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# Plant–pathogen interactions: genetic and comparative analyses

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**Abstract**

The interactions between plants and pathogens can be shifted to favor either plant of pathogen by small changes in the environment, primarily temperature and plant nutrition, and it leaves a quandary as to whether the plant on pathogen is most affected by the change in the environment. The stage of development of a plant can affect the resistance or susceptibility to a pathogen. A plant may be susceptible to a given pathogen a one stage of development but resistant at another stage of development. The view of the gene-for-gene hypotheses as a one-for-one relationship is not supported by experiments that ask whether avirulence genes and resistance genes function alone. The term genomics has been interpreted several different ways, but its most useful impact on studies of host–pathogen interactions will, most likely, be to find all the pieces to the puzzle of how plants and pathogens communicate.

## Introduction

It was 20 years, almost to the day, that I was last in Greece at a conference on active defense mechanisms in plants. My presentation at that meeting (Ellingboe, 1982) was somewhat stressful because what I had to say was not consistent with most of the other presentations. I felt somewhat the same at this meeting. The meeting 20 years ago, however, was very beneficial to me because it was at that meeting that I developed the strategy for cloning the genes in host and pathogen that affect their interactions. Even though I know I will disagree with many of the interpretations of data presented at this meeting, I look forward to this meeting with the hope that I might gain new insights that may change future research in host–pathogen interactions.

Progress in research in biology has commonly been dependent on the conceptual framework under which a phenomenon was pursued, and this is certainly true of studies of plant–pathogen interactions. It is not difficult to see the many different arguments that have been used for studies of plant–pathogen interactions, particularly when consideration is given to the hundreds of species of plants and thousands of species of plant pathogens.

For this paper, I was asked to make comparisons between genetic and comparative biochemistry (correlative) studies of host–pathogen interactions and this is how I plan to proceed.

I will begin with a brief discussion of the studies that have indicated that the interaction between a host and a pathogen can be shifted in favor of the host or of the pathogen. There have been more than 50 years of research in trying to determine what favors the pathogen and what favors the host. I consider these relevant to the discussions on acquired resistance. The studies that I will describe very briefly are observations from field studies. I mention certain examples to emphasize that there are probably as many examples of treatments that favor the pathogen as those that favor the host.

The nutritional status of the plant can favor either the host or the pathogen. Maize grown with low potash fertilization is more affected by stalk rots caused by *Fusaruim* species than maize grown in the presence of a more balanced fertility with adequate potash. Examples of questions that could be asked, but I doubt that they have been, are whether (1) the fertilization induced special resistance mechanisms, (2) the plant growing

with an adequate balance of nutrients has a different anatomical structure, (3) the high potash is toxic to the pathogen, etc. What has been demonstrated is that the fertilization of the plant can alter the balance in favor of either host or pathogen. The temperature at which an inoculated plant is held can also put the favor to the plant or the pathogen. Wheat becomes more affected by stem rust as the temperature is raised from 22 to 28 °C but is less affected by stripe rust at the higher temperature.

There is a long history of the concept that a resistant plant can resist the growth and development of a pathogen by the production of inhibitory chemicals in the plant. Phytoalexins have been studied extensively as a putative mechanism of resistance to a pathogen. But many studies have shown that the synthesis of phytoalexins occurs in both resistant and susceptible plants. Furthermore, mutations which Ellingboe and Poplawsky made in *Colletotricum lindemuthiam* to reduced sensitivity to phaseolin had no affect on the expression of virulence of that pathogen or resistance of the host. Thus the correlation between the synthesis of these secondary metabolites and the expression of resistance suggests to me that the synthesis of these metabolites is the result of the expression of resistance rather than the cause of resistance. Though the concept of synthesis of phytoalexins, for example, may be intuitively very pleasing, the critical testing of the biological significance of the correlations is almost always lacking.

The stage of development of the host can also have a dramatic effect on the development of symptoms by the pathogen. For example, maize is very susceptible to the yellow leaf blight pathogen, *Phyllosticta maydis*, as a seedling. It becomes very resistant to this pathogen when about a month old, and becomes very susceptible again about ten days after pollination. The results of resistance/susceptibility or avirulence/virulence are *very* dependent on the stage of development of the plant.

The above is but a brief set of examples where the advantage to the pathogen or host is altered. The questions that arise from these observations have relevance to the interpretations of these comparative studies, and how these affect future research efforts.

