Impact of Violated High-Dose Refuge Assumptions on Evolution of Bt-Resistance

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Abstract

Transgenic crops expressing *Bacillus thuringiensis* (*Bt*) toxins have been widely and successfully deployed for the control of target pests, while allowing a substantial reduction of insecticide use. The evolution of resistance (a heritable decrease in susceptibility to *Bt* toxins) can pose a threat to sustained control of target pests, but a high-dose refuge (HDR) management strategy has been key to delaying countervailing evolution of *Bt* resistance. The HDR strategy relies on the mating frequency between susceptible and resistant individuals, so either partial dominance of resistant alleles or non-random mating in the pest population itself could elevate the pace of resistance evolution. Using classic Wright-Fisher genetic models, we investigated the impact of deviations from standard refuge model assumptions on resistance evolution in the pest populations. We show that when *Bt* selection is strong, even deviations from random mating and/or strictly recessive resistance that are below the threshold of detection can yield dramatic increases in the pace of resistance evolution. Resistance evolution is hastened whenever the order of magnitude of model violations exceeds the initial frequency of resistant alleles. We also show that existence of a fitness cost for resistant individuals on the refuge crop cannot easily overcome the effect of violated HDR assumptions. We propose a parametrically explicit framework that enables both comparison of various field situations and model inference. Using this model, we propose novel empiric estimators of the pace of resistance evolution (and time to loss of control), whose simple calculation relies on the observed change in resistance allele frequency.

Keywords: insecticide resistance, refuge strategy, high-dose, partial dominance, non-random mating, fitness cost, incomplete resistance.

Introduction

Genetically modified crops, expressing insecticidal toxins of *Bacillus thuringiensis* (*Bt*), were first introduced in 1995, and have now been adopted worldwide; by 2010, they had been planted on ~ 66 Mha of agricultural crop land (James 2011). While *Bt*-expressing crops have met with considerable success, resistance can arise whenever a pest population develops a genetically-based decrease in susceptibility to the toxin (Tabashnik et al. 2009), which may lead in turn to drastic loss of *Bt* crop efficacy under field conditions (i.e., effective field resistance). While resistant mutations have been reported in many cases (Tabashnik et al. 2013), almost two decades after *Bt* crops were first deployed, clearly documented cases of effective field resistance have arisen in only four pests: *Busseola fusca* (South Africa, Van Rensburg 2007), *Spodoptera frugiperda* (Puerto Rico, Storer et al. 2010), *Pectinophora. gossypiella* (India, Dhurua & Gujar 2011), and *Diabrotica virgifera virgifera* (USA, Gassmann et al. 2011).

Much attention has been devoted to the pace of resistance evolution (Tabashnik et al. 2013), as well as to developing operational strategies that can delay (Alstad & Andow 1995) or eventually reverse it (Carrière et al. 2010). Among them, the high-dose / refuge (henceforth, HDR) strategy, resulting in a lowered selection pressure on susceptible individuals (Carrière et al. 2010), has generally been effective (Huang et al. 2011), particularly in the USA, where its proper implementation has seldom led to loss of control (Tabashnik et al; 2013). This strategy amounts to planting non-resistant cultivars within or surrounding *Bt*-crop plantings, allowing the survival of some susceptible individuals in a *Bt*-dominated environment. If susceptible alleles (S) in the pest are dominant and rare resistant mutants (R) are completely recessive, then rare resistant individuals (RR) emerging from *Bt* plants will mate preferentially with susceptible individuals (SS) emerging from refuge plants. Crosses between (RR) and (SS) parents yield (RS) progeny, so if the dose of *Bt* toxin

85 expressed is high enough to kill 100% of heterozygous (RS) larvae, the HDR strategy should
86 strongly delay evolution of pest resistance to *Bt* toxins. Recommended refuge fractions for *Bt*
87 crops have ranged from ~5% to 50% of crop acreage in the USA (Bates et al. 2005),
88 depending notably on whether or not they were also sprayed with insecticide.

89 Theory shows that optimal efficiency of the HDR strategy is guaranteed when: (*i*) the
90 genetic bases of resistance in natural populations and the dose of toxin expressed by the plant
91 result in functionally recessive expression in the pest; (*ii*) mating is random among pest
92 genotypes, with regard to *Bt*-resistance; and (*iii*) the frequency of resistant mutants is low.
93 The available data suggest that low background frequencies (q_0) of resistance alleles are
94 associated with sustained susceptibility to *Bt* toxin (Tabashnik et al. 2013), so most modeling
95 studies have explored cases where ($q_0 \leq 0.001$) (e.g., Tuytuynov et al. 2008).

96 Success of the HDR strategy depends on the dominance level of the resistance allele (1
97 $> h > 0$), with $h = 0$ corresponding to a recessive trait and $h = 1$ to a dominant trait (Wright
98 1934). It also depends on the rate of non-random mating for resistant genotypes ($F > 0$),
99 resulting in excesses of resistant homozygotes (RR), relative to panmictic expectation.
100 Success also depends on the background frequency of (or rate of mutation to) resistant alleles
101 ($q_0 > 0$), as well as to the proportion ($1 - \omega$) of the susceptible (refuge) crop that is planted.

102 The fraction of *Bt*-crop planted in the landscape (ω) is expected to scale with the
103 proportion of susceptible pest individuals killed by the toxin. A lack of refuge planting in
104 India and China has apparently allowed rapid evolution of *P. gossypiella* resistance to Cry1Ac
105 *Bt*-cotton (Tabashnik et al. 2013). Similarly, low compliance among South-African farmers in
106 planting the recommended fraction of refuge *Z. mays* crop might have hastened the evolution
107 of *Bt*-resistance in the stem borer (*B. fusca*) (Kruger et al. 2012).

108 A review of documented cases of field monitoring has shown that rapid evolution of
109 resistance occurs predominantly when the initial frequency of resistance allele (q_0) was above

110 the threshold of detectability (Tabashnik et al. 2013). It has also been shown, however, that
111 sustained susceptibility to *Bt* toxins can be achieved in the field, even when ($q_0 > 0.001$), as
112 long as coupled with a high fraction $(1 - \omega) > 40\%$ of refuge acreage (Tabashnik et al. 2013).
113 Either failure to achieve high-dose concentration of toxin in plant tissues and/or the
114 presence of partially dominant ($h > 0$) resistance alleles yields a surviving fraction of
115 heterozygous (RS) larvae on *Bt* plants, which compromises HDR success. Notwithstanding
116 the potential problems, recessive inheritance has been supported by numerous studies of
117 laboratory-selected as well field-evolved *Bt*-resistance (e.g., Ferré & Van Rie 2002,
118 Tabashnik et al. 2003). On the other hand, it is notoriously difficult to estimate dominance (h)
119 levels reliably, under field conditions (Moar et al. 2008, Tabashnik et al 2008), or even in the
120 laboratory, largely attributable to concentration-dependent effects of the toxin (Gould et al.
121 1995, Tabashnik et al. 2002). There have also been more striking cases, for which (strong)
122 partially dominant ($h > 0.5$) resistance has been observed, probably stemming from diverse
123 inheritance of and biochemical basis for resistance in a variety of different organisms (Zhang
124 et al. 2012, Campagne et al. 2013, Jin et al. 2013).
125 Likewise, any elevated tendency ($F > 0$) for resistant individuals (emerging from the
126 *Bt* crop) to mate with each other, rather than with susceptible individuals (emerging from the
127 the refuge crop), profoundly increases the frequency of resistant (RR) homozygotes among
128 the progeny [fr(RR progeny) = (q^2+Fpq)], compromising the efficacy of the HDR strategy.
129 Promoting mating between resistant and susceptible individuals depends on both, the spatial
130 structure of the *Bt* crop and refuge blocks, as well as individual post-emergence dispersal
131 patterns (Alstad & Andow, 1995). Many pest populations conform satisfactorily to Hardy-
132 Weinberg expectations for selectively neutral markers (Han & Caprio 2002, Endersby et al.
133 2007, Krumm et al. 2008, Kim et al. 2009), suggesting a mating regime close to random, but
134 whether that same condition obtains for genetic markers under strong and spatially structured

Bt selection remains unclear. In spite of extensive genetic mixing and low inbreeding levels in the moth *Ostrinia nubilalis* (Bourguet et al. 2000), Dalecky et al. (2006) have demonstrated that this species would be prone to positive assortative mating in *Bt*-crop context. Indeed, mating between resistant individuals originating from a single *Bt* planting could reach a few percent, as a consequence of limited pre-mating dispersal. The effects of the spatial structure of refuge plantings have been both contentious and extensive (Onstad et al. 2011). Some modeling studies have suggested that large block refuges could be more efficient in delaying resistance evolution than scattered refuges (Tyutyunov et al. 2008); others have suggested that seed blends (a spatial mixture of *Bt* and non-*Bt* plants in the field) could provide at least as much HDR durability as block refuges (Pan et al. 2011). In practice, we still know very little about the empiric rates of non-random mating under field conditions for most pests.

Other crucial factors that might delay the evolution of resistance have been assessed in different pest species (Gassmann et al. 2009), among them: incomplete resistance, fitness cost, and the dominance of the fitness cost. Incomplete resistance denotes situations where the fitness of resistant individuals on *Bt* plants (V_{RR}) is lower than the fitness of susceptible individuals on non-*Bt* plants (U_{SS}), i.e., when ($V_{RR} < U_{SS}$), which reduces the selective advantage of resistant individuals in mixed plantings of *Bt* and non-*Bt* plants (Carrière et al. 2006). Fitness cost arises when a resistance allele confers reduced fitness on homozygotes (RR) in environments that are toxin-free, so that ($U_{SS} - U_{RR} > 0$) (Tabashnik et al. 2014). Fitness cost may be inherited as a trait exhibiting a range of dominance levels ($0 \leq g = (U_{SS} - U_{RS}) / (U_{SS} - U_{RR}) \leq 1$) as shown in Table 1. Available data suggest that a recessive ($g \approx 0$) fitness cost of 25% ($U_{SS} = 1$, $U_{RR} = 0.75$, $U_{SS} - U_{RR} = 0.25$) might be a reasonable average (Gassmann et al. 2009). Management accounting for fitness cost may strengthen the effects of the HDR strategy in delaying the evolution of resistance (e.g., Higginson et al. 2005).

