2010- ACL-11

ORIGINAL ARTICLE

The skill and style to model the evolution of resistance to pesticides and drugs

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Abstract

Resistance to pesticides and drugs led to the development of theoretical models aimed at identifying the main factors of resistance evolution and predicting the efficiency of resistance management strategies. We investigated the various ways in which the evolution of resistance has been modelled over the last three decades, by reviewing 187 articles published on models of the evolution of resistance to all major classes of pesticides and drugs. We found that (i) the technical properties of the model were most strongly influenced by the class of pesticide or drug and the target organism, (ii) the resistance management strategies studied were quite similar for the different classes of pesticides or drugs, except that the refuge strategy was mostly used in models of the evolution of resistance to insecticidal proteins, (iii) economic criteria were rarely used to evaluate the evolution of resistance and (iv) the influence of mutation, migration and drift on the speed of resistance development has been poorly investigated. We propose guidelines for the future development of theoretical models of the evolution of resistance. For instance, we stress the potential need to give more emphasis to the three evolutionary forces migration, mutation and genetic drift rather than simply selection.

Introduction

The massive use of drugs in medicine and of pesticides in agricultural systems since the 1950's have led to the selection of highly adapted resistant biotypes in natural populations of microbes and pests (Georghiou 1986; Guillemot 1999; D'Alessandro and Buttiens 2001; Hastings 2004; Levy and Marshall 2004). The evolution of resistance is a serious issue worldwide and several experimental studies have been carried out on resistant microbes and pests collected from hospitals and agricultural fields. These studies focused principally on the physiological mechanisms of resistance (Powles and Holtum 1994; McGowan and Tenover 1997; Raymond et al. 1998; Hakenbeck 1999; Caprio 2001; Gahan et al. 2001; Hsiou et al. 2001; Morin et al. 2003; Courcambeck et al. 2006), the genetic determinism and mode of inheritance of resistance (Edgar and Bibi 1997; Gould et al. 1997; Tabashnik et al. 1997, 2000; Andow and Alstad 1998; Bourguet et al. 2000, 2003; Ferré and Van Rie 2002; Tran and Jacoby 2002; Génissel et al. 2003; Roux et al. 2004; Chen et al. 2007) and, to a

lesser extent, the relative fitness of resistant biotypes in the absence of drugs or pesticides (Groeters et al. 1993; Cohan et al. 1994; Bergelson and Purrington 1996; Frost et al. 2000; Oppert et al. 2000; Purrington 2000; Carrière et al. 2001; Gagneux 2009; Ward et al. 2009).

In addition to carrying out these experimental studies, the scientific community has developed theoretical approaches for investigating the way in which evolutionary forces – mutation, selection, migration and drift – govern the speed and outcome of resistance evolution. The resulting theoretical models, assessing the relative influence of different evolutionary forces, constitute a useful tool for comparing the efficacy of existing management strategies and for designing new strategies (Tabashnik 1986). We previously highlighted the structure of the scientific community developing these theoretical models (REX Consortium, 2007). We analysed co-authorship and co-citation networks on the basis of 187 articles published from 1977 to 2006 on models of the evolution of resistance to all major classes of pesticides and drugs. We identified two main groups of scientists that

collaborate very little: the first group consists of ecologists or agronomists working on pesticide resistance, whereas the second group includes medical scientists interested in drug resistance. The two groups publish their research in their own journals and have their own keystone references (REX Consortium, 2007). This structure of the scientific community may have led to marked differences between the two groups in terms of the modelling approaches developed for studies of the evolution of resistance to pesticides and drugs.

Actually, four major nonmutually exclusive hypotheses may account for differences in the approaches developed for modelling resistance evolution: (1) there may be a lack of exchange between the two main groups of scientists, leading to the development of different lineages of models; (2) the organism studied may affect the biological parameters included in the model and the management strategies tested. For example, the availability of a specific means of control for any particular organism may have influenced the choice of strategies assessed with the model, even though a much broader array of resistance management strategies (including those not applicable for economic, technical or ethical reasons at the time of the study) could be investigated with theoretical models; (3) the mathematical approach (MT) chosen by the modeller may constrain the resistance management strategies and the underlying evolutionary forces that can theoretically be explored. Indeed, two major MT have been used in the modelling of resistance evolution (Levin 2001, 2002): (i) the population genetics approach, which considers changes in the frequencies of resistant and susceptible individuals as a function of pesticide or drug (PD) use; (ii) the epidemiological approach, which is related to the compartment model tradition of the mathematical epidemiology of parasites (Anderson and May 1991) and (4) the features of the model may have changed over time, because of the accumulation of knowledge about the evolution of resistance and increases in computer power.

In this study, we analysed a panel of 187 articles published over the last 30 years and involving the use of a theoretical model to study the evolution of resistance to pesticides or drugs. We described the 187 models, by recording the parameters describing (i) the biology of the target organism, (ii) the technical properties of the model, (iii) the resistance management strategies tested and (iv) the criteria used to evaluate the evolution of resistance. We then determined which of the four hypotheses cited above best accounted for variations in the features of the model. We did this by assessing the relative effects of the scientific community structure, the class of PD, the MT and the year of publication on the variability of the model's features. Based on our results, we propose guidelines for the future development of theoretical models of the evolution of resistance.

Materials and methods

Construction of the bibliographical database

The database of models of the evolution of resistance to the most common classes of pesticides (insecticides, fungicides, herbicides, miticides and insecticidal proteins, such as *Bacillus* toxins) and drugs (antibiotics, antiviral, antimalarial and antihelmintic drugs) has been described in a previous study (REX Consortium, 2007). We used a three-step process to select relevant articles. We first searched for articles in three bibliographical databases (CABs 1973–2006, Current Contents 1998–2006 and Medline 1950–2006) with a formula containing the words model* and resistan* (REX Consortium, 2007). This first step identified 1894 articles. The summary and keywords of each article were then carefully and independently read by two of us, to select articles dealing with a mathematical model or a computer simulation of the evolution of resistance over time in response to selective pressure exerted by a pesticide or a drug. This second step identified 266 articles. In the third step, the seven authors of this study, all familiar with the field of resistance evolution, carefully read each of these 266 articles. Each author was given a randomly chosen set of 14 articles to be read by all the readers, plus a randomly chosen set of 36 articles to be read by that author alone. A reading grid of 34 questions was filled in for each of the 187 articles finally considered relevant for modelling the evolution of resistance to pesticides or drugs.

