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Spatial and temporal dynamics of gene movements arising from deployment of transgenic crops

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Abstract

Many of the pressing questions about whether or not to release a genetically modified crop can be resolved into population-dynamics questions concerning invasion and persistence of the transgenic crop itself, or of hybrids with other crops and wild relatives. Progress in assessing risk demands a coherent theoretical framework within which questions such as the reversibility of breakdown in pest resistance or ‘escape’ of herbicide-tolerance genes can be phrased and tested. Here we begin to sketch out a framework that provides some answers while taking account of the inherent spatial and temporal variability of agricultural and semi-natural systems. After a brief summary of the risks associated with the deployment of novel crop varieties, we define what we mean by invasion and persistence in variable environments. This leads to a discussion of stochasticity and spatial scales in which we distinguish between different sources of variability that affect the probability of invasion and persistence times. We illustrate this with the distributions of global extinction times of crop plants or hybrids that persist as local patches in the landscape following the introduction of a new variety. Empirical studies of persistence of transgenes are next discussed. We begin with pollen dispersal, which leads to the dispersal profiles from point sources from which separation distances can be calculated to minimize contamination between transgenic and conventional crops. Three important aspects that emerge from some work on the dynamics of feral populations of transgenic crops are identified: feral patches of some transgenic crops are transient with short persistence times; there is large environmentally-driven variability in ecological performance; the seed bank is an important reservoir from which plants may emerge even after a patch appears to have gone extinct. Next we show how such variability can be incorporated into stochastic life history models, from which it is possible to identify intrinsic, genetically controlled properties of crop plants that favour persistence. These analyses suggest how to design new crop varieties with characters that minimize the risk of spread and persistence in the landscape. Finally, we introduce landscape-dynamic models in which we show, using

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individual-based percolation and metapopulation models, how the spatial arrangement of crop plants and feral patches of ‘escaped’ plants in the landscape influence invasion and persistence.

Keywords: Invasion; persistence; stochastic variation; life-history parameters; metapopulation analysis

Introduction

The deployment of any novel crop variety is seldom risk-free. The objective of much recent economic and biological modelling is to assess these risks in order to decide whether or not it is profitable and environmentally safe to release a novel crop. Recent advances in molecular biology that allow greater genetic control in the design of modern crop varieties promoting enhanced yield, greater pest and disease resistance, tolerance to herbicides as well as novel crops for biofuel, biopolymers, nutrients and biopharmaceuticals all lead to increased urgency for a robust theoretical framework for decision-making. In this chapter we focus on the spread of genetically modified plants and their introduced foreign genes (transgenes) into the environment. We argue that stochasticity plays an important role in successful invasion and persistence of these transgenes. We also show how, using ecological analyses of the population dynamics under variable environments, it is possible to identify which life-history parameters favour invasion and persistence and, hence, which crop characteristics should be targeted in breeding programmes so as to minimize the risk of unwanted spread and persistence.

Our analyses hold for the deployment of any novel crop variety, whether conventionally bred or genetically manipulated by transformation. Conventional plant breeding has been remarkably successful in steadily increasing yields of agricultural crops (Evans 1998). The demand for yet more progress continues to rise, however, with global population growth, so that the global demand for cereals is expected to increase by 30% by 2020, with developing countries accounting for two thirds of this demand (Rosegrant et al. 2001). Recent developments in molecular biology are likely to have an important impact on breeding technology. Thus, instead of selective breeding and reassortment of 50 to 100 thousand genes with up to ten recurrent selective back-cross generations to recover an elite line, modern methods of marker-assisted breeding allow the identification of many fewer genes that must be changed to achieve a successful line. Although this may involve transfer of genes between species more often than not it will occur between cultivars within the same species. Still, there remains uncertainty about the economic and environmental benefits and costs of releasing these newly developed varieties into commercial agriculture. Will, for example, a new variety continue to out-yield the conventional varieties it replaces? How does this change from year to year? Will novel pest or disease resistance remain effective or will it be necessary to supplement weakened genetic control with pesticides?

Some consequences of the deployment of novel varieties are classified as ‘irreversible’ by economists but still subject to uncertainty from a biological – and economic – perspective. These include benefits such as reduced risk of resistance to pesticides or to accumulation of toxic pesticide residues in soil, water or crops, following the release of varieties that are genetically resistant to pests or pathogens. Still greater uncertainty surrounds the irreversible costs associated with the release of a new variety. The principal concerns here are focused on:

- unexpected human-health risks from genetically-modified crops in the food chain;
- loss of biodiversity or an unfavourable change in ecological balance because of enhanced persistence or invasiveness of a genetically-modified crop;
- escape of transgenes to other crops or wild relatives.

Irreversible costs may also include squandering of resistance or toxin genes by promoting premature build-up of counter measures in a pest population so preventing further use of the resistance or toxin gene.

Many of these irreversible costs can be resolved into population-dynamic questions concerning invasion and persistence.

- Will a gene for pest resistance or herbicide tolerance ‘escape’ from a crop plant into a wild relative and if it does will the resulting hybrid invade?
- Will it replace the wild type?
- Will both persist?
- Is the process irreversible whereby one replaces the other and what is the time to extinction of one or the other form and hence the time-frame for irreversibility?

Gilligan (2003) has recently discussed the epidemiological perspective of pest resistance for transgenic crops in the context of options analysis advocated by Morel et al. (2003) and Wesseler (2003). He showed that: (i) theoretical progress can be made in predicting the risk of invasion and persistence of resistant pests and diseases; (ii) deterministic models are useful in identifying crude criteria for invasion but stochastic population models are essential to understand the risk of invasion and to identify strategies to minimize risk. The focus there is on the effects of new varieties on the dynamics of microbial and pest populations. Here we consider the spread of transgenes from crop plants. The spread of genes from these crops can originate from hybridization with wild or cultivated relatives or from transgenic seeds that disperse and potentially establish a persisting feral population of transgenic plants. Essentially both depend on the fitness of the resulting population irrespective of whether or not it arose by pollen or seed flow. Accordingly, we present below some analyses that focus on the effects of fitness on invasion and persistence and how transgenic crops may be designed to minimize the unwanted spread. We do this without proposing recourse to ‘terminator genes’ which themselves pose environmental threats (Masood 1998).