Failure of standard HDR assumptions (Huang et al. 2011; Tabashnik et al. 2013) has led to occasional resistance development (Tabashnik et al. 2014), and the matter needs further explication, both theoretically and empirically. Using Wright's (1942) classical genetic model, we here explore the sensitivity of resistance evolution to assumptions of strict randomness in mating and strictly recessive resistance alleles. This study is aimed at: (*i*) testing the robustness of the model when F and/or h might be slightly higher than 0; (*ii*) assessing the extent to which non-recessiveness and non-random mating may balance the effects of fitness cost ($U_{SS} - U_{RR} > 0$ and $g > 0$) and incomplete resistance ($U_{SS} - V_{RR} > 0$); (*iii*) evaluating whether violations of model assumptions impact the expected time elapsed before buildup of resistance in the pest threatens the efficacy of the *Bt*-crop itself.

Modeling Evolution of *Bt*-Resistance

Resistance is considered to involve a single locus, with a susceptible allele (S), of frequency p, and a resistance allele (R), of frequency q. The survival probability of the genotypes RR, RS and SS is denoted by (U_{RR}, U_{RS} and U_{SS}) on refuge-plants and (V_{RR}, V_{RS} and V_{SS}) on *Bt*-plants (Table 1). The proportion of *Bt*-crop in the landscape (ω) determines the relative fitness of the three genotypes; for modeling purposes the spatial distribution of *Bt* and non-*Bt* plants is considered continuous and random. The net relative fitness values of the three genotypes, emerging from a spatially randomized blend of *Bt* (ω) and refuge ($1 - \omega$) plants are:

$$W_{SS} = (1 - \omega)\cdot U_{SS} + \omega\cdot V_{SS}$$

$$W_{RS} = (1 - \omega)\cdot U_{RS} + \omega\cdot V_{RS} \qquad [1]$$

$$W_{RR} = (1 - \omega)\cdot U_{RR} + \omega\cdot V_{RR}$$

Any tendency for preferential mating (to type), whether due to genetically-programmed behavioral or spatially-imposed dispersal patterns (to or from the refuge crop), will result in

assortative mating ($F > 0$) among these new emergents. Given these genotype fitness values, the parental genotypic frequencies, the value of (F) (Table 1), we define the average relative allelic fitness values on the refuge crop as:

$$\tilde{U}_S = \left[\left(p + qF \right) \cdot U_{SS} + q \cdot \left(1 - F \right) \cdot U_{RS} \right]$$

$$\tilde{U}_R = \left[\left(q + pF \right) \cdot U_{RR} + p \cdot \left(1 - F \right) \cdot U_{RS} \right] , \qquad [2a]$$

and on the *Bt* crop as:

$$\tilde{V}_S = \left[\left(p + qF \right) \cdot V_{SS} + q \cdot \left(1 - F \right) \cdot V_{RS} \right]$$

$$\tilde{V}_R = \left[\left(q + pF \right) \cdot V_{RR} + p \cdot \left(1 - F \right) \cdot V_{RS} \right]. \qquad [2b]$$

At landscape level, we can then define (see Table 1 and Appendix A) weighted average allelic fitness values $\left(\tilde{W}_R \text{ and } \tilde{W}_S \right)$ for the collective population, which take the forms (Table 1):

$$\tilde{W}_S = \left[(1 - \omega) \cdot \tilde{U}_S + \omega \cdot \tilde{V}_S \right] \quad \text{and} \quad \tilde{W}_R = \left[(1 - \omega) \cdot \tilde{U}_R + \omega \cdot \tilde{V}_R \right] \qquad [3]$$

[Table 1 – about here]

Standard theory (Wright 1942) shows that the change in the frequency of the (R) allele over a single discrete generation depends on the average fitness of the advantageous allele over the population average:

$$\Delta q = (q' - q) = q \cdot \left[\frac{\tilde{W}_R}{\overline{\tilde{W}}} - 1 \right], \text{ where } \overline{\tilde{W}} = \left[q \cdot \tilde{W}_R + p \cdot \tilde{W}_S \right] \qquad [4]$$

It is more convenient here to define an equivalent form, using $y = q / (1 - q) = (q / p)$, so that [4] can be replaced with a more convenient analogue:

$$\Delta y = \left[\frac{\tilde{W}_R}{\tilde{W}_S} - 1 \right] \cdot y = \left[\frac{\omega \cdot \tilde{V}_R + (1 - \omega) \cdot \tilde{U}_R}{\omega \cdot \tilde{V}_S + (1 - \omega) \cdot \tilde{U}_S} - 1 \right] \cdot y = \tilde{\Lambda} \cdot y \qquad [5]$$

where $\tilde{\Lambda}$ accounts for all the parameters in the model, in its most general form (Table 1). In practice, q may either increase ($\Delta y > 0$, when $\tilde{W}_R > \tilde{W}_S$) or decrease ($\Delta y < 0$, when $\tilde{W}_R <$

209 $\tilde{W}_S$), while the sets of parameters for which $\Delta y = 0$ ($0 < q < 1$) delineate two alternative
210 trajectories of the resistance allele frequency. Eq [5] expresses a balance between the selective
211 advantage of susceptible individuals on refuge and that of resistant individuals on *Bt* crop,
212 balanced against the refuge crop fraction (1 – ω). Comparing the values of $\tilde{W}_R$ and $\tilde{W}_S$
213 amounts to comparing $(\tilde{U}_S - \tilde{U}_R)/(\tilde{V}_R - \tilde{V}_S)$ with $\omega/(1-\omega)$. If $(\tilde{U}_S - \tilde{U}_R)/(\tilde{V}_R - \tilde{V}_S) >$
214 $\omega/(1-\omega)$, the resistant allele (R) increases in frequency. Conversely, if
215 $(\tilde{U}_S - \tilde{U}_R)/(\tilde{V}_R - \tilde{V}_S) < \omega/(1-\omega)$, the resistant allele (R) decreases in frequency. In practice,
216 the fitness of (RR) individuals on refuge plants may be lower than that of (SS) on refuge
217 plants ($U_{SS} - U_{RR} \geq 0$), labeled a "fitness cost" (e.g., Gassmann et al. 2009, Tabashnik et al.
218 2014). Moreover, heterozygote (RS) fitness on the refuge crop may also show partial
219 dominance, yielding ($U_{SS} > U_{RS} > U_{RR}$) on the refuge crop, counterbalanced by ($V_{RR} > V_{RS} >$
220 V_{SS}) on the *Bt*-crop. Finally, we must also consider incomplete resistance, cases where ($U_{SS} >$
221 V_{RR}).

222 **Time to Loss of Containment (Passage Time)**

223 An adaptive resistance allele (R) will increase in frequency from very low to very
224 high, in classic sigmoidal fashion. A convenient criterion used to assess evolution of
225 resistance is the number of generations (henceforth "passage time") for which the frequency
226 of the resistant (R) allele is lower than some critical frequency in the population (say, q_k =
227 0.1), as in (Tyutyunov et al. 2008). If we denote the initial frequency of the resistant allele (R)
228 as q_0 and that of the "critical' value as q_k, then the passage time (T^k) for the allele frequency to
229 increase from ($q_0 \rightarrow q_k$) may be obtained by iteration of Eq. [5]. Discrete models do not yield
230 closed form solutions for (T^k), but continuous approximations provide relatively simple (and
231 parametrically explicit) approximations (see Felsenstein 2007). We constructed differential

232 equations based on the difference equations, $\delta y/\delta t = \Delta y$, and used their solutions to derive an
233 approximate formula for passage times (Appendix B) for each of several models.

234 In the general form of Eq [5], the increase of resistance allele frequency for a single
235 generation can be calculated, based on the difference between y' and y. To solve the
236 differential equation based on the difference equation [5], $\tilde{\Lambda}$ may be easily re-written as a
237 ratio of two linear functions of y, so the passage time (time to loss of containment) may be
238 calculated for the general form of the model (Appendix B). When y_0 is small, the expression
239 for passage time can be further simplified (Appendix B), and we achieve a relatively simple
240 approximation of T_{Λ}^{k} for the case where $V_{SS} = 0$ and $U_{SS} = 1$ (the reference fitness values).
241 Given initial (q_0) and critical (q_k) frequencies of the (R) allele, the approximate passage time
242 can be written (in terms of $y = q / p$), as (see Appendix B for the full expression):

243 $$T_{\Lambda}^{k} \approx \frac{1-\omega}{\omega \cdot \varepsilon \cdot V_{RR} - (1-\omega) \cdot \chi \cdot (1-U_{RR})} \cdot \ln\left(\frac{y_k}{y_0}\right), \qquad [6]$$

244 where $\varepsilon = (F + h - Fh)$ and $\chi = (F + g - F \cdot g)$ capture the essence of deviations from classic
245 HDR assumptions on *Bt* and refuge crops, respectively.

