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Ecological genomics and epidemiology

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Abstract

The huge amount of genomic data now becoming available offers both opportunities and challenges for epidemiologists. In this "preview" of likely developments as the field of ecological genomics evolves and merges with epidemiology, we discuss how epidemiology can use new information about genetic sequences and gene expression to form predictions about epidemic features and outcomes and for understanding host resistance and pathogen evolution. DNA sequencing is now complete for some hosts and several pathogens. Microarrays make it possible to measure gene expression simultaneously for thousands of genes. These tools will contribute to plant disease epidemiology by providing information about which resistance or pathogenicity genes are present in individuals and populations, what genes other than those directly involved in resistance and virulence are important in epidemics, the role of the phenotypic status of hosts and pathogens, and the role of the status of the environmental metagenome. Conversely, models of group dynamics supplied by population biology and ecology may be used to interpret gene expression within individual organisms and in populations of organisms. Genomic tools have great potential for improving understanding of resistance gene evolution and the durability of resistance. For example, DNA sequence analysis can be used to evaluate whether an arms race model of co-evolution is supported. Finally, new genomic tools will make it possible to consider the landscape ecology of epidemics in terms of host resistance both as determined by genotype and as expressed in host phenotypes in response to the biotic and abiotic environment. Host phenotype mixtures can be modeled and evaluated, with epidemiological predictions based on phenotypic characteristics such as physiological age and status in terms of induced systemic resistance or systemic acquired resistance.

Introduction

The field of plant disease epidemiology has incorporated new technologies and perspectives on biology as they have become available, from computer simulation modeling to automated environmental sensing. Over the past decade, the study of DNA within all areas of biology has gone through a revolution, providing new types and new quantities of genomic data for epidemiological analyses. Given the advent of new technologies associated with rapid analysis and miniaturization, informatics, and molecular biology, it is now possible to expand the scale of studies of both agricultural and wild species to include entire genomes. The high-throughput advances associated with genomics and other "-omics" (e.g. proteomics, metabolomics) have allowed an unprecedented collaboration among scientists working at different biological scales and have fostered a new science, ecological genomics. In this "preview", we discuss how these new approaches may dovetail with plant disease epidemiology.

Epidemiology has already benefited from information about the population genetics of pathogens, as reviewed by Milgroom and Peever (2003). By simultaneously studying how pathogen gene frequencies change within and among populations as a result of both natural selection and gene flow, and how pathogen populations grow and spread, it has been possible to track disease outbreaks (e.g., Zwankhuizen et al., 1998),

develop predictions about sources of inoculum and pathogen life cycles (e.g., Cortesi et al., 2000; Cortesi and Milgroom, 2001), understand the evolution of virulence (Escriu et al., 2000a, b, 2003), and make predictions about the durability of resistance in crop genotypes (Escriu et al., 2000a, b). Ultimately, modeling plant disease epidemics and pathogen evolution depends on a complete understanding of both plant and pathogen traits that influence the dynamics between a pathogen and its host. To completely understand any trait and its significance in a dynamic interplay between species requires the simultaneous use of molecular, cellular, organismal, population and ecological approaches. Past efforts to combine epidemiology and population genetics have come up against an upper limit on the number of ecologically important genes that could be surveyed or lack of information on gene function and significance. Yet, host plants, as well as pathogens, exist in a matrix of hundreds or thousands of other taxa and their genes. Population changes in pathogens, reproduction and dispersal will all depend on the interactions among these organisms that can influence the dynamics of resistance evolution and direct effects on pathogen populations (Antonovics, 2003).

The developing synthesis of a functional genomics approach combined with a population and ecological perspective promises to lead to new avenues of research and understanding of plant/pathogen interactions. Evolutionary and ecological functional genomics or EEFG (Feder and Mitchell-Olds, 2003) has as a goal to understand ecological and evolutionary processes that maintain genotypes and phenotypes. The emphasis so far has been on wild species, but agricultural systems offer both an important application and relatively well-characterized systems for experimentation. The field of ecological genomics will address new types of questions beyond applications based on molecular markers. Microarrays allow synoptic measurements of gene expression in tens of thousands of genes. Real-time PCR allows highly accurate quantitative evaluation of gene expression at many time steps. It will also be possible to identify hundreds or even thousands of organisms simultaneously from individual samples as microarrays are developed with sequences representative of desired sets of species, potentially including non-culturable species. Advances in sequencing allow analysis of great numbers of "markers" with added information about their likely role through reference to databases such as GenBank (Black et al., 2001), thus revealing the gene content of particular organisms.

Functional genomics, or the use of genomic technologies (e.g. microarrays) to find genes and polymorphisms that affect traits of interest and to characterize the mechanisms underlying those effects, has been applied effectively in agricultural contexts and has potential in natural systems. Functional genomics moves beyond simple sequence analysis to evaluate the function of particular DNA sequences through, for example, gene knockout mutants or gene activation mutations. These techniques have natural applications for the study of resistance and virulence, but might also be usefully applied in the study of other epidemiological features. By simultaneously scanning thousands of plant genes for changes in expression in response to variables of interest (e.g. stress, infection) it has been possible to identify candidate loci or suites of genes and molecular mechanisms involved in the phenotypic expression of key traits of economically important crop species (Frick and Schaller, 2002; Jones et al., 2002; Mysore et al., 2003). A great deal has been learned about plant defense against disease through the use of functional genomics and model plant systems such as *Arabidopsis* (Wan et al., 2002; Schenk et al., 2003; Whitham et al., 2003a).