We first explain what we mean by invasion and persistence in variable environments and introduce some concepts of demographic and environmental stochasticity. Next we summarize some important empirical studies of pollen dispersal, with particular emphasis on separation distances between crops to minimize the risk of contamination and introgression, followed by a brief discussion of empirical studies of seed persistence. The importance of chance and space is then described for life-history models which leads naturally to landscape-dynamic models in which we consider the percolation of transgenes through a dynamically changing landscape.

Invasion, persistence, variability and spatial scales

Invasion and persistence of transgenic plants occur at a range of scales from the field through the farm to the regional scales. This resolves into a hierarchy of patches for which we distinguish two types of patch. A feral patch occurs on sites that have not previously grown the transgenic crop. Feral patches are often located around field

or road margins. They are typified by relatively small groups of plants following accidental deposition of grain as it is transported from fields to processing sites (Figure 1). They also occur at sites of cross-pollination with wild relatives. A volunteer patch arises, by contrast, from shed grain at sites within fields that have been sown to a transgenic crop. Volunteer patches often form large reservoirs of seed in the soil but they are regularly disturbed by agricultural cultivation, bringing buried seeds to the surface, where they may germinate or die. Invasion is determined by whether or not there is an increase in density of a transgenic plant or introgressed offspring in a region of interest following introduction. There are two processes, enhanced colonization of previously occupied feral and volunteer sites and colonization of new sites. Hence some analyses may treat the plant as an individual while others treat the patch as an individual. The identification of discrete patches leads naturally to the concept of a metapopulation comprising asymmetrically-sized volunteer and feral patches within a dynamic landscape of sites available for feral colonization (Gaston et al. 2000; Fahrig 1992) that may be supplied by seed and pollen from continued cultivation of a transgenic crop. Within sites, it is usual to define a 'pre-set threshold' density of plants above which a population is assumed to have become established. The identification of criteria for invasion in metapopulations is currently the subject of both empirical and theoretically-motivated research. Within metapopulations, criteria involve analogous threshold densities for occupied patches or for the balance between local extinctions and probabilities of transit between patches. Future work will examine the effects on invasion of geometrical balance and connectivity between field crops, volunteer and feral patches in the landscape. In this chapter, we focus on the stochastic description of patch size, distinguishing between volunteer and feral patches. This leads naturally to consideration of persistence and to the description and analysis of extinction times (Figure 2). Here by adopting a stochastic approach it is possible to derive a distribution for extinction times of a population of feral or volunteer patches (Figure 2). This yields substantially more management information than a simple mean, allowing us to estimate the proportion of patches that might persist for long periods of time.

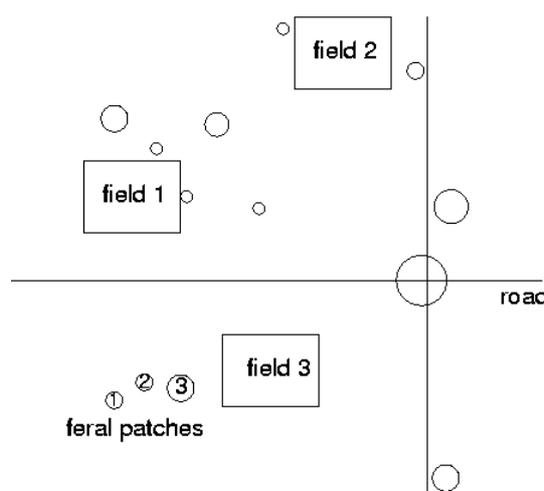


Figure 1. Schematic representation of the local mosaic for persistence of transgenes within fields as crops or as volunteer patches and as feral patches on field boundaries and along roads

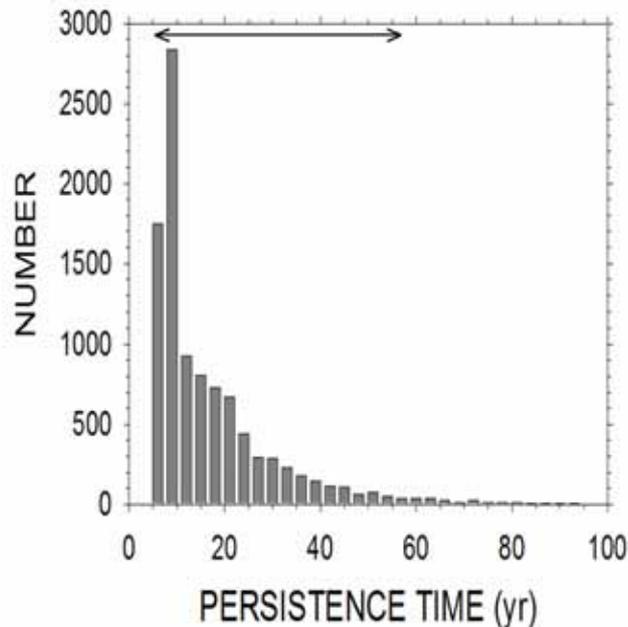


Figure 2. Distribution of persistence times in 10,000 model runs, measured as the time elapsed until the total population abundance falls below a pre-defined extinction threshold, given an initial condition of 100 seeds in a seed bank at $t = 0$. Extinction is defined as the total abundance < 1 individual (i.e., sum of above ground plants and seeds in seed bank). The arrow indicates the median 95% range of persistence times (6 yr – 57 yr), mean persistence time is 16 yr. The data were computed for a model similar to that in Box 1, with the probability of annual disturbance of $p=0.15$

Two broad types of variability affect the spatial and temporal dynamics of gene movement. One is demographic stochasticity, whereby the probabilities of individual seed germination, growth and subsequent seed production vary according to a set of probability-density functions that arise from the birth and death processes themselves. These have parameters that are fixed for a given set of environmental conditions. The second is environmental stochasticity, in which the underlying parameters for germination, growth and seeding fluctuate with environmental conditions. While this is largely driven by weather variables, it can also include other factors, notably disturbance of a volunteer site by cultivation or of a feral site by mowing, grazing or soil disturbance by rabbits or invertebrates.