## Conceptual framework of studies on host–pathogen interactions

I think it is reasonable to say that much of the research in plant pathology has followed the comparative biochemistry model that was developed in the 1930s. The conceptual framework for the research on the physiology and biochemistry of host–pathogen interactions can be illustrated as follows. Because many pathogens are successful in attacking relatively few host species, it has been assumed that the successful pathogen must be doing something to be successful on one host species and it is not doing what is necessary to be successful on another host species. Figure 1 illustrates host species–pathogen species specificity. *Leptosphaeria maculans* is a pathogen of canola but is not a pathogen of rice. *Magnaporthe grisea* is a pathogen of rice but is not a pathogen of canola. Following this model, the studies of virulent and avirulent pathogens have led to the discovery of host–specific toxins, and also compounds that are not so specific but nevertheless toxic to plants. Many studies on the mechanisms of resistance have correlated the expression of host resistance (and the concept of what this means is different for different people) with the presence of certain compounds that are present in the 'resistant' plants, but absent or in reduced concentration in the susceptible plants. An early-published example is the correlation of the presence of toxic phenolics in onions resistant to the onion smudge disease,

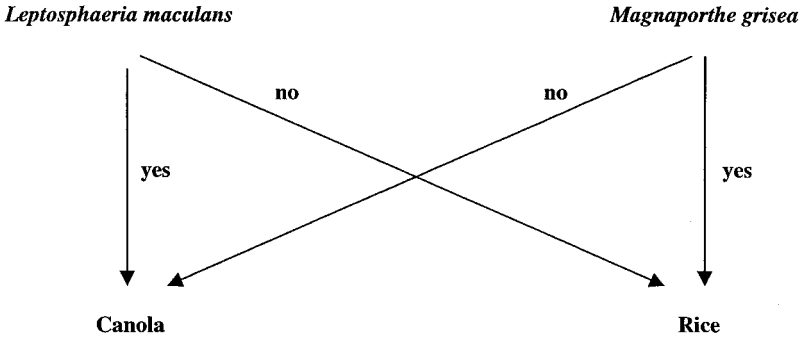


*Figure 1.* Pathogen species and specificity of interactions with hosts.

but absent or in low concentrations in susceptible cultivars (Walker and Link, 1935).

The arguments of comparative biochemistry (in these times it is comparative molecular biology) continue to be used extensively to determine the mechanisms of resistance/susceptibility or avirulence/virulence in pathogens. This argument is not consistent with much of the genetics of host–pathogen interactions. Let me illustrate this with the following discussion.

The work of Flor (1946, 1947) brought a new perspective to studies of host–pathogen interactions in that he studied the genetics of the host and the genetics of the pathogen together. His data suggested that the expression of resistance in a host is not dependent only on the genes (and other characteristics) of the host but is also dependent on the genes of the pathogen. The expression of avirulence or virulence of a pathogen was not dependent only on the genes (and other characteristics) of the pathogen, but also dependent on the genes in the host. It took many years for Flor's work to be accepted. Not all have accepted his work as being anything other that a peculiarity of certain host–pathogen combinations.

The evolution of the understanding and the scientific significance of Flor's work is an interesting example of how science develops. More than 30 years passed between the publication of Flor's results and the introduction of the recombinant DNA technology into studies of host–pathogen interactions. During this period, there is essentially no evidence that Flor's results affected research on the physiology and biochemistry of host–pathogen interactions. The results of Flor's work did evolve into the gene-for-gene hypothesis, and gradually evolved to a one gene for resistance and one gene for virulence hypothesis, despite much genetic evidence to the contrary.

## Recombinant DNA era beginning in 1980

The application of recombinant DNA technology in plant pathology changed the research on host–pathogen interactions. The first experiment that dealt with the specificity of the interaction was with *Xanthomonas syringe* pv. *glycinia*, a bacterial pathogen of soybeans. A library of one race of the pathogen was prepared and individual clones were transformed, one at a time, into a different race of the pathogen (Staskawicz et al., 1984). The question asked was whether any transformant had a change in reaction with a set of differential soybean lines. A clone was found that converted a virulent recipient bacterium to avirulence on a given soybean cultivar (Table 1). But no transformants were recovered that converted an avirulent recipient to virulence on a specific soybean cultivar (Table 2). This observation is consistent with the genetic expectations based on the dominance of resistance over susceptibility and the dominance of avirulence over virulence. It is, however, inconsistent with the intuitive argument that a virulent pathogen is doing something to be virulent that the avirulent pathogen cannot do. The clone that converted a virulent recipient to cultivar specific avirulence is considered to contain an 'avirulence' gene. This has become the definition of an 'avirulence' gene. The procedure, and the arguments, for cloning the first 'avirulence' gene have been used for several plant pathogens, and more than 50 'avirulence' genes have been cloned. The cloning of 'avirulence' genes is built on comparative biochemistry arguments and on the procedures used by Staskawicz et al.