246 We observe that mild deviations from HDR assumptions (e.g., $\varepsilon = 0.05$) dramatically
247 shorten passage time, even when fitness cost and incomplete resistance are substantial (Figure
248 1). Eq. [6] suggests that the role of fitness cost, in terms of both $(1 - U_{RR})$ and (g), as well as
249 incomplete resistance, denoted by $(V_{RR} < U_{SS})$, may have an impact for large fractions of
250 refuge $(1 - \omega)$ (Figure 1). When most of the acreage is planted to the transgenic crop (ω is
251 elevated), however, substantial levels of fitness cost and incomplete resistance are required to
252 delay resistance evolution substantially. Transgenic crops will presumably be dominant in the
253 landscape, so the sensitivity of passage time to the deviation parameter $\varepsilon = (F + h - F \cdot h)$ is
254 greater than are the protective effects of fitness cost and incomplete resistance. By inflating
255 the frequency of homozygotes (RR), F reinforces the role of fitness cost in the second part of

256 the denominator of Eq. [6], so that the parameter h is expected to have a larger effect on T_{Λ}^{k}
257 than will F, whenever fitness cost and incomplete resistance are sizeable.

258 [Figure 1 – about here]

259 We further explored the extent to which deviations from the idealized HDR
260 assumptions ($\varepsilon = 0$) could be compensated for by increasing the fraction of refuge, fitness cost
261 and incomplete resistance. We can calculate the minimal fraction of refuge required to
262 achieve a passage time greater than a given number of generations, based on Eqq. [5] - [6]
263 (see also Appendix B). Insisting on a passage time of at least 40 generations, we assessed
264 refuge requirements, based on our generalized model (Figure 2). Refuge requirement
265 appeared to depend more on ε than on any other feature of the model. While the amount of
266 refuge ($1 - \omega$) required to ensure that ($T_{\Lambda}^{k} > 40$) generations was typically lower than the
267 minimal requirement of 5% (unsprayed) refuge recommended for the classic model ($\varepsilon = 0$), a
268 suitable refuge fraction was higher than 40% ($F = 0.05 = h$) when incomplete resistance and
269 fitness cost were moderate ($V_{RR} = 0.9$) and ($1 - U_{RR} = 0.1$), respectively (Figure 2). A low
270 refuge requirement ($1 - \omega < 0.10$) was only appropriate for fairly incomplete resistance and
271 high values of fitness cost, e.g., ($q_0 = 10^{-4}$, $g = 0.4$, $\varepsilon \leq 0.05$, $(1 - U_{RR}) > 0.4$, $V_{RR} < 0.65$). In
272 overview, a sustained efficacy of *Bt*-crops over a time horizon of 20 years appears attainable
273 for most multivoltine species, but only with large fractions of refuge.

274 [Figure 2 – about here]

275 Along the same lines, some robust strategies might even be needed to ensure that (Δy
276 ≤ 0). In this case, the minimum fraction of refuge preventing an increase of resistance allele
277 frequency, i.e., which guarantees $(\omega \cdot \varepsilon \cdot V_{RR}) < (1-\omega) \cdot \chi \cdot (1-U_{RR})$ when ($q_0 \rightarrow 0$), would
278 constitute an interesting benchmark (via Eq [5]):

279 $$(1-\Omega)=\lim_{q\to 0}(1-\omega)_{[\Delta y=0]}=\frac{\varepsilon\cdot V_{RR}}{\varepsilon\cdot V_{RR}+\chi\cdot(1-U_{RR})} \quad [7]$$

280 According to Eq. [7], $\Delta y < 0$ may be achieved only if $9\times(\varepsilon\cdot V_{RR})<\chi\cdot(1-U_{RR})$, for a refuge
281 fraction of 10%; or $4\times(\varepsilon\cdot V_{RR})<\chi\cdot(1-U_{RR})$, for a refuge fraction of 20%, which clearly
282 refers to cases where incomplete resistance (V_{RR} << 1), fitness cost (1 – URR >> 0) and the
283 dominance of this cost (g >> 0) are considerable. Given that fitness cost might average at (1 –
284 U_{RR}) ≈ 0.25, and might be a rather recessive trait (g < 0.25), a decrease of resistance allele
285 frequency might not be obtained for (1 – Ω) < 0.3, in most cases.

286

287 **Some simpler cases**

288 While the general model illustrates the sensitivity of the pace of resistance evolution to
289 even mild deviations from the ideal HDR assumptions, it is also useful to examine some
290 special cases that elucidate particular features of the general problem, all involving relative
291 fitness of SS pest genotypes (U_{SS} = 1) on refuge plants and (V_{SS} = 0) on *Bt* plants, where
292 resistance is complete (V_{RR} = 1) and where there is no fitness cost (U_{RR} = U_{RS} = U_{SS} = 1).

293 *Basic HDR Model (h = F = 0)* - In a strictly recessive model, the (RR) individuals are
294 resistant to *Bt* (V_{RR} = 1), but both RS and SS individuals are fully susceptible (V_{SS} = 0, and
295 V_{RS} = h = 0). Mating is assumed to be random, with respect to the genetic locus in question (F
296 = 0). The proportion of *Bt*-crop in the landscape (ω) alone determines the relative fitness of
297 the three genotypes. Under such conditions, $\tilde{\Lambda}$ in Eq. [5] simplifies to:

298 $$\tilde{\Lambda}=\tilde{A}=\left(\frac{q\cdot\omega}{1-\omega}\right) \quad . \quad [8]$$

299 The rate of resistance increase is determined by the ratio of (*Bt* / Refuge) crop fractions, [ω /
300 (1 – ω)]. If y is initially low, the inflation due to the ratio ($\tilde{A}$) is moderate if the refuge
301 fraction is above 10% (i.e., as long as (ω < 0.9). There is very slow increase in the frequency

of the (R) allele, at least until ($q^2 > 0.01$). We use [7] as the reference frame, against which to gauge the impact of violated HDR assumptions on the rate of resistance evolution.

Non-random mating (F > 0) and non-recessive (h > 0) models - Next, we consider both the case of non-recessive resistance and non-random mating, due to mating of relatives or to 'mating to type'. Non-random mating ($F > 0$) elevates the frequency of rare resistant homozygotes (RR), while $h > 0$ increases the fraction of heterozygotes (RS) surviving on *Bt* plants. Either non-recessive resistance or non-random mating results in a dramatic increase in the rate of increase by the (R) allele, and a model with both yields an even more elevated rate of increase (see Appendix A):

$$\Delta \mathrm{y}_{\varepsilon > 0} = \tilde{\Lambda} \cdot \mathrm{y} = \tilde{\mathrm{D}} \cdot \mathrm{y} \text{, where } \tilde{\mathrm{D}} \approx \left[1 + \left(\frac{p}{q} \right) \cdot \left(F + h - F \cdot h \right) \right] \cdot \tilde{\mathrm{A}} \quad , \qquad [9a]$$

with

$$\frac{\Delta y_{\varepsilon > 0}}{\Delta y_{\varepsilon = 0}} = \left(\frac{\tilde{\mathrm{D}}}{\tilde{\mathrm{A}}} \right) \approx \left[1 + \left(\frac{p}{q} \right) \cdot \left(F + h - F \cdot h \right) \right] >> 1 \ . \qquad [9b]$$

The rate of *Bt*-resistance evolution is profoundly elevated whenever either F and/or $h >> q$. If both assumptions fail, the effect on the pace of *Bt*-resistance evolution is almost additive. As a consequence, the passage time expressions obtained for these two cases present striking differences. Solving the differential equation, for the basic HDR ($\varepsilon = 0$) case, yields:

$$T_{\tilde{\mathrm{A}}}^{k} \equiv T_{\varepsilon=0}^{k} \approx \left(\frac{1-\omega}{\omega} \right) \cdot \left[\frac{1}{y_0} - \frac{1}{y_k} + \ln \left(\frac{y_k}{y_0} \right) \right] , \qquad [10]$$

Eq. [10] typically yields long passage times, provided that ($y_0 < 0.01$). By contrast, for cases where ($h > 0$) and/or ($F > 0$), the ($1/y_0$) term disappears from the passage time equation, and:

$$T_{\tilde{\mathrm{D}}}^{k} \equiv T_{\varepsilon>0}^{k} \approx \left(\frac{1-\omega}{\omega} \right) \cdot \frac{1}{\varepsilon} \cdot \left[\left(\varepsilon - 1 \right) \cdot \ln \left(\frac{\varepsilon + y_k}{\varepsilon + y_0} \right) + \ln \left(\frac{y_k}{y_0} \right) \right] + \alpha \ , \qquad [11a]$$

where

$$\alpha = \frac{\varepsilon^2 + \varepsilon - \mathrm{h}}{\varepsilon} \cdot \ln \left(\frac{\varepsilon + y_k}{\varepsilon + y_0} \right), \qquad [11b]$$

with ($\alpha < 0.10$), provided (ε and q_k) < (0.1), reducing [10a] , relative to [9].

The shortening of passage time $\left[T_{\tilde{D}}^{k} - T_{\tilde{A}}^{k}\right]$ depends primarily on the product of [(1 – ω) / ω] and $(p_0/q_0) = (y_0)^{-1}$, and is dramatic when ($\varepsilon > 0$). For any value of ε and any starting value of y_0, reducing the refuge fraction (1 – ω) shortens passage time. As an example, 5% refuge (1 – ω = 0.05) shortens the passage time by a factor of five, relative to the rate for 20% refuge (1 – ω = 0.20), everything else being equal. Similarly, for any given value of ω, the passage time is drastically reduced whenever ($\varepsilon >> y_0$). For example, the set of parameters ($F = h = 0 \rightarrow \varepsilon = 0$, ω = 0.8, $q_0 = 10^{-4}$, $q_k = 10^{-1}$) yields a passage time of $T_{\varepsilon=0}^{k} \approx 2,500$ generations, which decreases for ($F = h = 0.025 \rightarrow \varepsilon \approx 0.05$, ω = 0.8, $q_0 = 10^{-4}$, $q_k = 10^{-1}$) to a value of $T_{\varepsilon\approx 0.05}^{k} \approx 30$ generations (see also Figure 1A). In view of the fact that many pest species are multivoltine, empirical loss of containment can be anticipated within 10 years. Even low levels of dominance and/or non-random mating can compromise current HDR management protocols, even with high refuge fractions (1 – ω).