Empirical studies of persistence

Empirical studies have been used to quantify the dispersal and persistence of transgenes following introduction in agricultural crops. They include quantification of dispersal gradients for the two important pathways of potential transgene spread via seed dispersal or via pollen dispersal (Wolfenbarger and Phifer 2000). They also involve measurements of seed survival as volunteer and feral patches within fields, along field boundaries or road margins as well as empirical tests for the feasibility of hybridization with other crop or weed species. The results of farm-scale field trials in the U.K. on the impacts of transgenic crops on biodiversity (Firbank et al. 2003; 1999) have recently been published (Squire et al. 2003). Here we focus on pollen and seed dispersal and the fate of feral populations, for which two important results emerge. The first is the importance of stochastic variation in determining whether or not a

transgene persists after release in an agricultural crop (Crawley et al. 2001; 1993; Hails et al. 1997). It follows therefore that the mere occurrence of hybridization with another species or of seed shed from a transgenic crop does not guarantee long-term persistence. We need instead to consider the distribution of extinction times (and the corollary persistence times: Figure 2) in order to assess the *risk* of persistence. Second is the challenge of scaling-up from individual dispersal gradients around single sites (comprising transgenic fields or feral patches) to the dynamics of invasion and persistence in a mosaic of loosely-coupled sites (Perry 2002; Colbach, Clermont-Dauphin and Meynard 2001a; 2001b). This leads naturally to the theory of metapopulations and landscape dynamics and is discussed briefly in the section on Landscape-dynamic models.

Pollen dispersal and hybridization

Hybrids of GM crops and wild relatives have been observed for a number of crop species, including maize, oilseed rape, rice and sugar beet (Messeguer 2003). The transfer of a transgene into a non-transgenic crop or wild relative is mediated by pollen transfer and the subsequent occurrence of hybrids finally leading to introgression of the transgene into the recipient population. Four broad risks can be identified with differing economic consequences and public acceptability.

- (i) Small-scale field testing of newly developed transgenic crops is usually done under semi-contained conditions to minimize dispersal of transgenic pollen, and the risk is considered to be small.
- (ii) Consumer preference increasingly stipulates that products from non-transgenic crops should not be contaminated with transgenic material. The main source of such contamination is influx of transgenic pollen into non-transgenic crops. The Advanta Seeds contamination incident (Advanta Seeds UK 2000), whereby large amounts of seed from non-transgenic crops was rejected because of contamination by transgenic hybrids, showed that this risk is real and has large financial side effects.
- (iii) Pollination of wild relatives with transgenic pollen can lead to hybridization. This has, for example, been observed in crosses between the crop species *Brassica napus* and the wild plant species *B. rapa* (Hauser, Shaw and Ostergard 1998). The hybrids have a low fitness but subsequent backcrosses with the wild plant species can lead to the development of a population where the transgene has introgressed into the wild species (Hauser, Shaw and Ostergard 1998). Introgression of transgenes into wild plant species is considered unwanted.
- (iv) Wild plant species that have introgressed a transgene might be more fit than their relatives increasing the risk of weed invasion.

In order to quantify the risk of pollen transfer and the risk of invasion of the transgene through hybridization with non-transgenic crops or wild relatives we need to know the dispersal pattern of transgenic pollen as well as the probability of hybridization and fitness of the offspring. Stochasticity enters via the dispersal range, viability of pollen, viability and fitness of the progeny as well as the relative spatial distributions of donor and recipient sites in the landscape. In this section, we focus on dispersal from a single site and summarize some of the principal considerations to analyse persistence.

Most work to date has focused on the empirical description of pollen dispersal gradients, usually for non-transgenic crops, and, in particular, on the tail of the distribution to assess the furthest extent of transport. This leads naturally to the

concept of a *separation distance* by which criteria are set for the separation of transgenic from other sensitive crops in order to reduce the probability of cross-pollination below a certain defined threshold (Perry 2002). There has therefore been considerable discussion in the general biological literature about the shape of dispersal kernels, particularly with respect to the thickness of the tail which can affect the probability of rare long-distance events and of the shapes of invasion wave-fronts (Ferrandino 1993; Shaw 1995; Kot, Lewis and Van den Driessche 1996; Shigesada and Kawasaki 1997). Two broad mathematical approaches have been used, one based on empirical description of dispersal gradients, the second on physical models for dispersal of particulates (Csanady 1973; Nieuwstadt and Van Dop 1982). Physical models include the effect of wind, turbulence, deposition and in some cases elements of landscape structure. These models have been applied to the dispersal of fungal spores, encysted bacteria and pollen (e.g. Aylor 1986) but so far relatively little exploitation has been made of the stochastic description of these models from which we may be able to ascertain risk of dispersal under a range of environmental conditions. Most attention has instead been directed towards the use of empirical models to describe dispersal profiles. Practical distinction of thick-tailed (typified by power-law, Pareto or Cauchy distributions) and thin-tailed (usually exponential) dispersal kernels, remains difficult, however, because of the problems of low detection rates at these long distances. These give few data-points in the critical range with which to distinguish models so that there may be considerable error in the estimation of rare distant events. Other factors can bias estimation. Recent reports of long-distance pollen transport leading to transgenic contamination of maize plants in Mexico (Quist and Chapela 2001; 2002) is now thought to have been due to illegal planting of GM seed by growers (Perry 2002). We argue below (Landscape-dynamic models section) that the tail of the distribution is rather less important than might be thought in predicting whether or not a transgene invades through a heterogeneous landscape, for which the magnitude of dispersal in the mid-ranges may be more important.

