), the frequency of the R allele decreased in many cases when the cost was additive or dominant, but not when the cost was recessive (table 1). Increased dominance of the cost favoured a decline in resistance because it increased the advantage of SS over RS individuals in refuges. As the initial R allele frequency increased, decreases in resistance were less likely (table 1) because an increased frequency of RR individuals raises the average fitness of the R allele (equation 2).

A 60% fitness cost favoured reversal of resistance and augmented the rate of decline of frequency of the R allele (table 1 and figure 1). With an initial R allele frequency of
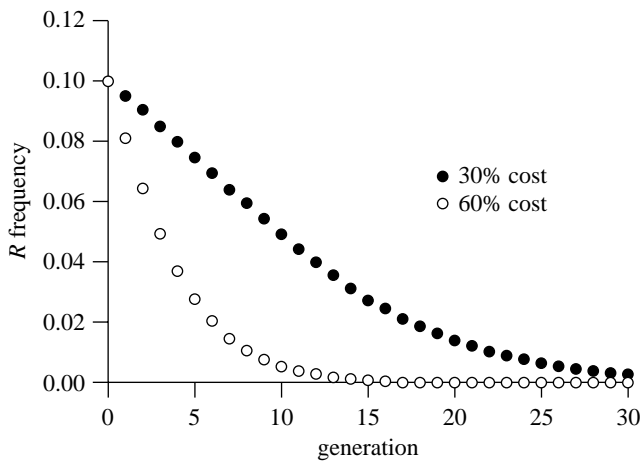
Figure 1. Effect of the magnitude of resistance costs on resistance evolution with a 50% refuge. The initial R allele frequency was 0.1, the survival of RR individuals on transgenics was 1.0 and the resistance was recessive. The survival of the SS genotype in refuges was 1.0 and the additive resistance costs were 30 or 60%.

0.3, a 50% refuge and an additive resistance cost of 60% (table 1), the frequency of the R allele remained at a stable equilibrium, as predicted by the value of $W_R - W_S$ ($W_R = W_S = 0.455$).

With an initial R allele frequency of 0.001 and refuges of 10–50%, additive or dominant resistance costs of only 1–3% prevented or delayed increases in resistance, but recessive costs did not (table 2).

(b) *Non-recessive resistance*

As expected, a decline in resistance was less likely when resistance was not completely recessive, i.e. the fitness of the RS genotype on transgenics was 0.1 rather than 0.0 (table 3). Nevertheless, a decrease in the frequency of the R allele was still possible, particularly if the initial frequency of the R allele was low.

(c) *Incomplete resistance*

It is often assumed (as above, see table 1 and figure 1) that the fitness of the RR genotype on transgenics is 1.0. However, experiments with the pink bollworm have shown that the fitness of putative RR individuals is considerably lower on transgenics than on non-transgenics (Liu *et al.* 1999; Tabashnik *et al.* 2000). As expected when resistance was recessive and refuges were large (equation 3), a decreased fitness of the RR genotype on transgenics made reversal of resistance more likely (table 4).

(d) *Mode of population regulation*

Although density-independent population growth is a realistic assumption for fields where transgenic and non-transgenic plants are mixed, it is less appropriate when external refuges are managed independently of transgenic fields in order to maintain pest populations below some threshold. Such control measures in external refuges would eliminate many SS individuals and favour faster evolution of resistance. We therefore compared resistance evolution with and without density-dependent population growth in refuges for a range of carrying

Table 2. Number of generations required for resistance evolution with small resistance costs.

(The initial frequency of the R allele was 0.001. See table 1 for a definition of the parameters.)

cost (%)	dominance of cost	refuge (%)		
		10	30	50
0	—		118	145	> 150
1	recessive	118	145	> 150
1	additive	> 150	∞	∞
1	dominant	∞	∞	∞
3	recessive	118	146	> 150
3	additive	∞	∞	∞
3	dominant	∞	∞	∞

Table 3. Number of generations required for resistance evolution with non-recessive resistance.