An intriguing area of epidemiology that will develop with the availability of new tools for studying gene expression is the study of phenotypic resistance and its responses to the biotic and abiotic environment. Infection with an incompatible pathogen, or a virulent pathogen that causes cell death, can make a plant more resistant to subsequent infection by the same or different pathogens, a phenomenon designated systemic acquired resistance (SAR; Durrant and Dong, 2004). The SAR response in *Arabidopsis* confers resistance to several diseases (Ryals et al., 1996). Resistance to pathogens can also be influenced by non-pathogenic organisms; systemic changes in disease resistance in response to colonization by rhizosphere-colonizing *Pseudomonas* bacteria have been well-documented and are commonly referred to as induced systemic resistance (ISR; Iavicoli et al., 2003; Cui et al., 2005). Dissection of the SAR and ISR signaling systems in *Arabidopsis*

indicate they are controlled by different pathways and signaling molecules with some common components. Understanding which genes are expressed during specific defense responses can provide indications of what pathways are activated in different biotic environments (Pieterse and van Loon, 1999). Tools are now available to begin studying these phenomena more widely in epidemiology.

This paper will address the following topics in ecological genomics. (1) Population genetics and population genomics can inform epidemiology to further our understanding of epidemics and to provide insights for disease management. We will also consider how studies of gene expression can potentially add predictive power at finer spatial and temporal scales than was possible in the past. (2) Models of populations and communities may apply to analogous systems of gene expression within organisms and in populations of organisms to inform a "population biology" of gene expression. (3) Genomics can contribute to understanding of resistance gene evolution and durability of resistance. (4) The landscape ecology of host populations and communities, in terms of both genotypic and phenotypic resistance, can now be studied more thoroughly as it affects epidemics. In addressing these topics, we will emphasize genes that influence the relationship between plant host and pathogen, but the same general concepts would apply to interactions between plant species, between plants and insect herbivores, etc.

How population genetics and population genomics can inform epidemiology

Epidemiology has traditionally used information about host species, pathogen and vector species, and environmental variables such as temperature and precipitation to predict epidemic progress. These models can be adapted to incorporate much more detailed information about the genomic status of the host and pathogen communities considered in the context of a broadly defined environment, i.e., defined to include abiotic components and potentially the complete community metagenome of soil (Rondon et al., 2000) or other systems (Figure 1). Information about the soil

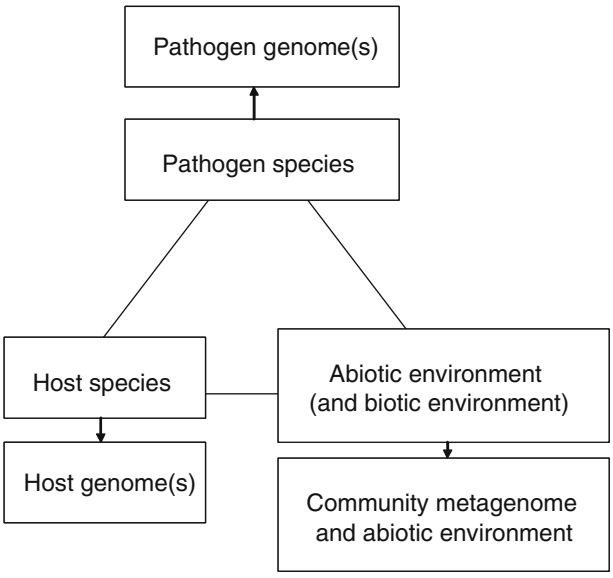

Figure 1. The traditional disease triangle depicts prediction of epidemics based on interactions between pathogen species, host species, and the abiotic environment. It is now much easier to obtain information about the complete genotype and current gene expression of host and pathogen, and there is even the potential to obtain this information for complete communities such as those in soil, the rhizosphere, and the phyllosphere, as well as endophytic communities. Models about a hierarchy of features of "genomic status" might be experimentally evaluated in this context. For example, "The host landscape is sufficiently described to predict epidemic features and outcomes by information about... ● ... host community composition (in terms of species)." ● ... a specific subset of the host genotype sequence(s)." ● ... the host genotype sequence(s)." ● ... a subset of host gene expression." ● ... complete profiles of host gene expression (now and/or in the past)."

Table 1. The temporal and spatial scale of variation in different components of host genomic status

Component of host genomic status	Temporal scale	Spatial scale
In annual monoculture		
Species	Cropping season	Size of field in many conventional systems
Genotype	Cropping season	Size of field in many conventional systems
Gene expression	Less than one hour to cropping season	Part of one individual to size of field
In unmanaged systems		
Species	Days to decades	One individual to majority of plant community
Genotype	Days to decades (potential for somatic mutation)	One individual to majority of species (in clonal species)
Gene expression	Less than one hour or until phenotype expressed (days for defense reaction, months for flowering, etc.)	Part of one individual to majority of area

metagenome may contribute to an understanding of disease suppressive soils that develop over time as microbial populations respond to the buildup of pathogen populations. For example, soils suppressive to the wheat take-all pathogen and potato scab have been described, with fluorescent pseudomonads and streptomycetes, respectively, being the likely causes of suppressiveness (Weller et al., 2002). Advances in genomics also make it possible to characterize the genomic status of host plants at a much finer temporal and spatial scale than in the past (Table 1). The addition of gene expression as a response variable or predictor variable in epidemiological models has the potential to shift the scale of inquiry to hours and millimeters. Monitoring the expression of genes in specific defense pathways, or individual genes that reflect the expression of the pathways, could be used to predict the outcome of pathogen infection in individual plants or plant organs. For most diseases, progress in determining the efficacy of different defense responses for controlling specific pathogens and how the responses become distributed throughout the plant must be made before this information is useful. Then epidemiologists will need to perform a range of exploratory field studies to identify the forms of predictors that are most useful for inclusion in more detailed follow-up studies. For example, if the early induction of senescence-related gene pathways were observed to occur, would this be related to reduced epidemic potential at a field scale?