The nature of the gradient depends on the method of dispersal. Distances range from 100 to 200 m for short- to medium-distance dispersal to approximately 10 km for long-distance dispersal by insects. The corresponding ranges for wind dispersal are < 1 km for short to medium distances but the long distances extend to hundreds of kilometres. Some crops have more than one dispersal mechanism, leading to bimodality in dispersal ranges. Simple dispersal profiles for pollen transfer can be obtained by sampling the air. Recent developments in molecular diagnostics and biosensing promise improved specificity in detection and automatic quantification. Mere arrival of the pollen is not enough to guarantee hybridization. In practice, trap plants are usually used to sample pollen yielding dispersal profiles for successful hybridization that automatically take account of the uncertainty in viability of pollen. Yet more uncertainty arises after hybridization, since Hauser, Shaw and Ostergard (1998) have shown that there is considerable variability in the fitness of F1 hybrids and further backcrosses with the wild relative. We conclude that stochasticity is therefore of key importance in pollen dispersal and hybridization and must be given greater prominence in evaluating risks of pollen dispersal.

Separation distance

Consideration of the risks described above, in combination with the pollen-dispersal models, has led to the idea of a *separation distance*, the distance between the transgenic pollen source crop and the nearest non-transgenic crop or population of

wild relatives. Methods are currently being developed to decrease the risks below a threshold that is perceived acceptable by regulations on minimum separation distance (Perry 2002; Firbank et al. 1999). For small-scale field testing of newly developed transgenic crops separation distances of 50 to 400 m and greater have been required in the past (Scheffler, Parkinson and Dale 1995). These separation distances were mainly based on those commonly used by plant breeders in the production of certified or basic seed. Experiments with *B. napus* showed that these distances of 400m result in hybridization ratios (fraction of hybrid seeds in the yield) of 0.3 - 4.0% (Scheffler, Parkinson and Dale 1995), which exceeds the threshold of 0.1% level that has been suggested to be publicly acceptable (Meacher 2001).

Perry (2002) discussed the relation between separation distance and the fraction of the agricultural land that can be used to grow a non-transgenic crop that has a hybridization ratio below the acceptable level, or conversely the fraction of agricultural land available to grow a transgenic crop given existing non-transgenic crops that must have this low hybridization ratio. He showed that the increase in separation distance currently under review by the UK government would have serious implications for future coexistence of non-contaminated non-transgenic crops and transgenic crops. A more involved treatment of the same problem including realistic pollen-dispersal models and realistic crop field spatial patterns can be found in Colbach, Clermont-Dauphin and Meynard (2001a; 2001b).

Hybridization rates between transgenic crops and wild relatives have been intensively studied for a variety of species when they are in close proximity (Wilkinson et al. 2000; 2003) Very little is however done on the relation between separation distance and the rate of hybridization. Moreover, the spatial spread of wild plant species with an introgressed transgene has not been studied so far.

Feral population dynamics

Many crops, such as oilseed rape and sunflower, are annuals that were originally selected from weedy ephemeral plants (Linder and Schmitt 1995). They both have a high seed yield but low competitive ability so that recruitment depends critically on disturbance of the vegetation cover to provide a site for invasion. The frequency of environmental disturbance therefore plays an important role in the invasion and persistence of these populations. Most European work has been focused on oil-seed rape and sugar beet. Crawley and Brown (1995) mapped the occurrence of feral populations of oilseed rape (i.e., patches of flowering plants) along the M25 motorway orbiting Greater London. Their results suggest that, in the absence of disturbance, the typical fate of a population of oilseed rape is local 'extinction' (i.e., absence of flowering plants) within 2 – 4 years, due to overgrowth by perennial grasses. However, absence of flowering plants does not imply true extinction of the population because viable seeds may still persist in the seed bank. In France, flowering plants from feral roadside populations were identified as an old cultivar which had not been cultivated for at least 8 – 9 years (Pessel et al. 2001). Unfortunately, it is unclear whether these populations had flowered every year or whether survival in the seed bank had allowed the populations to persist. Yet, seed-burial experiments show that oilseed-rape seeds, once dormant, have a high survival rate in the seed bank of >60% per year (P.J.W. Lutman, pers. comm.), suggesting that long-term persistence of the seed bank is possible. Hence disappearance of adult plants from a patch in one or more successive years does not necessarily mean that the seed bank is extinct.

Nevertheless, current evidence suggests that feral populations of oilseed rape are ephemeral. Further evidence for this and other crops comes from a large-scale field experiment in which Crawley et al. (2001; 1993) compared the performance of transgenic and conventional lines of oilseed rape, sugar beet, maize and potato in a range of 12 natural habitats in the UK over ten successive years. None of the transgenic traits was expected to increase fitness in the given environmental conditions. For oilseed rape, the field experiment was complemented with a seed-burial experiment in which the fate of transgenic seeds in the seed bank was compared with that of the conventional line (Hails et al. 1997). Overall, the transgenic lines did not out-perform the conventional line. Rather, there was a tendency for the transgenic lines that do perform less well than the conventional one, in particular in the seed-bank survival experiment (Hails et al. 1997).

Estimates of the population growth rate varied by more than two orders of magnitude between years and sites, but varied much less between conventional and transgenic lines in the same year and the same site (Crawley et al. 1993). This shows not only the importance of extrinsic factors such as disturbance for these crops, but also the importance of genotype x environment interactions. All seeded plots in the study by Crawley et al. (2001) went extinct within the 10 years of the monitoring period, most of them in two or three years. A smaller-scale field experiment with *Bt*-insecticidal oilseed rape shows that *Bt* transgenes can increase fitness when the plants are subjected to insect predation (Stewart et al. 1997). The differences between the results of Hails et al. (1997) for oilseed rape and Stewart et al. (1997) for maize show that the risk lies typically in those cases where the transgene *does* have an effect on life-history traits which increase fitness and favour persistence (see Life-history models section).