Determining Passage Time from the Evolutionary Trajectory

Based on the approximation of passage time for the generalized model Eq. [6], we note that the ratio $T_{\Lambda}^{k} / \ln\left(y_k / y_0\right)$ is a logarithmic mean; i.e., a constant that reflects the pace of resistance evolution. Many monitoring surveys of resistance evolution provided data on observed change in resistance allele frequency ($q_0 \rightarrow q_j$) over an observed time lapse of T^j generations. Consistent with Eq. [6], we can use T^{k*} and ξ^* as 1st approximations of passage time to q_k and the pace of resistance evolution, respectively:

$$\xi^* \approx \frac{\omega}{1-\omega} \cdot \varepsilon \cdot V_{RR} - \chi \cdot \left(1 - U_{RR}\right) = \frac{1}{T^j} \cdot \ln\left(\frac{y_j}{y_0}\right), \qquad [12a]$$

equivalently

$$T^{k}* \approx \frac{1}{\xi *} \cdot \ln\left(\frac{y_k}{y_0}\right) \quad [12b]$$

Because it relies on an approximation (Eq. [6]) of the general model, T^{k*} is an upper-bound estimate of passage time ($T^{k*} > T^{k}_{\Lambda}$), but for ($T^{k}_{\Lambda} < 100$), it is a suitable estimate (see Appendix B); i.e., ($T^{k}_{\Lambda}* \approx T^{k}_{\Lambda}$). The inverted logarithmic mean $\xi *$ defines the pace of resistance evolution; the higher the evolutionary rate, the shorter the passage time. The utility of such empirical estimates is that, while clearly related to Eqq. [5] - [6], their calculation does not require detailed knowledge of the system, seldom understood well enough together to translate into precise values of (ω, U_{RR}, V_{RR}, g, h and F).

Using published data reporting resistance evolution (Table 2), this exercise suggests a passage time (to $q_k = 0.1$) of about 10-15 years in the four cases for which suitable time-lapse data were available (i.e., $q_j < 0.1$, Table 2). These cases are acknowledged as situations where resistant mutations arose but for which control failure had not (yet) been observed (Tabashnik et al. 2013). In spite of some noticeable differences in terms of survey data, similar values of $\xi *$ have been observed in *H. armigera* in China and Australia, suggesting that this same approach may work reasonably well for similar examples of resistance evolution (Table 2).

[Table 2 – about here]

Discussion

While iterative genetic simulations of resistance evolution have been used to compare theoretical expectations and empirical data (e.g., Tabashnik et al. 2008, Jin et al. 2015), we have here defined parametrically explicit predictions of the rate of evolution. We embedded our analyses in a general model, which should be useful for modeling a variety of single gene responses to selection in diploid pest organisms. Our approach is complementary to complex simulation set up, notably demo-genetic and spatially explicit models, which may include additional levels of realism as well as a larger number of parameters. Our model reveals

373 contrasting outcomes that reflect the stringency of the HDR assumptions. Indeed, in the
374 simpler cases, the structure of passage time equations differ drastically, depending on whether
375 ε is assumed to be strictly '0' or not (see Eqq. [10] [11a]).

376 The equations reveal the parameters of primary importance in the generalized model to
377 be (q_0, ω, ε and χ). By lowering the selective pressure on pest populations, the refuge strategy
378 has been widely successful in delaying the evolution of *Bt*-resistance in some major pest
379 species since *Bt* crops were first deployed, 15 years ago (Huang et al 2011). Notwithstanding
380 that success, Tabashnik et al. (2013) have reported the field evolved resistance in 13 of 24
381 examined cases. Equations derived from a Wright-Fisher model show that passage time
382 depends primarily on the (refuge / *Bt*-crop) ratio (1 – ω) / ω, but also on the counter-balance
383 between the benefits (ε) of resistant (R) alleles on *Bt* crops and those of susceptible (S) alleles
384 on refuge crops (χ), highlighted in Eq.[12a]. The utility of incorporating these countervailing
385 adaptive payoffs in particular designs of the refuge strategy has been addressed by a number
386 of studies (see Gassmann et al. 2009, Tabashnik et al. 2009), but wherever crops expressing
387 insecticidal toxins dominate the landscape, the generalized version of the model is much more
388 sensitive to (ε) than to (χ). Indeed, the effects of a recessive fitness cost of 25% ($U_{SS} - U_{RR}$ =
389 1 – 0.25), which might be a reasonable average across species (Gassmannn et al. 2009),
390 appear limited whenever (ε > 0.01) and (ω > 0.7). Given the sensitivity of the model to low
391 values of ε, the question arises of how small deviations from classic HDR assumptions (ε) can
392 be empirically detected, especially with respect to that of recessive resistance and random
393 mating.

394 **Partial Dominance**

395 Foremost, the difficulty of accurately estimating degrees of partial dominance under
396 field conditions has been emphasized (Moar et al. 2008, Tabashnik et al; 2008). Although
397 laboratory bioassays are indisputably useful for monitoring resistance evolution, the extent to

which the dominance index (*h*), estimated under laboratory conditions, is an accurate indicator of field dominance is unclear (Bourguet et al. 1996). Indeed, both larval susceptibility to *Bt* toxin and the dominance level of any resistance are typically dosage-dependent (Gould et al. 1995, Tabashnik et al. 2002). It follows that an estimate of dominance is highly context-specific and its accuracy might be well below the standards that reliable predictions would require.

Assessing the partial dominance of R-alleles at early stages of resistance evolution remains a challenge, since such alleles are rare, of potentially different mutational origins, and may catalyze divergent biological functions (Zhang et al. 2012, Jin et al. 2013). In addition, seasonal variation in toxin concentration within plant tissues may translate into temporal variation in functional dominance (Carrière et al. 2010). In a recent review study (Tabashnik 2013), none of the 10 cases for which resistance had evolved to the point where more than 1% of individuals had become resistant could be considered "high-dose". In addition, there have been a few published cases of newly emerging resistance alleles showing partial dominance under field conditions (Campagne et al. 2013, Jin et al. 2013). We may yet discover that *Bt* strategies based on a strictly recessive resistance assumption are overly vulnerable to the range of empirical evolutionary responses under field conditions.

Non-Random Mating

Secondly, the amount of non-random mating entrained by refuge structure and individual pre-mating movement is not well understood. Generally, estimates of the randomness of mating often lack statistical power. The limited resolution of the genetic markers that have been routinely deployed in pest species (allozymes, AFLPs) or the frequent occurrence of null alleles in co-dominant genetic markers (microsatellites), have constrained our ability to detect small deviations from panmictic population structure, especially in Lepidopteran pests (Zhang 2004). For many population genetic studies of moth pests, the

analytical power has been sufficient to detect only substantial deviations (F-values > 0.1) from Hardy-Weinberg frequencies (e.g., Bourguet et al. 2000, Han & Caprio 2002, Endersby et al. 2007, Kim et al. 2009). As a consequence, low levels of local non-random mating, crucial for HDR strategy, could not really be detected in pest species. We have here assumed an unstructured refuge / *Bt*-crop distribution and therefore dealt with effective fractions of refuge and *Bt*-crop. The extent to which planted refuge within a field and landscape refuge (non-*Bt* farms) translate into comparable fractions of effective refuge is a pest-specific question that will need further clarification, particularly in terms of empirical data on actual pest species dispersal dynamics. As highlighted by Bourguet et al. (2000), high levels of gene flow within and among populations do not necessarily translate into a random mating pattern, either in general or with regard to genotypes at *Bt*-relevant loci. It is noteworthy that assortative mating regimes may only be evident for loci closely linked to the chromosomal segments containing loci under selection for resistance. In the European Corn Borer (*Ostrinia nubilalis*), although no significant departure from Hardy-Weinberg equilibrium was initially identified (Bourguet et al. 2000), mating was found to take place at restricted spatial scales (within 50 m), effectively translating into an assortative mating rate of perhaps 5% (Dalecky et al. 2006, Bailey et al. 2007). Low pre-mating movement is expected to increase the rate of assortative mating ($F > 0$) between individuals originating from the same block of *Bt* crop and has been suggested in few moths (see Cuong & Cohen 2003; Qureshi et al. 2006). In addition, some *Bt*-resistant pest strains evince slower larval development than *Bt*-susceptible con-specifics, potentially leading to emergence-asynchrony of resistant and susceptible genotypes (c.f., Gryspeirt & Grégoire 2012), which could increase assortative mating (in general) but also an elevated rate of mating with resistant siblings. We clearly need better information on pest ecology, and in particular, information on dispersal behavior, with respect to the various contexts within which transgenic crops are grown.

Pace of Resistance Evolution and Passage Time

Both the pace of resistance evolution and the passage time can be described by simple combinations of model parameters. On the one hand, the expected rates of resistance evolution can be obtained by evaluating the (V_{RR}, U_{RR}, ε, χ and ω) parameters. when estimates of those parameters are attainable. On the other hand, the observed rates of evolution can be obtained by observing allele frequency changes under field conditions. That duality provides us with a simple framework for explicitly connecting empirical data and theory. While Δq, as a measure of resistance evolution is completely dependent on the allele frequency q at any particular point on the trajectory the rate ξ^* offers a standardized measure of the general pace of resistance evolution, even when precise estimates of (ω, U_{RR}, V_{RR}, g, h and F) are not available, provided that ($q < 0.1$) is below the 'loss of containment' threshold.

