



## Landscape refuges delay resistance of the European corn borer to Bt-maize: A demo-genetic dynamic model

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### ABSTRACT

We constructed a reaction-diffusion model of the development of resistance to transgenic insecticidal Bt crops in pest populations. Kostitzin's demo-genetic model describes local interactions between three competing pest genotypes with alleles conferring resistance or susceptibility to transgenic plants, the spatial spread of insects being modelled by diffusion. This new approach makes it possible to combine a spatial demographic model of population dynamics with classical genetic theory. We used this model to examine the effects of pest dispersal and of the size and shape of the refuge on the efficiency of the "high-dose/refuge" strategy, which was designed to prevent the development of resistance in populations of insect pests, such as the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera, Crambidae). We found that, with realistic combinations of refuge size and pest dispersal, the development of resistance could be considerably delayed. With a small to medium-sized farming area, contiguous refuge plots are more efficient than a larger number of smaller refuge patches. We also show that the formal coupling of classical Fisher–Haldane–Wright population genetics equations with diffusion terms inaccurately describes the development of resistance in a spatially heterogeneous pest population, notably overestimating the speed with which Bt resistance is selected in populations of pests targeted by Bt crops.

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### 1. Introduction

Genetically modified crops containing a *Bacillus thuringiensis* (Bt) gene have been produced to control infestations of primary target pests in fields. Bt-transgenic plants are highly toxic to pest larvae and the high concentrations of Bt toxin they contain can almost entirely wipe out the pest during the growing season.

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However, following the work of McGaughey (1985), it has been recognised that target insect species will eventually develop resistance to Bt toxins, potentially decreasing the efficiency of transgenic technology. Consistent with this hypothesis, resistance to Bt-toxins and Bt-crops has already been selected in the laboratory, in several pest species (Tabashnik et al., 2003). Hence, before the first Bt crop was registered, it was already clear that a resistance management strategy would be required to avoid rapid genetic adaptation of the targeted pests.

Comins (1977a) provided the first theoretical demonstration that random gene exchange between insect subpopulations exposed and not exposed to an insecticide can delay the development of resistance if such resistance is recessive. Based on this demonstration, the "high-dose/refuge" (HDR) strategy has

been recommended as one of the best resistance management strategies for transgenic Bt crops (Alstad and Andow, 1995). Andow and Alstad (1998) stressed that the HDR strategy is particularly effective when (i) genes conferring resistance to Bt crops are initially present at low frequencies in natural populations, (ii) Bt crops are toxic enough (“high dose”) to kill both susceptible homozygous and heterozygous individuals in the pest population and (iii) homozygous resistant individuals emerging from Bt crop fields randomly predominantly mate with susceptible individuals emerging from non-Bt crop refuges.

Widespread (32 million ha of total area of Bt crops in 2006 (James, 2006)) and prolonged (since 1996) exposure to Bt toxin has exerted strong selection pressure, favouring the development of resistance in target pest populations. The US EPA therefore decided to implement the HDR strategy, with a view to reducing this selection pressure, and mandatory regulations were imposed in 1995 for Bt cotton and 2000 for Bt maize (Bourguet et al., 2005). The EPA stipulates that each grower planting Bt crops should also plant a non-Bt refuge area close to the Bt field. The current size of these mandatory refuges for Bt maize – between 20% and 50% of the area under Bt crops – was determined from a large number of computer simulations demonstrating that smaller refuges for Bt maize accelerate total population extinction and increase the rate of evolution of resistance. Different sizes of non-Bt refuges are recommended for different Bt crops: for example, refuges for Bt cotton may be as small as 5% of the area under the Bt crop (2008 Technology Use Guide, 2007). Almost all simulations carried out for the development of practical recommendations for growers have been based either on the classical population genetics model of selection (Fisher–Haldane–Wright (FHW) equations with additional terms for spatial diffusion), ignoring most demographic factors and focusing solely on genetic processes (e.g., Alstad and Andow (1995), Vacher et al. (2003), Cerda and Wright (2004) and Tabashnik et al. (2004)), or on highly detailed models making numerous biological assumptions (e.g., Peck et al. (1999), Guse et al. (2002), Ives and Andow (2002), Storer (2003) and Heimpel et al. (2005)). Complex spatially explicit simulation models that capture numerous important details of insect ecology and genetics have predicted that Bt resistance could be delayed by a period ranging from a couple of decades to more than 100 yr (Peck et al., 1999; Caprio, 2001; Ives and Andow, 2002; Guse et al., 2002; Sisterson et al., 2004, 2005), but the excessive complexity of such models and the huge number of parameters they include make difficulties for generalisation of modelling results. This problem is commonly encountered with detailed models: it is extremely hard, and sometimes impossible, to determine many parameters accurately from field data and, in some cases, there may even be no such field data (Ginzburg and Jensen, 2004). Even if all parameters of a complex model are carefully identified for a particular pest it can hardly be generalized for other pests. The distinction between crucial factors and factors of minor importance can also be problematic. The approach based on the FHW model also has several limitations. The main problem is that such models predict the very rapid development of resistance, within only a few decades, even if the initial frequency of the resistance allele is low (Vacher et al., 2003). Explanations of the delayed development of Bt resistance in natural populations of insects based on the FHW model require an additional assumption of a non-zero fitness cost of resistance (Vacher et al., 2003; Cerda and Wright, 2004). However, the existence of a fitness cost of Bt resistance in such pest targeted by Bt crops, as the European corn borer (ECB, *Ostrinia nubilalis* Hübner, Lepidoptera, Crambidae), has yet to be proven. The setting of this parameter of the model is thus also problematic.

We propose a new approach, complementing the classical approach based on FHW equations with the detailed ecological description of pest population dynamics, and providing support for

the results of previous studies based on analytical and simulation models. We have developed a conceptual demo-genetic diffusion (DGD) model to describe the spatio-temporal dynamics of the pest in a spatially heterogeneous environment (see also Zhadanovskaya et al. (2006) and Tyutyunov et al. (2007)). This model is based on a reaction-diffusion system of partial differential equations (PDEs) and the modified Kostitzin model, which describes the local kinetics of competing genotypes (Kostitzin, 1936, 1937, 1938a,b,c). This approach facilitates the explicit modelling of resistance gene evolution in a diploid population, providing an adequate description of the selection process close to or far from Hardy–Weinberg equilibrium (Svirezhev and Pasekov, 1982). Furthermore, using the DGD model, we were able to demonstrate that the conventional FHW model coupled with diffusion terms is a particular case of our DGD model and that Fisher’s diffusion model cannot correctly describe the spatial flux of genes in the pest population within a spatially heterogeneous habitat.

We focused on the effects of spatial factors, including pest dispersal, and of the size and spatial configuration of the refuge on the efficiency of the HDR strategy designed to reduce the selection of resistance in natural populations of ECB. We show that very different simulation results may be obtained with the DGD model and the Fisher–Haldane–Wright diffusion (FHWD) model, and provide explanations for this difference.

