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ORIGINAL PAPER

Proportions of different habitat types are critical to the fate of a resistance allele

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Abstract We describe a simple deterministic theoretical framework for analysing the gene frequency evolution of two alternative alleles at a single genetic locus in a habitat comprising two environments in which the genotypes have different relative fitnesses. We illustrate this for adaptation of pest insects, where one allele (resistance to toxins expressed in transgenic crops) is favoured in one environment (transgenic plants) and the other allele (susceptibility to toxins) is favoured in the other environment ('refuges' of non-transgenic plants). The evolution of allele frequencies depends on selection pressure because of relative sizes of the environments and relative fitnesses of the genotypes in each environment. We demonstrate that there are critical threshold proportions for habitat division that determine equilibrium allele frequencies. The stability of the system depends on relationships between the relative genotype fitnesses. In some cases, the division of the habitat in exactly the threshold proportions removes selection pressure and maintains polymorphism at all allele frequencies.

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Introduction

Natural populations live in heterogeneous habitats, and the interactions between environment and genotype can lead to differential selection. Investigating how selection can maintain genetic variation in adaptive phenotypic traits is a classic and contemporary issue in evolutionary biology (Byers 2005). Originally, Levene (1953) showed, theoretically, that diversifying selection over different habitats could maintain polymorphism. Using a framework based on soft selection (whereby a fixed number of individuals survive in each niche), the conditions promoting polymorphism depend on the harmonic mean fitness of heterozygotes being larger than that for homozygotes (Levene 1953). From this and later models, we now know that variable selection over space generally results in broader conditions for maintenance of genetic polymorphism, although it does not necessarily always ensure it (Hedrick 2006).

An alternative approach to explaining how diversifying selection and habitat heterogeneity affect the maintenance of polymorphism was introduced by Dempster (1955). In contrast to Levene's model, this theoretical framework involves hard selection (by allowing a constant number of zygotes in each niche of which variable number to survive to adulthood). In this situation, changes in the allele frequency of the whole population depend on arithmetic mean fitness values. Real populations are probably somewhat intermediate in character, perhaps depending on whether inter-specific or intra-specific competition is more important in limiting the number of fertile adults (Dempster 1955).

Levene (1953) noted that with preferential selection of niches and/or mating tending to occur within niches rather than at random across the whole population, conditions would be more favourable for polymorphic equilibria. Various authors have relaxed the model assumptions to investigate these effects (Hedrick 2006). For instance, Karlin (1977) determined conditions for the protection of a recessive allele (i.e. where its extinction is impossible from any initial polymorphic state) for almost any structure of migration between demes (as opposed to simple random mating and migration). This result holds true whether hard or soft selection is assumed. Further generalisations continue to be explored. For example, models of polymorphism for multiple alleles, with soft selection and arbitrary migration (Nagylaki and Lou 2001), have allowed the sufficient conditions for non-existence of a completely polymorphic equilibrium and the global loss of alleles to be deduced (Nagylaki and Lou 2006).

The gene frequency evolution of alternative alleles in a heterogeneous habitat is important to the broad questions of ecological generalism versus specialism. There are many examples of specialist species where at least one life stage develops in a particular environment. A rare allele or novel mutation might enable survival in a second environment. For example, a phytophagous insect whose larvae develop on a specific host plant might gain resistance to insecticides and so survive in a treated field. A parasite may extend its host range, by acquiring resistance to an antimicrobial drug and so be able to develop in treated individuals or by adapting to a novel host species. Developing resistance can have fitness costs, and these can be important in determining host range, with implications for public health, agriculture and conservation (Andersson and Levin 1999; Coleman and Welburn 2004; Hastings and D'Alessandro 2000; Levin 2001). Models can help predict or explain whether this resistance becomes predominant or disappears or some intermediate balance is maintained.

Some of the best documented examples of selection in heterogeneous environments involve recent human-induced changes to the environment, such as the use of chemicals to control pests or pathogens (Hedrick 2006). Alternative environments may be created or manipulated deliberately, to influence the evolution of the undesirable trait (such as resistance to these chemicals). For example, strategies for delaying the evolution of drug resistance in parasites include applying chemotherapy selectively, treating only those individuals with high worm loads (macro-parasitic infections) or high parasitaemias (micro-parasitic infections such as malaria), so that parasite populations in the untreated individuals dilute the parasite gene pool with genes for drug susceptibility (Anderson and May 1991). Similar approaches are used to delay resistance to conventional insecticides in disease vectors and agricultural pests (e.g. the 'stable zone strategy' where an area of critical size is left untreated [Lenormand and Raymond 1998]). Such strategies create a habitat sub-divided into two environments – one where the resistance allele is favoured (the treated area or hosts) and the other where it is not (untreated).

Population genetics models, such as those discussed above, typically allow parameters representing habitat variability to take arbitrary values and find conditions in terms of fitness parameters for stable equilibria that could maintain genetic polymorphism. Where the habitat is deliberately manipulated (or influenced), it could be more useful to express conditions for polymorphism in terms of habitat division, so that appropriate changes to the habitat proportions can be made to meet the objective of delaying or reversing resistance. Our aim in this study is to understand how habitat heterogeneity affects resistance evolution. Unlike many of the earlier studies, our interest is not in polymorphism per se but in understanding how the division of a habitat influences whether an undesirable trait (resistance) becomes universal, disappears or is able potentially to persist for a significant time at some intermediate level. We therefore consider all of the dynamical outcomes not just stable equilibria. We develop a population genetic framework that focuses on the relative sizes of two environments within a habitat and consider all potential steady states.

Biological motivation

One particular example of such a heterogeneous habitat that motivates our work arises in the context of transgenic crops that produce insecticidal toxins. These are now widely used to control insect pests (James 2006). We use this system, which has simple genetics and two habitat sub-types that differ in a single, important feature, as a model system to illustrate our framework for understanding resistance evolution.

Plants producing insecticidal proteins derived from *Bacillus thuringiensis* (Bt), principally maize and cotton, grew on 32 million ha worldwide during 2006 (James 2006). By reducing reliance on insecticidal sprays, decreasing production risk (insect damage) and/or improving yields, these can provide economic, health and environmental benefits (Brookes and Barfoot 2006; Huang et al. 2003; Shelton et al. 2002), which would be lost if resistance to the toxins spread to a significant proportion of the pest population. Although field resistance to Bt crops has not yet been documented, resistance to Bt sprays has been found in diamondback moth (*Plutella xylostella*) and cabbage looper (*Trichoplusia ni*), and strains of several pests that are substantially resistant to Bt toxins have been selected in the laboratory (Bates et al. 2005).