For the sake of illustration, we consider the case of *B. fusca* resistance in South Africa, for which no fitness cost has been observed (Kruger et al. 2014), and for which resistance seems complete and inherited as a dominant trait (Campagne et al. 2013). Moreover, the planted fraction of *Bt* maize averaged ($\omega < 0.30$) from 1998 to 2004 (~14 generations) in the area where this resistance evolved (Tabashnik et al. 2009). Assuming that initial allele frequency was low (i.e., $0.0001 < q_0 < 0.001$), the expected pace of resistance would then be $[\omega \cdot \varepsilon \cdot V_{RR}/(1 - \omega)] \approx [0.3 \cdot 1 \cdot 1/(1 - 0.3)] \approx 0.43$ (i.e., a passage time of ~11 to 16 generations, depending on q_0), roughly compatible with an empiric estimate of $\xi^* \approx 0.34$ (i.e., ~14 generations), based on the rate of change in resistance frequency (Table 2).

Implications for Resistance Management and Monitoring

The main option for delaying resistance evolution is to manipulate the fraction of refuge crop, either its proportion (1 – ω), lowering selection pressure, or its spatial organization, reducing the impact of limited dispersal on $F > 0$. In a context where resistance evolution is not expected to follow the trajectory of a "strictly recessive" allele (i.e., when

473 $\varepsilon > 0$), and where the estimation of some important parameters might not be achievable,
474 robust resistance management might have to involve substantial refuge fractions. Vacher et al.
475 (2003) suggested refuge fractions of ~25% to minimize pest density while efficiently delaying
476 resistance evolution. Similarly, our results show that ($1 - \omega < 0.20$) are not likely to result in
477 an effective expression of the fitness cost to increase passage time. Some strategies might
478 even be needed to ensure that ($\Delta y \leq 0$). In this case, the minimum fraction of refuge
479 preventing an increase of resistance allele frequency is expected to be ($1 - \Omega$) > 30%.
480 Unsprayed refuge requirements as low as (5%) of the total planted with *Bt* crops (Bates et al.
481 2005) do not appear to be sufficient with respect to the statements above (see also Vacher et
482 al. 2003). By contrast, in the Southern states of the USA, where cotton is grown, the decision
483 was taken to establish more generous refuge fractions, $(1 - \omega) \approx \omega \approx 0.5$, in areas were other
484 *Bt* crops were deployed.

485 The notion of high dose toxin, in the context of *Bt* crops, relies on a purely empirical
486 criterion, a dose that kills 99.99% of susceptible individuals in the field "to assure that 95% of
487 heterozygotes would probably be killed" (USEPA 1998, see also Gould et al. 1998), which
488 translates as ($\varepsilon > h > 0.05$). In this respect, our model results provides rationale to expect rapid
489 evolution of resistance (for $h = 0.05$), typically requiring a high refuge fraction ($1 - \omega > 0.25$)
490 to achieve a passage time of $T^k \approx 40$ generations (with $q_0 = 0.001$, $q_k = 0.1$, $F = 0$, $V_{RR} = 1$,
491 $U_{RR} = 0.75$, $g = 0.1$).

492 The model suggests that the definition of “high-dose” should depend explicitly on (the
493 unknown) q_0 (see Eq. [9b] and Appendix A), since the variation in frequency of the resistance
494 allele is inflated by a factor $[1 + (p/q)\ \varepsilon) \approx (1 + \varepsilon/q)$ whenever the system deviates from
495 idealized HDR behavior. Assuming the parameters just above, if we set our “dosage
496 requirement” high enough to ensure that $\varepsilon = (F + h - F \cdot h) < q_0$, which would reduce
497 ($\widetilde{\Lambda} \to 2 \cdot \widetilde{\Lambda}$) at most; we need a dose that kills a fraction p_0 of RS heterozygotes. Our model

(with the same parameters as above) shows that we can attain a passage time of $T^k \approx 40$ with a refuge fraction of only $(1 - \omega) = 5\%$, but only if we can assure that ($\varepsilon < 0.007$). Our findings suggest that, even with random mating, the current "high-dose" requirement is inadequate for low refuge fractions. The "dose" or the refuge fraction $(1 - \omega)$ need to be increased.

An efficient insect resistance management strategy must be based on robust assumptions that ensure sustained toxicity of *Bt* crops under a variety of circumstances. Notably, insect survival on transgenic crops expressing at least two *Bt* toxins, appeared to be higher than previously anticipated (Carrière et al. 2015). In this context, both, breeding programs and modeling studies may benefit from explicitly integrating other deviations from idealized situations in order to minimize the gap between theoretical expectations and empirical trends observed in the field. Better predictive models of resistance evolution may be a key for both designing sustainable strategies and anticipating eventual failures.

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Competing interests

The authors declare no competing interests.

Data Accessibility

Online Appendix A

Online Appendix B

Author's Contributions

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Figure Captions

Figure 1: Passage time T_{Λ}^{k} (generations) from $q_0 = 10^{-4}$ to $q_k = 10^{-1}$, the critical allele frequency of the resistance allele (R), under two different scenarios. (A) Effects of deviations from the assumption $\varepsilon = 0$ ($F = 0 = h$) on passage time T_{Λ}^{k} with varied *Bt*-crop fraction: $0.6 \leq \omega \leq 0.95$. The parameters of the model were set as follow: $V_{RR} = 0.75$, $U_{RR} = 0.75$, $U_{SS} = 1$, $g = 0.05$. (B) Combined effects of F and g ($\chi = F + g - Fg$) on passage time T_{Λ}^{k}. Parameters of the model: $V_{RR} = 0.75$, $U_{RR} = 0.5$, $U_{SS} = 1$, $F = h = 0.025$ ($\varepsilon \approx 0.05$) and $0 < g < 0.375$ ($0 < \chi < 0.4$).

Figure 2: Additional proportion of refuge required ($1 - \omega$) to keep passage time T_{Λ}^{k} above 40 generations (blue slices) when the model deviates from strict recessivity and strict random-mating ($\varepsilon = 0$, Eq. [10] red slices). Two scenarios were envisaged: (A) $q_0 = 10^{-3}$ and $g = 0.1$, and (B) $q_0 = 10^{-4}$ and $g = 0.4$. Additional refuge fractions, when deviation increases, were calculated based on Eqq [5][6]: light blue slices represent $\varepsilon \approx 0.02$ ($F = 0.01 = h$); $\varepsilon \approx 0.05$ ($F = 0.025 = h$), middle blue; $\varepsilon \approx 0.10$ ($F = 0.05 = h$), darker blue. Various combinations of parameters were used for incomplete resistance ($0.4 \leq V_{RR} \leq 0.9$) and fitness cost ($0.1 \leq (1 - U_{RR}) \leq 0.7$) with $U_{SS} = 1$.

Table 1: Summary of allelic fitness values, under the different parametric assumptions of the model

Model Parameters	SS	RS	RR	Planting Fraction
Frequencies	$p^2 + pqF$	$2pq(1 - F)$	$q^2 + pqF$	
Bt-Fitness	$V_{SS} < V_{RR}$	$V_{RS} = V_{SS} + h(V_{RR} - V_{SS})$	V_{RR}	ω
Refuge Fitness	U_{SS}	$U_{RS} = U_{RR} + g(U_{SS} - U_{RR})$	$U_{SS} > U_{RR}$	$(1 - \omega)$

Average Allelic Fitness Values – *Bt* Crop

$$\tilde{V}_S = \left[\left(p + qF \right) \cdot V_{SS} + q \cdot \left(1 - F \right) \cdot V_{RS} \right] \qquad \tilde{V}_R = \left[\left(q + pF \right) \cdot V_{RR} + p \cdot \left(1 - F \right) \cdot V_{RS} \right]$$

Average Allelic Fitness Values – Refuge Crop

$$\tilde{U}_S = \left[\left(p + qF \right) \cdot U_{SS} + q \cdot \left(1 - F \right) \cdot U_{RS} \right] \qquad \tilde{U}_R = \left[\left(q + pF \right) \cdot U_{RR} + p \cdot \left(1 - F \right) \cdot U_{RS} \right]$$

Weighted Average Allelic Fitness Values – Both Crops

$$\tilde{W}_S = \left[\left(1 - \omega \right) \cdot \tilde{U}_S + \omega \cdot \tilde{V}_S \right] \qquad \tilde{W}_R = \left[\left(1 - \omega \right) \cdot \tilde{U}_R + \omega \cdot \tilde{V}_R \right]$$

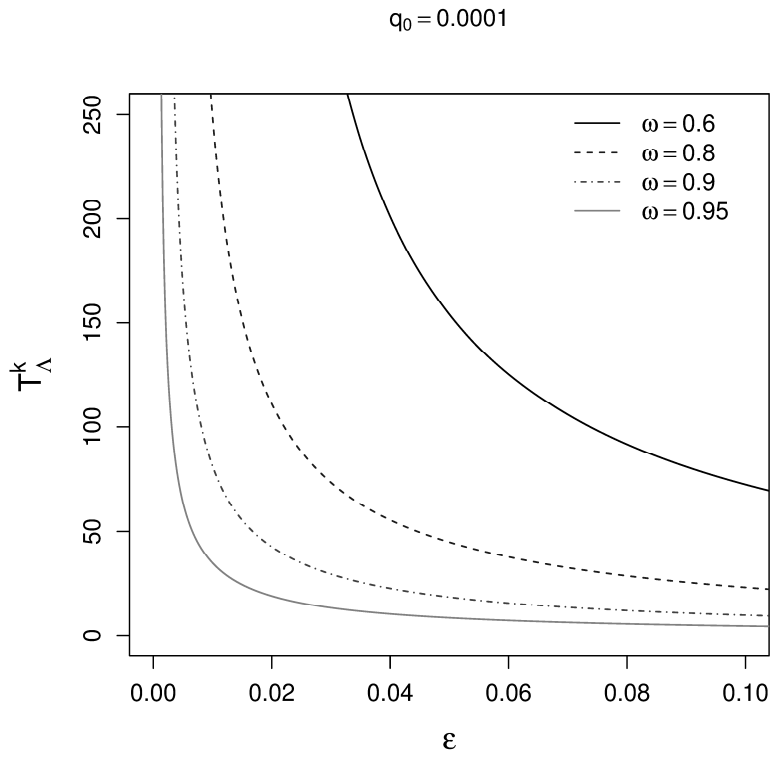
Table 2: Empirical estimates of pace of resistance evolution ξ^* and passage time T^{k*} (number of generations) from q_0 to $q_k = 0.1$, using survey data: q_0, the initial frequency of resistance alleles and qj, the allele frequency measured T^j generations later. Are considered, 11 cases for which field-evolved resistance or field resistance has been reported (see Tabashnik et al. 2013).

