Review

Heterogeneity of selection and the evolution of resistance

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The evolution of resistance to pesticides and drugs by pests and pathogens is a textbook example of adaptation to environmental changes and a major issue in both public health and agronomy. Surprisingly, there is little consensus on how to combine selection pressures (i.e., molecules used in the treatment of pests or pathogens) over space and time to delay or prevent this evolutionary process. By reviewing theoretical models and experimental studies, we show that higher levels of heterogeneity of selection are associated with longer-term sustainability of pest or pathogen control. The combination of molecules usually outcompetes other resistance management strategies, such as *Responsive alternation*, *Periodic application*, or *Mosaic*, because it ensures 'multiple intragenerational killing'. A strategic deployment over space and/or time of several combinations can ensure 'multiple intergenerational killing', further delaying the evolution of resistance.

The worrying issue of the evolution of resistance

Throughout history, humans have used a variety of strategies to control diseases and their vectors, as well as pests impacting crops and domestic animals. As far back as the 8th century BC, Homer referred to the use of sulfur to fumigate homes. Arsenic, an insecticide recommended by the Roman naturalist Pliny the Elder during the 1st century, was used during the 10th century by the Chinese to control garden pests. From the 1940s onwards, the discovery of modern pesticides, such as DDT, and most of the major classes of antibiotic, appeared to offer the key to controlling pests and pathogens. Most of these measures were relatively cheap and ensured high levels of control. During the two decades that followed, these pesticides were widely used in fields, farms, homes, and hospitals to treat crops, animals, and humans, saving yields and lives. Unfortunately, one of the drawbacks of these treatments is that they exert selection pressures on their target populations, leading to the evolution of resistance mechanisms (see Glossary) that reduce their efficacy [e.g., insecticides (Arthropod Pesticide Resistance Database; http://www.pesticideresistance.org/); herbicides [1]; antibiotics [2,3]; and HIV-1 protease inhibitors [4]].

The evolution of resistance to pesticides and drugs has offered several case studies of adaptive evolution and is a valuable example of other evolutionary changes that are more difficult to perceive and analyze. Hence, studies of the evolution of resistance to various pesticides have improved understanding of the molecular mechanisms involved in adaptation [5] and dominance [6], the epistatic relations between loci [7], and the fitness costs of adaptive mutations [8].

The evolution of resistance to pesticides and drugs is not only a textbook example of adaptation, but is also, and above all, a major issue for both public health and agronomy, because the number of drugs and pesticides with different mechanisms of toxicity and acting on independent targets has proved to be limited (e.g., for antibiotics see [9] and for pesticides see [10]; Box 1). Only a few new active molecules have been discovered during the past 30 years. A new wave of research and development (R&D) on

Glossary

Cross-resistance: a resistance to a pesticide or drug that also confers resistance to another pesticide or drug.
Degree of treatment heterogeneity (*DTH*): the probability that a set of resistance genes is confronted by more than one pesticide or drug during a certain amount of time, be it within or between generations.
Insecticidal toxins: toxins produced by bacteria, mostly *Bacillus thuringiensis* and *Bacillus sphaericus*, and used in sprays or in genetically engineered plants to control insects.
Multiple intragenerational killing: a strategy that uses a variety of pesticides or drugs on each pest or pathogen individual, to maximize the probability that each individual is killed. An individual that is resistant to molecule A but susceptible to molecule B will be killed if treated simultaneously by molecules A and B.
Multiple intergenerational killing: a strategy that uses a variety of pesticides or drugs on successive generations of pests or pathogens, to maximize the probability that the offspring of resistant individuals are killed. The offspring of an individual that is resistant to molecule A but susceptible to molecule B will be killed by molecule B.
Recessive resistance allele: an allele that confers resistance to diploid pests and pathogens only if present in a homozygous state. A dominant resistance allele confers resistance when it occurs in either a heterozygous or homozygous state.
Refuge: areas, fields, or group of pests or pathogens remaining untreated by pesticides or drugs.
Resistance: a heritable change in a population that is reflected in the ability of individuals to survive and reproduce in the presence of environmental conditions that once killed most individuals of the same species.
Resistance cost: a negative pleiotropic effect of a resistant genotype that results in a lower fitness of resistant individuals compared with susceptible ones in the absence of a pesticide or drug.
Resistance gene: a gene at which one or more alleles confer resistance to pesticides or drugs.
Resistance management strategy: a strategy devoted to delay or prevent the evolution of resistance in a population of pests or pathogens. *Mosaic*, *Periodic application*, *Combination*, and *Responsive alternation* (Box 2) are simple resistance management strategies using more than one pesticide or drug.