Individual reader error rate

We evaluated the individual error rate by using the set of 14 articles read by the seven authors of the present study. Only six of these 14 articles were considered relevant by all of us. These six articles were used to assess the agreement (congruence rate) between the answers to the questions on the reading grid given by the seven readers. For each question, the congruence rate was calculated as the proportion of the six relevant articles for which all the readers provided the same answer. This estimate of the congruence rate was then used to calculate the individual error rate, defined as the probability of a reader giving an 'incorrect' answer to the question. Assuming that the individual error rate P is identical for all readers, the congruence rate is $c = P^7 + (1 - P)^7$, where P^7 is the probability of all seven readers giving the incorrect answer and $(1 - P)^7$ is the probability of all the readers giving the correct answer.

Characterization of the models

Thirty one of the 34 questions of the reading grid were specifically used to characterize the range of diversity of model features, from the genetic features of resistance to the socio-economic criteria used to assess the efficiency of resistance management strategies. Each of the 187 models was characterized for these 31 parameters (further referred to as 'model parameters' and described in Table 1), which

can be classified as follows: (i) parameters describing the biology of the target organism and the genetics of resistance, (ii) parameters describing the technical properties of the models, (iii) parameters describing the management strategies for delaying or preventing the evolution of resistance studied and (iv) the output parameters used to assess the evolution of resistance. All model parameters had two levels ('taken into account' or 'not taken into account'; 'yes' or 'no'). We ordered them according to

Table 1. The 31 model parameters used to describe the 187 articles.

Category	Name	Description
Biological parameter	Diploidy	Concerns diploid organisms in which heterozygotes are identified or can be identified; excludes haploid models or models for which genetics is not trivial
	Quantitative resistance	Concerns cases in which resistance is a continuous trait (with a polygenic inheritance). Excludes situations where there is a single or a few resistance phenotypes
	Distance of migration	Distance of migration of the target individuals
	Mutation rate	Mutation rate of S → R and/or of S → R
	Resistance dominance	Rate of resistance dominance, i.e. difference in survival of resistant homozygotes and heterozygotes after treatment
	Initial resistance	Initial presence of resistant individuals
	Resistance cost	Fitness penalty linked to the resistance trait
	Migration	Migration or transmission rate of the target organism. A parameter specifically corresponding to the proportion of target organisms moving from one spatial unit to another (migration) or from one host to another (transmission)
	Cross-resistance	Cross-resistance between molecules
	Recombination	Recombination between loci
Modelling parameter	Model specificity	Specificity of the model, applied to one (or a few) species or diseases
	Simulation	Numerical simulation: the state of the system at time t or at equilibrium is obtained by successive iterations
	Stochasticity	Stochastic model (if the simulation is run at another time, the result is different)
	Resource dynamics	Resource dynamics over time: the model has parameters that are not linked to the target organism and that describe changes in the size or density of the resource over time
	Population dynamics	Population dynamics of the target organisms: models integrate equation parameters that take into account size or density variation of the target organism)
	Discrete time	Model in discrete time: time is divided into distinct units, often calculated as years or generations; equations give the state of the system at time $t + 1$, as a function of the state at time t
Strategies	No. of molecules	One or more than one active molecules
	Refuge	Spatial distribution of xenobiotics (refuge, reservoir): the model includes a spatial area in which the target is not treated
	Temporal distribution	Temporal distribution of xenobiotics: the model includes cases in which treatment is not continuously applied over time
	Mixture	Mixture of molecules, including associations, combinations, pyramiding, gene-stacking
	Rotation	Temporal distribution of treatments, including cycling, alternation, rotation
	Mosaic	Spatial distribution of treatments, including mosaic
	Alternative methods	Alternative methods of control, not using the xenobiotics, but having a direct or indirect impact on resistance
Output	No. of pests	Quantifies the size of the target organism population
	Resource	Quantity and quality of healthy resource (yields, patients…)
	Frequency of resistance	Frequency of resistant target organisms
	Economics	Economic gain. Follows an economic criterion
	Graph	A graph shows changes in resistance over time
	Finite time	Threshold is based upon a finite delay
	Frequency threshold	Threshold is based upon frequency
	Equilibrium	Comparison is based upon the situation at equilibrium (either analytical situation or stabilization of the resistance allele)

whether they were frequently (more than 80%) or rarely (lower than 20%) considered in the 187 models.

Characterization of the explanatory factors

We hypothesized that differences between the features of the models described in the 187 different articles could be accounted for by four factors (Table 2). The three remaining questions on the reading grid made it possible to define three of these factors: the class of PD studied, the year of publication and the MT used (population genetics model or epidemiological model). The last factor corresponds to the citation group (CG) to which the articles belonged. These CG were defined from the co-citation analysis performed in our previous study (REX Consortium, 2007).

We investigated whether these explanatory factors accounted for variations among the 187 models based on (i) the total number of model parameters taken into account, (ii) the nature of each model parameter taken into account and (iii) the combination of model parameters taken into account.

Identification of the factors accounting for the total number of model parameters

The total number of model parameters taken into account was counted for each model. Kruskal–Wallis rank sum tests were carried out with the *kruskal.test* function

of R (R_Development_Core_Team 2006), to assess the effects of the various explanatory factors on the total number of model parameters.

Identification of the factors best accounting for the use of each model parameter

Then we performed a set of statistical analyses to identify the factor best accounting for the use of each model parameter. We first tested the null hypothesis of independence between the various explanatory factors and each of the model parameters, using Fisher's pseudo-exact tests on contingency tables (with 10 000 permutations of the *chisq.test* function of R). False discovery rate correction was used to correct for multiple testing (Benjamini and Hochberg 1995). We then fitted generalized linear models to each model parameter, using binomial error and logit link (Venables and Ripley 2002). For each model parameter, we calculated the Akaike Information Criterion (AIC) of both the full model (model parameter = CG + PD + MT + year of publication) and each of the four linear models including only three of the four explanatory factors. We calculated the difference in AIC (ΔAIC) between the full model and each of the four linear models containing three factors each. A positive ΔAIC indicates that the three-factor model gives a worse fit (in terms of deviance explained and number of parameters used) than the full model. The three-factor model with the largest positive ΔAIC was selected and the factor excluded from this model was

Table 2. Distribution of the four explanatory factors among the 187 models analysed.