Case Summary					Survey Data			Projections		
Pest Species	*Bt* Crop	Toxin	Country	Gener / Year	q_0	q_j	T^j	ξ^*	T^{k*} Gener	Passage Time (yrs)
Busseola fusca	Corn	Cry1Ab	South Africa	2	*a*	> 0.1	< 16	> 0.336	*NA*	*NA*
Diatraea saccharlis	Corn	Cry1Ab	USA	4-5	0.0023	0.018	27	0.076	50.5	11.2
Helicoverpa armigera	Cotton	Cry1Ac	China	3-5	0.0058	0.075	36	0.069	40.5	10.5
Helicoverpa armigera	Cotton	Cry2Ab	Australia	3-5	0.0033	0.021	28	0.066	52.5	13.1
Helicoverpa punctigera	Cotton	Cry2Ab	Australia	3-5	0.0010	0.0091	28	0.093	54.5	13.6
Helicoverpa zea	Cotton	Cry1Ac	USA	3	0.0008	> 0.1	< 18	> 0.273	*NA*	*NA*
Helicoverpa zea	Cotton	Cry2Ab	USA	3	0.0004	> 0.1	< 12	> 0.471	*NA*	*NA*
Ostrina furnacalis	Corn	Cry1Ab	Phillipines	6	*a*	> 0.1	36	> 0.130	*NA*	*NA*
Pectinophora gossypiella	Cotton	Cry1Ac	China	3	*a*	> 0.1	39	> 0.120	*NA*	*NA*
Pectinophora gossypiella	Cotton	Cry1Ac	India	4-6	*a*	> 0.1	< 30	> 0.156	*NA*	*NA*
Spodoptera frugiperda	Corn	Cry1F	USA	10	*a*	> 0.1	< 30	> 0.156	*NA*	*NA*

a, no empirical estimate of q_0 is available; in such cases, $q_0 < 0.001$ was assumed to provide an estimate of ξ^*

NA, cases for which $q > 0.1$ occurred within T^j, no projections of passage time were performed.

A)


$q_0 = 0.0001$
$\omega = 0.6$
$\omega = 0.8$
$\omega = 0.9$
$\omega = 0.95$
T^k_Λ
0
50
100
150
200
250
0.00
0.02
0.04
0.06
0.08
0.10
ε

B)

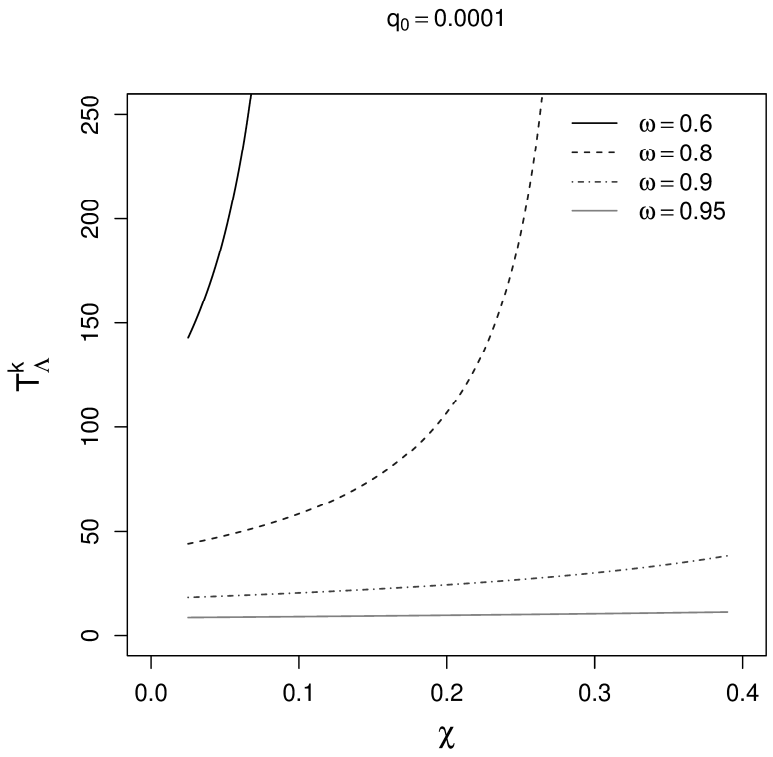

$q_0 = 0.0001$
$\omega = 0.6$
$\omega = 0.8$
$\omega = 0.9$
$\omega = 0.95$
T^k_Λ
0
50
100
150
200
250
0.0
0.1
0.2
0.3
0.4
χ

Figure 1:

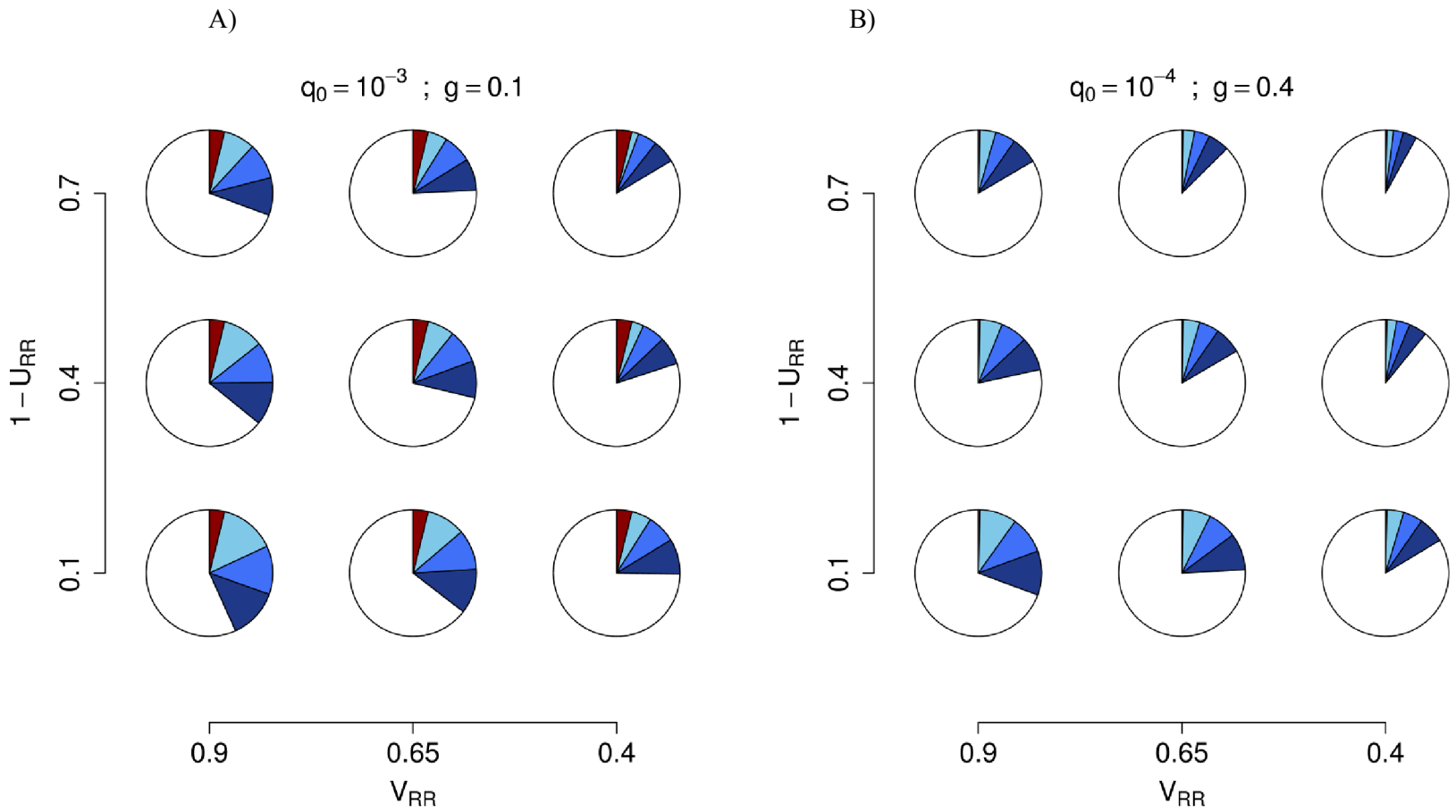


Figure 2

Supplementary Appendix A

Here we derive four different models, corresponding to cases where $F = 0$ or > 0 and/or where $h = 0$ or > 0, but holding homozygote fitness values to either 0 or 1 (see Tables below).