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Box 1. The evolution of resistance to pesticides and drugs

Almost 8000 cases of resistance to 300 insecticide compounds have been reported in more than 500 species of arthropods (Arthropod Pesticide Resistance Database; www.pesticideresistance.com). Similarly, 300 cases of field resistance to 30 fungicides have been reported in 250 species of phytopathogenic fungi (Fungicide Resistance Action Committee database; http://www.frac.info). The International Survey of Herbicide-Resistant Weeds (http://www.weedscience.com) has suggested that there are currently approximately 390 resistant biotypes in 210 weed species in 690 000 fields. The situation is most critical for antibiotic resistance. Genes conferring resistance to antibiotics are ubiquitous in bacteria and are highly diverse. The Antibiotic Resistance Genes Database (http://ardb.cbcb.umd.edu/), developed by Liu and Pop [61], lists more than 23 000 potential resistance genes of approximately 400 types, conferring resistance to 250 antibiotics in 1700 species of bacteria from 270 genera. Strains from highly pathogenic bacteria, such as tuberculosis bacilli, that are resistant to all known classes of antibiotic have recently been described [62].

In addition, most of the major classes of antibiotic were first isolated between 1940 and 1960 [63]. The more recently commercialized drugs and pesticides are often variants of previously isolated or synthesized compounds and, therefore, are not particularly effective against the prevailing resistance mechanisms (see e.g., herbicides [64], insecticides [65], antiviral drugs [66], and antibiotics [67]). The cost of developing new drugs and pesticides has been further increased by the tightening of requirements by regulatory authorities, necessitating a larger number of toxicological, clinical, and environmental trials [68]. Hence, according to Larson [69], it currently takes approximately 10 years and up to US$1 billion to develop a new antibiotic. Similarly, 10–12 years are required to develop and launch a new pesticide onto the market [70].

At the turn of the 21st century, the combination of approaches, such as genomics [71], proteomics [72], and metabolomics [73], with target-based high-throughput screening strategies [70,74] appeared promising for the discovery of new drugs and pesticides with little or no impact on the environment and health. However, these new methods and strategies have proved relatively unsuccessful, for both antibiotics [75] and pesticides [76]. The situation is different for insecticidal toxins, mostly proteins from *Bacillus thuringiensis*, whether formulated for application in sprays or produced by transgenic plants. The number of toxins identified is increasing [77] and the populations of most of the pests targeted remain resistance-free ([65], but see [78]).

drugs and pesticides, with the exception of that relating to insecticidal toxins, would be unlikely to yield substantial public health and crop protection options within the next 10–15 years [11]. In the meantime, there is a need to protect the existing molecules. Fortunately, most classes of pesticide and antibiotic [9] include several molecules that are still active and for which, at least in some cases, there is still no sign of resistance. This raises questions about how these molecules can be combined over time and space to preserve their efficacy for as long as possible.

In the literature, four principal basic strategies combining two (or more) molecules over time and/or space have been considered, to delay the evolution of resistance to drugs and pesticides: '*Responsive alternation*', '*Periodic application*', '*Mosaic*', and '*Combination*' (Box 2). Is one particular strategy intrinsically better than the others? Conversely, does the ranking of strategies depend on the target organism or the pesticide or drug being considered? Theoretical models predicting the outcome of selection pressures and experimental selection on pests and patho-

Box 2. Strategies for combining molecules over time and space

Four principal basic strategies combining two (or more) molecules over time and/or space have been considered for drugs and pesticides. These strategies differ in the way that the pesticides or drugs are combined. In the *Periodic application* and *Responsive alternation* strategies, molecule use is uniform over space but heterogeneous over time. *Periodic application* involves temporal cycles of pesticide or drug application, a strategy first suggested by Coyne [79]. By contrast, *Responsive alternation* corresponds to successive applications of molecules, but without a cycle. In this approach, a molecule is used repeatedly until the emergence of resistance, after which the second molecule is introduced, and so on. *Mosaic* (a strategy first suggested by Muir [80]) concerns a spatial pattern of application for at least two molecules. Molecule application remains uniform over time and the spatial distributions of the molecules used do not overlap. Finally, *Combination* is the concomitant use of two or more molecules over time and space. *Responsive alternation*, *Periodic application*, *Mosaic*, and *Combination* have been referred to by various names within and between the different classes of pesticides and drugs, as summarized in Table I.