Strains of Bt produce crystalline (Cry) proteins with insecticidal properties. Each different protein is usually

toxic to only a few species, and receptors on midgut epithelial cells are critical in determining Cry protein specificity. In Lepidoptera, the cadherin-like receptors are a major class of such receptors; several cadherin-like proteins confer susceptibility by binding to Cry toxins, and disruption in cadherin genes has been associated with resistance (Pigott and Ellar 2007). Different kinds of changes to cadherin genes imparting resistance have been identified, for example disruption by a retrotransposon (Gahan et al. 2001) or deletions (Morin et al. 2003) and genetic diversity of cadherin-specific genes of cotton bollworm (*Helicoverpa armigera*) populations in India differed more than 100-fold in their susceptibility to Cry1Ac (Gujar et al. 2007b).

Laboratory-selected resistant strains have been investigated in numerous studies. A recessive mutation of a single autosomal gene in diamondback moth populations in Hawaii and Pennsylvania, USA, confers high level resistance to four Bt toxins: Cry1Aa, Cry1Ab, Cry1Ac and Cry1F (Tabashnik et al. 1997a, b). Resistance to Bt Cry1Ab maize in sugarcane borer (*Diatraea saccharalis*) is determined by a nearly completely recessive allele at a single locus (Huang et al. 2007). *H. armigera* resistance to Cry2Ab toxin was found to be recessive (Mahon et al. 2007). In several independent strains of pink bollworm (*Pectinophora gossypiella*), resistance to Cry1Ac toxin is associated with a single locus at which three mutant alleles of a cadherin gene confer resistance in larvae having any combination of two resistance alleles (Morin et al. 2003; Tabashnik et al. 2005); the assumption of two alternative alleles seems a reasonable simplification here. However, a few studies have identified resistance to Bt toxins that is not due to a single major gene. Resistance in diamondback moth populations in the Philippines shows multilocus control and for some toxins is not recessive (Tabashnik et al. 1997b). Two major Cry1Ac resistance genes (either of which confers resistance) have been identified in tobacco budworm (*Heliothis virescens*), a cotton pest (Gahan et al. 2001, 2005); one of them, a cadherin mutation, was shown to be recessive and to account for 40–80% of resistance. However, one strain also exhibited resistance to Cry2Aa toxins, which is thought to be the cumulative result of several genes (Gahan et al. 2005). An analysis of inheritance of resistance in European corn borer (*Ostrinia nubilalis*) in laboratory-selected resistant strains showed that resistance was autosomal and suggested it was controlled by more than one locus, perhaps as many as 10–20 (Alves et al. 2006), with dominance of resistance decreasing as toxin concentration increased. Quantitative genetic variation has also been observed in resistance to both Cry1Ac and Cry2Ab toxins in bollworm (*Helicoverpa zea*; Jackson et al. 2006).

The main resistance management method currently used is the 'high-dose/refuge' strategy. This is based on the assumption that resistance to Bt crops is functionally recessive (because of the high levels of Bt toxins expressed, i.e. the 'high dose'), so that only those insects homozygous for a resistance allele can survive on Bt crops. Refuges are areas of non-Bt host plants in which susceptible insects can survive; if resistance has fitness costs (Bates et al. 2005), resistant insects would be at a selective disadvantage in the refuge. Susceptible homozygotes from the refugia mate with resistant insects from toxin-treated regions, producing heterozygous offspring that cannot survive on Bt crops, thereby tending to reduce the frequency of resistance alleles. Mathematical models predict that this strategy can slow or reverse the spread of resistance through wild pest populations (Alstad and Andow 1995; Carrière and Tabashnik 2001; Gould 1998; Tabashnik et al. 2005).

In this study, we show that the evolution of resistance allele frequency depends on the relative sizes of the two environments (illustrated by Bt crop areas and refugia) and on the relative fitnesses of the genes in each environment. We demonstrate that there are critical threshold proportions for the division of the habitat that determine the equilibrium allele frequencies and examine the behaviour of the system at these thresholds.

Materials and methods

Analytical model

We use a population genetic framework to examine the effects of refuges on the spread of resistance to Bt crops in an insect pest population (Hartl and Clark 1989; Tabashnik et al. 2005). We assume a closed homogeneous population, with random mating and no immigration, emigration or mutation and use a deterministic, discrete-generation model. We assume a 1:1 sex ratio. Larvae, the susceptible life stage, are assumed to spend their whole developmental time either on Bt crops or in the refuge, and we assume that migration occurs after emergence as adults and before mating (Fig. 1).

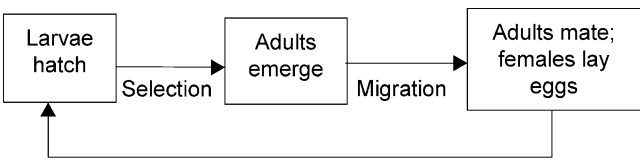

Fig. 1 Model. Fitness costs associated with the *r* allele and with Bt toxins take effect during the larval stage. Larvae spend that entire life stage either on Bt plants or in a refuge. Adults migrate before mating at random, and eggs are laid at random across Bt and non-Bt plants

Our canonical set of parameters is listed in Table 1, together with the symbols used and the constraints placed on their values.

We assume that the pest insect population is distributed at random across a fixed crop population, a proportion Φ of which expresses Bt toxins. The conventional (non-Bt) crop refuge size is therefore $1-\Phi$. Hard selection (Wallace 1968) acts in these two environments, with the number of larvae surviving to reproductive maturity from each environment depending on the relative fitness values of the eggs laid there. Larval susceptibility to Bt toxins is assumed to be controlled by a single autosomal locus with two alternative alleles at that locus: resistant r (frequency p in adults) and susceptible s (frequency q, $p+q=1$). There are three genotypes at this locus: *ss*, *sr* and *rr*.

We further assume that all fitness costs associated with the s and r alleles, in Bt and non-Bt crops, act during the larval stage (Fig. 1). We use a subscript i, taking any of the three values *ss*, *sr* or *rr*, to indicate the genotype to which each larval fitness parameter applies. We denote the relative fitness of larvae on Bt crops by ω_i, and υ_i is relative fitness of larvae on non-Bt crops. Fitnesses of these larvae are all relative to the *ss* genotype on non-Bt crops (set $\upsilon_{ss}=1$). With random distribution of larvae across Bt and non-Bt crops, the mean relative fitness of larvae of genotype i (i: *ss*, *sr* or *rr*) is

$$\Omega_i = \omega_i\Phi + \upsilon_i(1-\Phi) \quad (1)$$

We assume that the resistance allele may have a cost (on non-Bt plants $\upsilon_{rr} \le \upsilon_{sr} \le \upsilon_{ss} = 1$) and that on Bt crops, resistance is not over- or under-dominant ($\omega_{ss} \le \omega_{sr} \le \omega_{rr}$). By definition of resistance, $\omega_{rr} > \omega_{ss}$, but the more general assumption that $\omega_{rr} \ne \omega_{ss}$ is sufficient in conjunction with the constraint set out in the previous sentence.