Case 1: ($F = 0 = h$) **Case 2: ($F > 0 = h$)**

Case 3: ($F = 0 < h$) **Case 4: ($F > 0 < h$)**

Case 1: ($F = 0 = h$)

Model Parameters	SS	RS	RR	Planting Fraction
Frequencies	p^2	$2pq$	q^2	
Bt-Fitness	$V_{SS} = 0$	$V_{RS} = 0$	$V_{RR} = 1$	ω
Refuge Fitness	$U_{SS} = 1$	$U_{RS} = 1$	$U_{RR} = 1$	$(1 - \omega)$

Weighted Average Allelic Fitness Values

$$\tilde{V}_S = p\left[p \cdot V_{SS} + q \cdot V_{RS}\right] = 0 \qquad \tilde{V}_R = q \cdot \left[q \cdot V_{RR} + p \cdot V_{RS}\right] = q^2$$

$$\tilde{U}_S = p \cdot \left[p \cdot U_{SS} + q \cdot U_{RS}\right] = p \qquad \tilde{U}_R = q \cdot \left[q \cdot U_{RR} + p \cdot U_{RS}\right] = q$$

$$\tilde{W}_S = \omega \cdot \tilde{V}_S + (1-\omega) \cdot \tilde{U}_S = (1-\omega) \cdot p \qquad \tilde{W}_R = \omega \cdot \tilde{V}_R + (1-\omega) \cdot \tilde{U}_R = \omega \cdot q^2 - (1-\omega) \cdot q$$

Defining $\overline{W} = (\tilde{W}_S + \tilde{W}_R)$, we start with $p' = \tilde{W}_S / \overline{W}$ and $q' = \tilde{W}_R / \overline{W}$. Using the equivalent form $y = (q / p)$, we define $y' = (p'/q') = \tilde{W}_R / \tilde{W}_S$, which leads to:

$$y' = \left[\frac{(1-\omega) + q \cdot \omega}{(1-\omega)} \cdot \frac{q}{p}\right] = \frac{(1-\omega) + q \cdot w}{(1-\omega)} \cdot y = \left[\frac{q \cdot \omega}{(1-\omega)} + 1\right] = \left[\tilde{A} + 1\right] \cdot y \qquad [A1]$$

and thus

$$\Delta y = (y' - y) = \tilde{A} = \left[\frac{q \cdot \omega}{(1-\omega)}\right] \quad . \qquad [A2]$$

The pace of resistance evolution *increases* with the fraction of acreage planted to the *Bt*-crop.

Case 2: ($F > 0 = h$)

Model Parameters	SS	RS	RR	Planting Fraction
Frequencies	$p^2 + pqF$	$2pq(1 - F)$	$q^2 + pqF$	
Bt-Fitness	$V_{SS} = 0$	$V_{RS} = 0$	$V_{RR} = 1$	ω
Refuge Fitness	$U_{SS} = 1$	$U_{RS} = 1$	$U_{RR} = 1$	$(1 - \omega)$

Weighted Average Allelic Fitness Values

$$\tilde{V}_S = 0 \qquad \tilde{V}_R = q \cdot (q + pF)$$

$$\tilde{U}_S = p \cdot \left[(p + qF) + q \cdot (1 - F) \right] = p \qquad \tilde{U}_R = q \cdot \left[(q + pF) + p(1 - F) \right] = q$$

$$\tilde{W}_S = (1 - \omega) \cdot p \qquad \tilde{W}_R = q \cdot \left[(1 - \omega) + (q + pF) \cdot \omega \right]$$

That leads us instead to:

$$y' = \left[\frac{q}{p} \cdot \frac{(1 - \omega) + (q + pF) \cdot q\omega}{(1 - \omega)} \right] = \left[\frac{q^2 \cdot \omega}{(1 - \omega)} \cdot \left(1 + \frac{q}{p} \cdot F \right) + 1 \right] \cdot y = \left[\tilde{B} + 1 \right] \cdot y \qquad [A3]$$

It is then possible to define $\Delta y = (y' - y)$, and express it as an explicit function of $\tilde{B}$:

$$\Delta y = \tilde{B}, \quad \text{where} \quad \tilde{B} = \left[1 + \left(\frac{p}{q} \right) \cdot F \right] \cdot \tilde{A}, \qquad [A4]$$

which also yields:

$$\frac{\Delta y_{F>0}}{\Delta y_{F=0}} = \frac{\tilde{B}}{\tilde{A}} = \left[1 + \left(\frac{p}{q} \right) \cdot F \right] >> 1 \quad . \qquad [A5]$$

The frequency of R mutant is small at the outset, so ($q_0 << p_0$), and y_0 is very large. Even small values of ($F > 0$) exhibit profoundly elevated rates of resistance evolution, relative to **Case I**.

Case 3: ($F = 0 < h$)

Model Parameters	SS	RS	RR	Planting Fraction
Frequencies	p^2	$2pq$	q^2	
Bt-Fitness	$V_{SS} = 0$	$V_{RS} = h$	1	ω
Refuge Fitness	$U_{SS} = 1$	$U_{RS} = 1$	$U_{RR} = 1$	$(1 - \omega)$

Weighted Average Allelic Fitness Values

$$\tilde{V}_S = p \cdot \left[p \cdot V_{SS} + q \cdot V_{RS} \right] = p \cdot q \cdot h \qquad \tilde{V}_R = q \cdot \left[q \cdot V_{RR} + p \cdot V_{RS} \right] = q \cdot \left[q + p \cdot h \right]$$

$$\tilde{U}_S = p \cdot \left[p + q \cdot U_{RS} \right] = p \qquad \tilde{U}_R = q \cdot \left[q \cdot U_{RR} + p \cdot U_{RS} \right] = q$$

$$\tilde{W}_S = p \cdot \left[(1-\omega) + q \cdot h \cdot \omega \right] \qquad \tilde{W}_R = q \cdot \left[(1-\omega) + (q + p \cdot h) \cdot \omega \right]$$

The model leads to:

$$y' = \left(\frac{\tilde{W}_R}{\tilde{W}_S} \right) = \left[\frac{(1-\omega) + (q + ph) \cdot \omega}{(1-\omega) + qh\omega} \right] \cdot y \ . \qquad [A6]$$

Factoring by q and $(1 - \omega)$, and simplifying, we obtain:

$$y' = \frac{\left[1 + \left(1 + \frac{p}{q} \cdot h \right) \cdot \tilde{A} \right]}{1 + h \cdot \tilde{A}} \cdot y \approx \frac{\left[1 + \tilde{C} \right]}{1 + h \cdot \tilde{A}} \cdot y \ , \qquad [A7]$$

but for q and h both small, $(1 + h \cdot \tilde{A}) \approx 1$, which leads us to:

$$\Delta y \approx \tilde{C} \ \text{, where } \tilde{C} = \left[1 + \frac{p}{q} \cdot h \right] \cdot \tilde{A} \ , \qquad [A8]$$

and the rate of resistance evolution is again vastly elevated, relative to the HDR model, since

$$\frac{\Delta y_{h>0}}{\Delta y_{h=0}} = \frac{\tilde{C}}{\tilde{A}} \approx \left[1 + \frac{p}{q} \cdot h \right] >> 1 \ . \qquad [A9]$$

Case 4: ($F > 0 < h$)

Model Parameters	SS	RS	RR	Planting Fraction
Frequencies	$p^2 + pqF$	$2pq(1 - F)$	$q^2 + pqF$	
Bt-Fitness	$V_{SS} = 0$	$V_{RS} = h$	$V_{RR} = 1$	ω
Refuge Fitness	$U_{SS} = 1$	$U_{RS} = 1$	$U_{RR} = 1$	$(1 - \omega)$

Weighted Average Allelic Fitness Values

$$\tilde{V}_S = p \cdot \left[q \cdot (1-F) \cdot h \right] \qquad \tilde{V}_R = q\left[q + p\left(F + h - Fh\right) \right]$$

$$\tilde{U}_S = p \cdot \left[(p + qF) \cdot U_{SS} + q \cdot (1-F) \cdot U_{RS} \right] = p \qquad \tilde{U}_R = q \cdot \left[(q + pF) \cdot U_{RR} + p(1-F) \cdot U_{RS} \right] = q$$

$$\tilde{W}_S = p \cdot \left[(1-\omega) + (1-F) \cdot hq\omega \right] \qquad \tilde{W}_R = q \cdot \left[(1-\omega) + (q + pF) \cdot \omega + (1-F) \cdot hp\omega \right]$$

For this most general case, we discover again that y' may be written as a function of $\tilde{A}$:

$$y' = \frac{\left[1 + \left[1 + \frac{p}{q} \cdot \left(F + h - F \cdot h \right) \right] \cdot \tilde{A} \right]}{1 + (1-F) \cdot h \cdot \tilde{A}} \cdot y \qquad [A10]$$

and for q and h both small, the denominator $\rightarrow 1$, all of which leads to

$$\Delta q \approx \tilde{D} \text{ , where } \quad \tilde{D} = \left[1 + \frac{p}{q} \cdot \left(F + h - F \cdot h \right) \right] \cdot \tilde{A} \text{ ,} \qquad [A11]$$

from which we can also derive

$$\frac{\Delta y_{F>0<h}}{\Delta y_{F=0=h}} = \frac{\tilde{D}}{\tilde{A}} \approx \left[1 + \left(\frac{p}{q} \right) \cdot \left(F + h - F \cdot h \right) \right] >> 1 \text{ ,} \qquad [A12]$$

almost a doubly elevated rate of resistance evolution.