In practice, molecules in combinations are combined in variable ratios and at different doses. Strategies based on both *full-dose* and *half-dose* combinations have been proposed. In the *full-dose Combination* strategy, each pesticide or drug is applied at the dose at which it would be used if applied alone, whereas in the *half-dose* strategy, the dose of each pesticide or drug is half that used when the compound is applied alone. Consequently, the final overall dose of the *full-dose* strategy is equivalent to twice that applied if each molecule were to be used alone, whereas the final overall dose of the *half-dose Combination* strategy corresponds to the dose at which each molecule would be applied if used alone.

Practical recommendations on the strategy to be used depend on the target organism. For instance, *Combination* is currently recommended in the treatment of HIV [81], tuberculosis [82], and malaria [83]. Pesticides are also increasingly used in combination rather than as individual compounds, as exemplified by the new generation of *Bt* crops, which produce several independent toxins against the target pests [84]. However, *Combination* is not the current default strategy in antibiotic treatment, particularly in the community, and is not recommended for several pesticides (e.g., for the control of *Anopheles*, which is the malarial parasite vector [85]).

Table I. Names used to define strategies

Strategy	Antibiotics or antiviral drugs	Insecticides or Bt toxins	Fungicides	Herbicides
Responsive alternation	Sequential use	Sequence, sequential use, and serial use	_	Sequence and threshold strategy
Periodic application	Cycling, antibiotic rotation, *Periodic application*, and sequential use	Rotation, alternation, and sequential use	Rotation, alternation, and sequence	Rotation
Mosaic	Mixing, 50-50 treatment, antibiotic diversity, and multiple first-line therapy	*Mosaic*	_	*Mosaic*
Combination	*Combination*, antibiotic diversity, and simultaneous strategy	Mixture and pyramiding	Mixture and *Combination*	Mixture, *Combination*, and double knockdown

gens can be used to test such predictions. Here, we review the results obtained with theoretical models and in empirical studies for various pesticides and drugs (generally considered separately, by ecologists and agronomists on the one hand and medical scientists on the other [12,13]). We show that some consensus can be reached on the deployment of selection pressures over time and space to delay or prevent the evolution of resistance in pest and pathogen populations

Theoretical comparisons among strategies

We searched for articles that explicitly compared, in the same study, at least two of the four strategies [*Responsive alternation*, *Periodic application*, *Mosaic*, and *Combination* (whether *half*- or *full-dose*); Box 2] in terms of their efficacy for delaying the evolution of resistance to more than one pesticide or drug. Therefore, we excluded all studies that considered several molecules but modeled the evolution of resistance to only one molecule. A search of the Resistance to Xenobiotic (REX) bibliographic database [12,13] for articles relating to the modeling of resistance evolution identified 20 relevant articles. Further searches in the Web of Science and Google Scholar, and screening of the articles cited in the initial 20 articles, yielded an additional nine articles. Half of those articles were related to either insecticide or antibiotic resistance.

Based on the 29 articles retained (Table S1 in the supplementary material online), we identified a clear ranking of the strategies in terms of their efficacy for delaying resistance: *Combination* > *Periodic application* = *Mosaic* > *Responsive alternation* (Table 1). *Combination* was at least as good as, or outperformed *Responsive alternation*, *Periodic application* and *Mosaic* in more than 80% of the comparisons. *Half-dose Combination* was found to have been little studied and comparisons of *Combination* with other strategies were somewhat biased because a *full-dose Combination*, by doubling the dose of pesticide or drug used, increases overall selection pressure. *Responsive alternation* was less effective than *Periodic application* and *Mosaic* in all comparisons. The ranking of *Mosaic* and *Periodic application* was, by contrast, not straightforward. These two strategies were compared mostly to determine whether *Periodic application* (referred to as 'cycling' in clinical studies; Box 2) could delay resistance to antibiotics more effectively than could *Mosaic* (referred to as 'mixing' in clinical studies; Box 2) in hospitals or, more specifically, in intensive care units. All the epidemiological models gave the same answer: *Mosaic* > *Periodic application*. By contrast, Roush [14] and Lenormand and Raymond [15], who modeled the evolution of insecticide resistance, found *Periodic application* > *Mosaic*.

Combination and multiple intragenerational killing at the individual level

Combination is effective due to multiple intragenerational killing [16], a key feature that can be explained as follows: if resistance alleles at each of two independent loci are present at low frequency in the pest or pathogen population, then any given individual is extremely unlikely to carry resistance alleles at both loci. Moreover, if resistance is recessive, then diploid pests and pathogens are only resistant if they are homozygous for the resistance allele at both loci. When resistance alleles are at low frequencies, this probability is low. Thus, most individuals can be killed by each one of the pesticides or drugs *A* and *B*. This is described as multiple intragenerational killing, because most pest or pathogen individuals are susceptible to both molecules and, therefore, are 'killed twice' (Figure 1).