## 2. The model

### 2.1. Population genetics

As in most models of the development of resistance to Bt crops (e.g., Peck et al. (1999), Guse et al. (2002), Ives and Andow (2002), Vacher et al. (2003) and Cerda and Wright (2004)) and for several lepidopteran pests targeted by Bt crops, such as the pink bollworm (Tabashnik et al., 2002; Morin et al., 2003) and the tobacco budworm (Gould et al., 1995), we assume that resistance to Bt maize within ECB populations is recessive and governed by a single diallelic locus with a Bt-susceptible allele  $s$  and a Bt-resistance allele  $r$ . Assuming that the resistance allele is inherited in an autosomal manner, the pest population consists of the Bt-susceptible genotypes  $ss$  and  $rs$  and the Bt-resistant genotype  $rr$ . Mating is assumed to be random. Unlike the conventional approach based on the FHW model, we do not use the Hardy–Weinberg principle to determine the relationships between allele and genotype frequencies in the pest population.

### 2.2. Modelling the demo-genetic dynamics of the population

Taking into account both demography and genetics, and assuming that insect infestation in the crop field  $\Omega$  is controlled by the Bt plants, we propose a model based on a reaction-diffusion system of PDEs:

$$\begin{aligned} \partial N_{ss}/\partial t &= W_{ss}b(N_{ss} + N_{rs}/2)^2/N - \alpha N_{ss}N - \mu N_{ss} + \delta \Delta N_{ss}; \\ \partial N_{rs}/\partial t &= W_{rs}2b(N_{ss} + N_{rs}/2)(N_{rs}/2 + N_{rr})/N - \alpha N_{rs}N \\ &\quad - \mu N_{rs} + \delta \Delta N_{rs}; \end{aligned} \quad (1)$$

$$\partial N_{rr}/\partial t = W_{rr}b(N_{rs}/2 + N_{rr})^2/N - \alpha N_{rr}N - \mu N_{rr} + \delta \Delta N_{rr},$$

where  $N_{ss} = N_{ss}(\mathbf{x}, t)$ ,  $N_{rs} = N_{rs}(\mathbf{x}, t)$ ,  $N_{rr} = N_{rr}(\mathbf{x}, t)$  are the genotype densities at position  $\mathbf{x}$  at time  $t$ ;  $N = N_{ss} + N_{rs} + N_{rr}$  is the total population density. We assume that the birth rate  $b$ , the mortality rate  $\mu$ , the competition coefficient  $\alpha$  and the diffusion coefficient  $\delta$  are the same for all genotypes, but that genotypic fitness  $W_{ij}$  ( $i, j = r$  or  $s$ ) may differ between genotypes. Fitness  $W_{ij}$  may be interpreted as the survival coefficient of larvae of genotype  $ij$  as a function of location in the habitat (toxic or non-toxic plant tissues).

We assume a constant 1:1 sex ratio and Mendelian inheritance.

The diffusion coefficient characterises the intensity of the random movements of individuals in the habitat. We assume that there are no density fluxes across boundaries, i.e.,

$$\nabla N_{ij} \cdot \mathbf{n} = 0, \quad \mathbf{x} \in \partial\Omega, \quad (2)$$

where  $\mathbf{n}$  is the external normal to the boundary  $\partial\Omega$ .

The model (1–2) is autonomous, and therefore does not take into account seasonal variations in environmental conditions. It does not take into account the stage structure of the pest population either, assuming instead that death and reproduction occur continuously, at constant rates. In system (1), the random movements of individuals take place on the large spatio-temporal scale of lifetime dispersal. Micro-scale movements of pest insects are ignored.

Differential equations (1) are the continuous approximation of real ecological processes that are discrete in insect populations. However, the proposed model (1–2) is intended solely for long-term forecasting and continuity is a natural simplification that should be borne in mind when interpreting model outcomes.

In the non-spatial (point) case of (1–2), the model coincides with the classical Kostitzin demo-genetic model (Kostitzin (1937); see also Scudo and Ziegler (1976) and Svirezhev and Pasekov (1982)). In this case, in an entirely non-transgenic area (i.e., all  $W_{ij} = 1$ ), summing the three equations (1) generates the simple logistic equation for the growth of the whole pest population:  $dN/dt = bN - \mu N - \alpha N^2$ . If  $b > \mu$  then the ratio  $K = (b - \mu) / \alpha$  is the carrying capacity of the pest population.

The transformation of (1–2) from its density form to its frequency form provides the connection between the two modelling approaches: demo-genetic and conventional Fisher model.

Denoting the allele frequencies by  $p_s = (N_{ss} + 0.5N_{rs}) / N$  and  $p_r = (N_{rr} + 0.5N_{rs}) / N$  and taking into account that  $p_s + p_r = 1$ , we obtain an equivalent frequency form of the DGD model:

$$\begin{aligned} \partial p_r / \partial t &= b p_r (W_r - W) + \delta \Delta p_r + 2\delta \nabla \ln N \cdot \nabla p_r; \\ \partial N / \partial t &= N (bW - (\mu + \alpha N)) + \delta \Delta N, \end{aligned} \quad (3)$$

where  $W_r = W_{rs}p_s + W_{rr}p_r$  is the fitness of the resistance allele (although the concept of “allelic fitness” in itself has little sense in a diploid population). The mean population fitness  $W$  can be written as  $W = W_{ss}p_s^2 + 2W_{rs}p_s p_r + W_{rr}p_r^2$ . Of course,  $p_s$ ,  $p_r$ ,  $W_r$  and  $W$  are all functions of space and time  $(\mathbf{x}, t)$ .

Combining the equations (3) with the reflective boundary conditions

$$\nabla p_r \cdot \mathbf{n} = \nabla N \cdot \mathbf{n} = 0, \quad \mathbf{x} \in \partial\Omega \quad (4)$$

provides a complete description of changes in the frequency of the Bt-resistance allele  $r$  and the dynamics of total pest density (see the details of transformation of (1–2) into (3–4) in the paper by Tyutyunov et al. (2007)).

The local kinetics of system (3) is described by the continuous-time approximation of the classical FHW equations (Ginzburg, 1983). Model (3–4) differs from the FHW model coupled with diffusion (the FHW model used by Vacher et al. (2003) and Cerda and Wright (2004)) only by the term  $2\delta \nabla \ln N \cdot \nabla p_r$ , which, together with the diffusion term, describes the dispersal of the resistance allele. Clearly, this term can be interpreted as an ‘advective’ term describing a directed flow of the allelic frequency  $p_r$  with velocity  $-2\delta \nabla \ln N$ . This advection, which is never taken into account in spatial FHW models, results from the heterogeneity of the spatial distribution of  $N$  and  $p_r$  and disappears if either  $N$  or  $p_r$  is uniformly distributed.