Factors of article classification	Classes	n (%)	Mean no. of parameters per model (SD)	Kruskal–Wallis rank sum test
Year of publication	1976–1985	10 (5.3)	12.4 (2.4)	$\chi^2 = 1.257$
	1986–1990	29 (15.5)	13.3 (2.8)	d.f. = 4
	1991–1995	27 (14.4)	13.5 (3.0)	$P = 0.869$
	1996–2000	51 (27.3)	13.4 (3.2)	
	2000–2006	70 (37.4)	13.1 (3.1)	
Citation group	Ecologists and agronomists	44 (23.5)	13.7 (3.0)	$\chi^2 = 17.588$ d.f. = 2
	Medical scientists	138 (73.8)	11.9 (2.3)	$P < 0.001$
	Isolated	5 (2.7)	10.4 (3.8)	
Pesticide or drug	Insecticidal protein	39 (20.9)	14.8 (3.0)	$\chi^2 = 33.138$
	Insecticide	30 (16)	14.4 (2.4)	d.f. = 7
	Antibiotic drug	29 (15.5)	11.5 (2.8)	$P < 0.001$
	Others	25 (13.3)	13.7 (2.4)	
	Herbicide	18 (9.6)	13.6 (3.2)	
	Unspecific pesticide	17 (9.1)	11.4 (3.5)	
	Fungicide	15 (8)	12.0 (2.9)	
	Antiviral drug	14 (7.5)	12.4 (1.6)	
Mathematical approach	Population genetics	110 (58.8)	14.2 (2.9)	$\chi^2 = 35.536$
	Epidemiology	41 (21.9)	12.9 (2.5)	d.f. = 2
	Other	36 (19.3)	10.8 (2.5)	$P < 0.001$

considered to be the most explanatory according to the AIC. The best explanatory factor was the most explanatory according to the AIC if it was also significant according to Fisher's exact test. Finally, we determined the proportion of the total deviance accounted for by each of the four models including only one of the explanatory factors.

Identification of the factors accounting for the combination of model parameters

We assessed the effects of the various explanatory factors on the combination of model parameters, by hierarchical clustering of the 187 articles on the basis of pairwise 'Manhattan' distance (i.e. the sum of the differences for each of the model parameters) under the 'complete' clustering option of the *hclust* function of R. Bootstrap values were estimated for the nodes of the tree, with the *pvclust* function available in the *pvclust* library of R. The correspondence between this clustering and the classification of articles as a function of the four factors considered was assessed by reporting these factors on the leaves of the tree.

Results

Individual error rates for parameter assignment

Individual error rates were <2.6% for each of the three explanatory factors: PD, MT and Year of publication. They were also low for most model parameters. Mean error rates were 2.5%, 3.2%, 3.7% and 6.5% for the biological parameters, the modelling parameters, strategies and outputs respectively. Error rates exceeded 5% for

seven model parameters (Resource dynamics, Discrete time, Resistance cost, Migration, Temporal distribution, Number of pests and *Resource*). Some of these reading errors could be as a result of the lack of clarity with which some models were described. These errors may have decreased the statistical power of some of our analyses, but they probably had too weak an effect to change our conclusions significantly.

Frequently considered versus poorly investigated model parameters

Out of the thirty-one model parameters (Table 1), four were frequently taken into account whereas six parameters were poorly investigated (Fig. 1). Of the 11 model parameters describing the biology of the organism and the genetics of the resistance, Initial resistance was frequently considered (88%). Conversely, Cross-resistance, Quantitative resistance, Recombination and Distance of migration were seldom considered (5%, 7%, 9% and 11% respectively). All the parameters describing the technical properties of the models were used in more than 20% of models. The most frequently used parameters were Simulation (89%) and Stochasticity (80%). None of the strategies for delaying or preventing the evolution of resistance was investigated in more than 80% of the models. The management strategy was not specified in 15% of the models and the Mosaic strategy was studied in only 13 articles (7%). Last, among the eight parameters describing the output criteria used to assess the evolution of resistance, the final Frequency of resistance was considered in 80% of the articles, whereas the Economic criterion was rarely
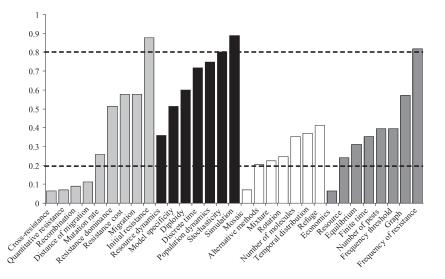

Figure 1 Frequency of the 31 model parameters of the reading grid in the articles. Light grey: biological parameters; black: modelling parameters; white: modelling strategies; dark grey: model output. The dotted lines indicate frequencies of 20% and 80%.

used (6%). These findings, especially the identification of poorly investigated parameters, are useful for deriving guidelines for future modelling efforts (see Discussion).

Factors accounting for the total number of model parameters

The number of model parameters taken into account ranged from 7 to 20 in the 187 articles. Among the four explanatory factors, three had a significant effect on the total number of parameters per model: CG, PD and the MT (Table 2). It is noteworthy that the number of model parameters was similar (13.2 ± 3.0) in all publication years. Hence, the complexity of the models did not increase over time through the addition of model parameters.

Factors best accounting for the use of each model parameter

The nature of the model parameters taken into account did not change over time, since Year of publication was never found to be the best explanatory factor for the use of each of the 31 model parameters. According to both Fisher's exact tests and the AIC, the factors PD, MT and CG were the best explanatory factors for ten, five and one model parameters respectively (Table 3; Fig. 2). These 16 parameters included five biological parameters, three parameters describing the technical properties of models, three parameters describing the resistance management strategies and five parameters related to the output of the models. They are presented in detail below.