Table 1 Parameters and symbols

Symbol	Parameter	Constraints
r	Resistant allele	–
s	Susceptible allele	
Φ	Proportion of crops expressing Bt toxins	$0<\Phi<1$
$1-\Phi$	Refuge size	
i	Genotype	*ss*, *sr* or *rr*
ω_i	Relative fitness of larvae on Bt crops	$0\le\omega_i\le1$ $\omega_{rr} \ne \omega_{ss}$ $\omega_{ss} \le \omega_{sr} \le \omega_{rr}$
υ_i	Relative fitness of larvae in non-Bt plants	$0\le\upsilon_i\le1$ $\upsilon_{rr}\le\upsilon_{sr}\le\upsilon_{ss}=1$
Ω_i	Average relative fitness of genotype i	
p	Frequency of resistance allele r	$0\le p\le1$
q	Frequency of susceptible allele s in current adult generation	$0\le q\le1$ $p+q=1$
p_0	initial r allele frequency	

Dominance of alleles can be assessed in different ways, and several measures have been used in insecticide studies (Bourguet et al. 2000). The most relevant to our model is in terms of the relative fitness of the three genotypes (rather than in terms of mortality levels, for example), either on Bt crops or in the refuge. The benefits of resistance affect fitness on Bt plants, and the costs of resistance affect fitness on both Bt and non-Bt plants. Special cases arise for our model when the resistance allele is dominant, recessive or co-dominant, in both environments, i.e. where the *sr* heterozygote has, respectively, the same fitness as the *rr* homozygote, the same fitness as the *ss* homozygote or fitness midway between those of the homozygotes.

Assumptions and estimation of parameters

There are problems with validating these population genetic models. Ascertaining appropriate parameter values to use is difficult as field-evolved resistance to Bt crops has not yet been observed (Gressel 2005). However, there is a range of knowledge and experimental data available from which to create an appropriate parameter set.

As explained in our introduction, in most known cases, resistance to Bt toxins is monogenic (Ferré and Van Rie 2002; ffrench-Constant et al. 2004), and the assumption of two alternative alleles is a reasonable simplification. Most economically significant cases of conventional pesticide resistance are believed to be due to allelic variations at one or two gene loci. Consequently, one-locus two-allele models are commonly used (Tabashnik 1990). These assumptions are also thought to be realistic for many instances of resistance to drugs within diploid parasite populations or to pesticides in populations of insect or molluscan intermediate hosts of infectious diseases (Anderson and May 1991).

There are field data for estimated Bt resistance allele frequencies in several pests: tobacco budworm (Gould et al. 1997), cotton bollworm (*H. armigera*; Li et al. 2004), pink bollworm (Tabashnik et al. 2005), European corn borer (Stodola et al. 2006) and sugarcane borer (Huang et al. 2007); broadly, these estimates are of order of magnitude one in 1,000 alleles (0.001), with one exception. Early results for pink bollworm in Arizona estimated resistance allele frequencies at 0.13 or 0.16 in 1997 (Tabashnik et al. 2000); however, a later study estimated a mean frequency of 0.004 across 1998–2004 (95% confidence limits 0 to 0.01), with annual estimates varying approximately from 0 to 0.08 (Tabashnik et al. 2005).

In the particular context of resistance to Bt crops, it would be appropriate also to assume that no genotype is fitter on Bt crops than non-Bt plants ($\upsilon_i \ge \omega_i$). However, we do not impose this additional assumption in our model.

Where the r allele is an adaptation, representing a change from the wild-type s allele, it is reasonable to assume that

there may be a fitness cost that will be at least as great where two copies are present as with one copy ($\upsilon_{rr} \leq \upsilon_{sr} \leq \upsilon_{ss} = 1$). The assumptions about heterozygote fitness ($\upsilon_{rr} \leq \upsilon_{sr} \leq \upsilon_{ss} = 1$ and $\omega_{ss} \leq \omega_{sr} \leq \omega_{rr}$) determine the directions of some inequalities when analysing the model and ensure that some divisors are non-zero. In principle, the analysis could be repeated assuming the heterozygote form is superior or inferior to homozygotes in either or both environments.

The potential for the high-dose refuge strategy to delay the evolution of resistance increases as the magnitude and dominance of fitness costs increase (Carrière and Tabashnik 2001). Empirical methods for estimating relative fitness parameters in wild populations are problematic, and estimated values vary considerably (Bourguet et al. 2000; Tabashnik et al. 2005, 2004). Exceptionally, some extremely resistant strains of diamondback moth are able to develop on Bt crucifers with no ill effects (for our purposes $\omega_{rr} \approx 1$; Ramachandran et al. 1998; Tang et al. 1999), but generally, resistance is incomplete (in our model $\omega_{rr} < \upsilon_{rr}$), and fitness costs have been observed in resistant strains of several species (Bates et al. 2005). In cotton bollworm (*H. armigera*), laboratory-selected resistance to Cry2Ab toxin was found to be recessive and to confer a very high level of resistance ($\omega_{rr} \approx 1$; Mahon et al. 2007). As noted earlier, most cases of resistance to Bt toxins have been shown to be recessive ($\omega_{sr} \approx \omega_{ss}$), especially at the high levels expressed in Bt crops. Fitness costs in pink bollworm resistant to Cry1Ac Bt cotton have been shown to be substantial, recessive and mainly affecting survival (Carrière et al. 2005a; Liu et al. 2001). One study found that fitness costs reduced survival of resistant larvae on non-Bt cotton by an average of 51.5% compared to susceptible strains (υ_{rr}= 0.485; Carrière et al. 2001a). Another study measured their survival on Bt cotton at 46% relative to survival on non-Bt cotton (ωrr=0.46υ_{rr}; Liu et al. 2001). An over-wintering cost in pink bollworm appeared to be recessive to some extent, and reduced emergence from diapause in spring was conservatively estimated at 71% (Carrière et al. 2001b). Fitness costs can affect traits other than survival; for example, paternity effect experiments showed that resistant pink bollworm males can have reduced success in competing for virgin females, and resistant males that mated first sired fewer offspring than first-mating susceptible males (Higginson et al. 2005). Resistance to Cry1Ac Bt cotton in *H. armigera* in Australia has been shown to have fitness costs whose dominance and magnitude vary with the host plant species deployed in refugia – cotton, pigeon pea or sorghum (Bird and Akhurst 2007). However, experimental evidence did not support a hypothesis that different cotton cultivars would affect the fitness costs of resistance to Bt cotton in pink bollworm (Carrière et al. 2005a). The presence of a nucleopolyhedrovirus can increase the fitness costs of resistance to Bt Cry1Ac sprays in diamondback moth (Raymond et al. 2007).

