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Dynamics of the Emergence of Genetic Resistance to Biocides among Asexual and Sexual Organisms

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ABSTRACT:

A stochastic, agent based, evolutionary algorithm, modeling mating, reproduction, genetic variation, phenotypic expression and selection was used to study the dynamic interactions affecting a multiple-gene system. The results suggest that strong irreversible constraints affect the evolution of resistance to biocides. Resistant genes evolve differently in asexual organisms compared with sexual ones in response to various patterns of biocides applications. Asexual populations (viruses and bacteria) are less likely to develop genetic resistance in response to multiple pesticides or if pesticides are used at low doses, whereas sexual populations (insects for example) are more likely to become resistant to pesticides if susceptibility to the pesticide relates to mate selection. The adaptation of genes not related to the emergence of resistance will affect the dynamics of the evolution of resistance. Increasing the number of pesticides reduces the probability of developing resistance to any of them in asexual organisms but much less so in sexual organisms. Sequential applications of toxins, were slightly less efficient in slowing emergence of resistance compared with simultaneous application of a mix in both sexual and asexual organisms. Targeting only one sex of the pest speeds the development of resistance. The findings are consistent to most of the published analytical models but are closer to known experimental results, showing that non-linear, agent based simulation models are more powerful in explaining complex processes.

Key words:

Insecticides, biocides, pesticides, antibiotics, resistance, sex, evolution

Short title: Dynamics of genetic resistance

INTRODUCTION

After a prolonged use of antibiotics or pesticides, often genetically resistant strains emerge in the treated population, rendering the treatment useless. This problem is becoming more serious as an increasing number of species become resistant. Metcalf (1980) for example wrote that "the concerted effect of the exponentially increasing costs of insecticide development, the dwindling rate of commercialization of new materials, and the demonstration of cross or multiple resistance to new classes of insecticides almost before they are fully commercialized makes insect pest resistance, the greatest single problem facing applied entomology". To understand and eventually solve this problem Georghiou (1972) predicted that in the future computer programming may provide a method for making reliable predictions regarding factors which influence the potential for development of resistance in a population. In fact, several models have been developed to study emergence of resistance (the more recent ones include Tabashnik 1990a,b, 1994, Moran 1992, Capiro and Tabashnik 1992, Comins et al. 1992, Follet et al. 1993, Curtis et al. 1993 for example) which have increased our understanding of the problem. But many aspects still defy our understanding. For example, McKenzie and Batterham (1994) showed differences in the evolution of insecticide resistance in the field from that in laboratory experiments, suggesting that the difference arises due to the fact that in the field, evolution of insecticide resistance tends to be based on an allele of major effect at a single locus, whereas resistance obtained in the laboratory is usually polygenically based. This difference might also be related to different costs of selection to pesticides (Groeters et al. 1994). The adaptation of phenotypic characters not related to the genes involved in resistance is generally thought of as irrelevant to the dynamics of emergence of resistance. This might not necessarily be true, as evolution in complex systems show irreversible behaviors which may produce counter-intuitive adaptation dynamics (Axelrod 1995, Jaffe 1996b for example) which are not apparent from analytical models or from simplified simulation models.

Several mechanisms may be involved in evolution of resistance (French et al. 1992, Valera et al. 1993, Clark et al. 1995, for example) and abundant experimental data (for the two most recent reviews see Tabashnik 1994 and Clark et al. 1995, for example) allow for the validation of theoretical models. It is widely accepted that the factors involved in the development of resistance to a chemical agent are the existence of a resistant gene in the population and the presence of an adequate selection pressure (Cochran 1993; Khayrandish and Wood, 1993a; Keiding et al., 1992 and McKenzie and Byford 1993). The rate at which resistance develops will depend on the frequency of the resistant genes, their dominance, and the history of selection pressure due to pesticides. Also important are the type of pesticide involved, the specific mechanism of resistance present (like delayed penetration of the toxicant, increased metabolism and nerve insensitivity), the eating habits, and the number of generations per year. Considering these factors, Georghiou (1983) suggested various strategies that can be used as counter-measures. These strategies are grouped in three general categories: management of resistance by moderation, by saturation and by multiple attack. Among the most common suggestions are the uses of low doses of pesticide, the use of chemicals with low persistence in the environment, the avoidance of compounds with gradual liberation rates and the use of synergic compounds or mixtures (Dong and Scott, 1992; Yano et al., 1992; Calderon, 1992; Khayrandish and Wood, 1993b and van Laecke and Degheele, 1993).