Five of the 11 factors describing the biology of the target organism and the genetics of resistance were significantly influenced by at least one of the four explanatory factors, according to both Fisher's exact tests and AIC. The PD factor best accounted for Diploidy (65% of the total deviance), Resistance dominance (58%), Initial resistance (18%) and Mutation rate (10%). Initial resistance was included in almost all the models, but to a lesser extent in those dealing with resistance to fungicides, antibiotics and antiviral drugs. About 50% of the models dealing with resistance to herbicides, antibiotics and antiviral drugs included Mutation rate, whereas this parameter was rarely considered in models dealing with fungicide resistance. The MT factor best accounted for Migration (19%) and Distance of migration (18%). Migration was more often taken into account in epidemiological (ca. 90%) than in population genetics (60%) models. The Distance of migration was considered in only 20% of population genetics models dealing with resistance to insecticides (including insecticidal proteins) and herbicides.

Half of the parameters describing the technical properties of the models were significantly influenced by at least one of the four explanatory factors, according to both Fisher's exact tests and AIC. The PD factor best accounted for Discrete time (31% of the total deviance) and Model specificity (18%). Discrete time was mostly used for modelling resistance of countable organisms, such as weeds or insects (90% of the corresponding articles). Conversely, this parameter was considered by <50% of the articles modelling resistance to fungicides or to antibiotics and antiviral drugs. As expected, general models of resistance to pesticides almost never specified a target organism, whereas most models of the evolution of resistance to antiviral drugs were specific (85% of the articles). The Resource dynamics parameter was best accounted for by the MT factor (13%).

Only three of the seven parameters describing the resistance management strategies were significantly influenced by at least one of the four explanatory factors, according to both Fisher's exact tests and AIC. Refuge, Rotation and Alternative methods were all largely accounted for by the PD factor (29%, 11% and 10% of the total deviance respectively). The Temporal distribution of a given molecule, and the Mixture and Mosaic strategies were not structured according to any of the four explanatory factors. The Refuge strategy was typically considered when modelling resistance to insecticidal proteins (>95% of the articles) or to insecticides (45%). The Rotation strategy was never considered in models dealing with the evolution of antiviral drug resistance. This strategy was also ignored in most models of the evolution of resistance to insecticidal proteins. Conversely, Rotation was frequently taken into account in models dealing with resistance to fungicides (60% of the articles dealing with fungicide treatments). Finally, more than half of the articles modelling the evolution of herbicide resistance considered strategies based on Alternative methods, such as crop rotation or the mechanical control of weeds.

Finally, five of the eight parameters related to the output of the models were significantly influenced by at least one of the four explanatory factors according to both Fisher's exact tests and AIC. The Resource and Frequency of resistance parameters were best accounted for by the MT factor (37% and 11% of the total deviance respectively). The proportion of articles including a Frequency of resistance parameter was slightly higher for population genetics (90%) than for epidemiological (72%) models. The Frequency threshold and Finite time parameters were best accounted for by the Pesticides or drug used (16% and 10% of the total deviance respectively). None of the articles used these two output criteria simultaneously to

Table 3. Effect of the four explanatory factors on the variation in the use of the 31 model parameters.

Model parameters	Citation group	Explanatory factors		Year of publication	Largest ΔAIC
		Pesticide or drug	Mathematical approach		
Biological					
Diploidy	0.00* (0.41)	**0.00* (0.65)**	0.00* (0.53)	0.01 (0.03)	Pesticide or drug
Mutation rate	0.01* (0.05)	0.00* (0.10)	0.08 (0.02)	0.43 (0.00)	Mathematical approach
Distance of migration	0.03 (0.09)	0.00* (0.23)	**0.00* (0.18)**	0.44 (0.00)	Mathematical approach
Resistance cost	0.07 (0.02)	0.36 (0.03)	0.24 (0.01)	0.04 (0.00)	Citation group
Resistance dominance	0.00* (0.30)	**0.00* (0.58)**	0.00* (0.51)	0.00 (0.03)	Pesticide or drug
Initial resistance	0.00* (0.10)	**0.00* (0.18)**	0.00* (0.09)	0.18 (0.04)	Pesticide or drug
Migration	0.00* (0.06)	0.00* (0.12)	**0.00* (0.19)**	0.01 (0.02)	Mathematical approach
Cross-resistance	0.37 (0.03)	0.08 (0.16)	0.60 (0.02)	0.42 (0.00)	Pesticide or drug
Recombination	0.29 (0.02)	0.05 (0.12)	0.94 (0.00)	0.29 (0.01)	Pesticide or drug
Quantitative resistance	0.25 (0.04)	0.07 (0.13)	0.03 (0.06)	0.44 (0.01)	Mathematical approach
Modelling					
Model specificity	0.30 (0.01)	**0.00* (0.18)**	0.01* (0.03)	0.29 (0.01)	Pesticide or drug
Population dynamics	0.02* (0.03)	0.30 (0.05)	0.14 (0.02)	0.92 (0.00)	Mathematical approach
Resource dynamics	0.00* (0.06)	0.00* (0.09)	**0.00* (0.13)**	0.01 (0.06)	Mathematical approach
Discrete time	0.00* (0.10)	**0.00* (0.31)**	0.00* (0.27)	0.46 (0.01)	Pesticide or drug
Stochasticity	0.38 (0.02)	0.46 (0.03)	0.76 (0.00)	0.23 (0.03)	Year
Simulation	0.85 (0.01)	0.01* (0.14)	0.01* (0.06)	0.23 (0.03)	Year
Strategies					
No. of molecules	0.82 (0.00)	0.09 (0.05)	0.21 (0.01)	0.29 (0.00)	Year
Refuge	0.00* (0.04)	**0.00* (0.29)**	0.00* (0.09)	0.56 (0.00)	Pesticide or drug
Temporal distribution	1.00 (0.00)	0.30 (0.04)	0.25 (0.01)	0.36 (0.01)	Mathematical approach
Mixture	0.11 (0.03)	0.00* (0.10)	0.21 (0.02)	0.11 (0.02)	Citation group
Rotation	0.21 (0.02)	**0.00* (0.11)**	0.08 (0.02)	0.11 (0.01)	Pesticide or drug
Mosaic	0.52 (0.01)	0.44 (0.10)	0.19 (0.06)	0.76 (0.00)	Mathematical approach
Alternative methods	0.04 (0.04)	**0.00* (0.10)**	0.63 (0.01)	0.47 (0.00)	Pesticide or drug
Output					
No. of pests	0.23 (0.02)	0.43 (0.03)	0.90 (0.00)	0.97 (0.00)	Citation group
Resource	0.00* (0.14)	0.00* (0.22)	**0.00* (0.37)**	0.47 (0.00)	Mathematical approach
Frequency of resistance	0.74 (0.00)	0.01* (0.11)	**0.00* (0.11)**	0.41 (0.00)	Mathematical approach
Economics	0.10 (0.09)	0.06 (0.16)	0.10 (0.05)	0.03 (0.02)	Mathematical approach
Graph	0.12 (0.02)	0.28 (0.03)	0.04 (0.03)	0.83 (0.00)	Mathematical approach
Finite time	0.15 (0.02)	**0.00* (0.10)**	0.53 (0.01)	0.44 (0.00)	Pesticide or drug
Frequency threshold	0.00* (0.08)	**0.00* (0.16)**	0.00* (0.06)	0.84 (0.00)	Pesticide or drug
Equilibrium	**0.00* (0.09)**	0.00* (0.09)	0.00* (0.05)	0.15 (0.01)	Citation group
No. of max. dev.	0	28	3	0	
No. of best ΔAIC	4	12	12	3	
No. of significant tests	12	19	14	0	