Characterizations of populations may include the composition of both qualitative features produced by different genotypes and quantitative features produced by different levels of gene expression in what may be the same genotype. Evaluation of qualitative features might be performed using marker or sequence studies, while evaluation of quantitative features might be performed using microarrays or real-time PCR. Studies of gene expression in agriculturally important host plants have expanded remarkably, with microarrays now available for several major crop species. These allow host resistance to be assessed as an outcome of gene expression. In addition, the expression of plant genes in response to non-pathogenic microorganisms may be highly relevant to epidemiology, as it may provide an understanding of how plants select for rhizosphere flora that are antagonistic to pathogens, for example (Smith and Goodman, 1999). Microarray analyses can be used to identify sets of coregulated genes and their common regulatory elements (e.g., Maleck et al., 2000; Chen et al., 2002), which may both reveal different response pathways and allow selection of smaller sets of indicator genes to represent particular stress response pathways. Microarrays developed using genes from one plant species may also be applied, with some caveats, in studies of related species; for example Travers et al. (in preparation) have applied maize microarrays to study gene expression in the related tallgrass prairie grasses *Andropogon gerardii* and

Sorghastrum nutans, and have identified statistically significant responses to simulated climate change in native field populations.

New genetic information can be used to refine state transition models such as Susceptible-Infected (SI) models (e.g., Otten et al., 2003). Rather than modeling host individuals as simply "susceptible and uninfected" or "infected", more details about the state of individuals could be included. The first simple modifications might include broad genotypic resistance and susceptibility. Further refinement could include transitional states of greater or lesser susceptibility based on physiological age, and probabilities of exposure to other organisms that would induce resistance. Matrix-based models of probabilities of transitions from one state to another could be applied to predict long-run states such as disease severity or survival of different genotypes. Such models could potentially be applied to develop both epidemiological theory and better disease management schemes. In the short-run, they could be used to ask questions about the incremental benefits of adding information about host phenotype to epidemic models. In the long-run, these models could contribute to a much deeper understanding of epidemic dynamics.

The more complete genetic information from DNA sequencing can be used to study long-standing questions of population structure, host specificity, and phylogenetics. Due to the growth of sequence databases and the reduction in PCR amplification and sequencing costs, determining the sequence of a specific gene in a microorganism is often the most efficient way to determine the species of the microorganism. Databases now carry information on a huge number of organisms, and matching an unknown sequence to the sequences in a database like GenBank takes only a few minutes, although one must keep in mind that not every sequence accession in GenBank is annotated correctly. Reduced sequencing and DNA amplification costs make the identification of components of large microbial populations feasible. Entire fungal or bacterial communities can be characterized taxonomically by incorporating new techniques such as "shotgun sequencing" of a community's collective genome and using genome database searches to identify species and predict gene function (Venter et al., 2004). At a finer scale, sequencing specific genes in pathogen mutants may give insight into cost of virulence (Vera Cruz et al., 2000; Ponciano et al., 2004). Sequencing can also be used to evaluate the potential repertoire of resistance genes available, to the extent that sequence similarity can predict functional similarity (Bai et al., 2002). Examples include NBS-LRR genes, the largest class of disease resistance genes. Plant genome projects have indicated there are approximately 150 in *Arabidopsis* and more than three times this number in rice. Information about the number of resistance genes available may contribute to resistance gene deployment strategies. The identification of sequences associated with resistance genes may also be applied to related plant species to answer long-standing questions about the number and type of resistance genes in natural populations (Gilbert, 2002). Microbial genome projects are providing similar estimates of the number and types of effector proteins in a single organism, such as the number of gene products transferred into plant cells by the type III secretion system of *Pseudomonas syringae* strain DC3000 (Collmer et al., 2002; Alfano and Collmer, 2004; Rohmer et al., 2004; Chang et al., 2005). These are not only important proteins that make the bacteria successful pathogens, but also the targets of plant disease resistance proteins. These are just a few examples of how partial and whole genome sequencing projects can contribute to understanding host–pathogen interactions.

Studies of gene expression in pathogens are still limited, but, where available, are being used to understand expression of genes during plant colonization, and under various cultural practices. As more whole genomes are sequenced, microarrays using various platforms are becoming available for several pathogens. As examples, arrays exist for the rice blast fungus and for several bacterial plant pathogens. Techniques other than microarrays are also being applied to understand gene expression; for example, serial analysis of gene expression (SAGE) has been applied to study gene expression in response to rice blast infection (http://www.mgosdb.org/). Microarrays can also be used in comparative genomics studies of closely related pathogens using full genome sequences. For example, the gene content of the human pathogen *Yersinia pestis* has been studied as an indicator for adaptation (Chain et al., 2004). Genomes have been evaluated to determine what is missing in a

fastidious, xylem limited species like *Xylella fastidiosa* by comparison to other less fastidious bacteria (Van Sluys et al., 2002). The genomes of *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* are being compared for insights into why the first is systemic in xylem while the second grows in mesophyll (A. Bogdanove, pers. comm.).