Most of the recommendations to avoid the emergence of biocide resistance are based on models. One of the most important criticisms to the use of models in biology, and in explaining genetic resistance in particular, is that biological and ecological systems are rather complex, and that simple models ignore that many relevant biological phenomena are emergent

properties from complex interactions. This criticism is difficult to refute as evidence of the emergence of unexpected properties from complex system simulations is mounting (Ruelle 1991, Prigogine 1996 for example). Complex simulations of sexual reproduction in virtual organisms, for example, have shown that the behavior of the system is dependent on complex interactions, not evident from simple simulations (Jaffe 1996b). Although sex is thought to be adaptive because it enhances genetic variability in organisms (Fisher 1930), no computer model to date has been able to demonstrate this advantage, weighed against a greater mutation rate in asexual organisms. Recently Hamilton et al. (1990) suggested that sexual reproduction might have evolved to reduce parasite pressure. A multi-locus dynamic simulation model (Jaffe 1996b) showed that sex is only adaptive if mate selection mechanisms exist and that mate selection is fundamental for complex organisms (i.e. organisms with a large number of genes) as it guides evolution in situations where multiple simultaneous selection pressures act on the organism. The simulation model allows for continuous monitoring in an evolving population of the genetic composition of multiple loci, responsible for a variety of phenotypic expressions, not necessarily related to pesticide resistance. It revealed that certain well-known biological features, such as sex, are the product of dynamic constraints of the evolutionary process, rather than adaptations to specific selective pressures. The model also showed that genetic drift was very strong in organisms, with various loci simultaneously subjected to selection. The genetic characteristics of a population, once evolved, were not normally reversed by reversing selection pressure, demonstrating an irreversible dynamics producing a kind of genetic "memory" in the population. Populations following an asexual reproductive strategy adapted faster to environmental constraints but failed to consistently stabilize the allelic frequency distribution at optimal values, showing a fast reduction of the variability of the gene pool. Sexual populations on the other hand, responded much slower to selective pressure, but had a higher probability of reaching optimal values in the fitness landscape. Sexual and polyploid populations maintained a larger variability of their gene pool, even after stabilizing the genetic frequency distribution and thus, were more resistant to the depletion of the variability of the gene pool due to selection, compared with asexual organisms. These simulations showed that sexual strategies required mate selection mechanisms to prevent asexual genes from invading and taking over the population. Thus, different evolutionary dynamics affect asexual and sexual organisms, suggesting that different mechanisms are involved in the emergence of genetic resistance to pesticides in sexual and asexual organisms.

The aim of this paper is to simulate organisms, as complex as possible, and to compare the results with those of more simple models, in order to assess if dynamics of the emergence of genetic resistance is affected by complex interactions in complex systems, with special reference to the various reproductive mechanisms used by pests and parasites.

METHODS

The main difference of the present model to previous ones is its complexity. This one studies the effect of various selection pressures on 17 different genes evolving simultaneously in a population of organisms. This agent based adaptive model, simulates a population of virtual organisms which suffers various kinds of processes as described below and is described in detail elsewhere (Jaffe 1996 a,b).

The numerical simulation model, based on the Monte Carlo method, consisted of a population matrix $M(i,j,k)$, representing a given number of organisms i , with gene loci j , represented by alleles or gene copies k (the number of k is dependent on the ploidy number). The phenotype at birth was defined by $M(i,j,1)$. At each time step, representing a reproductive cycle, each organism " i " was subjected to specific transformation rules defined by logical

algorithms simulating the 5 transformation steps described below. For example a female individual $i=n$ of species 1 it was referred to as $M(n,1,1)=1$ and $M(n,8,1)=1$ where $j=1$ indicate genes defining the species and $j=8$ defines the gene coding for sex. This individual, in order to reproduce, had to find a mate $i=m$ such that $M(m,1,1)=1$ and $M(m,8,1)=2$. Always allele $k=1$ was expressed phenotypically, whereas allele $k=2$ was absent in haploids and in diploids was never expressed phenotypically but could be inherited and eventually expressed as allele $k=1$ in the offspring. The genetic composition of the virtual population was monitored continuously, visualizing the dynamic properties of a complex interacting assemblage of genes evolving together in a population of organisms. An initial population of a given size (initial number of organisms) was created by randomly assigning in the matrix $M(i,j,k)$ to each individual i a specific set of genetic characteristics (alleles), corresponding to different genes, each codifying for a specific phenotypic character through the value of its allele. This initial population was then subjected to a transformation program consisting of: Mate selection, Reproduction, Variation, Phenotypic expression, and Selection of the phenotypes.