In our analysis, we considered the full range of theoretically possible values of fitness parameters and did not restrict ourselves to relative values that are plausible in the context of managing resistance to Bt crops. Similarly, we used a wide range of values for our simulations.

We assume that eggs are laid at random across the whole habitat. Clearly this would be a harmful strategy for a specialist able to survive only in a single habitat sub-type. Such a situation could occur and persist if two environment types are indistinguishable by the pest. This might be reasonable where the host plant has a form that is resistant to insects, giving two distinct plant phenotypes (rather than a spectrum of plant resistance levels). This could happen through genetic mutation in the plant or where the alternate variety is an invader (e.g. one-off seeding from another location) or was deliberately planted (e.g. Bt crops or a variety selected for resistance through classical horticultural breeding methods).

It is common to assume that the organism is in only one of the environments throughout the life stage that is subject to differential selection (Hartl and Clark 1989). US refuge requirements for Bt maize or cotton stipulate placement as well as size, to minimise larval movement between Bt and refuge but allow adult dispersal (Bourguet et al. 2005; Carrière et al. 2005b), so each larva should remain on either Bt or non-Bt plants.

Population genetics

The change in resistance allele frequency in a generation, Δp, is given by (Gillespie 1998; Hartl and Clark 1989):

$$\begin{aligned}\Delta p &= \frac{p^2\Omega_{rr} + pq\Omega_{sr}}{q^2\Omega_{ss} + 2pq\Omega_{sr} + p^2\Omega_{rr}} - p \\ &= \frac{pq(p(\Omega_{rr} - \Omega_{sr}) + q(\Omega_{sr} - \Omega_{ss}))}{q^2\Omega_{ss} + 2pq\Omega_{sr} + p^2\Omega_{rr}}\end{aligned} \quad (2)$$

Where $p,q \neq 0,1$ (resistance allele present but not at fixation), the sign of Δp is determined by the sign of $p(\Omega_{rr} - \Omega_{sr}) + q(\Omega_{sr} - \Omega_{ss})$. We assess stability using end-point analysis (Gillespie 1998).

Simulations

To confirm our analysis, we performed a series of simulations over a wide range of parameter values by iterating genotype frequencies using Eq. 2, reflecting the order of events illustrated in Fig. 1 and calculating allele frequencies at each generation from the genotype frequencies. We selected parameter combinations sufficient to cover all classes of outcome, without regard to whether

the values are plausible in the specific context of managing resistance to Bt crops. Values were chosen across the following ranges: p_0 0.1–0.9, υ_{sr} 0.4–1, υ_{rr} 0.4–0.7, ω_{ss} 0–0.2, ω_{sr} 0–0.4, ω_{rr} 0.1–0.6 and Φ 0.3–1. We ran further simulations with randomly generated values satisfying the model constraints, some of which fell outside the stated ranges.

Results

Equilibrium points

There are potentially three steady state points (Fig. 2): $p^*=0$, $p^*=1$ and $p^*=\rho$ where

$$\rho = \frac{-\Omega_{ss}+\Omega_{sr}}{-\Omega_{ss}+2\Omega_{sr}-\Omega_{rr}} = \frac{(-1+\Phi-\omega_{ss}\Phi+\upsilon_{sr}-\upsilon_{sr}\Phi+\omega_{sr}\Phi)}{(-1+\Phi-\omega_{ss}\Phi+2\upsilon_{sr}-2\upsilon_{sr}\Phi+2\omega_{sr}\Phi-\upsilon_{rr}+\upsilon_{rr}\Phi-\omega_{rr}\Phi)} \quad (3)$$

Equation 3 is only biologically relevant if ρ exists between 0 and 1.

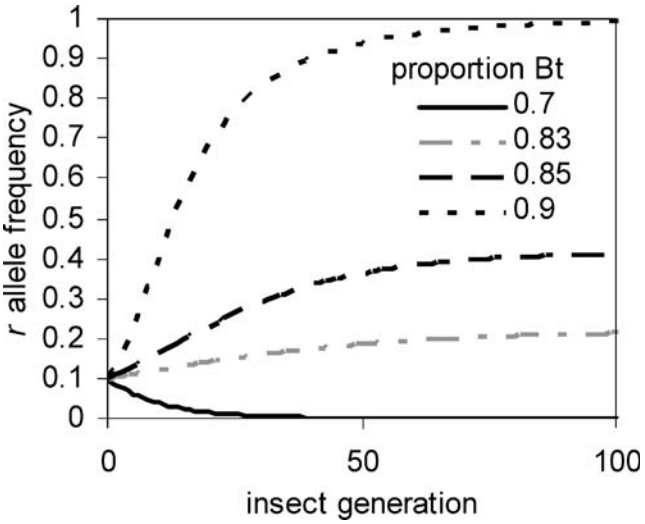

Fig. 2 Division of habitat affects allele and phenotype frequency evolution. The graph shows the frequency over time of the *r* allele for different divisions of the habitat and illustrates three possible outcomes: extinction, fixation or an intermediate steady state. The initial *r* allele frequency was 0.1. Relative fitnesses of the genotypes in the two environments were: $\upsilon_{ss}=1$, $\upsilon_{sr}=0.8$ and $\upsilon_{rr}=0.4$ in the refuge and $\omega_{ss}=0$, $\omega_{sr}=0.05$ and $\omega_{rr}=0.1$ on Bt crops. With these fitness parameters, the critical proportions are $\Phi_1=0.8$ and $\Phi_2=8/9\approx0.8889$. Where the proportion of Bt crops is below the lower threshold ($\Phi=0.7$, *solid line*), the *r* allele goes to extinction. Above the higher threshold ($\Phi=0.9$, *dotted line*), the *r* allele goes to fixation. In between the two ($\Phi=0.83$, *dashed grey line*, or $\Phi=0.85$, *dashed black line*), the *r* allele tends to an intermediate equilibrium ($\rho=15/68\approx0.2206$ or $\rho=5/12\approx0.4167$, respectively)

Threshold values for sizes of habitat sub-types (Bt crop area and refuge)

Special cases arise when the resistance allele is dominant, recessive or co-dominant, in both environments. These are dealt with separately below.

In the three special cases, there is no distinct third steady state ρ, so no internal equilibrium is possible. In other cases (partial dominance), there are two critical threshold values, Φ_1 and Φ_2, that determine whether a third steady state exists:

$$\Phi_1 = \frac{(1-\upsilon_{sr})}{(1-\upsilon_{sr}+\omega_{sr}-\omega_{ss})} \quad (4)$$

$$\Phi_2 = \frac{(\upsilon_{rr}-\upsilon_{sr})}{(\upsilon_{rr}-\upsilon_{sr}+\omega_{sr}-\omega_{rr})} \quad (5)$$

The third steady state ρ lies between 0 and 1 if and only if Φ lies between Φ_1 and Φ_2.