The greater availability of genetic information will allow plant pathologists to move "beyond the inoculation experiment" in studies of the genetic features of host–pathogen interactions. In the past, painstaking and expensive analyses of genetic expression in host–pathogen interactions have generally been applied to studies of pathogens introduced to hosts either in highly conducive environments, in the case of rust fungi, for example, or directly inoculated into host tissues, in the case of many bacterial pathogens. In contrast, it would be extremely interesting and valuable to have a greater understanding of the genetic basis for the broad range of other epidemiological features that are important in determining population-level interactions between host and pathogen. For example, from the standpoint of the pathogen, aside from direct effects on virulence or aggressiveness, what are the genes most important for features such as survival in soil or on plant surfaces, tolerance for temperature extremes, dispersal capability, or other specialized features such as conversion from production of urediniospores to production of teliospores in rust fungi? At larger epidemic scales, the genetic characteristics most important to dispersal might be those that affect survival of propagules under challenging environmental conditions. These characteristics would help determine whether the long-distance transport events so important to establishment of epidemics in new areas occur or not. From the standpoint of the host, what genes are most important for predicting epidemics aside from direct resistance genes, including features such as the probability of escape through faster or slower movement through developmental stages, "leaking" of compounds in the phyllosphere or rhizosphere, and architectural features that affect microclimate? Such information would be useful both for applied crop plant breeding programs and for understanding resistance profiles in natural plant populations.

There is the potential to identify genes predictive of epidemiological features using "comparative genomics" to inform "comparative epidemiology". For example, Kranz (2002) discusses several disease parameters influenced by host plant resistance that together predict epidemic rates and outcomes: disease intensity, incubation period, latent period, infection efficiency, disease efficiency, infection rate, lesion size, infectious period, and sporulation intensity. In comparative epidemiology, the differences in these parameters between host–pathogen systems can be evaluated both in terms of their typical values and the frequency distribution of these values in response to typical forms of resistance. The availability of gene expression data will also make it possible to study disease parameters as a function of measures of gene expression, given a particular genotype (Figure 2), in the same way that the expression levels of key genes associated with the initiation of flowering have been used to predict flowering time (Welch et al., 2003, 2005).

There is a basic need in epidemiology for improved diagnostic systems and genomic advances will greatly expand the tools available. For example, as models of the risk of invasion by particular plant pathogens are constructed, their validation depends on researchers' ability to determine precisely the abundance of pathogens in a range of environmental settings. In their simplest form, such studies require the ability to detect and identify particular species of pathogens. Diagnosis may also be taken to more sophisticated levels through the ability to detect particular genotypes,

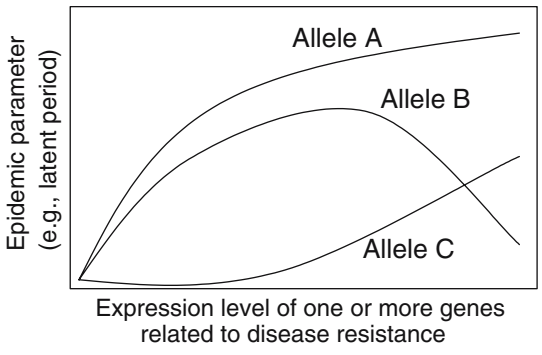

Figure 2. Schematic of possible relationships between gene expression levels and epidemic parameters such as latent period, infection rates, lesion size, etc. Such a relationship could be incorporated in models to refine predictions of epidemic outcomes such as disease severity or disease incidence. The different lines indicate possible differences in response for different alleles.

in particular, those that are capable of causing disease. Presence of genes for these traits, such as genes related to pathogenicity, toxin production, and other epidemiological features, if known, could be used to more reliably measure genotypes in a population responsible for disease. A particularly important application might be the identification of disease, through evaluation of host or pathogen, when infection is still at very low levels, to allow for early management that might, for example, allow an invasive pathogen to be eradicated before it has become well-established. Further refinement for successful diagnosis of gene expression may allow assessment of features such as quorum sensing status (van Bodman et al., 2003). The use of microarrays also opens the possibility of synoptic rapid-throughput diagnostic procedures for huge numbers of organisms for the study of the community characteristics of systems such as disease-suppressive soils, the phyllosphere, and endophytic communities. These approaches could bring great advances in understanding microbial biodiversity, including the potential to find new non-culturable putative pathogens through scans for microbial genes used for taxonomic classification or even genes associated with pathogenicity. Epidemiologists might also make good use of a genomic tool that would allow them to study the past presence of pathogens through on-going expression in host populations. Such a measure of pathogen ''footprints'' could support studies of long-term epidemics and changes in host resistance over time. But it appears that an indicator of past infection is not readily available in plants, or at least researchers have not yet discovered how to recognize it.

How models of populations and communities may apply to systems of gene expression to inform a ''population biology'' of gene expression

A null model for how models of populations and communities apply to the study of gene expression might be ''consilience''; that is, the null model might be that the same models will apply across scales, so tests could be developed to determine where population models do and don't adequately explain patterns of gene expression.

Models from population biology can be applied in the study of gene expression in three general ways. First, at the smallest scale, genes may be conceptualized to interact within a cell comparably to the way that species interact within an ecosystem (Mauricio, 2005). For example, it may be useful to apply such models to the interactions between different defense response pathways. There is evidence the jasmonate (or ethylene) and salicylic acid pathways affect somewhat different pathogens and pests but also interact with each other (Thomma et al., 1998; Glazebrook et al., 2003). Depending on the response examined, they may sometimes be viewed as complementary (van Wees et al., 2000) and in other cases as in competition (Spoel et al., 2003).