The surviving population continued to endure the five-step transformation, at each time step. The exact genetic composition of the population was plotted at each time step, using the actual number of surviving individuals. Thus we could assess when and if the population survived.

The model did not assumed any simplified expression of fitness but reproduction and individual survival were decomposed into different aspects using external and internal variables. The external or ecological parameters were set before the simulation and remained constant. These were: optimal size of the population (or size above which density dependent selection increased exponentially, ops), initial size of the population (ino), number of genes (ng), proportion of individuals randomly killed at each time step (density independent selection), proportion killed if not possessing the correct alleles for resistance (gene dependent selection with strength $pe1$ and $pe2$) optimal clutch sizes (or clutch size above which fitness of offspring is reduced, ocs), optimal age for reproduction (or age before and after which reproduction will decrease the fitness of mother and offspring, oar), reproductive system (monosexual or bisexual) and ploidy (haploid or diploid).

Internal or biological parameters were initially assigned at random among the individuals of a virtual population, and then varied (i.e., suffered variation and selection) according to the constraints of the five-step transformation process. The internal parameters were modeled as a distinct gene coding for specific phenotypic characteristics of the organisms subjected to selection. These genes j were: species (where one allele defined the species and which in this case were immune to mutations), maximum life span (with 10 possible allelic values of k coding for life spans from 1 to 10 time steps), maximal clutch size (11 alleles coding for clutch sizes from 0 to 10), minimum age for initiating reproduction for males and for females separately (5 alleles each), mutation probability (11 alleles coding for mutation rates from 10^{-7} to 0.2 mutations per gene with logarithmic increments), mutation intensity (11 alleles coding for new mutated values close or far from the original value of the allele suffering mutation), sex (2), sex ratio of newborns (10), two kinds of resistance to biocides (with 11 alleles each, where only allele 0 was resistant to that particular biocide), number of males screened in search of potential mates before mating ($mef = 1$ to 90) and two neutral genes (with 10 alleles each) whose phenotypic expression did not affect the fitness of the organism.

The five transformation steps consisted of the following:

Mate selection: Sexual individuals not finding the right mate after screening the given number of mates did not reproduce during that time step. Females choose a mate so that mates were of the same species and of different sex. In addition, when mate selection was simulated, females preferred males showing resistance to simulated pesticides but mated with

conspecific mature males if they could not find resistant mates during their mate search (mate search was limited by the value of the corresponding gene). Mate selection of resistant phenotypes aimed to simulate situations where pesticides affect the ability of males to court and or attract females. For example, we may imagine a common situation with insect pests, where control systems are sometimes based on pheromone traps, where males having a more attractive pheromone blend than that in a sub-optimal artificial pheromone trap have a relative reproductive advantage in the field.

Reproduction: Mated individuals produced offspring according to their phenotypically fixed clutch size, transmitting their genes to the offspring according to the following rules:

Asexual reproduction: genes of newborn were identical to those of the single parent.

Bisexual reproduction: each parent provided all (if haploid) or half (if diploid) of the genes to the newborn, which then had one or two copies of different alleles for each gene respectively, following the rules of segregation (i.e. meiosis). That is, if the first chromosome (allele) in the offspring was selected from the father, the second came from the mother, or vice versa.

We defined asexual reproductive systems as those in which organisms possess only one sexual form and individuals transmit a complete and single copy of genetic information to their offspring (haploid). Sexual reproductive systems were modeled as haploid or diploid bisexuals (individuals of two different sexual morphs), in which individuals possessed either one (haploid) or two (diploid) copies of genetic information.

Variation: Randomly selected genes mutated, changing their allelic value in a genetically determined range (mutation intensity) and with a genetically determined probability (mutation probability, or probabilistic frequency of mutations). For example, a mutation probability of 0.01 will in average produce a mutation, randomly, in one of every 100 genes passed over to the offspring.