Supplementary Appendix B

HDR Model (*F* = 0 = *h*):

We can approximate the difference equation for the simple HDR (ε = 0) model with a 1st order ordinary differential equation. Thus, we can write

$$\Delta y \approx \frac{\delta y}{\delta t} = \tilde{\mathrm{A}} \cdot y$$

where $\tilde{\mathrm{A}} = \left(\frac{q \cdot \omega}{1-\omega}\right) = \left(\frac{\omega}{1-\omega}\right) \cdot \frac{y}{1+y}$, which yields the following solution:

$$t = \left(\frac{1-\omega}{\omega}\right) \cdot \left[-\frac{1}{y_t} + \ln\left(y_t\right) + c\right] \qquad \text{[B1]}$$

where c is a constant of integration, determined by initial conditions, and t is expressed in continuous generations. Note that, as long as $y_t > 0$, Eq. [B1] can be re-written as:

$$\left(\frac{p_t}{q_t}\right) \cdot \exp\left\{-\frac{p_t}{q_t}\right\} = \exp\left\{-\left(\frac{\omega}{1-\omega} \cdot t + c\right)\right\} \qquad \text{[B2]}$$

which is equivalent to (see also Fig. B1):

$$y_t = \frac{1}{\mathbf{W}\left(e^{-\left(\frac{\omega}{1-\omega} \cdot t + c\right)}\right)} \qquad \text{[B3]}$$

where **W** is Lambert's W function which reduces $\mathbf{W}\left(y_t \cdot e^{y_t}\right)$ to y_t, and where c is the constant of integration. Based on (Figure B.1), which plots the temporal trajectory of $q_t = y_t / (1 + y_t)$, we define the "passage time" (T_0^k, in generations) required to go from (q_0 to q_k) for the classic HDR (ε = 0) model as:

$$T_0^k = t_k - t_0 = \left(\frac{1-\omega}{\omega}\right) \cdot \left[\frac{1}{y_0} - \frac{1}{y_k} + \ln\left(\frac{y_k}{y_0}\right)\right] \quad . \qquad \text{[B4]}$$

[Figure B.1 - about here]

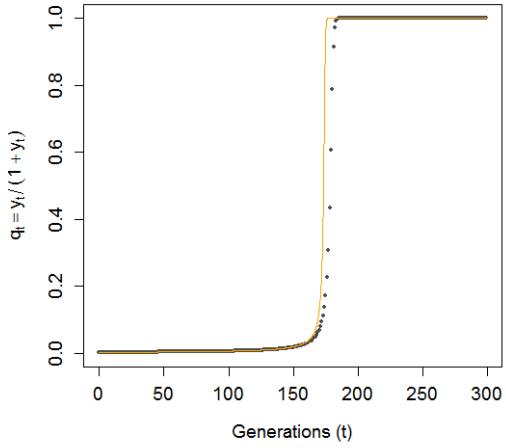


Fig. B1: Frequency of a recessive resistance allele as a function of time. Dots represent the trajectory obtained using iterations (see Eq. [3a,b]), continuous line represents the continuous-time approximation (Eq. [B3]). The parameters where set as follow: $\omega = 0.7$, $q_0 = 0.0025$.

Generalized Model

The increase of resistance allele frequency in a single generation can be calculated based on Δy (see Text Table 1 for the labels of the different parameters):

$$\Delta y = (y' - y) = \left[\frac{\tilde{W}_R}{\tilde{W}_S} - y\right] = \left[\frac{\omega \cdot \tilde{V}_R + (1-\omega)\cdot \tilde{U}_R}{\omega \cdot \tilde{V}_S + (1-\omega)\cdot \tilde{U}_S} - 1\right] \cdot y = \tilde{\Lambda} \cdot y \qquad [B5]$$

which is compatible with all the special cases, since $\tilde{\Lambda}$ translates into $\tilde{A}$, $\tilde{B}$, $\tilde{C}$ and $\tilde{D}$ for text Eqq. [5b], [6a], [7a] and [8a], respectively. To solve the differential equation based on the difference equation [B5], $\tilde{\Lambda}$ may be easily re-written as a ratio of two linear functions of y,

$$\tilde{\Lambda} = \frac{(\beta_1 \cdot y) + \gamma_1}{(\beta_2 \cdot y) + \gamma_2} - 1 \qquad [B6]$$

where

$$\begin{aligned}
\beta_1 &= \omega \cdot V_{RR} + (1-\omega)\cdot U_{RR} \\
\gamma_1 &= \omega \cdot \left[F \cdot V_{RR} + (1-F)\cdot V_{RS}\right] + (1-\omega)\cdot \left[F \cdot U_{RR} + (1-F)\cdot U_{RS}\right] \\
\beta_2 &= \omega \cdot \left[F \cdot V_{SS} + (1-F)\cdot V_{RS}\right] + (1-\omega)\cdot \left[F \cdot U_{SS} + (1-F)\cdot U_{RS}\right] \\
\gamma_2 &= \omega \cdot V_{SS} + (1-\omega)\cdot U_{SS}
\end{aligned} \qquad [B7]$$

leading to:

$$\frac{\delta y}{\delta x} = \tilde{\Lambda} \cdot y = \frac{(\beta_1 - \beta_2) \cdot y + (\gamma_1 - \gamma_2)}{\beta_2 \cdot y + \gamma_2} \cdot y \ , \qquad [B8]$$

Solution of the differential equation [B8] yields an expression for passage time:

$$T_{\Lambda}^{k} \approx \frac{\gamma_2}{\gamma_1 - \gamma_2} \cdot \ln\left(\frac{y_k}{y_0}\right) + \frac{(\beta_1 \cdot \gamma_2 - \beta_2 \cdot \gamma_1)}{(\beta_2 - \beta_1)(\gamma_2 - \gamma_1)} \cdot \ln\left(\frac{(\beta_2 - \beta_1) \cdot y_k + (\gamma_2 - \gamma_1)}{(\beta_2 - \beta_1) \cdot y_0 + (\gamma_2 - \gamma_1)}\right) \qquad [B9]$$

For y_0 small and $y_k < 0.1$, however, the expression can be simplified, since the 2nd order terms may be neglected in Text Eq.[11b]. Retaining only the first term of text Eq. [13], we achieve a further approximation of T_{Λ}^{k} for the case where $V_{SS} = 0$ and $U_{SS} = 1$ (the reference fitness values):

$$T_{\Lambda}^{k} \approx \frac{\gamma_2}{\gamma_1 - \gamma_2} \cdot \ln\left(\frac{y_k}{y_0}\right) = \frac{1 - \omega}{\omega \cdot \varepsilon \cdot V_{RR} - (1 - \omega) \cdot \chi \cdot (1 - U_{RR})} \cdot \ln\left(\frac{y_k}{y_0}\right) \ , \qquad [B10]$$

where $\varepsilon = (F + h - F \cdot h)$, as before, and where $\chi = (F + g - F \cdot g)$.

Performing a simple simulation, we show that the approximation in Eq [B10] is a suitable lower-bound estimate of the full expression Eq [B9] when the number of generations considered is ($T_{\Lambda}^{k} < 100$), as shown in (Figure B2 - see below).

[Figure B2 – about here]

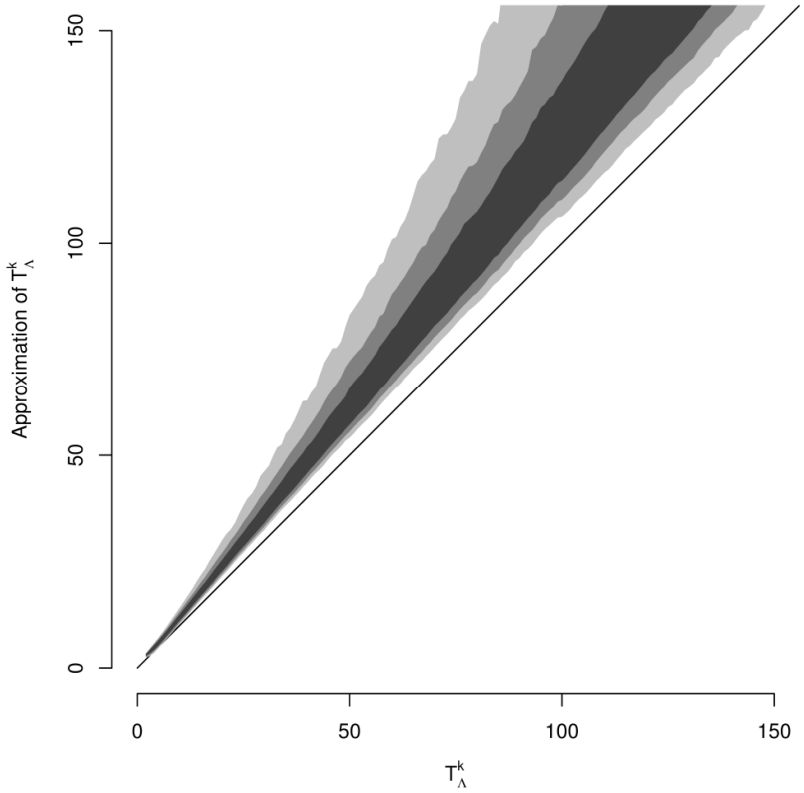


Figure B2: Approximation of passage time Eq. [B10] as compared to the numerical values of the full expression [B9]. 100,000 simulations were performed by randomly sampling values of the model parameters from uniform distributions: ($0.5 < \omega < 0.95$); ($0.5 < V_{RR} < 1$); ($0.5 < U_{RR} < 1$); ($0.0001 < q_0 < 0.005$); ($0 < g < 0.25$); ($0 < h < 0.1$); ($0 < F < 0.1$), while $U_{SS} = 1$ and $V_{SS} = 0$. Calculations were performed for both the approximation and the full expression, on each of the 100,000 parameters set. Grey envelopes represent symmetric simulation intervals: light grey envelope contains 90% of simulated T_Λ^k approximations; middle grey, 75%; dark grey, 50%.