Three important aspects of the dynamics of feral populations of crops emerge from the work by Crawley:

- (i) there is large environmentally-driven variability in ecological performance;
- (ii) many feral patches of transgenic crops are transient with relatively short persistence times;
- (iii) the seed bank is an important reservoir for long-term survival from which plants may emerge even after a patch has appeared to become extinct.

It follows from these empirical analyses that because there is rapid local extinction of feral patches, then global persistence of a transgenic crop can only occur if there is continual input to the system. Without this, feral patches, at least of oilseed rape, will die out. Reinvasion occurs through continual cultivation of the transgenic crop yielding a reservoir of volunteer patches from unharvested seed and a spatially dynamic reservoir of spillage from harvested seed to establish new feral patches.

Life-history models

Empirical studies show that variability is an important aspect of the spread and persistence of transgenes, both via seed dispersal and via pollen dispersal. Field-release experiments are the most reliable way to assess the fate of specific GM plants in cultivated or natural environments. Yet, such experiments do not get at mechanisms by which the risk can be controlled. One approach is to use life-history models to identify which features of transgenic plants ought to be selected to inhibit invasion

and persistence. The formulation of a simple life-history model is summarized in Box 1.

Box 1. The matrix population model

Deterministic formulation

Here we formulate a simple matrix model that relates the state of the population in year t , denoted by $\mathbf{n}(t)$, to the population next year, $\mathbf{n}(t+1)$. The elements of the vector $\mathbf{n}(t)$ represent the number of adult plants, $n_1(t)$, and the number of seeds in the seed bank, $n_2(t)$, respectively. The projection from year to year can be represented in vector-matrix notation as

$$\mathbf{n}(t+1) = \mathbf{A}\mathbf{n}(t)$$

(Caswell 2001). The projection matrix \mathbf{A} summarizes the contribution from each category to the population structure next year and is the mathematical representation of the life cycle of the studied population. In our model (Claessen et al. in press)

$$\mathbf{A} = \begin{pmatrix} F(1-\mu)[d\sigma_2G + (1-d)\sigma_1]S & \sigma_2GS \\ F(1-\mu)d\sigma_2(1-G)s_2 & \sigma_2(1-G)s_2 \end{pmatrix}$$

and the interpretation of the matrix \mathbf{A} corresponds to a description of our assumptions on the life cycle: adult plants each produce F seeds, of which a fraction μ disperses. Of the remaining fraction $(1-\mu)$, a fraction d is incorporated in the seed bank. In the seed bank, seeds have a probability of σ_2 to survive the winter while a fraction G germinates in spring. Of the seeds that did not enter the seed bank, i.e. the fraction $(1-d)$, a fraction σ_1 survives the winter as seedlings. Seedlings have a probability S to reach the flowering stage. Together, these assumptions are translated in the element a_{11} of \mathbf{A} : the contribution of adult plants to new adult plants. In addition, new adult plants can emerge from seeds in the seed bank that survive the winter, germinate and reach the flowering stage, i.e. σ_2GS , which equals element a_{12} of \mathbf{A} . Element a_{21} represents the seeds produced by the current adults which enter the seed bank and do not germinate next spring, while element a_{22} represents current seeds in the seed bank that survive and remain in the seed bank.

Stochastic formulation

We incorporate chance by letting some life history processes depend on a stochastic environmental variable. First, fecundity F is assumed to be high in meteorologically favourable years ('good' years), and low in others. Good and bad years are assumed to occur with equal probability but this can easily be adjusted to reflect different environments. Second, germination G and seedling survival S are assumed to be high in years when the habitat is disturbed, with probability p , which reduces competing vegetation, and low in others. The combinations of good and bad years, and of disturbed and undisturbed habitat, produce four different environmental conditions, each occurring with a fixed probability. For each condition we can write down the corresponding projection matrix by substituting the values of F , G and S . The stochastic matrix model is hence equivalent to drawing randomly a matrix out of four possible matrices, at each time step, and can thus be written down as

$$\mathbf{n}(t+1) = \mathbf{A}_i\mathbf{n}(t),$$

in which environmental stochasticity enters via the transition matrix.

Deterministic formulation

Transgenes in plants affect life-history traits such as fecundity, plant survival and survival in the seed bank (Wolfenbarger and Phifer 2000). Some changes are intentional, such as increased plant survival and fecundity conferred by a *Bt* transgene (Stewart et al. 1997) while others are unintended 'side effects' of the transgene, such as increased seed-bank survival conferred by a transgene for oil modification (Linder and Schmitt 1995). In the face of genetic mechanisms such as recombination,

however, it remains to be seen how stable such side effects are in successive generations.

The expected rate of population growth, $\log \lambda$, can be estimated from knowledge of the life-history traits using matrix population models (Caswell 2001). Assuming a small population size, as is realistic for an invading species, $\log \lambda$ is equivalent to fitness (Metz, Nisbet and Geritz 1992). It indicates whether a population is growing (if $\log \lambda > 0$) or declining (if $\log \lambda < 0$). In addition, with elasticity analysis the sensitivity of population growth to model parameters can be computed. The latter analysis identifies life-history traits which, if modified by genetic engineering, have the largest impact on population growth rate (Claessen et al. in press; Bullock 1999). Modifying such traits can hence either produce an invasive crop, or a GM crop with a reduced capacity to invade and persist.

Several studies have used mathematical models to estimate λ of transgenic cultivars in a particular habitat (Crawley et al. 1993; Bullock 1999; Parker and Kareiva 1996). The fact that these investigations use deterministic models is a major shortcoming, however, because the dynamics of feral-crop populations are inherently influenced by unpredictable environmental variability.

Introducing chance

Here we focus on environmental stochasticity because it introduces and captures the natural variability in weather and disturbance from season to season. Apart from influencing the long-term population growth rate, environmental stochasticity also affects the persistence of a population. A run of bad years may drive a population with a positive expected population growth rate to extinction while, in contrast, a run of good years may result in long persistence of a population which is expected to go extinct.