Phenotypic expression: A single, randomly chosen allele was expressed phenotypically. Complex life history traits, similar to what is known for most real organisms, were simulated. Here we restrict our study to three features, partially determined by three genes: Maximal possible life span, maximal possible clutch size and age for starting reproduction. The features simulated here correspond to the most common mechanisms known from the reproductive biology of insects and mammals. That is, the final clutch size of individuals was calculated based on the allelic characteristic of the gene coding for clutch size and the age of the reproducing individual, using a normal distribution, so that :

$$cs(i) = M(i,cs,1) / ((oar^{0.5})^{-((age(i)-oar)^2)} / oar)$$

where $cs(i)$ is the clutch size of individual i , $M(i,cs,1)$ is the allelic value for maximum clutch size in the part of the genome which is phenotypically expressed by individual i , $age(i)$ is the age of individual i , oar is optimal age for reproduction fixed as an external parameter in each simulation. That is, the maximal clutch sizes was genetically predetermined and occurred only at an optimal age of reproduction. The size of the clutch of newborns affected the probabilities of survival of the future adult, so that individuals born in clutches larger than optimal decreased their probability of survival exponentially. The equation here was :

$$\text{if } cs(i) < ocs \text{ then } fit(k) = 1 \text{ else } fit(k) = ocs/cs(i)^3$$

where $cs(i)$ is the size of the clutch individual k was born, ocs is optimal clutch size, fixed as an external parameter in each simulation, and $fit(k)$ is the extra probability for the offspring k being killed by random selection.

Selection: Individuals were excluded from the population when their age exceeded their genetically prefixed life span, when randomly selected by density dependent and density independent constants, when parents clutch size exceeded optimal sizes at high population

densities, or when a pesticide was applied to randomly trim the population from individuals not possessing the right resistant phenotype. Individuals with non resistant phenotypes of genes R1 and R2 were killed randomly according to the probabilities pe_1 and pe_2 .

Variables: We assessed the probability of the development of resistant genes in response to the application of pesticides (or illness) by repeating simulations under various specific conditions. The conditions were:

Sex: Could be asexual (monosexual-haploid), bisexual-haploid or bisexual-diploid.

Target of the pesticide: The pesticide affected both sexes or only males or females. Females mated once, whereas males could mate several times during each reproductive cycle.

Number of types of pesticide: Either 1 or 2.

Pesticide efficiency (pe): Indicated the proportion of non-resistant individuals killed during each cycle by the pesticide (0.15, 0.20, 0.30, 0.60 or 1.0).

Initiation of pesticide application (t): Either when pest population was expanding following colonization by wild forms (during the first generations), or after the pest population had stabilized its allelic frequency distributions as an adaptation to the new environment (during later generations).

Type of management: Pesticides were applied in three forms :

a- Continuously with an alternating frequency each second time step (i.e. reproductive cycle) during the whole simulation (Table 1,2) ;

b-Either simultaneously or the second pesticide was applied with a time lag (Table 3,4: with a time lag $tl = 0, 5$ or 10 reproductive cycles).

c- Each pesticide was applied during 5 time steps only, either simultaneously or alternating them (Table 5,6: timing of initiation of application for each pesticide is given by t_1 and t_2).

RESULTS

Several thousand simulations were performed, and thus only the most relevant results can be shown. Any specific scenario can be studied running the computer model (Jaffe 1996a). Our simulations confirmed previous findings that the likelihood of emergence of genetic resistance in a given population is related to the following parameters (Table 1): Size of the initial population, i.e. the more individuals submitted to the treatment, the likelier that resistant individuals survive (compare experiment Nr. 0, 1 and 3 or 4 and 5 in Table 1) ; Length of the treatment with pesticides, i.e. the longer the simulations where run under pesticide pressure, the likelier it was that a few resistant individuals emerged (see experiment Nr.4 to 9 comparing results for time step 15 to 60); Mutation rate, i.e. at high mutation rates, fewer individuals survived and thus few resistant individuals were present (compare experiment Nr 1 with 2 and 5 with 6 or 9), showing that most mutations were lethal. When mutation rates were allowed to be fixed by selection, values converged to less than 1 mutation for every 10,000 genes). The number of genes involved in conferring resistance and the number of genes subjected to natural selection (i.e. experiments Nr 7 and 8 compared to Nr 5) had little effect on the proportion of resistant organisms in the population but affected the total number of resistant individuals as they affected total population size. These results may serve as a cross validation of our agent-based simulation with other type of models. The model however suggested that the allelic diversity of genes potentially conferring resistance was dependent on the treatment given (Table 2). Allelic variance was reduced when high concentrations of pesticide were applied, even affecting the variance of genes with no direct selective pressure (compare experiment 3 with 1 and 2 in Table 2) affecting the effectiveness of an eventual treatment with a second pesticide. This effect was due to genetic drift, which increased with the complexity of the organism (compare experiments Nr. 2 and 4). The drop in genetic diversity was less pronounced or absent in sexual species compared to asexual ones, due to

the effect of sex in maintaining high genetic diversity (Jaffe 1996b). Once a population became resistant, elimination of pesticide treatment reduced the frequency of resistant alleles in the population only very slowly, suggesting a irreversible effect of biocide treatment. Recovery to original allelic variance in organisms where mutation frequency was left for selection to fix, was not achieved simulations running for up to 200 time steps. In organisms with fixed but high mutation frequencies (average 1 mutation for every 5 genes), recovery of allelic frequencies to pre-pesticide treatment levels was approximately two times slower relative to the speed resistance emerged (by running experiment 5 of Table 2), but of course varied with concentration of biocide used.