Second, an individual plant may be conceptualized as a population of cells or organs across which gene expression occurs. It is now possible to measure gene expression in individual plant cells (Kerk et al., 2003; Nakazono et al., 2003) so the spatial pattern of expression through an individual host can be measured and modeled at whatever spatial grain is motivated by the experimental questions. Spatial patterns of defense responses between cells are relevant both to how effective defense responses are to pathogen challenge and to how the host responds to adjacent or subsequent challenges by the same or other pathogens. Could models of the dispersal of individuals through ecological landscapes be usefully adapted to describe the dispersal of gene products within and between cells? State transition models could be applied to individual plants in cases where it makes sense to treat them as a set of units, such as different tissues and organs, each of which would have its own expression status. This could be addressed using a variation on SI models. Predictions based on these models might include the predicted infection level as well as the predicted plant growth rate.

Third, experiments in epidemiology might begin with models within individuals, predicting infection levels based on the expression of particular genes, and then expand on these to predict infection rates in plant populations based on the gene expression rates in individuals. A null model for such a study might be that the mean expression level of individual hosts is fully predictive of the level of infection in the population. In contrast to the null model, it would be interesting to determine whether the frequency distribution of the expression rates, and perhaps even their spatial pattern,

in the different host individuals would substantially improve predictions of epidemic features, just as different patterns of disease severity across individuals can result in different overall yields for the same mean disease severity (Hughes, 1996). Simulation modeling might be used for initial tests of the sensitivity of epidemic outcomes to such patterns of expression. Addressing questions such as these with an understanding of mechanism will require considerably greater understanding of the relationships between gene expression and gene product physiological function. This third scale is addressed further in a later section.

The study of gene expression offers a new method for measuring integrated effects of environmental variation (Figure 3). Environmental variables such as temperature and precipitation are standard predictors of disease progress in epidemiological models (Jeger, 2004), and integrated forms such as ''growing degree days'' are already commonly used to predict growth stage as a model component. Different types of host responses may be integrated over different time intervals. Growth stage, or more specialized responses like the development of sun and shade leaves, are the products of the cumulative effects of gene expression, as affected by environment, over a period of time. Younger tissues might only experience ''indirect'' effects from past environmental conditions, perhaps as an analog to maternal and grandmother effects in individuals. Induced systemic resistance might be an example of short-term gene expression in response to non-pathogens while systemic acquired resistance might be an example of short-term gene expression in response to pathogens or to chemical stimulants. The timing of infection and its effects on losses in productivity can also be evaluated through variations on time-of-infection models for predicting yield loss (Madden et al., 2000) that include explicit descriptions of host gene expression in response to infection. The schematic model in Figure 3 applies most directly in agricultural systems in which a genotype is generally maintained, at least for a season, through removal of competitors. A more complicated model might be developed in which host genotypes can be replaced by other plant genotypes. The schematic might also be adapted to take into account the possibility of thresholds such that long-term changes in phenotype could be produced by short-term gene expression at critical time points in development.

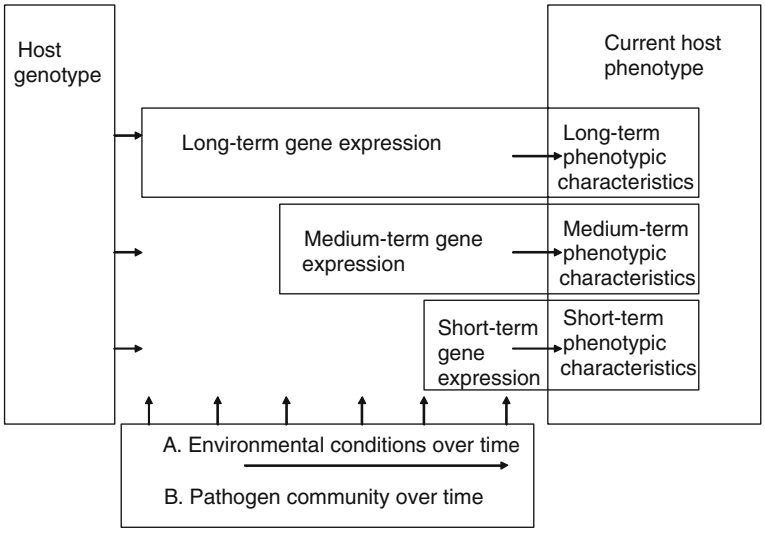

Figure 3. The current host phenotype, at any spatial scale within a host individual, is a form of integration of the individual's environment, including the composition of the pathogen community, acting on the host genotype. Long-term phenotypic characteristics would include features such as physiological age of leaves or roots, forms of specialization such as the development of sun or shade leaves, and other characteristics that may influence disease resistance. Short-term characteristics might include features such as upregulation of pathways contributing to induced resistance. Of course, host gene expression will also influence pathogen populations and even the abiotic microenvironment.