The superiority of *Combination* over the other strategies appears to be robust: in most models, this approach was effective for longer even if input and output parameters were varied. Its comparative advantage is particularly high when: (i) resistance to each pesticide or drug is initially rare [16–20]; (ii) resistance to each pesticide or drug in the combination are controlled by independent loci (no cross-resistance) [16,21–23]; (iii) there is a high rate of recombination between the loci [16,19,22,23]; (iv) in diploids, homozygous susceptible individuals have a high mortality [14,20]; (v) in diploids, resistance to each pesticide is functionally recessive [16,22,24–26]; (vi) the pesticides or drugs are of similar persistence [14,26]; and (vii) some of the population remains untreated [19,21,23,24]. Even if these conditions are not completely met, *Combination* appears at least as good as the three other strategies.

Degree of treatment heterogeneity and multiple killing

The most recent approaches in medicine focus on antibiotic heterogeneity [27,28], the idea being that higher degrees of treatment heterogeneity (*DTH*) are associated with slower evolution of resistance. Mani [29] explored this idea for insecticide resistance more than 20 years ago. He showed that, after *Combination*, the most promising strategy was not to vary applications of a given molecule over time (*Periodic application*) or space (*Mosaic*), but to alternate the insecticides used over both time and space, thereby maximizing the *DTH*.

Table 1. Side-by-side comparisons of the four strategies in terms of their relative efficacies for delaying or preventing resistance

Strategy		Theoretical studies					Empirical studies			
1	2	*n*[a]	1 > 2	1 = 2	1 < 2	Conditional[b]	*n*	1 > 2	1 = 2	1 < 2
Combination	*Responsive alternation*	14	11	0	0	3	10	8	2	0
Combination	*Periodic application*	16	14	0	1	1	8	2	5	1
Combination	*Mosaic*	7	5	0	1	1	1	1	0	0
Periodic application	*Responsive alternation*	7	3	4	0	0	9	7	2	0
Periodic application	*Mosaic*	11	2	3	5	1	3	2	0	1
Mosaic	*Responsive alternation*	3	2	1	0	0	2	1	0	1

[a] *n*, number of comparisons in all theoretical and empirical studies.

[b] The ranking of the strategies depends on the setting for one or several input or output parameters.