We studied a stochastic matrix population model of feral oilseed rape, taking into account that in any given year the vegetation cover in the habitat may be disturbed or not, as well as that seed production is high in favourable years and low in others (Claessen et al. in press). Short-lived plant species like oilseed rape are typically structured into a seed bank and established plants above ground. The matrix model projects the number of individuals above ground and in the seed bank in one year, to the number of individuals above ground and in the seed bank in the next year. The contribution of each individual to the next year is determined by the life-history parameters given the current environmental conditions. For example, the fraction of seeds in the seed bank that germinates and emerges is larger if the habitat is disturbed. Seedling survival is also positively affected by disturbance, while survival in the seed bank is assumed to be unaffected by environmental conditions. The model is parameterized for oilseed rape in a feral habitat, but can be adapted to represent other crops or other habitats, by choosing appropriate values of the life history parameters.

In a stochastic matrix model the population growth rate is computed as the geometric mean of annual growth factors, and denoted $\log \lambda_s$ (Caswell 2001). Invasion and establishment happens with a non-zero probability only if $\log \lambda_s > 0$ (Metz, Nisbet and Geritz 1992). If $\log \lambda_s < 0$, extinction is certain, although the timing of extinction is uncertain. Persistence can be measured as the time it takes to go extinct given a certain initial condition. For feral oilseed-rape populations the predicted persistence times, depending on $\log \lambda_s$, are shown in Figure 3a. The 95% confidence interval spans many decades for most values of $\log \lambda_s$, indicating that persistence is highly variable.

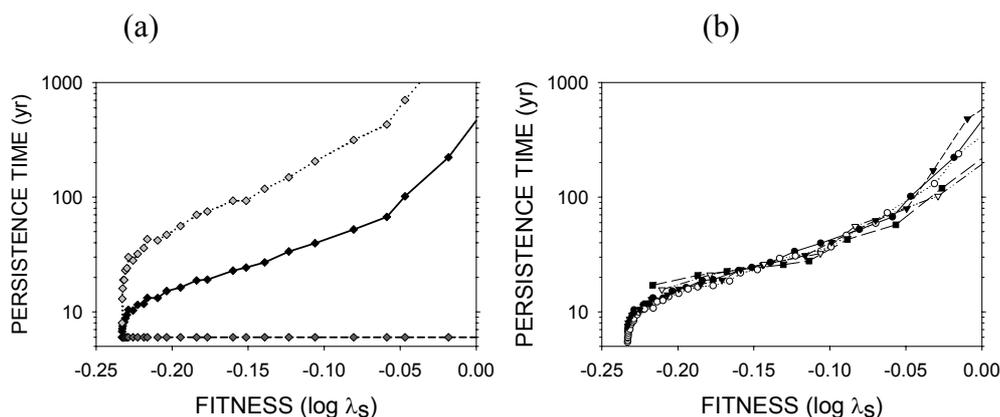


Figure 3. Persistence time computed as quasi-extinction time, versus fitness measured as $\log \lambda_s$. (a) Mean persistence time and the 95% confidence interval for the default parameter set for a feral oilseed-rape population. (b) Mean persistence time for five different parameter settings

Importantly, persistence depends on overall fitness and not on the details of the life-history parameters (Figure 3b). For the issues of invasion and persistence the basic parameter that matters is therefore the population growth rate. Figure 4 summarizes the relation between invasion, persistence and fitness.

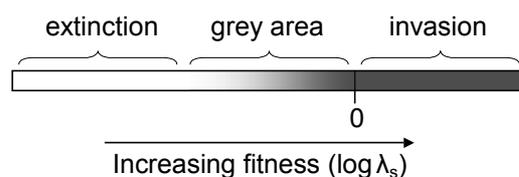


Figure 4. Schematic representation of the relation between fitness, extinction and invasion. If fitness is positive, invasion followed by establishment happens with a certain probability but environmental stochasticity may drive the population to extinction. If fitness is negative extinction is certain. However, if fitness is negative but close to 0 the time to extinction can be very large. In this 'grey area' transient persistence occurs

The determining role of $\log \lambda_s$ means that we can use elasticity analysis to refine the analysis, because it allows us to identify life-history parameters with large impact on fitness and hence on both invasion and persistence. From elasticity analysis we conclude that for oilseed-rape survival in the seed bank is of over-riding importance, while fecundity and plant survival appear less critical (Claessen et al. in press).

Landscape-dynamic models

Whether or not a transgene invades and persists in the landscape leads naturally to consideration of landscape-dynamic models in which spread occurs through a dynamic mosaic of habitable sites. Here we think of a dynamically changing mosaic of habitable sites and occupied sites. Some simple models that underlie the ideas are summarized in Box 2.

Persistence of a transgene in feral or volunteer patches within the landscape depends on the balance between the rate of occupation and extinction of these patches. These suggest simple criteria for invasion (Box 2) but they do not take explicit account of the spatial arrangement of patches nor of stochasticity in extinction and colonization.