How genomics can contribute to understanding of resistance gene evolution and durability of resistance

A major goal of agricultural plant pathology is the development of durable resistance to plant pathogens in agricultural species. ''Durable resistance'' has been defined as resistance that is still effective after it has been deployed over a wide area, over a long period of time, in a disease-conducive environment (Johnson, 1981). Without durable resistance, plant breeders are forced to continually incorporate new resistance genes in crop varieties as pathogen populations adapt to infect older varieties with previously deployed resistance genes. An understanding of the evolution of host and pathogen genes affecting host–pathogen interactions is needed to form strategies for the durable deployment of resistance in agriculture. It has long been thought that understanding of the relative importance of the disease effector proteins from bacterial and fungal pathogens that are detected by R genes (i.e., the products of avirulence genes) should provide insight into which R genes might have more durable effects, but this idea has had limited impact because of the difficulty of identifying and characterizing these effector protein genes. Comparative genomic approaches for identifying these genes and functional genomic approaches to obtain 'knocking-outs' of their function is making this increasingly feasible (Leach et al., 2001). Some resistance genes, like *mlo* of barley (Buschges et al., 1997), may confer resistance without interacting with specific pathogen effector proteins. These genes may provide non-specific resistance by changing the physiology and gene expression of the plant before pathogen challenge (Wolter et al., 1993). Gene expression analysis has indicated other resistance genes with suspected non-specific effects may alter expression of defense genes before pathogen challenge (Bowden and Hulbert, unpublished). Such analysis should be useful in identifying genes controlling non-specific and thus durable resistance and also provide insight into the possible physiological cost of the resistance.

The isolation and sequence analysis of several resistance genes has provided insight into the evolution of disease resistance in plants (Hulbert et al., 2001). Some of the results of these analyses are consistent with a classical evolutionary arms race model, while others are not (Hulbert, 1998). High levels of sequence variation have been observed at most R gene loci examined. This is consistent with the arms race model's prediction that R genes would evolve rapidly, creating novel alleles with new specificities in response to pressure imposed by rapidly evolving pathogen populations. Loci like *L* of flax (Ellis et al., 1999), which is structurally simple but has multiple resistance alleles, exhibit extremely high levels of polymorphism compared to most genes. At some R gene loci, the patterns of nucleotide substitution between alleles or family members show evidence of the types of diversifying selection that might be predicted by an arms race model. While polymorphic nucleotides are usually synonymous (not affecting the encoded amino acid) at most loci, the opposite is true of certain regions of some R gene loci. This is most often true in regions of R genes thought to code for the ligand recognition part of the protein, like the leucine-rich repeat regions (Parniske et al., 1997; Meyers et al., 2003). Evidence of diversifying selection in other regions of R genes, like the TIR domain-encoding regions of the L alleles, has suggested they may also be involved in recognition (Luck et al., 2000).

One interpretation of an arms race evolutionary progression is that there should be little variation at a given R gene locus at one point in time and that most R gene alleles should be fairly recent in their evolutionary origin. This would be expected if new highly effective R genes arose periodically and replaced the older 'defeated' alleles. The polymorphic nature of many R gene loci indicates this is apparently not the case for most of them. In fact the partitioning of polymorphism between functional alleles and non-functional alleles at the *Rpm1* and *Rps5* loci of *Arabidopsis* indicated that the classes of alleles have co-existed for a long period (Bergelson et al., 2001; Tian et al., 2002), probably the result of some form of balancing selection. While actual estimates of the age of specific resistance gene alleles are not available, this may be an indication that some R gene alleles are ancient. In contrast, no evidence that resistance alleles are ancient has been obtained by sequencing the same resistance allele from multiple germplasm accessions. If resistance alleles are indeed ancient, it should be possible to identify versions that have accumulated extensive neutral sequence polymorphisms. This has not yet been the case in the limited experiments that have been

conducted (Caicedo et al., 1999; Jia et al., 2003; Smith et al., 2004). The low nucleotide diversity among the functional alleles of these loci is consistent with the idea that they could be recently evolved, although other explanations are possible.

The sequence evidence collected to date implies that different R gene loci are evolving in different manners. For example, some appear to be under strong diversifying selection while others do not. The implications of an R genes' evolutionary history for the stability of the resistance it confers is not clear, but the ability to predict durability based on genomic analysis would be quite useful for crop improvement strategies. Molecular analyses of resistance proteins and their corresponding avirulence proteins have indicated that some physically interact directly (Scofield et al., 1996; Tang et al., 1996; Jia et al., 2000, Deslandes et al., 2003) while others detect modifications of other host components (Mackey et al., 2003; Axtell and Staskawicz, 2003). It is possible that whether an R gene recognizes effector (avirulence) genes directly or protects host targets from modification by effector proteins affects the type of selection pressure driving its evolution (Ponciano et al., 2003). This association, however, is not yet clear due to the small number of interactions in which this type of information is known. For R proteins that guard other host components, it is not clear if the nature of the host protein being guarded affects the durability of the R gene, but it might be expected that some targets are more important to the pathogens ability to cause disease than others. The nature of the effector gene, particularly how essential it is to pathogenicity, has been proposed by many to affect R gene durability and recent data indicates this is true (Vera Cruz et al., 2000).

One response to the problem of rapid "breakdown" of resistance in agricultural systems has been a shift by some plant breeders toward greater use of minor resistance genes that each contribute a small amount of resistance and are generally thought to be more durable (Leung et al., 2003; Liu et al., 2004). However, these genes, because of their small effects, are more difficult to study in the field and even to recognize by the phenotypes of individual plants. The use of genetic markers has made the incorporation of minor genes easier, but the problem remains that, because we do not know what genes are responsible for quantitative traits, the association of the markers with the traits is not absolute. Genomic tools will allow discovery of the genes responsible for quantitative traits, and may make it easier to determine whether resistance governed by quantitative traits is truly more durable; whether the effects of QTL are actually less pathotype specific, or whether an apparently more durable effect may be mediated by a weaker selection on individual pathogen genes. To the extent that function can be inferred from sequence, the response of pathogens to particular minor genes may be better predicted as this information becomes available. It will be particularly useful if comparative genomics would allow predictions of the interactions between minor resistance genes and their responses to abiotic and biotic environments. Functional genomics may also contribute to the identification of new minor resistance genes. QTL analysis or the identification of quantitative trait loci provides a powerful tool for assessing the fitness consequences for genes including resistance genes. For example, Newcombe and Bradshaw (1996) used it to identify genes of large effect that changed the resistance of poplar to pathogenic *Septoria populicola* with community level effects.