These thresholds Φ_1 and Φ_2 are calculated from the various fitness parameters, so the relative fitnesses of the three genotypes in the two environments determine the range of possible outcomes. The areas actually planted to Bt crops and refuge are important in determining which of those possible outcomes arises in practice.

Φ_1 is defined in terms of the fitness parameters of *sr* and *ss* genotypes. The proportion of crops expressing Bt toxins Φ relative to the threshold Φ_1 determines whether *sr* heterozygotes are fitter on average than *ss* individuals. Above that threshold ($\Phi>\Phi_1$), heterozygotes have higher average relative fitness than susceptible homozygotes ($\Omega_{sr} > \Omega_{ss}$). Below it ($\Phi<\Phi_1$), the heterozygotes are less fit on average ($\Omega_{sr}<\Omega_{ss}$), and at the threshold ($\Phi=\Phi_1$), they are equally fit ($\Omega_{sr} = \Omega_{ss}$).

Similarly, Φ_2 is defined in terms of the fitness of *sr* and *rr* genotypes. The Bt crop proportion Φ relative to the threshold Φ_2 determines whether *sr* heterozygotes are fitter on average than *rr* individuals. Above ($\Phi>\Phi_2$), below ($\Phi<\Phi_2$) or at ($\Phi=\Phi_2$) that threshold, *sr* individuals are, respectively, less fit on average ($\Omega_{sr} < \Omega_{rr}$), fitter ($\Omega_{sr} > \Omega_{rr}$) or equally fit ($\Omega_{sr} = \Omega_{rr}$).

These thresholds are determined by the interplay between the costs and benefits of resistance. If the difference in fitness between two genotypes is large on Bt crops compared to the difference between them in the refuge, the relevant threshold will be relatively small. Conversely, if the fitness difference is large in the refuge compared to the difference on Bt crops, the threshold will be nearer to one. For example, if the *r* allele gives better resistance with two copies ($\omega_{sr} < \omega_{rr}$) and the fitness penalties of one or two copies are about the same ($\upsilon_{sr} \approx \upsilon_{rr}$), Φ_2 will be close to zero (Eq. 5), and almost any planting of

Bt crops will mean that Φ is above Φ_2 and resistant homozygotes will be fitter on average than heterozygotes. If the fitness difference is the same in both environments, the relevant threshold is half of the habitat.

Eventual fate of resistance allele and stability of equilibria

In the general case (partial dominance), the stability of the system depends on the relative fitness of each genotype on Bt and non-Bt plants, in particular whether $\Phi_1<\Phi_2$ or $\Phi_1>\Phi_2$.

Where $\Phi_1<\Phi_2$:

- If the proportion of Bt crops is below or equal to Φ_1, the resistance allele will decline to extinction (stable equilibrium $p^*=0$).
- If the proportion of Bt crops is above or equal to Φ_2, the resistance allele will go to fixation (stable equilibrium $p^*=1$).
- If the proportion of Bt crops lies between Φ_1 and Φ_2, the frequency of the resistance allele will settle at a stable internal equilibrium $p^* = \rho$ lying between 0 and 1.

Figure 2 illustrates these three possible patterns of frequency evolution of the resistance allele (extinction or fixation or intermediate equilibrium) for a given set of fitness parameters and shows that the intermediate equilibrium frequency (ρ) depends on the value of Φ. Figure 3a shows the regions of outcomes on a plot of initial allele frequency (p_0) against the proportion of Bt crops (Φ). These equilibria are unique and globally stable over the range in question; for a given set of fitness values and a particular value of Φ, a unique equilibrium is determined, and the r allele frequency will tend asymptotically towards that equilibrium from any value in the range $0<p<1$.

Where $\Phi_1>\Phi_2$, the fixed point ρ is unstable (where it exists), and the resistance allele will eventually go to either extinction (stable equilibrium $p^*=0$) or fixation (stable