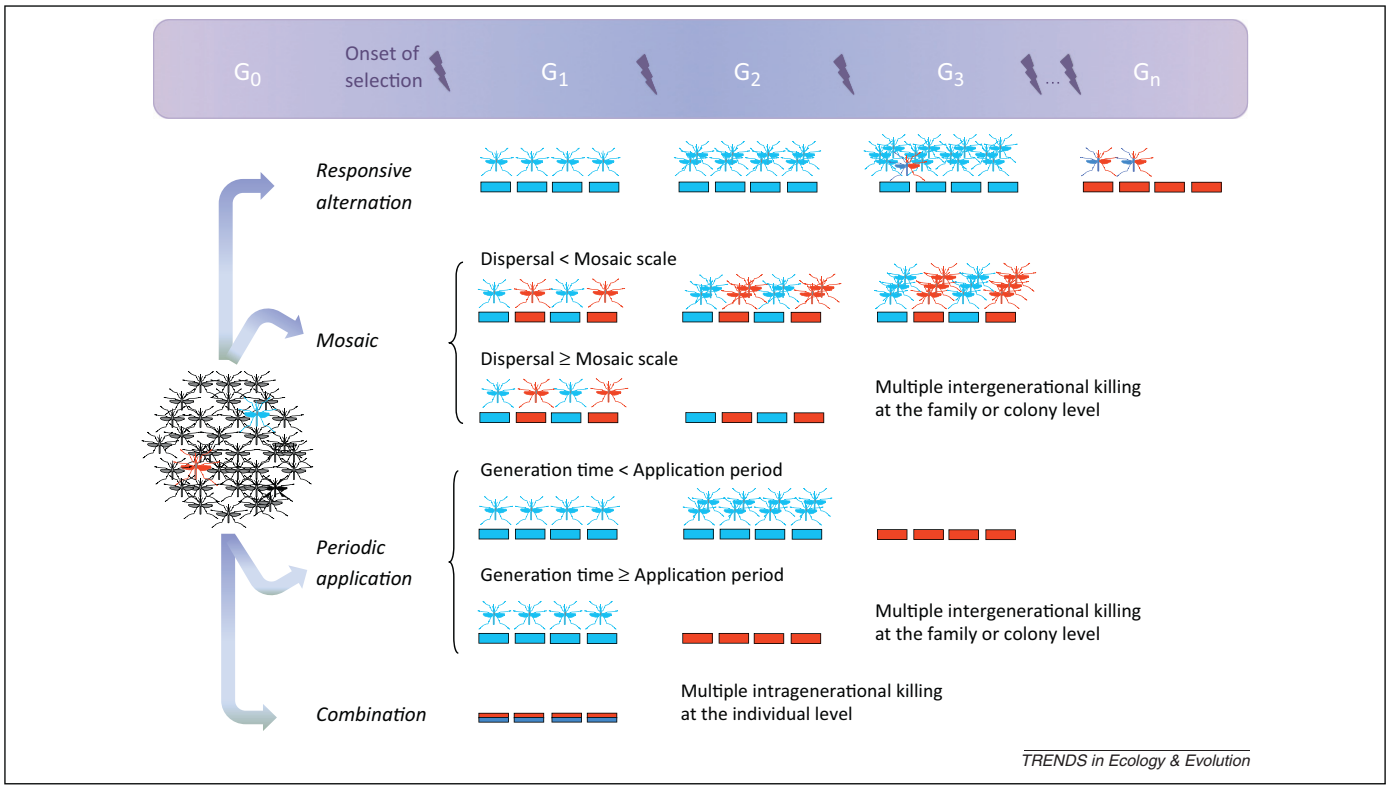

Figure 1. Schematic representation of the effect of the different strategies (*Responsive alternation*, *Mosaic*, *Periodic application*, and *Combination*) on the targeted pests or pathogens, here, a mosquito. These strategies can lead to multiple intragenerational killing at the individual level (for *Combination*) or multiple intergenerational killing at the family or colony level (for *Periodic application* and *Mosaic*). This depends on the balance between the spatial and temporal scales of the treatments and the dispersal capacities and generation time of the targeted pests or pathogens. At each generation (G), pests or pathogens are selected by molecule 1 (in blue patches), molecule 2 (in red patches), or a combination of these two molecules (in blue-and-red patches). Individuals S (black mosquitos), R_1 (blue mosquitos), and R_2 (red mosquitos) are susceptible, resistant to molecule 1, and resistant to molecule 2, respectively. Individuals R_{12} (blue-and-red mosquitos), harboring genes conferring resistance to molecule 1 as well as genes conferring resistance to molecule 2, can survive in a patch treated with a combination of these two molecules. The degree of treatment heterogeneity (*DTH*), defined here as the probability that a set of resistance genes is confronted by more than one pesticide or drug, varies among the strategies. *Combination* displays the largest *DTH*, followed by *Periodic applications* and *Mosaic*, depending on the generation time, dispersal distance, period, and spatial scales of application, and by *Responsive alternation*.

To our knowledge, the relation between *DTH*, temporal or spatial selection heterogeneity, and the sustainability of efficacy for a given molecule has never been clearly formalized. We suggest that *DTH* should be defined as the probability that a set of resistance genes is confronted by more than one pesticide or drug within or between generations. In case of *Periodic application*, offspring from individuals resistant to one molecule will be treated with another molecule depending on the generation time of the pathogen or pest and on the period of application of the drug or pesticide. These offspring would be expected to be susceptible to the second molecule, particularly in the absence of cross-resistance and if resistance genes are independent of each other. In this case, *DTH* therefore ensures multiple intergenerational killing at the colony or family level (Figure 1), because the first molecule kills most individuals in the parental generation and the second molecule then kills the offspring of the few survivors. As explained above, *Combination* ensures multiple intragenerational killing and a maximal *DTH* because every individual suffers both molecules simultaneously. In a *Mosaic* set up, the survivors to the first molecule can disperse and then be killed by the second molecule. If dispersal distances are larger than the scale of *Mosaic* unit, then *Mosaic* can also lead to multiple intergenerational killing.

All things being equal, higher *DTH* should be associated with longer-term sustainability of pesticides or drugs. *Responsive alternation* systematically results in the lowest *DTH*, because the offspring are treated with the same molecules as their parents until the population size (or disease severity or yield loss) reaches unacceptable levels. Depending on the pattern of pest or pathogen dispersal, its generation time, and the temporal and spatial scales of treatment, higher *DTH* can be achieved with either *Periodic application* or *Mosaic* strategies, or through the use of a combination of these two extreme strategies.