The study of pathogen and host co-evolution in natural plant populations is also important for understanding what role pathogens may have played in structuring plant communities. In studies demonstrating the importance of genetic variation in host plant species within a larger community that includes pathogens, hybridization of host plants (e.g. willows, sagebrush, oaks) has led to fundamental changes in the species composition of the entire community (reviewed in Whitham et al., 1999). This "extended phenotype" effect would be reflected in the context of epidemiology by the dying out of some pathogens and replacement with others (Whitham et al., 2003b). Agricultural systems and unmanaged systems offer an interesting contrast, because the selection pressures in unmanaged systems are "direct" while selection pressures in agricultural systems are mediated by human decision-making.

The landscape ecological genomics of host populations and communities, in terms of both genotypic and phenotypic resistance

Once meteorological measurements could be collected using automated systems, epidemiologists

were faced with the question of what temporal scale of resolution was needed for understanding epidemic progress. Information about variation in temperature at the scale of minutes is not generally needed for predicting epidemic features. But whether predictions are improved by resolving differences in temperature at the scale of days or weeks will vary from one host–pathogen system to another, based on characteristics such as pathogen generation time, and requires attention for the construction of good models. Information about variation in meteorological features across space is still not so readily collected at very fine scales, though the increasing availability of "smart dust" and other tiny wireless sensor networks may change that (e.g., http://webs.cs.berkeley.edu/). The same question of appropriate scale of variation to include for modeling will arise for the new spatial maps of meteorological features. Similarly, the potentially huge amount of information about gene expression will require research to determine what scale of variation is important to include in prediction of epidemics for particular host–pathogen systems. The cost of microarray analyses limits the number of samples in time and space for now, but as costs become less limiting, epidemiological research will focus more on determining optimal scales of variation in expression data to include in predictive models.

Plant disease epidemiology has developed models of disease foci and how these foci expand in time and space (Zadoks and Vandenbosch, 1994; Waggoner and Aylor, 2000), including studies of the spatial pattern of disease used to draw inference about modes of dispersal and to devise optimal sampling strategies. Landscape ecology also offers methods for studying spatial features with models for describing the relationships between organisms in landscapes and for describing the connectivity of features (With, 2002). In agricultural systems, the spatial pattern of host genotypic resistance is sometimes manipulated through the construction of intercropping systems and/or use of mixed genotypes within a crop species (Garrett and Mundt, 1999; Zhu et al., 2000; Mundt, 2002). And, of course, most unmanaged systems include a mixture of plant species that, with few exceptions (e.g., *Phytophthora cinnamomi*), do not tend to share the same pathogen species. Mixtures of susceptible and other genotypes make models of disease spread through space somewhat more complicated. Some models have assumed that epidemic "waves" move out from an initial point with constant velocity to simplify the modeling of the system, while other researchers predict that epidemic waves accelerate (Scherm, 1996; Cowger et al., 2005).

A genomics approach applied to epidemiology could explore multiple spatial and temporal scales as well as levels of detail in genomic status, perhaps employing cellular automata models (e.g., Kleczkowski et al., 1997; Figure 4). Within a host individual, the local phenotype might be at the scale of a leaf or of a cell. Local gene expression might be at the point of infection; for example, within compared to beyond a green island of host tissue formed around an infection by a rust fungus. Regional gene expression within an individual might be expression in tissues adjacent to infection. Within a host individual and its immediate environment, a wide range of pathogens may be present, specializing on different host tissues. Competition between particular pathogens may play out differently depending on the time of infection and the type of plant tissue (Adee et al., 1990; Al-Naimi et al., 2005). The question for epidemiologists will be what spatial and temporal resolution is needed for predicting epidemics with the new and upcoming abundance of data, as opposed to averaging over host and pathogen individuals' genomic status across space and time.

In host populations, "expression foci" in which host individuals share altered gene expression patterns may form around inoculum sources, with properties related to those of disease foci. Gene expression changes in hosts in response to exposure to pathogens and other microbes may range from increased resistance through SAR or ISR to increased susceptibility because of weakened tissue integrity. The effect of exposure to pathogens that do not infect has the potential to be substantial, at least temporarily; Calonnec et al. (1996) estimated that the infection efficiency of *Puccinia striiformis* was reduced by 44% when plants were previously exposed to an "inducer race" of the pathogen. At increasing distances from a primary inoculum source, exposure to inoculum may have occurred at more recent time points, potentially resulting in waves of different expression patterns surrounding the initial source areas. Spatial patterns of abiotic features, such as differences in topography that produce cooler or wetter local conditions, may