(The dominance of resistance was 0.1. The survivals of the RR, RS and SS genotypes on transgenics were 1.0, 0.1 and 0.0, respectively. The resistance cost was 30%. Other parameters are as in table 1.)

initial R allele frequency	dominance of cost	refuge (%)		
		10	30	50
0.01	recessive	6	15	20
0.01	additive	6	16	∞
0.01	dominant	7	∞	∞
0.1	recessive	3	6	12
0.1	additive	3	6	24
0.1	dominant	3	8	∞

Table 4. Effect of incomplete resistance on the number of generations required for resistance evolution.

(The resistance was recessive, the cost of resistance was recessive and the initial frequency of the R allele was 0.1. The survival of SS individuals on non-transgenics was 1.0.)

cost (%)	survival of the RR genotype on transgenics	refuge (%)		
		10	30	50
30	0.1	19	∞	∞
30	0.2	9	64	∞
30	0.4	5	18	104
60	0.1	36	∞	∞
60	0.2	11	∞	∞
60	0.4	5	33	∞

capacities. Populations that surpassed the carrying capacity in refuges were set back to 10% of the carrying capacity by reducing the number of each genotype in refuges by an equal proportion. While the frequency of the R allele rapidly decreased with density-independent population growth, it initially decreased but sharply increased again under density-dependent growth in refuges as soon as the carrying capacity was reached in
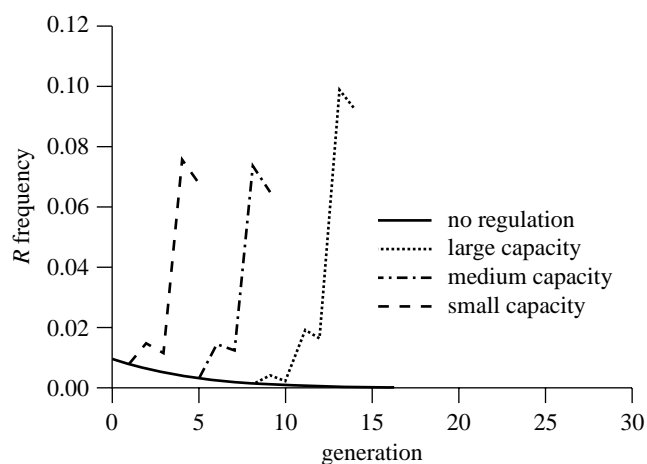
Figure 2. Effect of hard and soft selection on the evolution of resistance with a 50% refuge. Regulated populations that surpassed the carrying capacity were set back to 10% of the carrying capacity. The initial R allele frequency was 0.01 and the survivals of the RR, RS and SS genotypes were 1.0, 0.1 and 0.0 on transgenics and 0.7, 0.7 and 1.0 in refuges. The initial population size was 10^4 and the net reproductive rate (R_0) was 30. The carrying capacities in refuges and transgenic fields were $10^{12}/2$ (large), $10^9/2$ (medium) and $10^6/2$ (small).

refuges (figure 2). The jagged pattern of resistance evolution occurred because mortality of the predominantly SS individuals in refuges increased abruptly each time the carrying capacity was reached. Massive elimination of SS individuals in refuges was followed by a more rapid increase of the S than the R allele, until the carrying capacity was reached again in refuges. In transgenic fields, carrying capacities were not exceeded. In refuges, populations exceeded smaller carrying capacities sooner than larger ones. Thus, resistance evolved fastest with the smallest carrying capacities.

4. DISCUSSION

We found a range of conditions under which the frequency of the resistance allele did not increase, even when the initial frequency was much higher than 0.001. Assuming random mating and either completely recessive or nearly recessive inheritance of resistance, the factors favouring prevention or reversal of resistance were non-recessive costs of resistance, low initial R allele frequency, large refuges, incomplete resistance (RR genotype fitness on transgenics less than on non-transgenics) and density-independent population growth in refuges. As expected (Rausher 1993), evolution of resistance was more likely when density-dependent population growth occurred in refuges. This implies that management of refuges with control tactics that are not used on transgenics can accelerate resistance evolution.