As might be expected, a populations ability to produce resistant genes varied with sexual strategy (Table 3,4). Asexual organisms were more susceptible to various types of manipulations (greater variance of the mean probability for development of resistance in Table 3), due to the drop in genetic variability induced by strong selection. Sexual selection produced strong genetic resistance if sexual selection criteria were linked to their susceptibility to pesticides. Haploid sexual organisms (or genes with dominant resistant alleles) consistently evolved resistance faster than diploids under the same circumstances. The type of management of pesticide application (i.e. either initiating the application of both pesticides simultaneously ($tl = 0$) or initiating the application of a second pesticide with a time lag ($tl = 5$ or 10)) was more important in predicting emergence of resistance for asexual populations.

When the data in Table 3 was analyzed isolating the effect of a single factor at a time, we found: Independent of the sexual strategy used by organisms, timing of the application of pesticides was important. Two pesticides applied simultaneously reduced very slightly although significantly the odds of emergence of resistance to either of them when compared with the situation were first only one pesticide was used, and later a second pesticide was added to the treatment ($P < 0.001$, paired binomial test when $t = 20$, $tl = 0$ versus $t = 20$ $tl = 5$ in Table 3 for example). Again, this effect can be ascribed to the evolutionary dynamics regulating genetic variability rather than to specific aspects of the model.

An analysis of variance (Table 4) pinpointed the relevant parameters for the emergence of resistance. These showed to be different for sexual populations compared with asexual ones. Unlike sexual populations, asexual populations reduced significantly their odds of developing resistance when pesticides were applied long after populations stabilized in the new environment. A shorter time lag between the initiation of application of two pesticides reduced the development of resistance much more in asexual compared with sexual populations. Asexual populations were much more susceptible to multiple pesticides compared with sexual ones. Sexual populations in turn reduced significantly their odds of developing resistance if pesticide concentrations were high, but not so much the asexual populations. For sexual populations, polyploidy (or recessive genes) reduced significantly the odds for development of resistance and so did, of course, relationships between pesticide and mate selection.

A different scenario is shown in Table 5. Here pesticide applications lasted only for short periods (5 time-steps). This table again shows that sexual and asexual organisms have a different dynamics for developing resistance. Asexual organisms showed the lowest odds and variance for resistance to emerge. Again, sexual haploids (or resistance determined by dominant genes) showed that they are more prone to develop resistance compared with diploids (or recessive genes). Sequential use of toxins increased the speed for emergence of resistance in sexual but not asexual organisms.

Table 5 also shows the effect of targeting one sex in a population where males can copulate several times but females only once for each reproductive cycle. When only males were targeted, the odds for the development of resistance increased significantly ($P < 0.001$, matched pairs test), compared with situations where both sexes were targeted by the pesticide. When only females were targeted, the odds for developing resistance were not

always higher compared with situations targeting both sexes. Targeting both sexes with pesticides or only females changed the effect of reduced dose (efficiency) of pesticides on resistance. If only females were the target of a pesticide, the odds of resistance emergence in the pest populations were affected negatively at increased pesticide concentration. These results were confirmed statistically in Table 6.

DISCUSSION

Clearly, many factors determine the odds of a pest population becoming resistant to a certain treatment. For example gene amplification (Tabashnik 1990b, Devonshire and Field, 1991); biochemical factors (Rivet et al., 1994; Sakata and Miyata, 1994; Yu and Nguyen, 1994); ecological aspects (Roush and McKenzie, 1987); gene flow (Capiro and Tabashnik 1992) have been shown to affect genetic resistance. Dynamic constraints may not be the most important, but occasionally they may be manipulated without much additional cost to the treatment, and thus have particular practical importance. As our simulations showed, there are ways of reducing the odds of making a pest species resistant to a pesticide, and we may predict situations where genetic resistance will emerge with very high probability (i.e., pesticides affecting mate choice). This information could be useful in reducing the odds or delaying the appearance of resistance and could have an economic and environmental impact by reducing the quantities of pesticides applied.