Empirical comparisons between strategies

In 1983, Georghiou [30] stated that: 'Perusal of pertinent literature reveals that there are more papers discussing the value of mixture [i.e., *Combination*] (as well as rotation [i.e., *Periodic application*]) than those that report actual research on the subject'. Unfortunately, this remains true in 2012. Using the Web of Science, Google Scholar, and the references cited in recent articles on this topic, we found only 17 empirical studies (half of them being on insecticide resistance) comparing at least two strategies under laboratory, greenhouse, care units, or field conditions (Table S2 in the supplementary material online).

In the 17 empirical studies identified, the ranking of efficacy was: *Combination* = *Periodic application* > *Responsive alternation* (Table 1). Indeed, in five out of eight comparisons, *Combination* was found to be as good as *Periodic application*, and *Responsive alternation* never

outperformed either of these two strategies. It was not possible to rank *Mosaic* reliably, because too few comparisons included this strategy. *Mosaic* outperformed *Periodic application* and *Responsive alternation* in two independent studies, but was found to be less effective than *Combination*, *Periodic application*, and *Responsive alternation* in the other four comparisons (Table 1).

Although *Combination* appeared to be the best strategy in theoretical models, it did not clearly outperform *Periodic application* in empirical studies. This discrepancy between theoretical and empirical results can simply reflect time constraints. Indeed, in most experimental studies, treatments were applied during a fixed number of generations. In most cases, resistance emerged when molecules were used singly, but not when they were combined over space and/or time (*Periodic application*, *Mosaic*, or *Combination*). Thus, several studies reported an absence of resistance development for at least one molecule for both *Combination* and *Periodic application* strategies (e.g., [31–35]), making it impossible to draw any firm conclusions concerning possible differences in efficacy between these two strategies. The conclusion that these two strategies are similar in efficacy is thus valid for the number of generations over which selection took place, but might not hold absolutely true *per se*.

The discrepancy between theoretical and empirical results can also result from the use of experimental settings that decrease the advantage of *Combination* over other strategies. As mentioned above, empirical studies are limited by the number of generations that can be run. They are also limited by the number of individuals per generation that can be manipulated. These experimental constraints have two important consequences. First, empirical studies focus on the evolution of resistance alleles already present in populations rather than on resistance alleles acquired *de novo* by mutation or horizontal transfer. Hence, in all but one of the experimental studies (Table S2 in the supplementary material online), a deliberate decision was taken to have a high frequency (i.e., $>10^{-3}$) of resistance to at least one pesticide or drug at the start of selection, thereby decreasing the efficacy of *Combination* by violating one of the favoring conditions [16–20]. Second, a sufficiently large number of individuals must survive pesticide or drug treatments to establish the next generation. Consequently, the selection pressure applied in such experiments generally varies between 0.5 and 0.8, corresponding to low doses. In such cases, resistance can be functionally dominant, further decreasing the comparative advantage of *Combination* over the other strategies [16,22,24–26]. Experimental settings with high initial frequencies of resistance alleles and low selection pressures can, in some cases, approach real conditions. Molecules newly released onto the market are sometimes used in combination with other molecules for which resistance has already been selected in the targeted pest or pathogen populations, for economic purposes. Selection at low doses can also occur in field conditions because of the dilution of the molecules and their degradation over time.

Nevertheless, one specific feature of empirical studies clearly differs from practice. As pointed out above, the presence of untreated individuals from refuges increases the success of *Combination*. However, nine of the 17 empirical studies were conducted without such refuges (Table S2 in the supplementary material online). This is unfortunate, because such refuges could easily be included in studies of the selection of resistance. Leaving a fraction of the population free of pesticide exposure would have better mimicked the conditions in fields, hospitals, and care units. Indeed, a significant proportion of the pests or pathogens often remain untreated unintentionally. Dormant weeds, resting spores of fungi, hidden mosquito breeding sites, soil seed banks or field borders, alternative hosts, or humans outside the medical system are common and constitute unplanned refuges of pests and pathogens.

Can *Combination* be outcompeted?

One particular condition can render *Combination* inferior to other strategies. This condition is the occurrence of fitness costs, resulting in resistant individuals being less fit than susceptible individuals in the absence of the pesticide or drug. Such costs might lead to the counterselection of resistance alleles and, therefore, would delay, if not prevent, the development of resistance. The expression of this cost would require spatial or temporal variation in pesticide or drug selection, with locations or periods of time in which one of the pesticides or drugs is absent. *Combination* is the only strategy combining two molecules that does not generate such variation and, therefore, it is the only strategy that does not allow the expression of a resistance cost. Consequently, fitness costs can facilitate the mitigation of resistance in all strategies except *Combination*. This can explain why Dobson *et al.* [36] (theoretically) and Immajaru *et al.* [37] (experimentally) found *Combination* to be less effective than *Periodic application* and *Mosaic* (Tables S1 and S2 in the supplementary material online). Indeed, their theoretical and biological models were characterized by high fitness costs and an absence of refuges.