P-values for Fisher's exact tests of the effect of the four explanatory factors on the variation in the use of the 31 model parameters, deviance (%) accounted for by the factor (in brackets), and factor best accounting for article classification according to the Akaike Information Criterion (AIC). The asterisks indicate significant Fisher's exact tests on contingency tables after false discovery rate correction (calculated on the basis of 31 tests and at the 5% level). Characters in bold typeface indicate that the best explanatory factor according to the AIC was significant in Fisher's exact test.

evaluate the evolution of resistance. More than 50% of the articles dealing with insecticide resistance (*sensu lato*) used the Frequency threshold criterion, while articles dealing with resistance to fungicides and herbicides were more likely to use the Finite time to reach a threshold criterion. Most articles modelling the evolution of drug resistance considered none of these criteria, focusing instead on the Equilibrium output criterion. This model

parameter was best accounted for by the CG factor (9% of the total deviance).

Factors best accounting for the combination of model parameters

Finally, our analyses reveal that all 187 articles used different combinations of model parameters. Globally, the
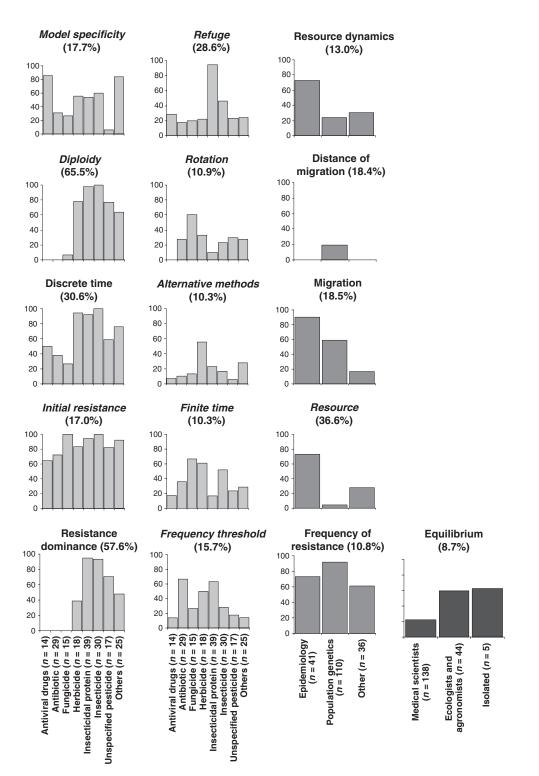
Figure 2 Frequencies of articles considered positive for the various model parameters. Data are presented as a function of the explanatory factor giving the best Δ Akaike Information Criterion (light grey: Pesticide and drug; medium grey: Mathematical approach; dark grey: Citation group). Details of the model parameters are presented in Table 1 and the per cent in brackets are the proportion of the deviance accounted for by the most explanatory factor (Table 3).

hierarchical clustering tree did not reveal any clear structuring of the articles based on the combinations of model parameters they used (Fig. 3). The deep nodes of the tree were supported by very low bootstrap values, suggesting that the information supplied by the 31 model parameters was highly heterogeneous. However, many intermediate and superficial nodes were supported by bootstrap values above 50%. The correspondence of this clustering as a function of the four explanatory factors was also assessed. Our findings suggest that the factors CG, MT and PD were not randomly distributed among the leaves of the tree, indicating that these factors account for the clustering.

Discussion

In this study, we describe the state-of-the-art for modelling of the evolution of resistance to pesticides and drugs, based on a bibliographical analysis of 187 models. In this discussion, we will begin with identifying and discussing the model parameters which were either rarely or frequently taken into account in the 187 models considered. We will then present and discuss the parameters for which none of the four factors potentially accounting for variability in the features of the models (scientific community structure, class of PD, MT and year of publication) actually accounted for the observed heterogeneity. Finally, we will discuss the extent to which each factor accounted for the use of the remaining model parameters. Based on our results, we propose in conclusion guidelines for the future development of theoretical models of the evolution of pesticide resistance.