The model presented here confirmed several previous conclusions from other models (Curtis et al. 1993, Tabashnik 1990a, for example) regarding the effect of concentration of insecticides, initial genetic variance or population size for example, which have been discussed extensively before. The new feature illuminated by our model is about the strictly dynamic aspects of evolution of resistance. In this respect, we found no contradiction between our simulations and experimental results reported in the literature. On the contrary, experimental data from sexual organisms help verify the model and lead support to our predictions. For example, with our simulation of sexual organisms, alternating insecticides delayed the development of resistance similarly compared with mixing pesticides. McKenzie and Byford (1993) demonstrated a significant delay, but for only one to seven generations, when using either alternating or mixtures of insecticides, compared with what occurring with single insecticide treatments. Curtis et al. (1978) found that selection against resistance was inversely related to the initial frequency of the susceptible genotypes, which could also be proven with our model. Strong et al. (1995) showed that the decline in genetic resistance of German cockroaches occurs slowly and linearly in finite populations in the laboratory, but its reemergence is very fast, showing up significantly in F1 populations of insects subjected again to the pesticide. The model produced equivalent data as presented above.

Our results show that evolution under a complex assemblage of selection pressures is different from evolution driven by a single environmental factor such as a pesticide. This may explain the differences in the evolution of resistance observed between laboratory and field studies (McKenzie and Batterham 1994). It is reasonable to assume that in the field, organisms have to adapt to a series of environmental constraints, not necessarily related to resistance, which are absent in a more uniform laboratory environment. Direct fitness cost of resistance, such as reported by Groeters et al. (1994) will affect evolution of resistance; but here we claim that even adaptation of phenotypic expression of genes, which are independent to those related to the emergence of resistance, will affect the dynamics of the evolution of resistance.

When cross-resistance is absent, a sequence of two insecticides is expected to be more durable than a mixture. Despite the lack of convincing experimental evidence for this (Tabashnik 1989, Denholm and Rowland 1992, Curtis and Kasim 1992) mixtures are often

recommended as a means to avoid or delay resistance (Stone et al. 1991, Georghiou 1990, van Rie 1991, Feitelson et al. 1992, Gill et al. 1992). Tabashnik and McGaughey (1994) seem to have convincingly demonstrated that a mixture of *Bacillus thuringiensis* strains do not slow resistance in the moth *Ploidia interpunctella* compared with a sequential application. Our model showed that increasing the number of pesticides reduces the probability of developing resistance to any of them in asexual organisms but much less so in sexual organisms. In addition, sequential applications of toxins, contrary to common believe, were slightly less efficient in slowing emergence of resistance compared with simultaneous application of a mix. Thus, this result would give a theoretical base to the actual experimental findings by Tabashnik and McGaughey (1994). Curtis et al. (1993) reached a similar conclusion studying vector control (but see Curtis and Kasim 1992), recommending the use of mixtures from the outset instead of rotation of insecticides. These authors however think that insecticides to which the vector developed resistance may eventually be used again as regression of resistance is likely to occur. Thus they use the term rotation instead of sequential application of insecticides. Our model would predict that such a regression is unlikely to occur due to the irreversible nature of the evolutionary process. Experimental confirmation of this irreversibility was provided, among many others, by Strong et al. (1995) as described above.

Our agent based simulation model provides the following conclusions:

