Disease Resistance and Pathogen Population Genetics

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Abstract

To understand the process that leads to breakdown of resistance genes, we need to understand the processes that govern pathogen evolution. We ranked these risks associated with each evolutionary process and developed a quantitative framework to predict the risk that a pathogen will evolve to overcome major resistance genes. Our hypothesis is that much of the durability of resistance genes is due to the evolutionary potential of the pathogen population. The principles of the developed framework can be tested individually or in combination according to the available population genetics knowledge for any pathogen. We show how this framework can be used to design breeding strategies to lead to durable resistance.

Keywords: risk assessment, gene flow, mating system, boom-and-bust cycle, durable resistance

Plant pathologists have seen many boom-and-bust cycles following the deployment of resistant varieties. These cycles result when pathogen populations adapt to the presence of a major resistance gene by evolving a new population that can overcome this resistance gene. In these cases, the breakdown of genetic resistance is due to the evolution of the local pathogen population because of selection for mutants, recombinants, or immigrants that are better adapted to the resistant cultivar. To understand the process that leads to breakdown of a resistance gene, we need to understand the processes that govern pathogen evolution. Population geneticists have identified five evolutionary forces that interact to affect the evolution of organisms. In Table 1, we summarize the risks associated with each of these forces. We ranked these risks and developed a quantitative framework to predict the risk that a pathogen will evolve to overcome major resistance genes (McDONALD & LINDE 2002a,b). Our hypothesis is that much of the durability of resistance genes is due to the nature of the pathogen population rather than to the nature of the resistance gene. The framework we developed (McDONALD &

Table 1. Extremes of evolutionary risk posed by plant pathogens and examples of factors that affect risk assessment

Highest risk of pathogen evolution	Lowest risk of pathogen evolution
High mutation rate	Low mutation rate
Transposable elements active	No transposons
Large effective population sizes	Small effective population sizes
Large overseasoning population	No overseasoning propagules
Extinction of local populations rare	Extinction of local populations common
No genetic drift, no loss of alleles	Significant genetic drift, alleles lost
High gene/genotype flow	Low gene/genotype flow
Asexual propagules dispersed by air over long distances	Asexual propagules soilborne
Human-mediated long-distance movement common	Quarantines effective
Mixed reproduction system	Asexual reproduction system
Annual sexual outcrossing and asexual propagules produced	Only asexual propagules produced
Efficient directional selection	Disruptive selection
R-gene deployed in genetically uniform monoculture	*R*-genes deployed in mixtures/multilines
R-gene deployed continuously over large area	*R*-genes deployed as rotations in time or space