In practice, fitness costs might only rarely make *Combination* worse than other strategies. First, the multiple intragenerational killing provided by *Combination* approaches might be sufficient to ensure the superiority of this strategy in many cases, even in the presence of fitness costs. Second, although mutations conferring resistance are often costly (see, e.g., herbicides [38], insecticidal proteins [39], antibiotics [40], and antivirus [41]), decreases in fitness can be attenuated or even completely abolished by compensatory mutations (see, e.g., herbicides [42], antimicrobial drugs [43], antibiotics [44], and antivirus [45]) or through interactions with other resistance mutations [46]. Over time, costly resistance mutations can also be replaced by resistance mutations associated with lower fitness costs [47]. Finally, when part of the population remains untreated, fitness costs counteract the selection of resistance alleles, even for *Combination*. Untreated individuals can be actively preserved by the use or maintenance of refuges for pests and pathogens. The use of refuges is not possible in hospitals, because it would be unethical not to treat infected humans with antibiotics or other drugs. However, the community outside hospitals constitutes a refuge for most pathogen populations and individuals carrying pathogenic bacteria or viruses but

displaying no symptoms, or only minor symptoms, are left untreated. Finally, pathogens or pests generally escape treatments even within the host or the field, because treatment coverage is rarely complete.

Increasing the *DTH* of *Combination*

The number of molecules that can actually be used in a *Combination* is limited by resistances that have already developed (Box 1) and by several challenges outlined in Box 3. Generally, the concomitant use of a large number of molecules entails higher costs, which can outweigh the benefits of delaying or preventing resistance in the eyes of the stakeholders. Thus, combinations containing all the available molecules are unlikely to be used. However, it might be possible to use several different combinations to treat a given pest or pathogen. These combinations would ideally be used to ensure the highest *DTH*, yielding multiple intragenerational killing (at the individual level) and multiple intergenerational killing (at the colony or family level). Depending on the distances over which dispersal occurs and on generation time, the highest *DTH* can be provided by a complex temporal and spatial arrangement of the various combinations.

This might have practical consequences. For example, in antibiotic resistance management, treatment heterogeneity is currently defined at the level of the hospital rather than the pathogen. The theoretical and empirical studies reviewed here show that diversity in antibiotic use between care units or beds at a given time (i.e., a *Mosaic* strategy) is more sustainable than is cycling different antibiotic regimens over time (i.e., a *Periodic application* strategy). This is because, at the scale relevant to bacterial populations, *Mosaic* imposes greater *DTH* than does *Periodic application* [28,29,48,49]. This is particularly true when the cycle of each antibiotic regimen is long, extending over several months. Indeed, due to its short generation time, a bacterial colony is more likely to encounter the second antibiotic in a *Mosaic* implemented at the scale of the bed or at the scale of the care unit than in a *Periodic application* based on the cycling of antibiotics over several months. Another hypothesis has been put forward by Boni *et al.* [50] to explain the higher performance of *Mosaic* over *Periodic application*: *Periodic application* degrades the mean fitness of the parasite population more quickly than does *Mosaic*, making it easier for new resistant types to invade and spread in the population.

Although difficult to implement, we suggest that *Periodic application* at the level of the patient, rather than the hospital (or care unit), might result in greater *DTH* than using a *Mosaic* approach. Alternating antibiotics to treat patients would increase the likelihood of multiple intergenerational killing (i.e., the probability of colonies resistant to a given antibiotic being treated and, therefore, killed by another antibiotic in the next generation).

Beyond *Combination* and *DTH*: protecting populations against the emergence of resistance alleles

The question of how to combine pesticides and drugs over time and space is only one part of the overall debate on resistance management. The dose of the molecules used must also be considered. Resistance management strategies sometimes include the use of high doses of pesticides and drugs. For bacterial and HIV infections, this has been referred to as the 'Hit hard and early' approach [51]. Interestingly, different rationales are applied to pesticides and drugs. For drugs, the reason for treating 'hard' is to decrease the size of the pathogen population as much as possible, to prevent the appearance of resistance alleles. For pesticides, high-dose strategies are designed to avoid not only the emergence of new resistance alleles [48,49], but also the building of polygenic resistance [52], and to make resistance of diploid pests functionally recessive [53]. The use of a high dose can also enlarge the spectrum of pests targeted. This is particularly true for herbicides,

Box 3. Challenges with combinations

Imagine that two molecules are available and that all conditions are satisfied for their combination to outperform all other strategies for delaying the evolution of resistance. Would *Combination* become the optimal strategy for use with any given set of pesticides and drugs? This is unlikely because there are several obstacles to the universal recommendation and implementation of this strategy.