- 1- Emergence of genetic resistance is an irreversible process. Once genetic resistance developed, it is unlikely to disappear.
- 2- Increasing the number of pesticides reduces the probability of developing resistance to any of them in asexual organisms. This relationship is less significant in sexual organisms
- 3- In particular cases where the susceptibility to a given pesticide is related to mate selection mechanisms (for example pesticides might kill differentially individuals with different "sex appeal" such as more mobile or larger males for example), emergence of resistance is accelerated. This is relevant in cases where sex pheromones are used to control pests. If sex pheromones are used to capture and kill insects, resistance to this control method (i.e. to the use of pheromones but not necessarily to the pesticide) will appear faster compared with emergence of resistance to classical pesticides, as the sex pheromone is clearly related to the mate selection mechanisms of that species. That is, mutants which use a different blend of compounds of the pheromone to attract mates or mutants which respond to different blends of the mate, will have a selective advantage compared with their con-specifics which are captured in the traps.
- 4- Pesticides used in high doses will increase the odds of developing genetic resistance in asexual organisms but not necessarily in sexual ones, although this may vary in sexual species with three or more resistant alleles (Tabashnik 1990b). In sexual organisms the life history of the pest will determine the direction of the effect of pesticide concentrations on resistance development. This is contrary to what has been suggested before (as discussed in the introduction) as low doses of insecticides are widely recommended as a strategy to reduce resistance (Georghiou, 1983, but Curtis et al. 1993).
- 5- Weakening an asexual population with one biocide or with any stress or selective pressure (e.g. predators, temperature, moisture or lack of it), and then applying a second biocide should improve the efficiency of the second biocide and reduce the odds for emergence of genetic resistance to it. This kind of approach might improve treatments of bacterial or viral infections but seems irrelevant to most insect pest management.
- 6- Control of only one sex of the pest may change the conclusions above. Differential susceptibility to pesticides among the sexes has been related to increased resistance (Rathman et al., 1992), confirming the prediction of our model. Thus, the natural history of the specific pest has to be simulated if methods for delaying resistance want to be found.
- 7- Although Aphids are often considered as asexuals, they occasionally reproduce sexually and represent a special case which we did not cover in our simulation presented here.

We believe that our results suggest strongly that pest or health problems caused by asexual organisms (viruses, bacteria) should receive a different treatment than those produced by sexual organisms (some parasites, insect pests), as both differ in their dynamics in developing genetic resistance. Toxic mixtures have shown to be successful in controlling asexual organisms (i.e. AIDS for example) but not sexual pests. Here we present a theoretical rationale for this difference. Clearly, experimental work should in the end confirm or discard our conclusions, but the biodynamic approach developed here should help in focusing on the relevant questions and showed that agent based simulation models seem to be more powerful in analyzing real situations compared with traditional analytical models used so far.

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Table 1 : Percentage of alleles showing resistance to pesticide 2 and 1 in simulated sexual populations. The results are the average of 200 simulations for each experiment.

Nr	ino	ng	pe1	pe2	mp	tstep	% alleles res 2			% alleles res 1			Nr res 1 60
							15	30	60	15	30	60	
0	1600	10	0	0	0.2	9	9	9	9	9	9	99	
1	800	10	0	0	0.2	10	10	9	9	9	9	42	
2	800	10	0	0	0.002	10	10	9	10	10	10	65	
3	400	10	0	0	0.2	8	9	9	9	9	10	19	
4	400	10	0.2	0	0.2	10	8	9	15	21	27	40	
5	800	10	0.2	0	0.2	9	8	9	15	21	28	112	
6	800	10	0.2	0	0.002	8	8	9	17	30	55	321	
7	800	10	0.2	0.2	0.2	15	19	27	15	22	29	80	
8	800	2	0.2	0	0.2	9	9	9	15	20	26	172	
9	800	12	0.2	0	free	9	8	8	18	31	56	330	

Nr. Indicate experiment Nr.; ino = initial number of organisms, ng = number of genes subjected to selection; pe 1 and pe 2 indicate the proportion of non resistant organisms killed by the pesticide each alternative time step; mp = mutation probability in mutations per gene; and tstep = number of reproductive cycles simulated.

Number in bold fonts are statistically different from the respective control number (from experiments 1 or 5 respectively), as assessed by a chi-squared test ($p < 0.01$).

Table 2. Variance of allelic values for genes for resistance under various conditions for sexual populations.

Nr.	Ng	pe1	pe2	variance r1	variance r2	p
1	12	0	0	8.12	8.12	ns
2	12	0.2	0	8.47	8.58	ns
3	12	0.6	0	0.92	8.35	<0.001
4	4	0.2	0	10.30	9.67	<0.01
5	4	0.6	0	1.32	9.67	<0.001

Nr. Indicate experiment Nr. ; ino = 800, ng = number of genes subjected to selection ; pe1 and pe2 indicate the proportion of non resistant organisms killed by the pesticide each alternative time step ; variance r1 and r2 gives the average variance at time step 60 of the alleles in genes for resistance 1 and 2 respectively.

Experiment 2 is identical to experiment Nr. 9 in Table 1. Each data is the average of 200 simulations run for 60 time steps.

p indicate probabilities for statistically differences between values for r1 and r2, as assessed by a t-test.