## Is the one gene for resistance and one gene for avirulence correct?

My initial reason for encouraging the cloning and sequencing of genes involved in host–pathogen interactions was to gain an understanding as to what were the crucial interactions that determine the fate of the

*Table 1*. Transformation of virulent pathogen strain 2 with a clone of strain 1, and the recovery of a transformant avirulent on host with $R_1$

| Pathogen | | Host | |
|---|---|---|---|
| | | $R_1$ | $r_1$ |
| 1 | $P_1$ | −[a] | + |
| 2 | $p_1$ | + | + |
| *Transform pathogen 2 with library of pathogen 1* | | | |
| Transformant | | − | + |

[a] − =incompatible interactions. + =compatible interactions.

*Table 2*. Only transformants with reduced virulence were recovered. No transformants with increased virulence were recovered

| Pathogen strain | Soybean | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Donor | +[a] | − | + | − |
| Recipient | − | + | − | + |
| Transformant | − | − | − | − |

Data from Staskawicz et al. (1984).

[a] − = incompatible interactions. + = compatible interactions.

interactions, how do genes interact with each other, and to do this with genes that are known to be determinants of the interactions. Since the genetics of host–pathogen interactions has evolved into a one-for-one concept, one of the first questions of interest to me was: do these genes functions alone? To answer this question, a pathogen was needed in which all types of genetic analyses were possible. To this end, more than a decade ago, we developed populations of *Magnaporthe grisea* that are pathogenic on rice and are sexually competent so that crosses could be made at will. The first discovery was that each 'avirulence' gene had a second locus that functioned as a dominant suppressor of the 'avirulence' allele. The crosses that illustrate how the other genes were discovered is presented in Table 3. Two genes, *P* and *S*, are important in cultivar specificity (Lau et al., 1993). Table 4 gives the example of one pair which demonstrates that the $S_{12}$ locus has no phenotype in the absence of the $P_{12}$ gene. Table 5 illustrates the specificity and universality of the *P* and *S* gene interactions. Avirulence genes do not function alone. Mutations of the suppressor gene can make the *S* locus look like a dominant gene for cultivar-specific virulence.

The second question was whether each mutation to increased virulence was at the 'avirulence' locus. This helped to identify loci that were necessary for the expression, not suppression, of an 'avirulence' gene (Lau and Ellingboe, 1993) (Table 6). Thus, it has become very clear that the 'avirulence' gene (the gene in the pathogen that determines the specificity with the host) does not function alone (an observation that is not surprising to a geneticist). Table 1 represents the framework for cloning avirulence genes by function. Table 7 shows what can happen depending on the genotype of the recipient. If the virulent recipient is strain 2 or strain 3, the clone that converts strains 2 or 3 to cultivar specific avirulence is likely to contain the gene $P_{12}$. If the virulent recipient in the genetic transformation is strain 4, the clone that converts strain 4 to cultivar specific avirulence is likely to contain *M1*. If the virulent recipient is strain 5, the clone that converts strain 4 to cultivar specific avirulence is likely to contain *M2*. Thus a genetic transformation with a clone of a cultivar-specific avirulent strain into a virulent strain and select for cultivar-specific avirulence does not guarantee that the transformation was with a clone that contains a gene that determines specificity with the host genes. So what

*Table 3.* The crosses and test crosses of *M. grisea* that identified the interaction between *P* (avirulence) and *S* (suppressor) genes

| Pathogen | | Host | |
|---|---|---|---|
| | | A | B |
| | | $R_1$[a] | $r_1$ |
| A | $P_1$ | − | + |
| B | $p_1$ | + | + |
| Cross made | A × B → 21 A : 20 V | | |
| assumption | $P_1$ $p_1$ $P_1$ $p_1$ | | |
| Test | A × A → avirulent plus virulent | | |
| interpretation | $P_1p_2 \times p_1P_2 \rightarrow P_1P_2$ avirulent | | |
| | $P_1p_2$ avirulent | | |
| | $p_1P_2$ avirulent | | |
| | $p_1p_2$ virulent | | |
| Test | V × V → Virulent plus avirulent | | |
| interpretation | $P_1S_1 \times p_1s_1 \rightarrow P_1s_1$ avirulent | | |
| | $P_1S_1$ virulent | | |
| | $p_1s_1$ virulent | | |
| | $p_1S_1$ virulent | | |

[a] − = incompatible interactions. + = compatible interactions.