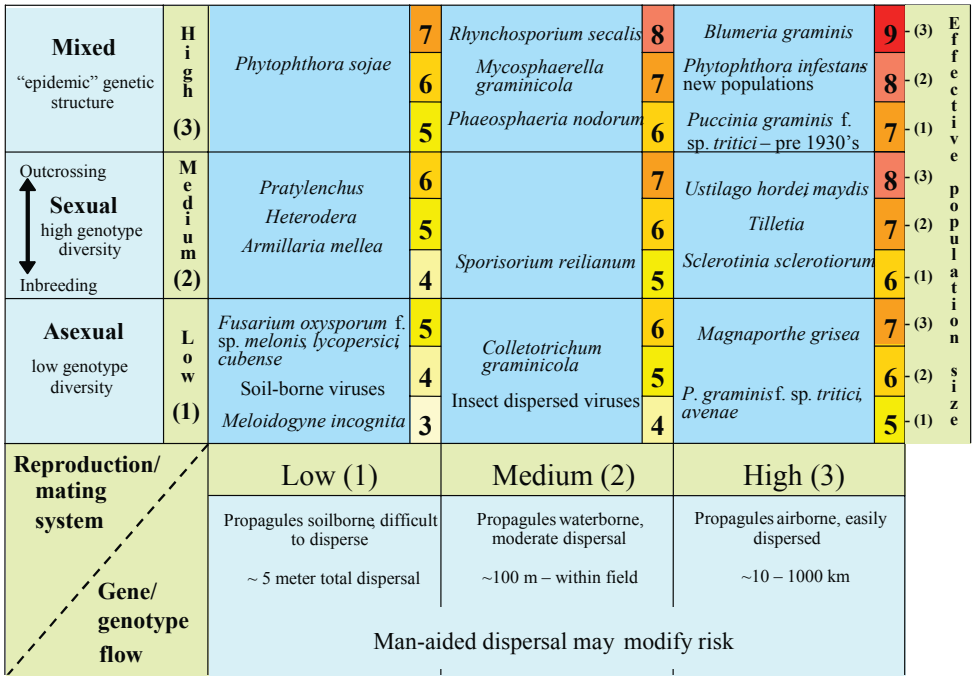

Figure 1. A risk assessment model for quantifying the evolutionary risk posed by different plant pathogens. The scale of evolutionary risk is organized according to the risk factors reproduction/mating system, gene/genotype flow and effective population size. Assignment of total risk value assumes that all effects are additive. Placement of example pathogens is according to principles explained in McDonald and Linde (2002a,b)

Linde 2002a,b), which is described briefly here, can be used as a hypothesis to test against a large number of plant pathosystems. The underlying principles of the framework can be tested individually or in combination according to the available knowledge of the population genetics for any pathogen. We propose that this framework can be used to design breeding strategies to break the boom-and-bust cycle and lead to durable resistance (McDonald & Linde 2002a,b).

Figure 1 is a simplified diagram that we propose as a model framework for assessing the evolutionary risk posed by most plant pathogens. The evaluation of evolutionary risk is relevant not only for breakdown of resistance genes, but also for development of resistance to fungicides or antibiotics. Figure 1 considers only the evolutionary risk due to differences in reproduction/mating system, gene/genotype flow, and effective population size. Mutation rate was not included in this diagram because we assumed that mutation rates would be low and relatively constant across pathogens. For pathogens known to have very high mutation rates, or for bacteria and viruses where mutation is likely to play a more important role in evolution, these risk values can be increased accordingly. Selection was not included in this diagram under the assumption that selection is likely to be efficient in the genetically uniform monocultures that dominate modern agricultural ecosystems. Selection risk is increased by increasing the land area covered to the same resistance gene, or decreased through resistance gene deployment strategies, such as gene rotations or mixtures. We expect that the population size for most pathogen populations is large, so it is likely that virulent (or fungicide-resistant) mutants will be present and the effects of genetic drift will be small. However, we recognize that some pathogen populations have smaller effective population sizes because of a founder effect, regular bottlenecks, or short-lived overseasoning propagules. Other pathogen populations have larger effective sizes due to year-round multiplication, short latent periods, and production of long-lived overseasoning propagules. This results in a range of values for each cell in the matrix (Figure 1). The proposed risk categories may need to be adjusted in many cases as a result of anthropogenic activities. For example, gene/genotype flow may be increased beyond the normal biological limits of spore dispersal by movement of inoculum or infected plant material through international commerce and travel.

The risk values presented in Figure 1 are on a 3–9 scale. This ranking system assumes that reproduction/mating system, gene/genotype flow, and effective population size affect evolutionary potential equally. A further assumption is that these effects are additive. The proposed scale offers many possibilities for developing

testable hypotheses and assigning relative evolutionary risks. For example, pathogens that have exclusively asexual reproduction and little potential for gene flow are assigned to the lowest risk category. This category includes some bacterial pathogens and the *Fusarium oxysporum* formae speciales (Figure 1). At the other extreme, pathogens that have mixed reproduction and asexual spores that are disseminated over long distances by wind are assigned to the highest risk category. This category includes pathogens such as the powdery mildew fungi. In the intermediate risk categories are pathogens that we expect to have more limited evolutionary potential as a result of lack of an asexual propagule that has high gene flow potential, or lack of regular outcrossing that produces new recombinants (Figure 1). Figure 1 hypothesizes that pathogens with regular sexual cycles will evolve faster than pathogens without recombination. It also hypothesizes that pathogens producing asexual propagules distributed over long distances will break down resistance genes faster than pathogens with short distance dispersal of asexual propagules.