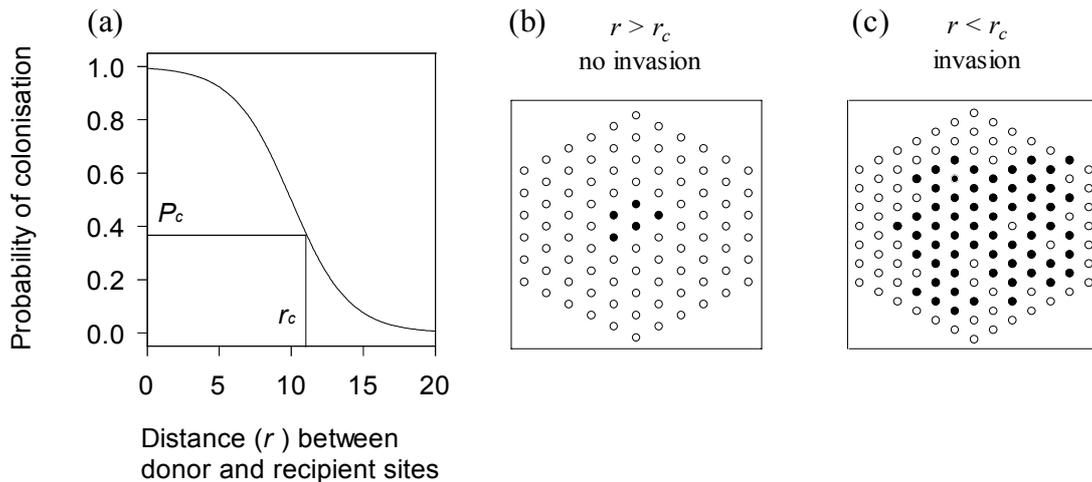


Figure 5. Use of percolation thresholds to predict whether or not a transgene invades across a lattice of contiguous sites. (a) Dispersal profile for transmission from donor to recipient sites. For a given lattice shape (here shown as triangular) there is a characteristic probability (P_c) for percolation that can be used to predict the critical distance (r_c) between sites on the lattice for percolation to occur in a population of sites. (b) Example of restricted spread for inter-site distances $r > r_c$. (c) Percolation through the landscape for $r < r_c$. The hollow sites are uncolonized and the dark sites colonized

The patch-occupancy rate depends upon the density of transgenic crops within a region, from which volunteer or feral patches arise. The extinction rate is influenced by the fitness of the plants as well as disturbance rate and weather. Although it is arguably a useful, if simplistic, starting point this model is naïve in that it assumes β_i (Box 2) to be one constant value for all patches. The model thus does not take into account that movement throughout the landscape is restricted. The probability of transmission of both seed and pollen decreases away from the source, so that dispersal is localized. This leads to a stepping-stone model for patch occupancy, whereby the transgene spreads through a spatially defined set of sites. One appealing simplification of this approach is to consider the system first as movement through a lattice, adapting models from statistical physics and epidemiology (Gilligan 2002; Grassberger 1983). Under static conditions (in which the locations of habitable sites do not change), percolation theory predicts that there is a critical probability (P_c) that corresponds with a threshold inter-patch distance above which the system is connected and invasion occurs (Figure 5). Below the threshold, contiguous patches may be colonized but the patches occur in isolated fragments and invasion is restricted. (At the notional threshold, theory predicts that the system will be self-similar, having clusters of all sizes from single sites right up to connect the entire lattice (Stauffer and Aharony 1991).) Hence the critical probability marks a phase transition between isolated sites with a very low probability of invasion below the threshold, while above the threshold the probability abruptly increases. Clearly habitable patches do not occur on a lattice but the concept can be extended to spread on a random graph. Moreover, the dependence of the phase transition on values for $p > 0.35$ (depending on the nature of the lattice) rather than on the tail of the distribution shows again the importance of considering the mass of the dispersal profile well above the tail.

Box 2. Patch-occupancy models

One of the simplest models for patch occupancy derives from the first metapopulation model proposed by Levins (1969) in which each site is either occupied (I) or unoccupied ($1 - I$) and there is a colonization rate (β) and an extinction rate (δ), giving for continuous time,

$$\frac{dI}{dt} = \beta I(1 - I) - \delta I .$$

This yields a simple expression for the equilibrium density of occupied patches, $\hat{I} = 1 - \delta / \beta$, with a criterion for invasion $\beta / \delta > 1$. Many systems have two sources of occupation, one from transgenic crops (β_1) and the other from feral or volunteer patches (β_2), giving

$$\frac{dI}{dt} = (\beta_1 I + \beta_2)(1 - I) - \delta I ,$$

and an equilibrium density of occupied patches of $\hat{I} = m \pm (m^2 + n)^{1/2}$, where $m = (\beta_1 - \beta_2 - \delta) / 2\beta_1$ and $n = -\beta_2 / \beta_1$.

Here the number of patches is treated as fixed. Keymer et al. (2000) circumvented this by adapting earlier models for landscape dynamics and patch occupancy in which patches appear and disappear. They have three classes of patch, ‘habitable and empty’ (S), ‘habitable and occupied’ (I) and ‘non-habitable’ (R). (The variables S, I, R are used because of analogy with epidemiological models, where S, I, R refer to susceptible, infected or removed states.) Keymer et al. (2000) introduced two parameters for patch dynamics, representing the rates of patch creation (λ) and patch extinction (e) as well as two parameters for biological dynamics of colonization (β) and local extinction (δ), analogous to those above. The mean-field form of the model is now given by:

$$\text{Empty habitable patches} \quad \frac{dS}{dt} = \lambda R - \beta SI + \delta I - eS ,$$

$$\text{Occupied habitable patches} \quad \frac{dI}{dt} = \beta SI - (\delta + e)I ,$$

$$\text{Non-habitable patches} \quad \frac{dR}{dt} = e(S + I) - \lambda R .$$

Analysis of this simple mean-field model shows that, when allowance for patch dynamics is made, the criterion for invasion becomes $R'_0 = R_0 \bar{n} \gamma > 1$, where $R_0 = \beta / \delta > 1$ is the criterion derived above for fixed patches (and $\beta_2 = 0$) above. The new criterion depends on the inherent life history ($R_0 = \beta / \delta$, the balance of colonization to extinction), the long-term proportion of habitable sites ($\bar{n} = \bar{S} + \bar{I} = \lambda / (\lambda + e)$) and $\gamma = \delta / (\delta + e)$, which is the ratio of intrinsic extinction to effective extinction because of patch extinction in addition to biological extinction (Keymer et al. 2000). Moreover, it is possible to identify the minimum amount of suitable habitat (n_{\min}) that a dynamic landscape needs in order to support the colonizing agent:

$$n_{\min} = \frac{1}{\beta} \left(\delta + \frac{1}{\bar{\tau}} \right),$$

where $\bar{\tau} = 1/e$ is the average life span of a habitable patch. Stochastic analyses show that while the mean field model captures the qualitative behaviour of the spread it grossly underestimates the time taken to achieve dispersal (Keymer et al. 2000).