In the general case, system (3) does not necessarily evolve to Hardy–Weinberg equilibrium  $u_{ss}^* = p_s^2$ ,  $u_{rs}^* = 2p_s p_r$ ,  $u_{rr}^* = p_r^2$ , where  $u_{ij} = N_{ij} / N$  are the genotype frequencies. Introducing the additional variable  $\xi = u_{ss}u_{rr} - u_{rs}^2/4$  to quantify the deviation

of system (3) from Hardy–Weinberg equilibrium (see Svirezhev and Pasekov (1982)) and expressing the genotype frequencies as  $u_{ss} = p_s^2 + \xi$ ,  $u_{rs} = 2p_s p_r - 2\xi$ ,  $u_{rr} = p_r^2 + \xi$ , we obtain an equation describing the spatio-temporal dynamics of the deviation  $\xi$ :

$$\begin{aligned} \partial \xi / \partial t &= b (p_s^2 p_r^2 (W_{ss} + W_{rr} - 2W_{rs}) - \xi W) + \delta \Delta \xi \\ &\quad + 2\delta \nabla \ln N \cdot \nabla \xi + 2\delta |\nabla p_r|^2. \end{aligned} \quad (5)$$

The variable  $\xi$  indicates the deviation from panmixia, as does the F statistic of S. Wright (1922, 1930), to assess inbreeding in the population. We can see that, for genotypes differing in fitness, the deviation  $\xi$  tends to zero (i.e. system (3) tends to the Hardy–Weinberg equilibrium) only if the frequency of one of the two alleles –  $r$  or  $s$  – tends to zero. Otherwise, in a general polymorphic case (e.g., if  $W_{rs}$  exceeds  $W_{ss}$  and  $W_{rr}$ ) the system (3) evolves beyond Hardy–Weinberg equilibrium. Moreover, the deviation  $\xi$  increases because of the spatial heterogeneity of allelic frequencies.

### 2.3. Modelling the HDR strategy

Spatial heterogeneity was taken into account by assuming that the whole pest habitat  $\Omega$  is a rectangle composed of several plots, each planted with a Bt or non-Bt crop.  $\Omega_{Bt}$  denotes the set of plots of the Bt crop and  $\Omega_{Ref}$  the refuge domain of in which the non-Bt crop is planted.  $\Omega_{Bt}$  and  $\Omega_{Ref}$  are disjoint. Any pattern of refuges can be considered.

In line with previous studies (e.g., Vacher et al. (2003) and Tabashnik et al. (2004)), we assume that the susceptible pest genotypes differ in fitness in the Bt area and in the refuge:

$$\begin{aligned} W_{rr} &= 1 - c, \quad \text{everywhere in } \Omega; \\ W_{rs}(\mathbf{x}) &= \begin{cases} 1 - h_c c, & \mathbf{x} \in \Omega_{Ref}; \\ 1 - \sigma + h_\sigma (\sigma - c), & \mathbf{x} \in \Omega_{Bt}; \end{cases} \\ W_{ss}(\mathbf{x}) &= \begin{cases} 1, & \mathbf{x} \in \Omega_{Ref}; \\ 1 - \sigma, & \mathbf{x} \in \Omega_{Bt}, \end{cases} \end{aligned} \quad (6)$$

where  $\sigma$  is the fitness loss due to the Bt toxin;  $c$  is the fitness cost of resistance;  $h_\sigma$  is the dominance level of Bt-toxin selection;  $h_c$  is the dominance level of the fitness cost (Bourguet et al., 2000). Parameters  $\sigma$ ,  $c$ ,  $h_\sigma$ ,  $h_c \in [0; 1]$ . Note that  $h_\sigma = 1 - h$ , where  $h$  is the effective dominance level estimated by Tabashnik et al. (2004) (see also Vacher et al. (2004)).

The difference between the refuge and the Bt domains is therefore determined exclusively based on differences in pest survival coefficients between these two types of domain. Note that we set conditions (2) and (4) only for the outer boundary  $\partial\Omega$ , whereas the boundaries between adjacent refuges and Bt domains are permeable.

### 2.4. Estimation of demographic parameters

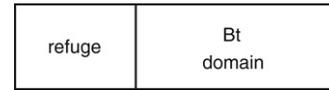
We determined the parameters of the continuous model (1–2) and its equivalent (3–4), based on the parameter values estimated by Guse et al. (2002) in a detailed discrete model taking into account seasonality and stage transitions in the life history of the ECB. All parameters are listed in Table 1.

The ECB is assumed to produce two generations per year. We also assume that the life span of each generation covers the period from the egg stage to the end of the adult stage (egg-to-egg cycle). The life span of the second generation is longer than that of the first as it includes overwintering of the larval stage.

According to Guse et al. (2002), one adult ECB female lays an average of 288 eggs during her lifetime. Thus, taking into account the production of two pest generations per year:  $b = 2 \ln \lambda$ , where

**Table 1**  
Parameter values and initial conditions used in the DGD and FHWD models

Parameter	Value	Description
$b$	$9.94 \text{ yr}^{-1}$	Birth rate
$\mu$	$6.84 \text{ yr}^{-1}$	Mortality rate
$\alpha$	$2.1 \times 10^{-6} \text{ ha yr}^{-1} \text{ ind}^{-1}$	Competition coefficient
$K$	$147.4 \times 10^4 \text{ larvae/ha}$	Carrying capacity
$\delta$	Varied	Diffusion coefficient
$\Omega$	5 km by 5 km	Whole pest habitat
$\Omega_{Ref}$	Varied	Refuge domain of non-Bt crop
$\Omega_{Bt}$	$\Omega \setminus \Omega_{Ref}$	Set of Bt crop plots
$W_{ss}(x)$	$\begin{cases} 0, & x \in \Omega_{Bt}; \\ 1, & x \in \Omega_{Ref}. \end{cases}$	Fitness of <i>ss</i> genotype
$W_{rs}(x)$	$\begin{cases} 0.05, & x \in \Omega_{Bt}; \\ 1, & x \in \Omega_{Ref}. \end{cases}$	Fitness of <i>rs</i> genotype
$W_{rr}(x)$	$1, x \in \Omega$	Fitness of <i>rr</i> genotype
$p_r^0$	0.0015	Initial frequency of the resistance allele
$N_{ss}^0$	$2.9392 \times 10^4 \text{ ind/ha}$	Initial density of <i>ss</i> genotype
$N_{rs}^0$	88 ind/ha	Initial density of <i>rs</i> genotype
$N_{rr}^0$	0	Initial density of <i>rr</i> genotype
$N^0$	$2.948 \times 10^4 \text{ ind/ha}$	Initial total density of the pest
		$N^0 = N_{ss}^0 + N_{rs}^0 + N_{rr}^0$



**Fig. 1.** Single-strip border refuge pattern for the modelled farming area.

$\lambda$  is lifetime fecundity and assuming that the sex ratio is 1:1 (i.e.,  $\lambda = 144$ ), we obtain  $b \approx 9.94 \text{ yr}^{-1}$ .