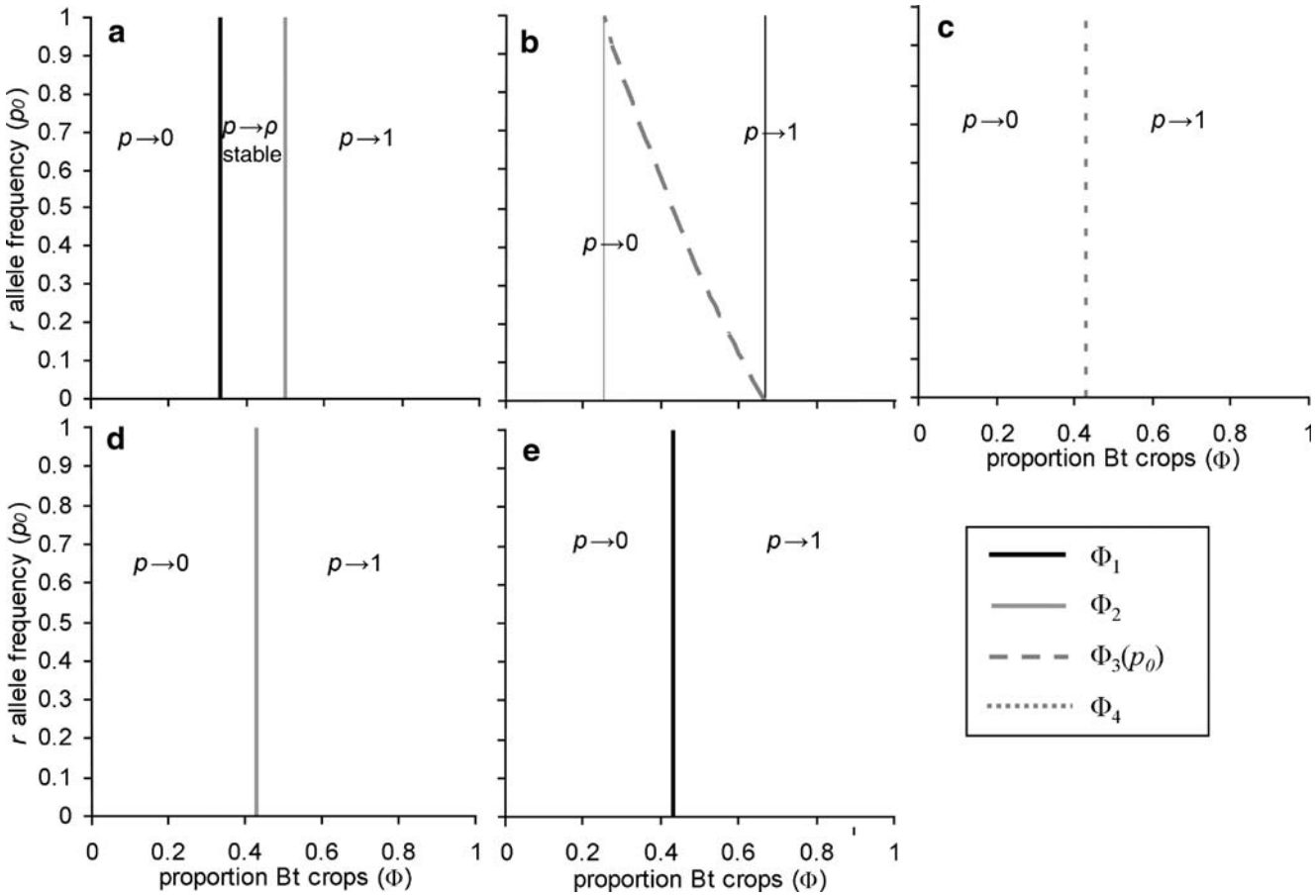

Fig. 3 Critical thresholds determine allele fates and the nature of steady states. The graphs show regions of outcomes for r allele frequency, plotting initial allele frequency (p_0) against the proportion of Bt crops (Φ). Resistant homozygotes have 0.7 relative fitness on non-Bt plants (υ_{rr}) and 0.4 fitness on Bt crops (ω_{rr}), and other genotypes are fully susceptible to the toxins ($\omega_{ss}=\omega_{sr}=0$). The relative fitnesses of heterozygotes are varied between panels. **a** Incomplete dominance with $\Phi_1<\Phi_2$ ($\upsilon_{sr}=0.9$, $\omega_{sr}=0.2$): With Φ at the thresholds, the r allele goes to extinction ($\Phi=\Phi_1$) or fixation ($\Phi=\Phi_2$); ρ is a stable equilibrium. **b** Incomplete dominance with $\Phi_1>\Phi_2$ ($\upsilon_{sr}=0.8$, $\omega_{sr}=0.1$): At the threshold ($\Phi=\Phi_3$), the r allele is at an unstable equilibrium (at frequency p_0). **c** Co-dominant ($\upsilon_{sr}=0.85$, $\omega_{sr}=0.2$). **d** Complete recessiveness ($\upsilon_{sr}=1$, $\omega_{sr}=0$). **e** complete dominance ($\upsilon_{sr}=0.7$, $\omega sr=0.4$). In **c**–**e**, with Φ at the threshold, the r allele remains constant at any frequency. The only situation where the eventual r allele fate is dependent on p_0 is in case **b** where Φ lies between Φ_1 and Φ_2

$p^*=1$), depending on whether the proportion of Bt crops is, respectively, above or below a third threshold Φ_3, the value of which is dependent on the initial frequency of the resistance allele p_0 (Fig. 3b):

$$\Phi_3 = \frac{(-1 + p_0 + \upsilon_{sr} - 2p_0\upsilon_{sr} + p_0\upsilon_{rr})}{-1 + p_0 + \omega_{ss} - p_0\omega_{ss} + \upsilon_{sr} - \omega_{sr} - 2p_0\upsilon_{sr} + 2p_0\omega_{sr} + p_0\upsilon_{rr} - p_0\omega_{rr}} \quad (6)$$

This threshold is a function of all the genotype fitness parameters and the initial r allele frequency. There is an unstable internal equilibrium ($p^*=\rho=p_0$), which is only achieved if the resistance allele frequency and proportion of Bt crops are at the appropriately related values from the start. If the proportion of Bt crops is equal to Φ_3, the frequency of the resistance allele will remain constant at p_0, but slight perturbations above or below that allele frequency (vertical movement away from the Φ_3 line on Fig. 3b) will result in its fixation or extinction, respectively. As the value of the equilibrium ρ is a function of Φ, any perturbation in refuge size (horizontal disturbance on Fig. 3b) will also lead to extinction or fixation. The equilibria $p^*=0$ or 1 are locally stable; any perturbation that does not push the allele frequency to or beyond ρ (Eq. 3, the allele frequency p at which $\Phi_3(p) = \Phi$) will not alter the r allele's ultimate fate.

The high-dose/refuge strategy aims to make the resistant phenotype functionally recessive ($\omega_{ss} = \omega_{sr}$) by having toxins expressed at sufficiently high levels. If the r allele is recessive to the s allele for resistance but the cost of resistance is not also recessive (so $\upsilon_{ss} > \upsilon_{sr}$), the threshold Φ_1 is equal to 1 (Eq. 4). In these circumstances, Φ_1 (=1) is always greater than Φ_2, and so an unstable steady state will exist at $\Phi=\Phi_3$ (which depends on the value of p_0), and the resistance allele will go to fixation or extinction according to whether the proportion of Bt crops is above or below this limit. If the r allele is initially rare ($p_0\approx0$), Φ_3 is also close to 1, so the allele will go extinct unless there is virtually no refuge.

Similarly, if the r allele is dominant to the s allele for resistance ($\omega_{rr} = \omega_{sr}$) but the cost of resistance is not dominant ($\upsilon_{sr} > \upsilon_{rr}$), the threshold Φ_2 is equal to 1 (Eq. 5). This means $\Phi_1<\Phi_2$ (=1), and so a stable internal equilibrium can exist. The resistance allele will decline to extinction or tend to the stable intermediate equilibrium ρ (defined by Eq. 3) according to whether the proportion of Bt crops is below or equal to Φ_1 (Eq. 4) or between Φ_1 and 1, respectively. Such dominant resistance with non-dominant fitness penalties would never reach fixation. The outcome is independent of the initial frequency of the resistance allele, p_0.

Early tests on Bt cotton plants that achieved a high dose against tobacco budworm showed that the same plants only caused 75–90% relative mortality of susceptible bollworm (*H. zea*) larvae; a high dose was not achieved for that insect (Gould 1998). There are indications that cotton bollworm (*H. armigera*) is relatively more tolerant to Cry1Ac toxins than tobacco budworm, and inheritance of Cry1Ac resistance in *H. armigera* has been reported to vary from recessive to semi-dominant depending on the strain (Gujar et al. 2007a). If resistance to Bt cotton plants were non-recessive for such pests, one of Fig. 3a and b would depict the relevant outcome. If relatively few heterozygous larvae could survive a life cycle on Bt cotton ($\omega_{sr}\approx0$ and $\omega_{ss}=0$), Φ_1 would be close to 1 (Eq. 4), and so probably $\Phi_1>\Phi_2$, and the situation shown in Fig. 3b is likely to apply.

Special cases: co-dominant resistance, complete recessiveness or complete dominance

There are three special cases to consider, for which the outcomes are broadly similar.