Table 3. Probabilities of developing genetic resistance to pesticides for sexual and asexual populations.

t	tl	np	pe	Asexual Hapl	Sexual Dipl Hapl	Msel			
10		0	1	30	1.0		.33	.91	1.00
10		0	2	30	.14		.12	.44	.94
10		0	2	100	.13		.05	.14	.85
10		10	2	30	.20		.14	.70	.98
20		0	2	30	.10		.18	.51	1.00
20	0	2	100	.07		.07	.26	1.00	
20	5	2	30	.14		.34	.72	.99	
20	5	2	100	.12	.21	.45	1.00		
Mean				.24	.18	.52	.97		
sd				.31	.11	.25	.05		

Pesticides were applied continuously during the 30 time steps of each simulation. Values are calculated by counting the number of simulations in a which a reproductive viable resistance emerged out of 250 simulations for each data point.

t, Timing of the initiation of the first application (in number of generations). Early timings capture the population having high genetic variability but low adaptability, i.e. frequency distributions of alleles are sub-optimal. Late timings capture the population with stabilized genetic variability and optimal allelic frequency distributions.

tl, Time lag between first application of insecticide and initiation of second (in number of generations).

np, Number of pesticides applied and evaluated for resistance (1 or 2)

pe, Proportion of non-resistant individuals affected by pesticide application (30 or 100%)

Dipl, sexual diploids; Hapl, sexual haploids; Msel, sexual diploids where pesticides affect mate selection.

Any two probabilities differing in more than 0.05 units differ significantly ($P < 0.05$) as assessed by a chi-squared test.

Table 4. T values for relations between dependent variable and the probabilities of developing resistance

Variable	All	Asexuals	Sexuals:
Start of first application (10 or 20)	1.53	-5.11*	1.91
Time lag for second application (0, 5, 10)	1.78	5.86**	2.23*
Efficiency of pesticides (30 or 100%)	-2.10*	-2.17	-2.48*
Polyploidy (haploid or diploid for sexuals)	-4.43***	---	6.25 ***
Sex (asexual or sexual)	3.70***	---	---
Number of different pesticides (1 or 2)	-4.07***	-66.4***	-3.26**
Mate selection (random or directed)	8.71***	---	12.20***

Statistical significance is indicated as *, ** and *** for $P < 0.05$, 0.01 and 0.001 respectively and was assessed using a Kruskal-Wallis analysis of variance.

Table 5. Probabilities of developing genetic resistance to any of two different pesticides for sexual and asexual populations. Pesticides were applied during 5 time-steps. (n = 150 simulations for each value)

t1	t2	pe	Haploids		Sexual-diploids		
			Asex	Sexual	Both	Fem	Male
10	10	15	.09	.39	.20	.15	.36
10	10	30	.15	.47	.15	.09	.37
10	10	60	.17	.40	.11	.02	.50
10	15	15	.08	.49	.24	.54	.84
10	15	30	.10	.66	.36	.41	.91
10	15	60	.22	.62	.37	.28	.94
15	15	15	.08	.39	.25	.50	.81
15	15	30	.11	.50	.27	.36	.87
15	15	60	.16	.51	.23	.16	.84
15	20	15	.04	.46	.36	1.0	1.0
15	20	30	.13	.62	.45	.99	1.0
15	20	60	.18	.64	.46	.71	1.0
Mean			.13	.51	.29	.43	.79
sd			.05	.10	.11	.33	.24

t1, t2, Timing of the application (in number of generations) of pesticide 1 and 2 respectively. Each pesticide was applied for 5 subsequent generations.

pe, Proportion of non-resistant individuals affected by pesticide application (15, 30 or 60%)

Both, Fem, Male indicate sexual diploids where pesticides target both sexes or only females or males respectively. In sexual haploids both sexes were targeted.

Any two probabilities differing in more than 0.11 units differ significantly ($P < 0.05$) as assessed by a chi-squared test.

Table 6. Coefficients of correlation between dependent variable and the probabilities of developing resistance

Variable	Asexuals	Sexuals			
		Sex targeted: Both Males Females			
Start of first application (10, 15 or 20)	-.28	.36	.58*	.58*	
Time lag for second application (0, 5)	-.11	.69*	.71*	.70*	
Efficiency of pesticides (15, 30 or 60%)		.88**	.20	.12	-.35
Polyploidy (haploid or diploid for sexuals)	-	-.76**	-	-	

Statistical significance is indicated as * and ** for $P < 0.05$ and 0.001 respectively and was assessed using a Kruskal-Wallis analysis of variance.

All data for sexuals are for diploids, except for the value of polyploidy which was calculated by comparing diploid and haploid sexuals where both sexes were targeted with pesticides.