The possibility of antagonistic effects between molecules (which may seriously reduce pest or pathogen control) constitutes a first obstacle to the use of the *Combination* strategy [86]. Synergistic molecule combinations can be advantageous in controlling pests and pathogens. However, resistance to such combinations can evolve faster than can resistance to antagonistic molecule combinations and, in some cases, to individual molecules themselves [87].

A second obstacle for using *Combination* is that the molecules prescribed by physicians and used by farmers not only control pests and pathogens, but may also injury crops and have adverse effects on non-target organisms and human health. The WHO has reported that there are approximately three million human cases of pesticide poisoning annually, resulting in 220 000 deaths worldwide [88], as well as hepatoxicity, neurotoxicity, and lipodystrophy [89–91]. Chemical pesticides have a significant impact on non-target plants, fungi, and arthropods [92]. Pesticide use can disrupt biological control through direct toxicity [93], indirectly changing the community structure [94] and natural predators or parasitoids [95]. A trade-off thus exists between controlling the pest with the right dose and limiting the adverse effects of treatment. Adverse effects occur when single molecules are used, but they are probably worsened by the use of combinations, because synergy between molecules [96] can increase the threat to the environment [97] and human health [98].

Stakeholders (i.e., companies, users, prescribers, and public authorities) diverge on their respective interests, goals, and their sensitivity to the costs of the strategy, depending on the policy implemented. For example, refuges increase the risk of pest and/or pathogen damage and, in the short term, this cost is met directly by users. Similarly, *Combination*, by multiple intragenerational killing, can be more efficient for controlling pests and/or pathogens, but because of the higher dose applied, implies financial costs to farmers or patients (or public authorities, if there is social health coverage), as well as an increased magnitude of adverse effects on health and the environment and, thus, the costs to be covered by public authorities.

The willingness of the various stakeholders to share the costs depends directly on the extent to which they are likely to be affected by or considered responsible for the emergence of resistance. Hence, users are confronted with the so-called 'Tragedy of the Commons' when exploiting a common property resource [99], even if they are likely to be strongly affected by the evolution of resistance. In most cases, by not playing their part in the management of resistance, each user maximizes their own short-term benefit but favors the selection of resistant pests and/or pathogens, thus having potentially long-term negative effects for the community.

because fields generally contain more than one weed species that must be controlled.

The drawback of hitting hard is that it increases the costs associated with resistance management (Box 3). This strategy can be counterproductive if resistant pathogens are already established [49,54]. However, in the case of new molecules for which no resistance has been detected, this approach can be the most appropriate, provided that the costs are sustainable. Unfortunately, most mathematical models of the evolution of pesticide resistance assume that population size is infinite, but see, for example, [52,55]. Consequently, resistance alleles are generally assumed to be initially present at all resistance loci in the population. In fact, natural populations are limited in size and might contain no resistance alleles. In such cases, the appearance and early increase in the frequency of resistance alleles is a stochastic process that is dependent on the balance between mutation rates and population size. This stochasticity is also largely ignored in empirical studies. As indicated above, empirical studies are always performed at locations or using strains in which resistance to at least one molecule occurs at a relatively high frequency, which constitute an unfavorable situation for high-dose strategies.

Therefore, there is a need for both theoretical and empirical studies to further investigate the evolution of resistance in conditions allowing stochastic events [56]. In such situations, 'hitting hard' probably results in a greater efficacy of *Combination* than of other strategies and *full-dose Combination* certainly provides populations with the highest level of protection against the emergence of resistance alleles. The use of high-dose *Bt* crops could be seen as a life-size experiment testing this hypothesis. Interestingly, in the USA, populations of the pink bollworm, *Pectinophora gossypiella*, targeted by *Bt* cotton, and of the European corn borer, *Ostrinia nubilalis*, targeted by *Bt* maize, have been declining from year to year [57,58]. For the pink bollworm, sterile moth releases have been successful in suppressing the emergence of resistance alleles to *Bt* cotton [59], a cornerstone for the multi-tactic eradication program of this pest [60]. This provides some hope that pest populations could be eliminated over a wide area before resistance alleles emerge and spread.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tree.2012.09.001.

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