In the case where both resistance and costs of resistance are co-dominant ($\omega_{sr} = \frac{1}{2}(\omega_{ss} + \omega_{rr})$ and $\upsilon_{sr} = \frac{1}{2}(\upsilon_{ss} + \upsilon_{rr})$, so $\Omega_{sr} = \frac{1}{2}(\Omega_{ss} + \Omega_{rr})$), there is no third steady state (Eq. 3 denominator would be zero), and the resistance allele will eventually go to either extinction or fixation, depending on whether the proportion of Bt crops is above or below a fourth threshold Φ_4.

$$\Phi_4 = \frac{(1 - \upsilon_{rr})}{(1 - \upsilon_{rr} + \omega_{rr} - \omega_{ss})} \quad (7)$$

This formula for Φ_4 is analogous to those for Φ_1 (Eq. 4) and Φ_2 (Eq. 5) but is defined in terms of the fitness parameters of the homozygous genotypes. For co-dominance (and for the completely recessive or dominant cases), the fitnesses of the heterozygotes in each environment are defined by the fitnesses of the homozygotes in that environment. The smaller the fitness difference between homozygotes on Bt plants ($\omega_{rr} - \omega_{ss}$) compared to the fitness difference in the refuge ($1-\upsilon_{rr}$), the nearer the critical threshold will be to 1.

Where the costs and benefits of resistance are fully recessive ($\upsilon_{ss}=\upsilon_{sr}=1$ and $\omega_{ss} = \omega_{sr}$, so $\Omega_{ss} = \Omega_{sr}$), Eq. 3 is equal to zero, so there is no distinct third steady state ρ, and Φ_1 does not exist. Φ_2 is the only critical threshold. Substituting $1=\upsilon_{sr}$ and $\omega_{ss} = \omega_{sr}$ into Eq. 5 shows that in this particular case, Φ_2 is equal to Φ_4 as defined in Eq. 7.

Similarly, Φ_2 also equals Φ_3 as defined in Eq. 6, and in this case, Φ_3 is independent of p_0.

Where the costs and benefits of resistance are fully dominant ($\upsilon_{rr} = \upsilon_{sr}$ and $\omega_{rr} = \omega_{sr}$, so $\Omega_{rr} = \Omega_{sr}$), Eq. 3 is equal to one, there is no distinct third steady state ρ and Φ_2 does not exist. Again, the sole critical threshold, which here is Φ_1, is equal to Φ_4 (shown by substituting $\upsilon_{rr} = \upsilon_s$ and $\omega_{rr} = \omega_{sr}$ into Eq. 4) and also equal to Φ_3, which is now independent of p_0.

In all three special cases (Fig. 3c–e), if the proportion of Bt crops (Φ) is below the relevant threshold Φ_4, the resistance allele will decline to extinction (sole equilibrium $p^*=0$, globally stable over the range $0<p<1$). If Φ is above Φ_4, p will tend to go to fixation (sole globally stable equilibrium $p^*=1$). These results are consistent with Karlin's (1977) finding in a multi-deme model that, with a dominant or a recessive trait, fixations of the two alleles cannot both (simultaneously) be locally stable. If Φ is equal to Φ_4, the frequency of the resistance allele will remain constant regardless of its initial value, i.e. for any p_0.

At this critical threshold Φ_4, the steady state is not a conventional equilibrium value for the r allele frequency. At a stable (or unstable) equilibrium point, the frequency would not change, but at values of p at either side of the equilibrium, the frequency would move towards (or away from) the equilibrium value. In this case, if $\Phi=\Phi_4$ the frequency p does not change, whatever its value ($\Delta p=0$ for all p). The relative fitness of the various genotypes on conventional and Bt crops (υ_i, ω_i) and the refuge size ($1-\Phi$) are such that the average fitnesses of all three genotypes are equal ($\Omega_{ss} = \Omega_{sr} = \Omega_{rr}$), and so there is no selective pressure, and the frequency of the r allele will remain constant at any value. Any perturbation in allele frequency (assuming no change in refuge size) will merely result in the allele frequency staying constant at the new value. Any perturbation in refuge size will lead to extinction or fixation of the r allele. Therefore, this steady state is unstable with respect to the proportion of Bt crops (Φ) but not with respect to the frequency of the resistance allele (p).

As noted above, resistance to Bt toxins is thought to be recessive for many pests; therefore, if fitness costs are also recessive (as observed in pink bollworm, for example), the situation represented by Fig. 3d is the most likely in practice.

Dependence on initial conditions

Only in the case of partial dominance where $\Phi_1>\Phi_2$ is the critical threshold proportion of Bt crops, Φ_3, dependent on the initial frequency of the resistance allele p_0. However, Φ_3 always lies between Φ_2 and Φ_1 (Fig. 3b). In both the general cases of partial dominance (Fig. 3a and b), a sufficiently large or sufficiently small refuge will determine the ultimate fate of the resistance allele independently of initial conditions. If Φ is below the lower of Φ_1 and Φ_2, the resistance allele will go to extinction regardless of p_0. If it exceeds the higher of Φ_1 and Φ_2, the r allele will go to fixation irrespective of its initial frequency.

Where resistance is co-dominant, completely recessive or completely dominant, the threshold proportion of Bt crops (Φ_4) is independent of the initial frequency of the resistance allele. Thus, the eventual fate of the r allele is not sensitive to its initial frequency.

Comparison of completely dominant and completely recessive resistance

Note that the thresholds for extinction or fixation are the same for completely recessive resistance and completely dominant resistance (Φ_4 see Eq. 7). For any particular homozygote fitnesses (rr and ss), a given refuge size will lead to the same eventual frequency of the resistance allele (i.e. fixation or extinction according to whether the proportion of Bt crops is above or below Φ_4) for both (Fig. 3d and e). However, the rate of approach to fixation or extinction, which depends on the initial resistance allele frequency p_0 and the magnitude of Φ, would be different (compare values of Δp in Eq. 2 with either Ω_{rr} or Ω_{ss} substituted for Ω_{sr}). Either the recessive version or the dominant version can be faster to extinction or faster to fixation, depending on the parameter values and initial conditions.

Discussion

In this study, we have shown from analysis and simulations of a simple population genetic model that there are critical threshold proportions for dividing a habitat, which will determine the equilibrium allele frequencies. We also identified the way in which the nature of the steady states at these thresholds depends on the dominance of resistance and of fitness costs. Our motivation for this was to understand how insect resistance might evolve in response to areas planted with transgenic (Bt) and conventional crops.

Arthropods are well-known to cause major devastation of crops, destroying around 18% of the world annual crop production, and act as vectors for many human and veterinary diseases (Nicholson 2007). Widespread resistance to insecticides among arthropod populations limits the efficacy of pest control. Resistance has now been reported in a wide variety of important insects and against every chemical class of insecticide, including microbial drugs and insect growth regulators (Nicholson 2007).