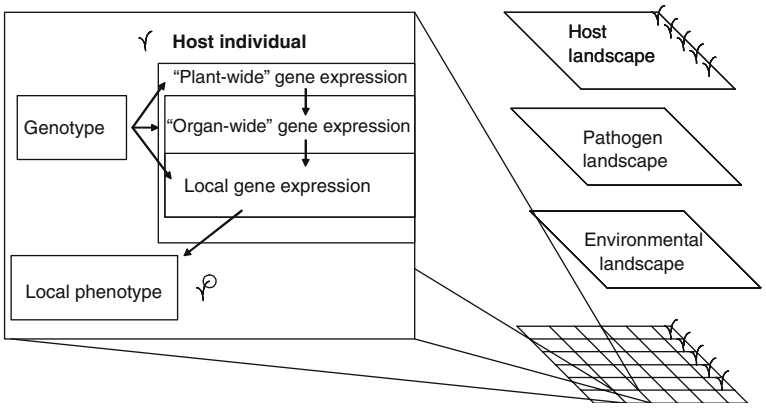

Figure 4. Each host individual is potentially influenced by the landscape of hosts, pathogens, and other biotic and abiotic environmental features. Within a host individual, these influences may play out through "plant-wide", "organ-wide", or more local gene expression, depending on the scale of variation of each feature in the landscape and how it acts upon the host individual. "Plant-wide" gene expression might include responses to factors such as drought and disease that alters water relations within the host. "Organ-wide" gene expression might include responses to factors such as stem or petiole lesions. Local gene expression might include responses such as localized forms of induced resistance. Models related to spatial scale and scale of genetic detail that could be experimentally evaluated as predictors of epidemic features include the following, presented in a hierarchy of increasing complexity. "The host landscape is sufficiently described to predict epidemic features and outcomes by information about... ● ... the abundance of host species" ● ... the abundance of host genotypes" ● ... the abundance and spatial pattern of host species" ● ... the abundance and spatial pattern of host genotypes" ● ... the mean level of gene expression among host individuals" ● ... the spatial pattern of gene expression among host individuals" ● ... the spatial pattern of gene expression within host individuals."

also produce expression foci relevant to epidemics. Studies of gene expression in landscapes may develop distinctions analogous to the distinction between a dispersal gradient and a disease gradient. Disease gradients may differ markedly from dispersal gradients if the success rates per unit of inoculum are low, particularly if the reproductive rates of the pathogen are density dependent (Garrett and Bowden, 2002). There may be similar effects for gene expression, such that thresholds of exposure to pathogen inoculum, for example, must be exceeded before substantial gene expression results. At much smaller spatial scales, gene expression in bacterial populations may give insights into quorum sensing and its implications for density dependent reproduction (van Bodman et al., 2003).

Epidemiologists have developed the terms autoinfection and alloinfection to describe infection of a target host individual by inoculum produced on the same target host individual vs. infection by inoculum produced on other host individuals, respectively (Robinson, 1976). The rate of autoinfection can be an important predictor for epidemics of non-systemic disease in mixed genotype host populations. If some host genotypes are susceptible and others are not, the reduction in epidemic rates on susceptible genotypes that would be predicted by the presence of other genotypes will be reduced if autoinfection rates are high; more inoculum will land on susceptible host individuals rather than being lost through dispersal to non-hosts (Garrett and Mundt, 1999; Mundt, 2002). It may prove useful to develop analogous concepts for gene expression, so that "autoinduction" of gene expression would occur when microbes associated with a particular plant individual disperse to other organs within that individual to induce SAR, ISR, or other reactions. By comparison, "alloinduction" would occur when microbes are dispersed to a different plant individual to induce these reactions. Higher rates of alloinduction compared to autoinduction would tend to result in higher mean levels of SAR or ISR in populations, and the rate of alloinduction would be a function of host size and the dispersal properties of the relevant microbe populations.

Feedback between host and pathogen would occur as pathogens disperse, infect or elicit other responses in plants, and then disperse further through a landscape of phenotypic resistance that has potentially been altered in response to previous dispersal. Good models of such a system would require the ability to predict plant

phenotypic resistance levels in response to the biotic and abiotic environment, pathogen phenotypes in response to "non-host" environmental features, plant phenotypic responses to exposure to pathogens, etc. (Figure 1). Of course, one challenge is simply to be able to describe the current level of phenotypic resistance of a host individual. Adding the spatial pattern of host genotypes provides additional modeling challenges. The level of aggregation of susceptible hosts will produce a particular "genotype unit area" (Mundt and Leonard, 1986), or area occupied by a single host genotype, and help to determine the extent to which microbial populations are spread between host species/genotypes or tend to be dispersed within host species/genotypes. This pattern of host genotypes will also influence the pattern of expression in response to microbes associated with a particular host genotype. The combination of host genotype spatial patterns and the spatial pattern of the biotic and abiotic environment will result in a host "phenotype mixture". Just as the effects of genotype diversity vary for different host–pathogen systems (Lannou et al., 1994; Mundt et al., 1995; Ngugi et al., 2001; Mitchell et al., 2002; Cox et al., 2004), the complex communities of plants and microbes involved in induced resistance may experience different patterns of spatial effects on induction. Models of epidemics in genotype mixtures will be useful in this context, but new models will also be needed.

Conclusion

Epidemiology will benefit from new genomic technologies in several ways. New diagnostic techniques will make the development of a "community epidemiology" more practical, through providing the ability to characterize thousands of microorganisms simultaneously as well as identifying particular genes and alleles. New techniques will make it easier to extend genetic analyses of pathogens beyond virulence genes, by facilitating the study of the population structure and evolution of genes important for other important features such as the ability to survive in non-conducive environments. Functional genomic analysis of pathogen virulence genes and host resistance and defense response genes will enable better predictions of the durability of resistance. New genomic tools will also allow great advances in the study of phenotypic resistance. It will finally be possible to thoroughly evaluate the many ideas put forward about age-related resistance and the effects of the biotic and abiotic environment on phenotypic resistance. Conversely, epidemiology provides the context for understanding the role and significance of pathogen genes and plant genes related to pathogen reproduction and also provides models for evaluating landscapes of plant phenotypes.

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