Similarly, we estimated the mean instantaneous mortality rate of ECB  $\mu$  as the sum of the mortalities of both generations:  $\mu = -\ln(0.077) - \ln(0.077 \cdot 0.18) = 6.84 \text{ yr}^{-1}$ , with natural ECB survival coefficients of 0.077 in the summer, through the larval stages of both generations, and 0.18 in the winter diapause, for the diapausing larvae of the second generation (Guse et al., 2002). Like Guse et al. (2002), we also ignore mortality at the egg and pupal stages.

In line with Guse’s model (Guse et al., 2002), we set a maximum of 22 ECB larvae/plant and 67,000 plants/ha, giving an estimate of carrying capacity  $K = 147.4 \times 10^4 \text{ larvae/ha}$ . The competition coefficient is therefore determined by the formula  $\alpha = (b - \mu) / K$ , so  $\alpha = 2.1 \times 10^{-6} \text{ ha yr}^{-1} \text{ ind}^{-1}$ .

As the large-scale diffusion coefficient  $\delta$  of insect species is difficult to estimate from a small number of field observations, we varied this coefficient from 0 to  $\infty$ , with irregular increments.

### 2.5. Simulation setup

We discretised the space with a regular grid, approximating the spatial derivatives at each node by the central difference. The obtained system of ordinary differential equations (ODEs) was then integrated by the fourth-order Runge–Kutta method with an automatic time step. The number of nodes in each spatial direction was set at 100. The accuracy of calculations was checked by doubling the spatial grid.

We focused on the development of Bt-resistance in populations living on large farms. Models (1–2) and (3–4) can be used with any field configuration of any size, but we fixed the modelled field  $\Omega$  as a square of 5 km by 5 km, considering various refuge configurations.

In all our simulations, the initial total density of the pest was taken as 2% of the carrying capacity  $K$ , corresponding to  $N^0 = 2.948 \times 10^4 \text{ ind/ha}$  (or 0.44 ind/plant). We assumed that, as a result of recurrent mutations, the resistance allele was already present in the pest population (Scott, 1995), at an initial frequency of  $p_r^0 = 0.0015$ , which is somewhat higher than the estimate of  $p_r < 10^{-3}$  for the ECB population (Andow et al., 2000; Bourguet et al., 2003). These initial conditions correspond to a pessimistic view of the situation, and therefore more cautious forecasts for resistance development. We also assume that, before the planting of Bt maize, there were no resistant homozygous individuals and

only a small number of heterozygotes possessing one copy of the Bt-resistance allele. These assumptions are consistent with the results of field investigations in natural ECB populations (Andow et al., 2000; Bourguet et al., 2003). If we assume that the initial genotype densities are uniformly distributed in space, given that  $p_r = (N_{rr} + 0.5N_{rs}) / N$ , we obtain the following initial densities of pest genotypes:  $N_{ss}^0 = 29392 \text{ ind/ha}$  (0.439 ind/plant),  $N_{rs}^0 = 88 \text{ ind/ha}$  (0.001 ind/plant),  $N_{rr}^0 = 0$ .

Assuming that some ECB heterozygotes survive in Bt areas and that there is no resistance cost, we used the genetic parameter values adopted by Vacher et al. (2003):  $\sigma = 1, c = 0, h_\sigma = 0.05$ , evaluating genotype fitness  $W_{ij}$  according to (3).

All values used in simulations are given in Table 1.

### 3. Simulation results

The proposed model (1–2) was used for three purposes:

1. To investigate the spatio-temporal patterns of resistance development in the ECB population;
2. To study the influence of refuge size and ECB dispersal on delaying resistance;
3. To compare the effectiveness of refuge configurations.

We considered two criteria for assessing the efficiency of resistance management and pest control strategies:

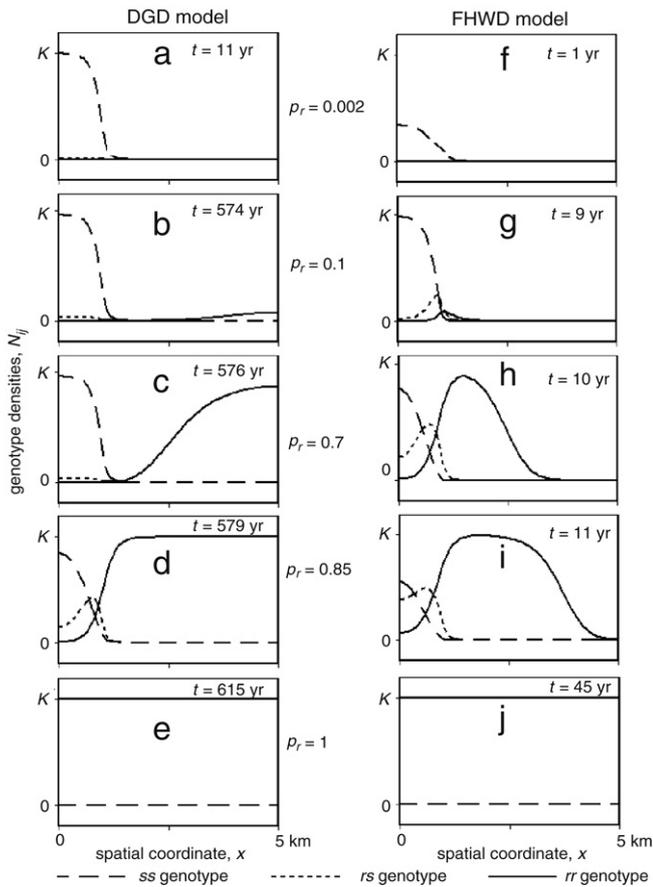
1. Maximisation of the time taken to develop resistance, evaluated as the time  $T_{10}$  required for the frequency of the resistance allele to reach 10% over the entire ECB population;
2. Minimisation of ECB density over the entire cultivated area  $\Omega$ .

#### 3.1. Spatio-temporal patterns of resistance development

Using a fixed refuge percentage (20% of  $\Omega$ ) and a fixed diffusion coefficient  $\delta = 1 \text{ ha yr}^{-1}$ , we analysed the genotype dynamics and the rate of resistance invasion, using a single-strip pattern for the refuge (Fig. 1).