*Table 4.* Relationship of the *P* and *S* genes affecting avirulence/virulence on a host with $R_{12}$

| | Host | |
|---|---|---|
| | $R_{12}$ | $r_{12}$ |
| $P_{12}S_{12}$ | +[a] | + |
| $P_{12}s_{12}$ | − | + |
| $p_{12}S_{12}$ | + | + |
| $p_{12}s_{12}$ | + | + |

[a] − = incompatible interactions. + = compatible interactions.

*Table 5.* The specificity between 19 different *P* and *S* genes

| |
|---|
| $P_1$-$S_1$ |
| $P_2$-$S_2$ |
| $P_3$-$S_3$ |
| $P_4$-$S_4$ |
| $P_5$-$S_5$ |
| $P_6$-$S_6$ |
| $P_7$-$S_7$ |
| $P_8$-$S_8$ |
| $P_9$-$S_9$ |
| $P_{10}$-$S_{10}$ |
| $P_{11}$-$S_{11}$ |
| $P_{12}$-$S_{12}$ |
| $P_{13}$-$S_{13}$ |
| $P_{14}$-$S_{14}$ |
| $P_{15}$-$S_{15}$ |
| $P_{16}$-$S_{16}$ |
| $P_{17}$-$S_{17}$ |
| $P_{18}$-$S_{18}$ |
| $P_{19}$-$S_{19}$ |

*Table 6.* Mutagenesis of *M. grisea* to increased virulence on a rice line with $R_{12}$

| | Host | |
|---|---|---|
| | A | B |
| | $R_{12}$ | $r_{12}$ |
| Pathogen | | |
| $p_{12}$ | +[a] | + |
| $P_{12}$ | − | + |
| Mutagenesis↓ | | |
| $P_{12}^{*}$[b] | + | + |
| $P_{12}M^{*}1$ | + | + |
| $P_{12}M^{*}2$ | + | + |

[a] − = incompatible interactions. + = compatible interactions.
[b] * = mutant gene.

*Table 7.* Identification of different genes by transformation; depending on the genotype of the virulent recipient

| Pathogen | | Host | |
|---|---|---|---|
| | | $R_{12}$ | $r_{12}$ |
| 1 | $P_{12}s_{12}\,M1\,M2$ | −[a] | + |
| 2 | $p_{12}s_{12}\,M1\,M2$ | + | + |
| 3 | $P_{12}^{*}{}^{b}s_{12}\,M1\,M2$ | + | + |
| 4 | $P_{12}s_{12}\,M^{*}1\,M2$ | + | + |
| 5 | $P_{12}s_{12}\,M1\,M^{*}2$ | + | + |

[a] − = incompatible interactions. + = compatible interactions.
[b] * = mutant gene.

does this tell us about all the 50 plus cloned 'avirulence' genes? It tells me that probably 80–90% of the cloned 'avirulence' genes are probably not the genes that determine the specificity with the host, but are genes that interact with the genes that determine the specificity with the host. If we clone and sequence *M1* and *M2*, will it tell us anything about the interaction with host lines with $R_{12}$ or $r_{12}$ if we don't also clone and sequence $P_{12}$? The point I am trying to make is that correlations can get one started on a biological phenomenon, but a geneticist will likely ask whether we have all the pieces to the puzzle for which a solution is sought.