Validation of the risk assessment model

To test the model, we considered 34 plant pathosystems and used Spearman rank order correlation analysis to determine the correlations between the four "expected" risk factors and the "observed" risk values for all 34 pathosystems (McDonald & Linde 2002a). When the analysis was conducted using the sum of mutation risk values and gene/genotype flow risk values, the correlation was 0.35 ($P = 0.044$, two-tailed t-test). When the largest outlier in the correlation matrix, the nematode *Meloidogyne incognita*, was removed from the analysis, the correlation rose to 0.46 ($P = 0.007$). This preliminary analysis suggests that the contributions of the evolutionary forces may not be equal. It also indicates that gene/genotype flow and mutation may be the dominant forces driving pathogen evolution in the 34 plant pathosystems we considered.

A decision diagram to aid resistance breeding

We proposed guidelines based on the evolutionary potential of the pathogen to choose appropriate types of resistance and to decide how to deploy major resistance genes in a breeding program (McDonald & Linde 2002a). The simple decision diagram is shown in Figure 2. This diagram offers some broad guidelines to consider before embarking on a resistance-breeding project, with the objective of choosing the appropriate type of genetic resistance and then applying a resistance gene management strategy that will match the pathogen's biology and minimize the likelihood that the pathogen population will evolve to overcome the resistance. The outcome of the decision diagram is a general recommendation for choosing the type of resistance to use and the optimum deployment method with the aim of maximizing the useful lifespan of the resistance genes. At one end of the decision diagram are pathogens that have strictly asexual reproduction, a low potential for gene/genotype flow, and small effective population sizes. In our risk model, these are pathogens with the lowest evolutionary potential. For these pathogens, a breeding strategy that relies on single major resistance genes is likely to be durable because the mutation to virulence will occur in a limited number of genetic backgrounds and the virulent lineages that inevitably arise are unlikely to move quickly to new fields planted to the same major resistance gene. An example of a

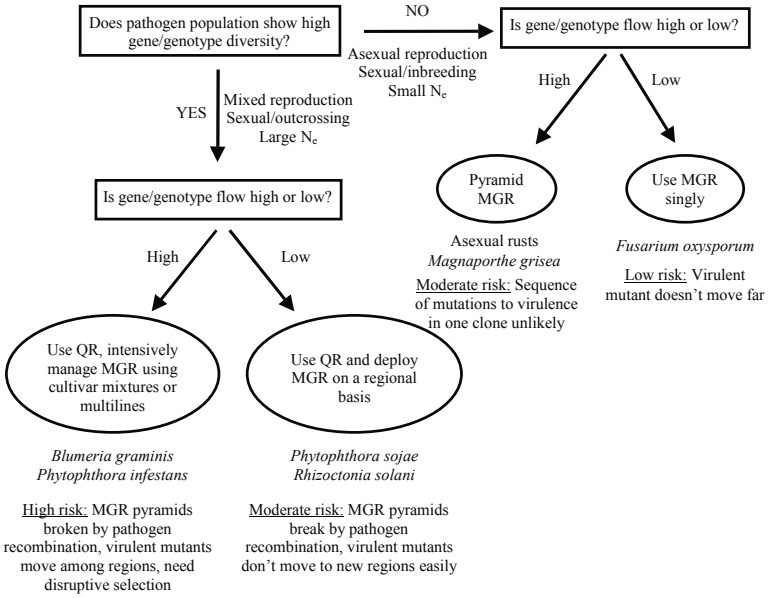

Figure 2. A simplified decision diagram to assist with developing resistance-breeding strategies to achieve durable disease resistance based on knowledge of pathogen population genetics. Major gene resistance (MGR): resistance that has large effects, is based on the hypersensitive response and follows the receptor-elicitor model of the gene-for-gene interaction. Quantitative resistance (QR): resistance that has, on average, small, nearly equal, and additive effects that are equally effective against all strains of the pathogen

class of pathogens that follow this life history is the *Fusarium oxysporum* wilts on many crops.