Simulations indicated that the HDR strategy has an immediate effect, disrupting the homogeneity of genotype densities. However, the frequency of the resistance allele  $p_r$  slowly increases, reaching 0.002 after only 11 yr (Fig. 2a). At this stage, pest density in the refuge reached levels slightly below the carrying capacity, whereas the pest is almost absent from the Bt area. In the refuge and in the cultivated area as a whole, the pest population consists principally of insects of the *ss* genotype (about 99%). This distribution persists for 570 yr. Nevertheless, the *rr* genotype has a high coefficient of survival on Bt plants and, despite the influx of large numbers of Bt-susceptible potential mates from the refuge, the system tends ultimately to complete invasion by insects with the resistant *rr* genotype (Fig. 2e). Once  $p_r$  reaches 10%, complete invasion of the Bt area by the *rr* genotype takes only five years (Fig. 2d), the susceptible genotypes from the refuge being completely replaced by the resistant genotype within 36 yr (Fig. 2e).

We carried out similar simulations with the FHWD model. By contrast to the DGD model, when  $p_r$  reaches 0.002 in the FHWD model, susceptible genotypes occupy only 30% of the carrying capacity of the refuge (Fig. 2f). However, the spatially heterogeneous distribution of susceptible genotypes (as in Fig. 2a) emerges very rapidly (within 4 yr) but persists for only five years (as opposed to 570 yr in the DGD model). Complete invasion by the resistant genotype requires a further 36 yr (Fig. 2j).



**Fig. 2.** Density distribution of ECB genotypes in a one-dimensional area 5 km long at the time (years) at which the frequency of the resistance allele  $p_r$  sequentially reaches 0.002, 0.1, 0.7, 0.85 and 1, for a 20% refuge area, in both the DGD and FHWD models.

The difference between the DGD and FHWD approaches lies in both the duration of the process and the nature of the spatio-temporal dynamics. In the DGD model, the conditions favouring the appearance of  $rr$  individuals are at the extreme right of the Bt domain (Fig. 2b). In the FHWD model, these conditions occur at the border between the refuge and the Bt field areas (Fig. 2g).

### 3.2. Influence of refuge size and ECB dispersal

We determined the period of time  $T_{10}$  during which the frequency of the resistance allele remained below 10% in a series of simulations with the DGD and FHWD models, varying the size of the single-strip refuge (Fig. 1) and the diffusion coefficient  $\delta$ . The results are reported in Table 2.

The data for the DGD model show that, in cases of low pest dispersal ( $\delta \leq 0.1$  ha yr<sup>-1</sup>) or panmixia ( $\delta = \infty$ ), increasing the size of the refuge has little effect, as it does not delay the development of Bt resistance (Bt resistance develops within 7 to 25 yr). For intermediate values of  $\delta$  (0.1 to 1 ha yr<sup>-1</sup>),  $T_{10}$  increases rapidly with increasing refuge size in the DGD model, and may even reach several hundreds or thousands of years, providing strong evidence that the HDR strategy is efficient. For a given refuge size,  $T_{10}$  increases considerably at a particular value of the diffusion coefficient, then decreases monotonically with further increases in  $\delta$ . At high levels of pest dispersal ( $\delta \geq 1$  ha yr<sup>-1</sup>),  $T_{10}$  increases only slightly with refuge size.

According to the FHWD model,  $T_{10}$  increases monotonically, but weakly, with the size of the refuge, over the whole range of the diffusion coefficient  $\delta$ . However, the HDR strategy cannot

**Table 2**

Time  $T_{10}$  (years) in the DGD and FHWD models, for various combinations of refuge size and diffusion coefficient, with a single-strip border refuge as in Fig. 1

Diffusion, $\delta$ (ha yr <sup>-1</sup> )	Refuge percentage (%)									
	5	10	15	20	25	30	35	40	45	50
DGD model										
0	24	24	24	24	24	24	24	25	25	25
0.1	24	24	24	24	25	25	25	25	25	26
0.2	24	24	24	25	25	25	26	2034	2483	2968
0.4	24	25	485	720	979	1262	1567	1894	2242	2610
0.5	92	266	473	700	947	1213	1496	1798	2116	2451
1	67	205	382	574	776	988	1208	1437	1675	1920
5	20	69	140	229	329	435	545	657	771	881
10	13	37	78	130	192	261	335	410	487	564
50	8	12	20	32	48	66	86	108	132	156
$\infty$	7	7	8	8	9	9	10	11	12	13
FHWD model										
0	24	24	24	24	24	24	24	25	25	25
0.1	8	9	9	9	9	9	9	10	10	10
0.2	8	9	9	9	9	9	9	9	9	9
0.4	8	8	9	9	9	9	9	9	9	9
0.5	8	8	9	9	9	9	9	9	9	9
1	8	8	8	9	9	9	9	9	9	9
5	7	8	8	8	8	8	9	9	9	9
10	7	7	8	8	8	8	9	9	9	9
50	5	7	8	8	8	9	9	10	11	12
$\infty$	7	7	8	8	9	9	10	11	12	13

necessarily be considered to be efficient based on the  $T_{10}$  values obtained, because no more than 25 yr was required for the frequency of the resistance allele to reach the 10% threshold, for all combinations of pest dispersal and refuge size considered.

### 3.3. Effectiveness of refuge configurations

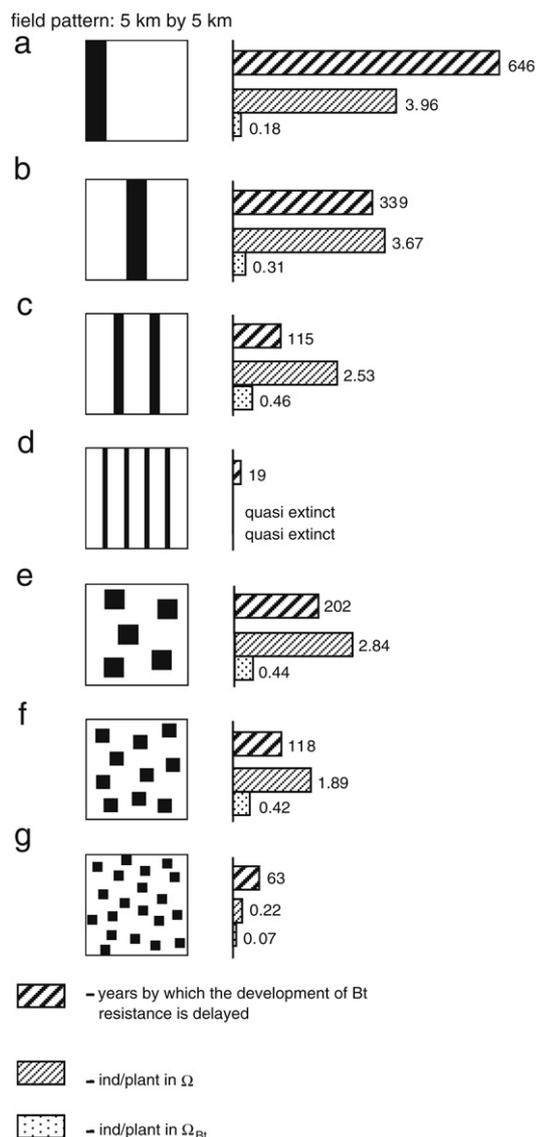
We investigated the effects of refuge shape and arrangement on the efficiency of the HDR strategy, with fixed values of refuge percentage and pest dispersal (Fig. 3). Simulations with the DGD model showed that, for a field size of 5 km by 5 km, the single-strip border refuge (Fig. 3a) was the most effective of the refuge forms tested (Fig. 3a–g). Locating the single-strip refuge in the middle of the maize field (Fig. 3b) approximately halved  $T_{10}$ , resulting in significantly higher levels of ECB infestation in the Bt area than with the refuge at the border, as in Fig. 3a. Splitting a one-strip refuge into several strips also greatly decreased the efficiency of the refuge (Fig. 3c,d).