Poorly investigated model parameters

A small number of models simulated quantitative resistance, recombination and cross-resistance between molecules. When more than one molecule was considered (35% of the models), the resistance mechanisms considered tended to be monogenic, independent and nonepistatic. This may be a reasonable assumption, because there is considerable evidence to suggest that resistance to pesticides and drugs mostly evolves through the selection of alleles with a major effect, and this view is supported by theoretical models (Roush and McKenzie 1987; Neve 2007). However, in some cases, resistance is clearly because of genes located on several chromosomes (Denholm and Rowland 1992) or has emerged from the addition of several mechanisms of small effect such as limited detoxification, sequestration and/or translocation (Park and Brown 2002), thus evolving as a quantitative genetic trait. The assumption that resistance is monogenic may thus reflect a reluctance to increase model complexity. Whatever the reason, quantitative multiple gene resistance

has not been the subject of any modelling approach by the 187 articles selected. Furthermore, although multi-drug resistance is frequent and despite the fact that many pesticide programs use a combination of nonindependent chemicals, cross-resistance is seldom considered into the models.

The distance of migration has also been largely ignored in models. In epidemiological models, microbes or viruses are considered to be transmitted from host to host. In these models, the distance over which the microbes are able to disperse depends on the hosts' movements and is therefore not a relevant parameter. The very small number of spatially explicit models is more surprising for population genetics models. Indeed, the distance of migration of pests is a key parameter determining the speed with which resistance spreads. It is a key factor in the management of Bt crops as part of an High-Dose-Refuge strategy (Peck et al. 1999; Caprio 2001; Ives and Andow 2002; Vacher et al. 2003; Cerda and Wright 2004; Sisterson et al. 2005; Tyutyunov et al. 2008).

The least studied of the basic strategies commonly used to delay the evolution of resistance was the mosaic strategy. Conversely, rotation was considered in about 25% of the models. Thus, when two molecules were considered, their distribution was more often considered over time, with nonoverlapping treatments, than over space, although these two dimensions could be symmetrically and even simultaneously explored. One reason for this lack of consideration of the mosaic strategy may be the greater complexity the introduction of this parameter would induce as such models are spatially explicit. Moreover, the mosaic strategy does not necessarily reflect current practices in agronomy or human health. Indeed, this strategy requires spatial management extending beyond the level of a single producer or a single hospital. Furthermore, as molecules often differ in efficacy, it is ethically unthinkable to adopt a strategy in which a proportion of human patients are not given the most effective cure. However, it would be possible and pertinent to evaluate the effect or consequences that an unequal access to medical care has on the evolution of resistance in human parasite.

The last model parameter poorly considered to date is the economic criterion for the comparison of efficiency between strategies. This absence is puzzling, because economics is one of the most important criteria, particularly in agronomy. One potential explanation for this is the selection of articles from life sciences databases, without considering articles referenced only in social sciences databases. It is also difficult to estimate both yield losses from pest densities (but see Ojiambo et al. 2002) and indirect economic costs, such as medical care, and their variability over a long-time period (Fleßa and Marschall 2009).
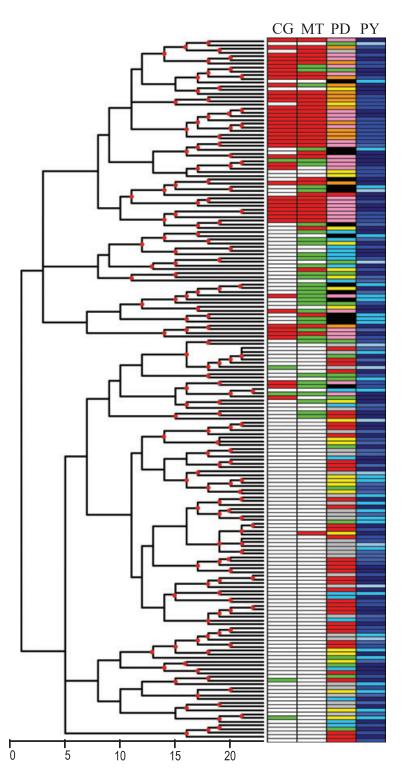
Figure 3 Tree of the 187 articles, showing their similarities based on the grid parameter values and their classification according to the four factors used for article classification. 'CG' is the citation group (the 'ecologists and agronomists' group in white, the 'medical scientists' group in red, and 'isolated' in green), 'MT' is the mathematical approach (population genetics in white, epidemiology in red and other in green), 'PD' is pesticide or drug (antiviral drugs in orange, antibiotics in pink, unspecified pesticides or drugs in green, fungicides in black, insecticides in grey, *Bt* toxin in red, herbicides in blue, others in yellow) and 'PY' is the publication year class (from light to dark blue, before 1986, 1986–1990, 1991–1995, 1995–2000 and 2001–2006). Red dots on the nodes indicate bootstrap values above 50%.

The use of an economic criterion for the management of drug resistance is also clearly limited by ethical considerations. Conversely, the introduction of economic criteria into models focusing on the development of pesticide resistance would favour the emergence of more sophisticated strategies. For example, the definition of an economic threshold below which the cost of treatment exceeds direct yield losses and other indirect side effects could prohibit treatment. The use of conditional treatments based on economic criteria each year could affect the dynamics of resistance evolution and might lead to the selection of different best strategies. Finally, we believe that the lack of reference to economic criteria highlights the contradiction between short-term return and long-term benefit. Calculations of the economic loss associated with the evolution of resistance would provide a clearer long-term view.

Frequently considered model parameters

Almost all the models used simulations. We expected the proportion of simulations to increase over time with increases in both the complexity of the models and the power of computers. However, the number of biological parameters included in models did not increase over time. Instead, the proportion of specific models – models using a large number of parameters to fit an existing situation – remained constant at about 50%.

The models clearly identified selection as the most important of the four key processes involved in the build up of resistance as an adaptive trait. This would appear to be logical, as pesticides exert a very strong selection pressure, decreasing the impact of migration, mutations and genetic drift on the evolution of resistance. Moreover, the poor accounting for genetic drift (and more generally stochasticity) had at least one consequence: the models did not consider situations in which elimination of the pest was a potential strategy for pest populations of limited size or in restricted areas (but see Boni et al. 2008a,b). Furthermore, the genetic drift may have important impact on the evolution of resistant phenotypes in the absence of drug selection (Levin et al. 2000).