The next category in the decision diagram is asexual or inbreeding pathogens that exhibit a high potential for genotype flow. These pathogens also exhibit low genotypic diversity, but when the virulent lineage arises by mutation, it is moved efficiently to neighboring fields or adjacent agricultural regions. For these pathogens, a breeding strategy that pyramids major resistance genes is likely to be durable because it is unlikely that a sequence of multiple mutations to virulence (loss of several elicitors simultaneously) will occur in the same clonal lineage. Examples of pathogens that follow this life history are the asexual rusts and *Magnaporthe grisea*. Pathogens that have a sexual cycle, but appear to be mainly inbreeding, such as *Sclerotinia sclerotiorum*, may also fall into this category.

Pathogens that exhibit mixed reproduction that includes regular recombination exhibit higher genotype diversity as a result of recombination and have greater potential for local adaptation to a changing environment. After a mutation to virulence occurs, it can be recombined into many different genetic backgrounds, and it can be recombined with other virulence mutations that occur at unlinked loci. Thus pyramids are not an optimum approach for these pathogens. Pathogens with a mixed reproduction system and a low potential for gene/genotype flow are placed in an intermediate risk category. For these pathogens, breeders should focus on quantitative resistance instead of major gene resistance. If quantitative resistance is not available, then major gene resistance can be deployed in rotations through time or space. The rationale for these choices are explained in McDonald and Linde (2002a,b).

The highest risk pathogens have a mixed reproduction system and a high degree of gene/genotype flow. We believe that these pathogens will require the greatest effort to achieve durable resistance because the mutations to virulence can be recombined into many genetic backgrounds until a pathogen clone with high fitness appears, and then this adapted genotype can be dispersed across long distances and into new populations. For pathogens in this risk category, we suggest the breeding effort should concentrate on quantitative resistance that will need to be renewed regularly to stay ahead of the pathogen. If quantitative resistance is not available, then major gene resistance should be managed aggressively, including development of cultivar's mixtures and multilines that can be used in combination with regional and temporal deployment strategies.

Genetically engineered resistance and the risk assessment for pathogen evolution

Genetic engineering technologies offer great potential, but present a number of uncharacterised risks that require further investigation. One risk is that genetically engineered resistance genes will face the same boom-and-bust cycles as the major resistance genes incorporated by traditional breeding methods. Our present knowledge indicates that plants evolved leucine-rich repeat (LRR)-types of receptors to recognize a diverse array of pathogen elicitors, and it is likely that pathogens coevolved with these receptors for millions of generations before agriculture arose. With this long history of coevolution, it seems unlikely that we will be able to eliminate plant diseases by engineering new receptors or combinations of receptors and putting them into our crops. Pathogens will continue to evolve. However, genetic engineering offers new opportunities to stay a few steps ahead of the pathogen. Genetic engineering can be used to create novel pyramids of major resistance alleles that can be transferred into plants as a cassette of linked genes. It may become possible to create a pyramid more quickly through a single transformation step than through a series of hybridizations and backcrosses. Of course, plants already have evolved cassettes of linked resistance genes over evolutionary time scales, and pathogens are still with us. Genetic engineering also could be used to synthesize multilines quickly and efficiently by inserting different resistance alleles into superior agronomic genotypes as they are developed. This approach may allow us to impose disruptive selection that slows pathogen evolution, but it is unlikely to eliminate the pathogen. It is most likely that pathogen populations will continue to evolve and respond to the new forms of genetic resistance that we deploy through genetic engineering. But with careful management of these new, engineered resistance genes, we may be able to create truly durable forms of genetic resistance. The best way to insure the durability of these new engineered resistance genes is to manage them wisely using knowledge of the evolutionary potential of the pathogen population.

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