Simulations with square refuge structures also indicated that, for the same proportion (20%) of refuge, increasing the number of refuge patches by decreasing their size greatly decreased the efficiency of the HDR strategy (Fig. 3e–g).

In all simulations, the pest population was much smaller within the Bt area, but the density of the pest over the entire area of cultivated maize remained high, essentially due to high levels of ECB infestation in the refuge.

## 4. Discussion

We have shown here that the use of the FHW model coupled with diffusion terms can lead to substantial errors when predicting the evolution of genetic structure in a spatially distributed diploid population. The source of the error is clear. The FHW model was initially developed for species with life cycles involving an alternation of non-overlapping haploid and diploid phases (Abroso and Bogolyubov, 1988; Neal, 2004). This alternation provides an opportunity to describe the evolution of an advantageous gene in the FHW model at the haploid level only, in terms of allelic frequencies. However, in most diploid



**Fig. 3.** Time  $T_{10}$  (years) required for the frequency of the resistance allele to reach 10% over the whole farming area and the levels of ECB invasion (ind/plant) of the whole maize area and the Bt area, with  $p_r < 0.1$  (during  $T_{10}$ ), in the DGD model. The refuge area is shown in black and the Bt area is shown in white. Refuges account for 20% of total area for all patterns. Pest dispersal  $\delta = 0.7 \text{ ha yr}^{-1}$ .

organisms, including insects, gametes cannot persist outside the diploid organism, so, diploid and haploid generations overlap. Inclusion of the assumption of panmictic reproduction (perfectly mixed gametic pool) is required to extend the area of applicability of the haploid FHW model and to describe the evolution of a diploid population with reduced haploid phase correctly. This assumption requires homogeneity of the spatial distribution of the population in space and an absence of density fluxes. Since works of Wright (1922, 1930, 1943), relaxation of the hypotheses of the panmixia and spatial homogeneity has made it possible to use the classical FHW model correctly under the assumption of deviation of the genetic structure of the population from Hardy–Weinberg equilibrium. Nevertheless, many models of the evolution of resistance assume Hardy–Weinberg equilibrium (e.g., Laxminarayan and Simpson (2002), Vacher et al. (2003) and Cerda and Wright (2004)). Our results demonstrate that considerable spatial heterogeneity of the pest population invalidates the FHW model for the biological system studied. Strong selection of the advantageous genotype also makes application of the FHW model

to diploid species problematic even in spatially homogeneous case. Despite these limitations, diffusion models of gene dispersal based on the FHW equations have been used to model the spatio-temporal dynamics of Bt-resistance genes in insect populations (e.g., Vacher et al. (2003) and Cerda and Wright (2004)).

The demo-genetic approach provides a natural generalisation of the classical frequency-based FHW model applied to diploid species. Kostitzin (1937) was the first to develop this approach in non-spatial cases, using Volterra's competition theory to describe interactions between genotypes in a diploid population and the evolutionary selection of more adapted genotypes as a direct result of intraspecific competition. In our DGD model, we have modified Kostitzin's equations, so as to assess genotype fitness in terms of larval survival rather than overall genotype fecundity. We have also considerably simplified Kostitzin's original model, assuming that pest genotypes differ only in fitness coefficients (see the general demo-genetic model of Kostitzin in Appendix). The concept of the integration of ecological and evolutionary processes into a population under strong selection has been considered in a number of other studies. Comins (1977a,b, 1979) noted the importance of population dynamics and density-dependent processes in spatially structured populations exposed to pesticide selection. Other studies have also examined this issue (Gould et al., 1991; Roush, 1994; ILSI-HESI, 1999). Attempts have been made to develop demo-genetic diffusion models of Bt-resistance evolution in pest populations based on Lotka–Volterra competition equations (Hillier and Birch, 2002a,b; Richter and Seppelt, 2004). However, these models are incorrect, as they are based on a genetic structure of the pest incompatible with Mendelian inheritance.

The transformation of the density form of the proposed DGD model (1–2) to its frequency form (see model (3–4)) reveals the common traits and key differences between our approach and the conventional FHW-based approach. The frequency form (3–4) of our DGD model contains a density-dependent advective flux of genes in the selection equation (the first equation of system (3)). In addition to the diffusion flux in the frequency DGD model, a directed gene flux is induced when the spatial distribution of total population density is heterogeneous. Thus, the FHW model describes spatial gene dispersal in a diploid population only in the idealistic and unrealistic case of homogeneous total population density distribution over the entire farming area, eliminating the effects of density dependence. Unlike model (3–4), the general Kostitzin model (A.1) and (A.2) (in which genotypes differ in all parameters) must include density dependence, even in the point model (Svirezhev and Pasekov, 1982). Thus, the process of selection cannot be separated from population growth (unless selection is weak, i.e. there is little difference between the genotypic parameters).

A comparison of simulation results for the two models provides information about how strongly the advective term  $2\delta \nabla \ln N \cdot \nabla p_r$  in the frequency DGD model affects the dynamics of the biological system studied. As shown in Table 2 for the DGD model, with particular combinations of refuge size and pest dispersal, the HDR strategy can delay the development of Bt resistance by several hundreds or even thousands of years. No such delay can be achieved with the FHW model unless there is a strong cost of resistance. The  $T_{10}$  values predicted by our DGD model may therefore account for the absence of Bt-resistant homozygous ECB in transgenic maize fields despite the intensive use of Bt maize in the USA and other countries over the last ten years.