Most models considered resistance alleles to segregate in populations before the introduction of selection pressure. This assumption may be correct (Génissel et al. 2003; Wenes et al. 2006), but probably not in all cases. For instance, the absence of glyphosate resistance in weed populations treated over a period of 25 years suggests a lack of pre-existing resistance alleles for this molecule in these populations (Dyer 1994; Bradshaw et al. 1997). Most models defined not only an initial frequency of the resistance allele but also set this frequency to a value several orders of magnitude above the frequency predicted under the hypothesis of mutation–selection balance.

Initial frequency of resistance alleles is generally not measured *in natura*. Moreover, the paucity of the measurement of their cost in the literature may prevent the computation of this frequency at mutation–selection balance. Therefore, this approach may be seen as conservative, as resistance is predicted to appear more rapidly than it would in natural situations, but it may also preclude the exploration of strategies in which resistance alleles may be lost by genetic drift.

Almost all models calculated changes in the frequency of the resistance allele over time. However, 10% of the models surprisingly ignored this output parameter. In 40% of the articles, the frequency of resistance was the only criterion used to compare strategies in terms of efficacy. As highlighted above, demography, yield loss or patient recovery and economic criteria are equally important alternative outputs for facilitating stakeholders to choose the best strategies for efficient chemical control.

Model parameters independent of the explanatory factors

Although taken into account heterogeneously in the models considered, several model parameters were found to be independent of the four explanatory factors. For instance, the cost of resistance was included in most models, regardless of the year of publication, scientific community, MT or class of PD. This finding is consistent with the early identification of the fitness cost of resistance being a key feature in the evolution of resistance to many pesticides and drugs. Fitness cost is not only the most directly obvious selective force counteracting the selection pressure exerted by pesticide treatments, but also underlies some of the possible control strategies, such as the stable zone strategy (Lenormand et al. 1998).

Similarly, it is fairly obvious why the maximal number of active molecules and the temporal distribution of these molecules were not linked to any of the four explanatory factors. For all the classes of PD, different molecules can be combined. Conversely, there is no obvious reason why the inclusion of strategies should differ as a function of the type of modelling or for different scientific communities investigating these opportunities in very different manners.

Effect of explanatory factors on model parameters

Year of publication had no impact on the use of each of the 31 model parameters. This suggests that new models were rarely developed through more detailed analysis of previous models and that most of the parameters, including those referring to space processes, had been considered from the earliest efforts to develop such models.

We expected scientific community to be a key factor accounting for variability between models. We had previously shown that the two major modelling communities ('ecologists and agronomists' and 'medical scientists') were isolated from one another (REX Consortium, 2007). This lack of exchange between groups could result in strong differences in the model parameters considered by each community. Twelve parameters were indeed considered differently by the two modelling communities, including, in particular, the ploidy of the target organisms and the dominance of the resistance alleles. This is consistent with the notion that the first community includes ecologists or agronomists preferentially working on diploid pests, whereas the second includes medical scientists focusing mostly on haploid microorganisms (bacteria and viruses). The difference in the modelling approaches developed by the modelling communities is therefore more likely to reflect differences between the organisms studied rather than differences in school of thought.

Population genetics and epidemiological models differed greatly in the ways in which they considered host 'quality'. Physicians and veterinary surgeons readily distinguish three classes of patients: healthy, infected and immune. Conversely, the quality of the resources in population genetics models (essentially the host plant for insects and fungi) is considered to be constant over time. This assumption is not always true. The physiological defences of attacked plants have both direct and indirect effects on pest dynamics, because of secondary secretions that are either directly toxic, limiting further attacks, or attract natural enemies of the pests, increasing the rates of parasitism and predation of the phytophagous pests (Despres et al. 2007). Similarly, large-scale germination of an uncontrolled weed may reduce or delay the development of new cohorts (Marushia and Holt 2008). Finally, pest damage may also promote the arrival of other pests (Landolt et al. 2000). A rapid review of papers published after the building of our database shows that alternative MT for modelling the evolution of resistance can now alleviate some of the limits described in this paper (Boni et al. 2006, 2008a,b; Day and Gandon 2007; Debarre et al. 2009). For instance, Boni et al. (2008b) presented a SIR (for susceptible, infectious, and recovered compartments) model of malaria drug resistance taking into account all the parameters classically used in population genetics models (mutation, fitness and allele frequencies). Similarly, a recent study by Debarre et al. (2009) not only mixes epidemiology and population genetic concepts but also incorporates distance of migration in their model.

Furthermore, the output of the models mirrored the differences between MT. Epidemiological models tended to focus strongly on the quantity and quality of healthy resources, whereas population genetics models often focused exclusively on changes in resistance allele frequencies. Indeed, population genetics models generally ignored the impact of pesticide treatments on pests and yields. At best, they modelled the population dynamics of the target pests while ignoring its effect on the resource on which the pests were living. This is clearly a pitfall, because the link between population density and damage is often not linear (Mitchell et al. 2004). We also know that pests not only reduce yields, but may also reduce the yield quality. For instance, maize may be contaminated by mycotoxins from fungi, the development of which is favoured by the damage caused by European corn borer larvae (Papst et al. 2005). Thus, population geneticists rarely include in their models the possible avoidance of treatment as a reasonable strategy.

Class of pesticide or drug as the best explanatory factor

Model classification on the basis of clustering analysis (based on the 31 questions of the grid) was clearly linked to factors such as the MT used and the scientific community. However, the PD factor was found to account for the largest proportion of the deviance explained, and was also considered the most explanatory on the basis of AIC and the number of significant Fisher's tests for the 31 model parameters considered.

Fungicides and antibiotics were the two classes of PD most frequently included in general models, for which the problem of resistance management is considered before specific cases arise. By contrast, drug resistances in populations of viruses, such as human immunodeficiency virus, were mostly explored on a case-by-case basis.

The models developed for herbicides, antibiotics and antiviral drugs frequently included mutation rate, whereas models developed for fungicides, insecticidal proteins and insecticides seldom introduced this parameter. The underlying rationale is probably that short-lived organisms have large effective population sizes and experience several generations under selection pressure, so mutations can indeed appear during the selection process.