Patches, of course, are not fixed. They are ephemeral. Habitable patches appear and disappear, thereby making and breaking local connections across which a transgene can spread, and now the problem becomes one of directed percolation. The approach of Keymer et al. (2000) offers a promising way forward to examine extinction in this framework. Fahrig (1992) had earlier shown that when the rate of creation and extinction of patches is fast, the details of dispersal distance and inter-

patch distance are relatively unimportant in influencing persistence. That work considered the density of plants within patches. Keymer et al. (2000) instead consider simple patch occupancy. By analysing the model first as a mean-field (Box 2) and then as a stochastic, patch-based model with dispersal amongst nearest neighbours Keymer et al. (2000) were able to show how the criterion for invasion was affected by the appearance of patches. This in turn allows prediction of the amount of suitable habitat that is required for persistence of a transgene in the landscape.

So far we have been considering presence or absence of the transgene at a habitable site. Clearly, the density of plants carrying the transgene will influence the chance of local extinction as well as the force of colonization for new sites. This leads naturally to consideration of structured metapopulations (Gyllenberg, Hanski and Hastings 1997) in which the stochastic dynamics within and between patches are explicitly modelled in order to identify criteria for invasion as well as persistence times. Recent work on the analogous problem of invasion of animal and plant disease in epidemiology, has shown how the number and size of patches affect the time to extinction of the entire population (Park, Gubbins and Gilligan 2001; 2002; Swinton et al. 1998). The theory shows that median extinction times pass through three regimes as patch population size increases. For small patch population sizes, few individuals make it to another patch and the extinction time scales with the log of population size. As population size increases, so spread occurs to some but not all available patches and median extinction times (across the entire population) tend to be longer but very variable. In the third regime, extinction time scales (T_E) with $T_E = a + bn^{1/2}$, where a is the local extinction time, b is the transit time from arrival in one patch till spread to the next and n is the patch population size (Park, Gubbins and Gilligan 2002).

The models introduced above all assume some overt over-simplification such as distributions of habitable sites on a lattice, or if in free-space, then that the patches are uniform in size. Colbach, Clermont-Dauphin and Meynard (2001a; 2001b) took a more challenging route to modelling reality in devising a detailed simulation model to map fields and feral patches. Our preference is to search for parsimonious metapopulation models in which we simplify the rules for spread in order to predict the risk of persistence. This may involve restriction of dispersal to local contiguous populations and habitable patches or perhaps dual sources of spread with a predominance of short-distance dispersal and some occasional long-distance dispersal analogous to small world mixing (Strogatz 2001). Some support for restricted dispersal in the regional spread of transgenic hybrids from oilseed rape comes from empirical work by Wilkinson et al. (2000). *Brassica napus* can hybridize with several species, notably *B. rapa*, which tends to be found in the U.K. near river courses, and *B. oleracea*, which is found close to coasts. Earlier work has shown that hybrids with *B. rapa* may exceed 90% when the recipient occurs as a weed in an oilseed-rape crop but this plummets to < 2% in natural populations where most pollen is from *B. rapa* (Scott and Wilkinson 1998). Wilkinson et al. (2000) concluded that hybridization would therefore be restricted to sympatric populations. An initial survey of a 100 x 120 km region in Southeast England revealed that only two *B. rapa* populations were found adjacent to *B. napus* fields, and out of the 305 plants in those populations, only one individual appeared to be a hybrid (Wilkinson et al. 2000). Subsequent work at the national scale has suggested that this inference may have been overly conservative and that widespread, relatively frequent hybrid formation is inevitable from male-fertile GM rapeseed in the UK (Wilkinson et al. 2003). Hybridization, however, does not necessarily lead to invasion, not least because of stochastic effects on spatially

isolated events. We conclude that, because of this, the metapopulation framework, with allowance for stochastic extinctions, is well suited to the analysis of landscape-dynamic models of transgene invasion and persistence.

Conclusions

Our intention has been to outline a theoretical framework for the spatial and temporal dynamics of gene movements arising from the deployment of transgenic or other novel crops. In doing so we have sought to emphasize the importance of stochasticity in invasion and persistence of transgenic crops and wild hybrids. We have also tried to keep models sufficiently parsimonious so that on balance they shed more 'light' than 'darkness' on the dynamics of transgene spread. Consideration of empirical data for pollen dispersal and the persistence of feral patches of transgenic crops underlines the importance of chance variation in influencing invasion and persistence, although data remain scarce. We have distinguished environmental from demographic stochasticity. Environmental stochasticity is characterized by year-to-year and site-to-site variation as well as the frequency of disturbance of feral patches. Demographic stochasticity reflects chance variation in seed set, seed survival or in hybridization with another crop or wild relative, when relative population densities of donor and recipient become important in influencing successful reproduction and survival. One of the most important results of stochastic analysis is the derivation of probability distributions for persistence times for a population distributed amongst feral, volunteer or other patches (Figures 2 and 3). These allow assessment of the risk of long- or short-term persistence. Despite the complexities associated with plant growth in variable environments, we have shown that it is possible to model the population dynamics of transgenic plants. The use of life-history models, in which the growth, reproduction and survival of transgenic plants are characterized by relatively few parameters, shows how transgene-induced life-history changes affect persistence times. This suggests a challenging and attractive possibility of designing crop varieties that would have low persistence as feral colonizers. Such design of crop ideotypes is not new (Donald 1968). Finally, landscape models, including percolation and metapopulation models provide a framework together with stochastic life-history models not only to assess risk but ultimately to minimize risk by optimizing the spatial deployment of novel crop plants in the landscape.

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