To what extent do our modelling results help in explaining the decrease in the estimated frequency of resistance to CrylAc that occurred in Arizona field populations of the pink bollworm between 1997 and 1999 (Tabashnik *et al.* 2000)? Pink bollworm inheritance of resistance to Bt cotton is completely recessive and the survival of putative RR individuals on Bt cotton relative to non-Bt cotton is *ca.* 0.4 (Liu *et al.* 1999, 2001; Tabashnik

et al. 2000). Substantial survival costs on non-Bt cotton and overwintering costs are associated with resistance to Bt cotton (Carrière *et al.* 2001b; Y. Carrière, C. Ellers-Kirk, Y.-B. Liu, M. A. Sims, A. L. Patin, T. J. Dennehy and B. E. Tabashnik, unpublished data). Under the experimental conditions used, the costs were recessive in three cases and dominant in one. Thus, three factors examined with the models here (recessive inheritance, incomplete resistance and fitness costs) might have contributed to decreases in resistance allele frequency in the field.

If the frequency of resistance increased to relatively high levels by the end of the growing season in 1997 and subsequently declined, as the empirical estimates imply (Tabashnik *et al.* 2000), temporal variation in one or more key factors influencing resistance evolution must have occurred. Variation in statewide use of Bt cotton cannot explain the observed pattern (see Lenormand & Raymond 1998) because use of Bt cotton did not decrease from 1997 to 1999. Bt cotton accounted for less than 20% of cotton grown in Arizona in 1996, followed by *ca.* 50% in 1997 (Sims *et al.* 2001), 56% in 1998 and 51% in 1999 (Carrière *et al.* 2001a). We suspect that environmental changes, such as changes in the weather, altered the magnitude or the dominance of fitness costs or fitness of RR individuals on transgenics or some combination of these factors. In principle, change in the extent of density-dependent population regulation in refuges might have also contributed to the fluctuations in resistance frequency (figure 2). An alternative hypothesis is that, despite the stable or increasing statewide percentage of Bt cotton from 1997 to 1999, movement of susceptible moths into Bt cotton increased. In any case, a return to conditions prevailing before 1998 could promote increases in resistance. Along with recent empirical findings (Tabashnik *et al.* 2000), the present paper indicates that the evolution of resistance does not necessarily proceed monotonically despite strong selection.

In most cases studied so far, the inheritance of high levels of resistance to Bt toxins in field- and laboratory-selected populations has been recessive (Tabashnik *et al.* 1998; Frutos *et al.* 1999). Few studies have provided reliable information about the degree of dominance of resistance costs. The acethylcolinesterase allele Ace^R was found to induce a dominant survival cost in *Culex pipiens* (Chevillon *et al.* 1997), while a reduction in fecundity, survivorship and body weight due to resistance to cadmium was dominant in *Drosophila* (Shirley & Sibly 1999).

Expression of resistance costs depends on environmental conditions (Bergelson & Purrington 1996). The Rdl allele involved in dieldrin resistance conferred a survival cost that varied from being large and dominant to moderate and slightly additive in different seasons in *Lucilia cuprina* (McKenzie 1990). The cost of resistance to parasitism was absent at low density but increased under high competition in *Drosophila* (Kraaijeveld & Godfray 1997). Resistance to insecticides in *Myzus persicae* was increasingly costly with decreased temperature, increased rainfall or greater windspeed (Foster *et al.* 1996). Factors that modulate the expression of herbicide resistance costs include nutrient levels, population density, temperature, light intensity and management practices (Warwick 1991; Purrington & Bergelson 1997; Jordan 1999).

We identified the types of resistance that are least likely to evolve with a refuge–high-dose approach. It would be useful to assess experimentally the frequency of resistance mutations that are more likely to evolve when this strategy is used in order to evaluate the long-term potential of the refuge–high-dose approach. Such advantageous mutations would confer complete resistance to high doses of toxin, with non-recessive inheritance of resistance and fitness costs that are minimal, recessive or both. Resistance mutations that reduce mating between resistant and susceptible adults could also be favoured (Liu *et al.* 1999).