Moreover, under certain conditions, during the transition from the initial distribution of genotype densities to the homogeneous steady state (Fig. 2e), the system in the DGD model may remain close to the unstable heterogeneous stationary state corresponding to the absence of the  $rr$  genotype for long periods (Fig. 2a). The existence of such “temporal equilibrium” at low resistance

gene frequency was previously demonstrated by Comins (1977a). During this period, the spatial gradients  $\nabla \ln N$  and  $\nabla p_r$  operate in opposite directions within the field, so that the advective flux of  $p_r$  limits the local growth of  $p_r$ , because  $2\delta \nabla \ln N \cdot \nabla p_r < 0$ . At the same time, when  $rr$  individuals migrate to the refuge, in which the ecological characteristics of all three genotypes are equal and pest population is close to carrying capacity, the initial majority of Bt-susceptible individuals results in reducing the density of the  $rr$  genotype due to intraspecific competition among pests (although the allelic frequencies remain constant). Within the Bt area, close to the refuge border, the  $rr$  genotype is eliminated by the intensive diffusive flux of the  $ss$  and  $rs$  genotypes from the refuge. When the density of resistant homozygotes in the Bt area becomes sufficiently high (Fig. 2b), the advective flux of  $p_r$ , which initially opposes the diffusive flux, changes direction, accelerating the spatial dispersal of the resistance allele and, thus, resistance invasion (Fig. 2e). Similar spatio-temporal patterns of resistance development were predicted with stochastic computer models by Peck et al. (1999) and Sisterson et al. (2004). Peck et al. (1999) found that a focus with high resistance frequency appearing in a cluster of Bt fields located far from refuges produces a “travelling wave” of resistance that takes over the whole region. In our DGD model, such a focus occurs at the extreme right-hand side of the Bt domain for the single-strip border refuge pattern in the one-dimensional pest habitat (Fig. 2b). Sisterson et al. (2004) demonstrated that the regional  $r$  allele frequency gradually increases from its initial value over a long period of time and then increases rapidly, exceeding a threshold of 50%.

The FHWD model is described with the same system of PDEs as the frequency DGD model, with the exception of the advective term. The absence of the advective term results in the absence of the additional opposite flux preventing the diffusive dispersal of the resistance allele in the habitat, so Bt resistance is predicted to develop much faster in the FHWD model than in the DGD model (Table 2, Fig. 2).

Refuge size plays an important role in ensuring the efficiency of the HDR strategy. If refuges are too small, they cannot delay the development of resistance for very long (Table 2, Fig. 3d and g). The intensity of the diffusive density flux due to the spatial heterogeneity of the habitat acts as a negative force, sweeping out all susceptible individuals from the small refuge, increasing the probability of their replacement by Bt-resistant individuals invading from the Bt area. The results presented in Table 2 and Fig. 3 are qualitatively consistent with the results of Ives and Andow (2002), Caprio et al. (2004) and Carrière et al. (2004). Varying the refuge percentage from 0 to 25% in the simulation model, Ives and Andow (2002) showed that the speed with which the susceptible population becomes extinct and the rate of resistance development increase with decreasing refuge size. Caprio et al. (2004) determined egg densities for cotton pests as a function of the width of refuges embedded within cotton fields. They found that narrow (less isolated) refuges produced fewer insects per unit area than wider refuges and were therefore less effective at managing resistance to Bt toxins. Our results, demonstrating that the influence of the refuge as a source of susceptible insects declines with distance from the refuge, are also consistent with those reported by Carrière et al. (2004), who revealed such a dependence relationship for Arizona populations of pink bollworm by means of geographic information system (GIS) technology coupled with data obtained from pheromone traps. The inefficiency of small refuges is related to the classical problem of critical habitat size, below which populations spreading by diffusion cannot persist.

Another key factor determining the efficiency of the refuge is pest dispersal. Using a two-patch model, Comins (1977a) showed that there is a critical rate of migration for susceptible alleles from

populations not exposed to selection, above which the resistance alleles cannot reach high frequencies. Our spatially explicit DGD model predicts more complex dynamics (Table 2). If there is too little pest dispersal, then the intensive flux of susceptible insects from the refuge required to eliminate the resistance allele in the Bt area cannot be maintained. However, a similar decrease in refuge efficiency is observed if the rate of diffusion between the Bt area and the refuge is infinitely high, resulting in homogenisation of the system and panmixia. These results are consistent with those of Caprio (2001). Using a source-sink framework in a spatially complex landscape model, he found that complete isolation between refuges and transgenic fields resulted in rapid resistance evolution, whereas some level of refuge isolation gave rise in significant delays in local adaptation to Bt toxin. Nevertheless, Ives and Andow (2002) concluded that resistance is likely to evolve more slowly if the rate of movement of adult pest between Bt and refuge fields is low. Conversely, other modelling studies have maintained that pest dispersal rates should be high to maintain a sufficient flux of susceptible insects toward Bt fields to prevent or reduce significantly the evolution of resistance (Peck et al., 1999; Storer, 2003). Sisterson et al. (2005) suggested that these conflicting results concerning the influence of pest dispersal result from dispersal being influenced not only by the relative abundance of refuges and Bt fields, but also by the spatio-temporal distribution of refuges and Bt fields.

Pest dispersal and field area are related. The diffusion coefficient  $\delta$  can be normalised with respect to field size, if a decrease in  $\delta$  is considered equivalent to an increase in field size. Thus, our conclusions concerning the maximal efficiency of a one-strip refuge aggregated along the field border (Fig. 3a) depend on the fixed value of  $\delta$  and/or field size. With lower levels of pest dispersal (or a larger farming area, at the regional scale for example) the refuge patches (e.g., Fig. 3c,d,f,g) may delay Bt resistance more efficiently. We cannot really manipulate pest dispersal to increase the efficiency of the HDR strategy, but our results suggest that the same goal could be achieved by controlling the size, proportion and spatial arrangement of Bt areas and refuges.

In this study, we considered only the case of fixed temporal locations of refuges. Temporal refuge distribution within Bt fields probably affects the rate of resistance evolution. Recent modelling studies based on spatially explicit stochastic simulations have reported that maintaining refuges at the same locations from year to year may delay resistance more effectively than rotating Bt and non-Bt fields every year (Peck et al., 1999; Storer, 2003; Sisterson et al., 2005). In regions in which the location of Bt and refuge fields differs every year, Bt resistance develops very quickly because the offspring of susceptible insects growing in refuges and emerging during the spring are more likely to be exposed to Bt-toxin selection after field rotation. However, Peck et al. (1999) showed that the greater efficiency of fixed locations may not apply to heavily mixed populations. Using a classical FHW panmictic model, Cerda and Wright (2004) also came to the conclusion that, for panmictic populations, temporal refuges are more efficient than fixed refuges at preventing the evolution of Bt resistance. Storer (2003) demonstrated that for a given refuge size, the adaptation rate of western corn rootworm populations is lowest when the refuge is planted in the same location each year, with a uniform distribution. Moreover, a 5% fixed refuge is, on average, as effective as a 20% refuge rotated annually. However, Sisterson et al. (2005) confirmed certain previous results by showing that a refuge rotation strategy may be beneficial, providing better insect control and reducing the need for insecticide sprays.