## Pieces to the puzzle

I will use two examples of how to find the pieces to a puzzle; one is with *E. coli* and the other is with type 1, juvenile onset diabetes in humans. Fred Blattner is a geneticist at Wisconsin whose research group sequenced the entire *E. coli* genome (Blattner et al., 1997). Almost a decade ago, when they had about 2/3 of *E. coli* sequenced, Fred put one of his graduate students on a problem of gene regulation. Of the hundreds of genes known and mapped in *E. coli*, the sequencing had revealed three or more open reading frames (ORFs) between each of the known genes. The functions of these ORFs were unknown. The student's project was to prepare a grid in which to look at the simultaneous regulation of 800 genes. The basis for comparison was to first determine which genes are transcribed at 37 °C using a standard medium. When the same conditions were used except that the temperature was dropped to 34 °C as a comparison to transcription at 37 °C, approximately 50 genes were up-regulated and 50 were down-regulated. Which of these ca. 100 changes is crucial to the growth at either temperature? When the temperature for growth was increased to 40 °C, approximately 50 genes were up-regulated and 50 down-regulated compared to growth at either 37 or 34 °C. One comparison was of great interest to me. They compared the growth of the culture at 37 °C, but with two different sugars, the same sugars used by Jacob and Monod more than 40 years ago to demonstrate the existence of operons. Comparison of gene regulation with two sugars (both cultures grown at 37 °C) also revealed about 50 up-regulated and 50 down-regulated gene differences. Which changes are crucial to growth on each of the energy sources? There are so many correlations in these experiments that it is hard to know where to begin, and it illustrates to me the difficulties to a comparative biochemistry approach to biology, whether it is at the level of secondary metabolism or primary metabolism.

## Genomics

The now abundantly used term genomics has many meanings to researchers. To some it means a determination of a complete DNA sequence of an organism that may also include an identification of all open reading frames. To others, it represents the use of a series of different approaches and arguments to identify all the pieces to a given array of biological phenotypes. To illustrate the latter, the second example that I want to present is with juvenile onset, Type 1 diabetes. Diabetes in humans has been known and studied for most of this century. Insulin was one of the first proteins characterized. Though there have been extensive studies of diabetes, relatively little progress has been made in understanding the onset of this disease. The

delivery, dose, and sources of insulin have improved considerably. Problems associated with use of porcine insulin have been overcome through cloning of the human gene for insulin.

Juvenile onset (Type 1) diabetes has been treated, until recently, as a relatively simple disease. The pancreas either does or does not secrete enough insulin and the receptor for the insulin is either normal or abnormal. The conceptual framework for research on this disease has changed greatly with the introduction of genetic analyses into the studies. First, there has evolved a great restraint in the over-interpretation of results from correlations. Secondly, diabetes is no longer treated as having only two phenotypes, either diseased or healthy, and the different phenotypes (e.g. time of onset, gene regulations of synthesis of insulin, apotosis of cells producing insulin, receptor mutant or covered up, insulin resistance, etc.) are recognized. The third is a wise use of genomics to identify all the pieces to the puzzle. The use of an extensive set of molecular markers, an array of family pedigrees, and a resistance to jump to quick conclusions, have now lead to the identification of 17 DNA segments that are correlated with particular phenotypes (Eisenbarth). Of the ORFs on each DNA segment, there is now an effort to determine which are crucial to a given phenotype. As segments of DNA are correlated with a given phenotype of the onset, there is always the question as to which segment is a crucial piece to the puzzle, and always a questioning as to whether there is another piece to the puzzle. Of the 17 DNA segments that are correlated with the different phenotypes, there are questions as to how these segments (genes?) interact with each other to affect age of onset, gene regulation, apoptosis, insulin resistance, etc.

## Summary

When I look at many areas of biology, and plant pathology in particular, I see a great temptation to over-interpret results. Phenomena that are correlated are immediately conjectured to have a cause and effect relationship. Rarely do I see the following questions: Is this interpretation correct? What are the alternate interpretations?, etc.

When I read papers and hear lectures on systemic acquired resistance, what do I see? I see many correlations and many quick conclusions. There are few questions about alternative interpretations for the data. The argument is frequently presented that there are many ways to induce the 'resistance response.' The implications are that the 'resistance response' is a general phenomenon that gives resistance (or an amelioration of symptoms) to most, if not all, of the pathogens. This is not consistent with the genetics of host–pathogen interactions in host species, such as wheat, in which there have been extensive analyses of the inheritance of resistance to many different pathogens. There are very few examples in which a single gene is even suspected of giving resistance to two pathogens. The expression of resistance to one pathogen usually has no direct effect on the reaction of a plant to a second pathogen. What is the scientific value of the conceptual framework of the 'resistance response'?

To make progress in plant pathology, and host–pathogen interactions in particular, it will probably be necessary to ask questions as to whether we have an understanding of all the pieces of the puzzle, avoid over interpreting the data, and ask more questions about alternate interpretations of the observations. The technologies are available to ask some very penetrating questions, but they will be most useful if they are used together.

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