A recent study (Brookes and Barfoot 2006) highlights global benefits attributed to GM crop technology used by

millions of farmers worldwide, most of them resource-poor farmers in developing countries. Insect-resistant crops have had positive economic effects, increasing global agricultural income, achieving environmental gains, improving health and safety and increasing crop quality. The evolution of resistance to plant-incorporated insecticidal toxins would be of economic and environmental concern.

Analysis of our population genetic model illustrates that the evolution of resistance allele frequency depends on the relative sizes of Bt crop areas and refugia and on the relative fitnesses of the genotypes in each environment. The relative advantages of the genotypes are dictated by the two threshold values Φ_1 and Φ_2 (which depend on the genetic parameters) and the proportion of the habitat that is planted with Bt crops (Φ) relative to those thresholds. A necessary and sufficient condition for stable polymorphism is that $\Phi_1<\Phi<\Phi_2$. This is equivalent to heterozygotes having the highest average fitness across the entire habitat, i.e. 'marginal over-dominance' (Wallace 1968). Similarly, the condition for unstable polymorphism, $\Phi_2<\Phi<\Phi_1$, is equivalent to heterozygotes having lower average fitness than both homozygotes, i.e. 'marginal under-dominance.' The potential for existence of a stable intermediate equilibrium decreases if the *r* allele is increasingly recessive to the *s* allele for resistance (as ω_{sr} approaches ω_{ss}) or with increasingly dominant costs of resistance (as υ_{sr} approaches υ_{rr}).

For all parameter combinations except where there is marginal under-dominance, the outcome is not sensitive to the initial allele frequency. Under the model assumptions, for any set of genetic parameter values, resistance can be reversed, even from very high initial frequencies, by arranging for a sufficiently large refuge to exist for a sufficiently long time (although this may be an impractical or uneconomic solution to managing an actual resistance event). The key assumption here is that resistance has fitness costs, so the *r* allele is always at a disadvantage in untreated areas (or in untreated hosts, in the case of resistant pathogens).

Where both resistance and fitness costs are recessive, dominant or co-dominant, a habitat divided in exactly the critical threshold proportions will maintain allele frequencies whatever their initial prevalence (i.e. not just at a single equilibrium allele frequency but at all allele frequencies). Habitats are usually dynamic systems, so the proportions into which the habitat is divided may fluctuate over time, and the allele frequencies would not be expected to remain constant indefinitely. However, where the mix of environments is at or near a critical threshold proportion or fluctuates around such a threshold, apparent stability might be observed for a number of generations. In resistance management, the wrong size of refuge, untreated area or group could effectively maintain the frequency of resistance alleles, at least for the short term.

The extent to which habitat proportions are amenable to control or influence or even to measurement varies with context. Planting of Bt or non-Bt crops can be controlled with reasonable accuracy, although less so if the pest is a more generalist species and alternative host plants persist nearby. However, the level of drug usage in a population might vary between areas of different transmission intensity, and a policy to limit a new drug to small fraction of cases can be impossible to implement because of preferred use by health professionals and self-treatment by the general public (Hastings and D'Alessandro 2000). Nevertheless, if fitness values can be estimated, a model such as this can indicate the likely limitations on the relative size of the untreated group or area, even if that division cannot be implemented precisely in practice.

Models such as the one presented here identify factors that should be considered in the design of resistance management programmes but that remain unknown and need to be measured in the field or laboratory. It can be difficult, expensive and inaccurate to measure crucial parameters such as baseline frequencies of resistance alleles or small fitness differences between genotypes; rather than attempting to measure these factors directly, practitioners are often limited to considering their implications in principle when designing a control or surveillance project (Hastings and D'Alessandro 2000). Mathematical models provide a powerful tool, setting out key relationships between factors that can assist in the planning and management of insect resistance.

The central assumptions in any mathematical model need careful evaluation. For instance, our assumption of random mating and egg-laying might break down over time. In the long term, genetic traits might arise that limit gene flow, and potential consequences include isolation leading to speciation (Templeton 1981). In the context of resistance to Bt crops, there is no isolating mechanism, but there might be selection for mating barriers between resistant and susceptible forms if the combination of fitness parameters and refuge size makes heterozygotes the inferior form on average (Kirkpatrick and Ravigné 2002). However, the potential for speciation in this manner is likely to be low, as the conditions under which it can arise are restrictive and indirect selection (as here, where an assortment trait would probably be influenced by other genes that are only genetically correlated with the resistance gene, on which direct selection is acting) is a weaker force than direct selection (Kirkpatrick and Ravigné 2002; Templeton 1981). Note that habitat divergence is not an issue in these circumstances because it is unlikely that the resistant phenotype would evolve to be fitter on the toxic crops than in the refuge (for example), so all phenotypes would prefer the same habitat if they were able to identify it.

Although numerous models predict that refugia can delay the evolution of resistance to high-dose Bt crops (Alstad and Andow 1995; Carrière and Tabashnik 2001; Gould 1998; Tabashnik et al. 2005), few of them (Mohammed-Awel et al. 2007; Tabashnik et al. 2005; Vacher et al. 2003) attempt to find a threshold value for refuge size. Typically, a small number of discrete values for refuge size are simulated, and of course, experimental evidence supporting the prediction was likewise restricted to a small number of refuge sizes. Most published models on Bt resistance management are sophisticated simulations, often of specific crops and their target pest insects; the underlying models are too complicated to allow mathematical analysis so that a threshold refuge size could be expressed in terms of fitness parameters and habitat division, as we have done.

Our model provides a framework that is useful for gaining overall insight into complex allele dynamics in heterogeneous habitats. Key conclusions may extend to more sophisticated models. Other numerical simulation models that take account of spatial structure also indicate that there is a critical size of refuge or untreated area determining whether resistance evolves against pesticides (Lenormand and Raymond 1998) and, in a stochastic framework, against Bt crops (Vacher et al. 2003). A simple model including constant immigration of susceptible pests, emigration and density-dependent population growth also identified critical refuge size thresholds, although it showed that in some circumstances with intermediate dominance values for the resistance allele, a refuge could spoil the resistance management benefit from immigration of susceptible insects (Mohammed-Awel et al. 2007). Although we have examined the case of a diploid organism reproducing sexually, population genetics models of insecticide resistance in haplodiploid insects have found results similar to those for diploids (Crowder et al. 2006), and other models suggest that bacteria resistant to antibiotics can only become established when the rate of treatment or prophylaxis exceeds a threshold value (Levin 2001). In the case of pathogens, a fuller understanding would require consideration of epidemiological factors as well as a population genetics approach.

We have presented a model that provides a generic framework with which to gain insights into the evolution of allele frequency in heterogeneous habitats that can be applied in a range of ecological and public health settings. In principle, it could be broadened further by extension to more than two alternative alleles and more than two environments within the habitat.

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