We focused here on the qualitative forecasting of Bt-resistance evolution. Although no resistant ECB have yet been detected in natural populations, we assume the worst-case scenario in our simulations, with a relatively high initial frequency of the Bt-resistance

allele and a sufficiently high (5%) rate of survival assumed for ECB heterozygotes. Clearly, the lower the real values for these two factors, the slower the evolution of resistance is likely to be in the ECB population. This is true for both FHW and demo-genetic models. However, FHW models consistently predict much faster resistance evolution than demo-genetic models, and this difference cannot be ignored. Accurate comparisons of the DGD and FHW models demonstrated a theoretical need to take into account both the diffusion and advective fluxes of genes in FHW models.

We consider here the demo-genetic processes occurring in the ECB population, but the proposed model (1–2) is universal and could be applied to any diploid population under pesticide or drug selection. The theoretical value of the DGD model lies in its ability to reveal the role of spatial heterogeneity in maintaining genetic diversity in dispersing populations. The practical use of this model requires field observations and the identification of parameters for a particular agro-ecosystem.

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### Appendix. General form of the Kostitzin non-spatial (point) model

Vladimir Kostitzin proposed applying the competition theory developed by Vito Volterra to interactions between genotypes in a diploid population (Kostitzin, 1936, 1937, 1938a,b,c). This approach allows explicit description of the selection of the fittest genotype as a result of intraspecific competition;

$$\frac{dN_i}{dt} = f_i(N) - \mu_i N_i - N_i \sum_{j=1}^n \alpha_{ij} N_j, \quad i = 1, \dots, n, \quad (\text{A.1})$$

where  $N_i = N_i(t)$  is the density of genotype  $i$ ;  $\mu_i$  is the mortality rate of genotype  $i$ ;  $\alpha_{ij}$  is the competition coefficient, intragenotypic ( $i = j$ ) and intergenotypic ( $i \neq j$ ); the reproduction function of genotype  $i$ ,  $f_i$ , satisfies the conditions for Mendelian inheritance;  $n$  is the number of genotypes. For a diploid population in which some heritable trait is coded with a diallelic gene, the total number of genotypes  $n = 3$ . The reproduction functions  $f_i$  ( $i = 1, 2, 3$ ) are thus:

$$\begin{aligned} f_1 &= \frac{1}{N} \left( \varphi_{11} N_1^2 + (\varphi_{12} + \varphi_{21}) \frac{N_1 N_2}{2} + \frac{\varphi_{22} N_2^2}{4} \right); \\ f_2 &= \frac{1}{N} \left( (\varphi_{12} + \varphi_{21}) \frac{N_1 N_2}{2} + \frac{\varphi_{22} N_2^2}{2} + (\varphi_{13} + \varphi_{31}) N_1 N_3 \right. \\ &\quad \left. + (\varphi_{23} + \varphi_{32}) \frac{N_2 N_3}{2} \right); \\ f_3 &= \frac{1}{N} \left( \varphi_{33} N_3^2 + (\varphi_{23} + \varphi_{32}) \frac{N_2 N_3}{2} + \frac{\varphi_{22} N_2^2}{4} \right), \end{aligned} \quad (\text{A.2})$$

where  $\varphi_{ij}$  is the birth rate for matings of pairs consisting of an individual of genotype  $i$  and an individual of genotype  $j$  ( $i, j = 1, 2, 3$ ); here it is implicitly assumed that all populations consist of males and females and the birth rates of pairs  $\varphi(i), \sigma(j)$  and  $\varphi(j), \sigma(i)$  may be different;  $N = N_1 + N_2 + N_3$  is the total population density. The numerical coefficients in A.2 correspond

to the proportions of individuals of genotype  $k$  in the progeny of each pair.

Kostitzin's model (A.1) and (A.2) can be used to consider the influence of various ecological selection factors on the genotypic dynamics of a population. Here, the absence of an ecologically independent haplophase for the modelled biological species is crucial. It is also reasonable to assume that the sex ratio is 1:1. Given the complexity of Eqs. (A.1) and (A.2), displaying rich dynamic behaviour, the complete Kostitzin model has yet to be thoroughly explored.

Unfortunately, Kostitzin's work (highly commended by V. Volterra himself, see his preface in Kostitzin (1937, pp. 6–7)) is poorly known nowadays (Bogolyubov, 2002; Scudo and Ziegler, 1976).

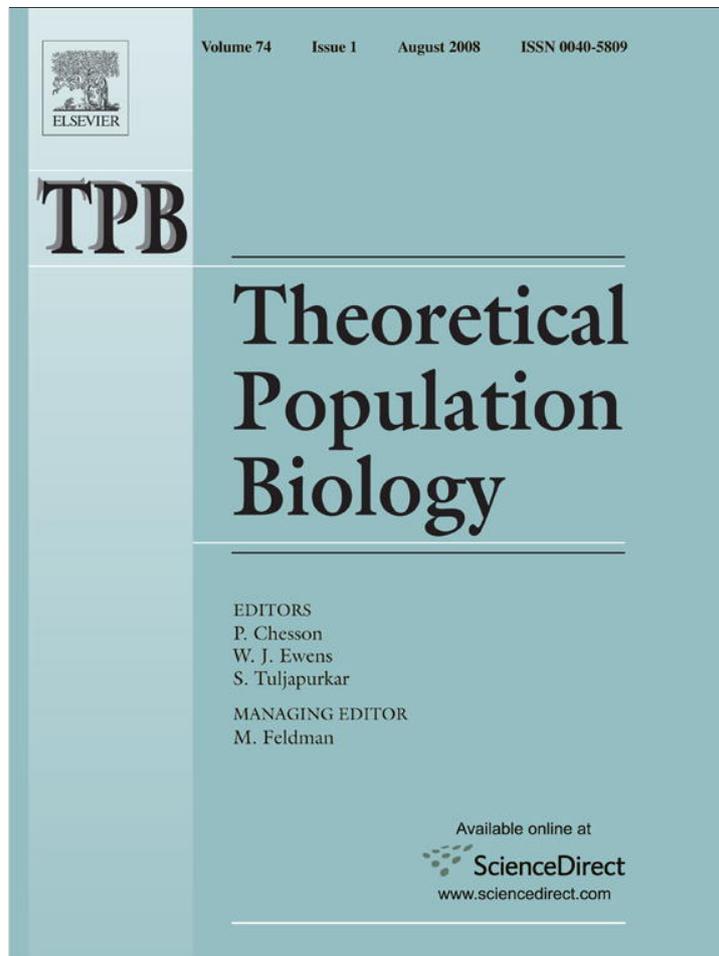
Unlike FHW frequency-based models, the Kostitzin model directly describes population dynamics in terms of genotype densities and is therefore more appropriate for spatial reaction–diffusion modelling. We hope that this manuscript will attract the attention of researchers to approach that was developed by Kostitzin, feeding the current growth in interest in studies on spatially distributed ecosystems.

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