The use of refuges – areas free of treatment – was considered in 94% and about 50% of the models developed for *Bt* resistance and insecticide resistance, respectively, but only in about 20% of the models of resistance to fungicides and antibiotics. As pointed out above, ethical reasons may preclude some strategies. The absence of refuges in models exploring antibiotic resistance illustrates this point: one can hardly imagine risking the patient's life by establishing 'untreated refuges' to delay the evolution of resistance. However, it should be stressed that the use of models can overcome this problem, making it possible to analyse potential scenarios without consequences. We must also keep in mind that untreated

populations – e.g. populations that are excluded, notably for economical reasons, from medical cares – actually constitutes involuntary refuges for bacteria and viruses. Hopefully, the increase of medical care would decrease the number of untreated people. This would in turn decrease the per cent of refuges for susceptible bacterial strains and therefore reinforce the selection for resistance.

The rotation strategy was completely ignored in models investigating resistance to antiviral drugs. This is probably because viruses reproduce rapidly, over time scales much shorter than the duration of treatment. Rotation would therefore mimic a mixture strategy in practice. Rotation of molecules was also poorly investigated in models of the evolution of *Bt* resistance. This is unfortunate, because several *Bt* toxins with different target sites are often active against the targeted pests. This lack of consideration of this strategy may be related to practical problems. For *Bt*

crops, rotation would require a general agreement at regional scale, potentially requiring federal legislation (Bourguet et al. 2005; Vacher et al. 2006). The addition of rules on the types of *Bt* crops farmers must grow, in addition to mandatory refuges, would be a challenging political issue. Finally, the high level of consideration of rotation in models of the evolution of fungicide resistance (60%) may be accounted for by the number of treatments per year. Indeed, the need for successive treatments during the season makes it possible for the owner of the field to adopt a rotation strategy unilaterally, without the need for concerted deployment at the regional scale. This underlines that the rotation strategy can finally correspond in practice to rotation of the mosaic strategy. Increasing the heterogeneity of the selection using several molecules independently can also be an effective strategy delaying the emergence of resistances (e.g. Boni et al. 2008a,b).

Table 4. Guidelines for further modelling the evolution of resistance.

Class of parameters	Observations and recommendations	Pesticides or drugs concerned
Biological parameters	Like models dealing with resistance to herbicides, antibiotics and antiviral drugs, models exploring the evolution of fungicide resistance could include mutation rate allowing resistance alleles to appear by mutation from susceptible alleles during the selection process	Fungicides
	The influence of pest migration on the evolution of resistance could be further explored by developing spatially explicit population genetics models	All pesticides expect fungicides
	While resistance sometimes involves several genes (such as detoxification), models considered almost exclusively monogenic resistance. Models could therefore consider cases of quantitative multiple genes resistance	All pesticides and drugs
	Among the evolutionary processes involved in the build up of resistance as an adaptive trait, models clearly emphasized the selection process. Models could give more emphasis to migration, mutation and genetic drift	All pesticides and drugs
Strategies	The mosaic strategy is rarely considered probably because the greater complexity the introduction of this parameter would induce. The development of spatially explicit models would allow a comparison of this strategy with the other strategies	All pesticides and drugs
	The rotation strategy was ignored in most models of the evolution of resistance to insecticidal proteins. The development of transgenic crops with different proteins would make this kind of models useful	Insecticidal proteins
	Probably for ethical reason, the refuge strategy – i.e. the maintenance of untreated areas/ patients – have not been consider in human epidemiological models. The investigation of this strategy would be a mean to evaluate the effect or consequences that an unequal access in medical care has on the evolution of resistance in human parasite	Antiviral and antibiotics
	More than half of the articles modelling the evolution of herbicide resistance considered strategies based on alternative methods, such as crop rotation or the mechanical control of weeds. Models on other kind of pesticides could also considered alternative methods for controlling pest	All pesticides except herbicides
Outputs	Among the criteria used for comparing strategies, the economic criterion was rarely used. Models could include demography, yield loss or patient recovery and economic criteria as outputs for facilitating stakeholders to choose the best strategies for efficient pest control	All pesticides and drugs
	Epidemiological models tended to focus strongly on the quantity and quality of healthy resources, whereas population genetics models often focused exclusively on changes in resistance allele frequencies. Population genetics models could consider (i) the impact of pesticide treatments on pests and yields in population genetic models and (ii) the effect of variation in pest demography on the resource on which the pests are living	All pesticides except fungicides

Conclusions and recommendations

Analysis of the possible causes of model diversity was highly informative. In a previous article analysing the scientific community of resistance evolution modellers through its citation and co-citation networks, we concluded that the scientific community was highly structured. We show here, that the main factor explaining the diversity of the models is the class of PD linked to the target organism, either than the structure of the scientific community.

Along the discussion, we have identified some lacks in biological parameters, strategies and outputs considered so far. They are summarized in Table 4. In this table, we provide guidelines for further modelling of resistance evolution.

Overall, we ended up with three main intermingle conclusions. First, among the four evolutionary processes involved in the build up of resistance as an adaptive trait, models clearly emphasized the selection process. Migration, mutation and drift are by far too rarely integrated in the models impeding the exploration of new strategies. For example, the fact that drift is not well accounted for has the consequence that models did not test the situation where a strategy would be pest or pathogen elimination at least over restricted area.

The second conclusion is that there is a clear asymmetry between space and time as sources of heterogeneity in the selection pressure. Much more emphasis has been made over time than over space processes. The increased capacity of computers to integrate complexity has not so far resulted in a better account of space in models. As a consequence, strategies like rotation, mosaic or refuges would remain clearly under-analysed. More generally, migration has been insufficiently considered in its double consequence of delaying the build up of resistance in a given space while transferring this resistance into new previously unscathed places.

Our last important conclusion is that many papers explored a situation of poor potential strategic interest. Most of the models analyse the performance of already used strategies in case of already present resistance rather than exploring new domains such as coupling rotation and mosaic in a more or less complex design. In parallel, models would also gain in extending the use of economic criteria or pest consequence that would allow test acceptance or integrate treatment. The paucity of the consideration that models make of the consequence of the evolution of resistance on the quality of the resource and its economical viability would contribute to delay the transfer from simulations to their experimental validation and use.

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