Four lines of evidence indicate that the spectrum of resistance mutations is constrained. First, some pests are unable to evolve resistance in isolated parts of their range even if they have done so elsewhere under similar selection intensity and time of exposure (Raymond & Marquine 1994). Second, the same mutation has spread in different species in response to exposure to the same toxicant (Guillemaud *et al.* 1998). Third, pests exposed worldwide to strong selection from pesticides only possess a small number of mutations that confer resistance (Chevillon *et al.* 1999). Finally, susceptible strains mutagenized and selected in the laboratory for resistance to three different insecticides yielded the same three mutations as those found in field populations (McKenzie & Batterham 1998). Therefore, the pool of mutations conferring resistance in particular species appears to be limited (ffrench-Constant *et al.* 1998). A high dose would further restrict such a pool because few mutations could generate effects sufficient to overcome the physiological challenge of the toxin.

When fitness costs are associated with resistance, the expected initial frequency of resistance alleles is low (Carrière *et al.* 1994). As demonstrated here, a refuge–high-dose approach can block the spread of resistance alleles with certain characteristics (recessive resistance, non-recessive fitness costs and incomplete resistance), particularly when their initial frequency is low or refuges are large. Such resistance alleles would rapidly decline to a new equilibrium between mutation and selection when transgenics are deployed (figure 1). Under these conditions, the selective advantage of modifier alleles that increase the magnitude or dominance of resistance or decrease the magnitude or dominance of the fitness costs would be very small (Otto & Bourguet 1999). Thus, an important question for resistance management is how frequently resistance mutations occur (see Bradshaw 1991) with characteristics that promote increases in their frequency when a refuge–high-dose approach is used.

APPENDIX A

(a) *General conditions for a decrease in resistance*

Resistance decreases when $W_S > W_R$. When r and s are the frequencies of the R and S alleles and W_{ij} is the fitness of the genotypes, the fitnesses of the R and S alleles are

$$W_R = rW_{RR} + sW_{RS} \tag{A1}$$

and

$$W_S = sW_{SS} + rW_{RS}. \tag{A2}$$

Assume no survival of the RS and SS individuals on transgenics. When tra $= 1 -$ ref and ref is the proportion of transgenic and refuge plants, the above inequality becomes

$$s(\text{ref}\,W_{SS/\text{ref}}) + r(\text{ref}\,W_{RS/\text{ref}})$$
$$> r((1-\text{ref})W_{RR/\text{tra}} + \text{ref}\,W_{RR/\text{ref}}) + s(\text{ref}\,W_{RS/\text{ref}})$$
$$- r((1-\text{ref})W_{RR/\text{tra}} + \text{ref}\,W_{RR/\text{ref}})$$
$$> (s - r)(\text{ref}\,W_{RS/\text{ref}}) - s(\text{ref}\,W_{SS/\text{ref}}). \tag{A3}$$

Because $W_{RR} = (1-\text{ref})W_{RR/\text{tra}} + \text{ref}\,W_{RR/\text{ref}}$, we obtain

$$-rW_{RR} > \text{ref}[(s-r)W_{RS/\text{ref}} - sW_{SS/\text{ref}}] \tag{A4}$$

or, equivalently,

$$rW_{RR} < \text{ref}[sW_{SS/\text{ref}} - (s-r)W_{RS/\text{ref}}]. \tag{A5}$$

(b) *Evolution of resistance with a recessive resistance cost*

With a recessive resistance cost, the equation above becomes

$$rW_{RR} < \text{ref}[sW_{SS} - (s-r)W_{SS}] \tag{A6}$$

and

$$rW_{RR} < \text{ref}[rW_{SS}]. \tag{A7}$$

Explicitly detailing fitness components of the genotypes, we obtain

$$r((1-\text{ref})W_{RR/\text{tra}} + \text{ref}\,W_{RR/\text{ref}}) < r\,\text{ref}^2 W_{SS/\text{ref}} \tag{A8}$$

or

$$(1-\text{ref})W_{RR/\text{tra}} < \text{ref}(\text{ref}\,W_{SS/\text{ref}} - W_{RR/\text{ref}}). \tag{A9}$$

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