CURRENT REVIEW

Population Genetics of Fungal and Oomycete Effectors Involved in Gene-for-Gene Interactions

Eva H. Stukenbrock¹ and Bruce A. McDonald²

¹Bioinformatics Research Center, Aarhus University, C. F. Møllers Alle Bldg. 1110, DK-8000 Aarhus C, Denmark; ²Plant Pathology, Institute of Integrative Biology, ETH Zurich, CH-8092 Zurich, Switzerland

Submitted 15 September 2008. Accepted 15 December 2008.

Antagonistic coevolution between plants and pathogens has generated a broad array of attack and defense mechanisms. In the classical avirulence (Avr) gene-for-gene model, the pathogen gene evolves to escape host recognition while the host resistance (*R*) gene evolves to track the evolving pathogen elicitor. In the case of host-specific toxins (HST), the evolutionary arms race may be inverted, with the gene encoding the pathogen toxin evolving to maintain recognition of the host sensitivity target while the host sensitivity gene evolves to escape binding with the toxin. Pathogen effector genes, including those encoding Avr elicitors and HST, often show elevated levels of polymorphism reflecting the coevolutionary arms race between host and pathogen. However, selection can also eliminate variation in the coevolved gene and its neighboring regions when advantageous alleles are swept to fixation. The distribution and diversity of corresponding host genes will have a major impact on the distribution and diversity of effectors in the pathogen population. Population genetic analyses including both hosts and their pathogens provide an essential tool to understand the diversity and dynamics of effector genes. Here, we summarize current knowledge about the population genetics of fungal and oomycete effector genes, focusing on recent studies that have used both spatial and temporal collections to assess the diversity and distribution of alleles and to monitor changes in allele frequencies over time. These studies illustrate that effector genes exhibit a significant degree of diversity at both small and large sampling scales, suggesting that local selection plays an important role in their evolution. They also illustrate that Avr elicitors and HST may be recognizing the same *R* genes in plants, leading to evolutionary outcomes that differ for necrotrophs and biotrophs while affecting the evolution of the corresponding *R* genes. Under this scenario, the optimal number of *R* genes in a plant genome may be determined by the relative abundance of necrotrophic and biotrophic pathogens in the plant's environment.

Plants and their associated pathogens have coevolved for many millions of years. As a result of this coevolution, natural selection has generated a diverse array of recognition and resistance mechanisms in plants to prevent or limit infection by pathogens. Selection simultaneously drove the evolution of pathogen genes to coopt or counteract plant defenses as well

as to promote virulence. This antagonistic coevolution of plants and pathogens remains a major force for creating and maintaining genetic diversity in both host and pathogen populations.

Largely as a result of the increasing availability of genome data, an increasing number of genes involved in gene-for-gene (GFG) interactions have been identified and their respective roles in virulence and resistance are being characterized (Bos et al. 2003; Dean et al. 2005; De Wit 1992; Huang et al. 2005; Kämper et al. 2006; Ridout et al. 2006). Patterns of nucleotide diversity in both target genes and their neighboring genomic regions can be analyzed to infer the molecular basis of host–pathogen coevolution (i.e., to determine how selection has shaped the diversity and distribution of particular alleles within and among pathogen and plant populations). Most studies to infer patterns of selection for pathogen genes have used a random sample of gene sequences originating either from the same species or homologous sequences from related species (Bishop et al. 2005; Damasceno et al. 2008; Liu et al. 2005; Staats et al. 2007). Additional studies have investigated the position of sites in the folded proteins to link patterns of selection with protein structure (Dodds et al. 2006; Staats et al. 2007; van den Burg et al. 2003). Results from these studies often support an evolutionary model in which host and pathogen have undergone continuous antagonistic coevolution with diversifying selection operating to increase protein diversity in both partners.

How does this evidence for molecular coevolution over millions of years relate to contemporary pathogen populations and their interactions with corresponding host populations in agroecosystems that are less than 10,000 years old? The finding of great heterogeneity in disease patterns in contemporary natural ecosystems and agroecosystems suggests that variation in host resistance and pathogen virulence traits continues to be an important component of the pathogen–plant interaction and further supports the premise that pathogens can respond rapidly to genetic changes in host populations. In highly homogeneous agroecosystems, local selection imposed by host cultivars with different resistance (*R*) genes can select for very different effector genes in the corresponding pathogen populations, creating a mosaic of different pathogen haplotypes in different geographical regions. This variability in disease patterns is not revealed by studies that consider only a small fraction of the total gene diversity within a single species or among related species. A population genetics approach that includes a broad and representative sampling strategy encompassing contemporary populations can be used to reconstruct patterns of selection in both time and space, leading to a more complete understand-

Corresponding author: Bruce A. McDonald;
E-mail: bruce.mcdonald@agril.ethz.ch

ing of how pathogen–plant coevolution operates in agroecosystems and providing novel insights into the coevolutionary process.

Very few studies have used large pathogen population samples from within species to infer geographical distributions of effector genes or coevolutionary patterns on population scales, and all of these studies are very recent (Brunner et al. 2007; Gout et al. 2007; Schürch et al. 2004; Stergiopoulos et al. 2007; Stukenbrock and McDonald 2007). The few studies conducted thus far analyzed effector gene sequences in geographical populations to determine how selection on pathogen effectors operates in the field in response to local host populations. Patterns of allele frequency and distribution can, for example, reveal how selected alleles become fixed by selective sweeps and how favorable mutations spread from one population to another. This kind of insight not only has scientific importance but also is relevant in an applied context because it can assist the development of sustainable management strategies in agroecosystems.

The speed with which a pathogen population responds to an altered host population is determined by its evolutionary potential (McDonald and Linde 2002). Evolutionary potential ultimately is correlated with the additive genetic variance for traits affecting fitness, with evolutionary potential increasing in proportion to the additive genetic variance associated with these traits. After introducing a new selective agent such as an *R* gene or fungicide, new mutations in the corresponding avirulence (*Avr*) gene or fungicide target gene can increase the genetic variance for virulence or fungicide sensitivity in the affected pathogen population. Genetic variance could also increase through immigration of pathogen strains carrying novel alleles at the affected loci. Effective population size, mutation rate, reproductive mode (sexual versus asexual reproduction), and dispersal patterns interact to influence the rate at which pathogens can respond to selective agents by determining the level of genetic variance present in a given population. By measuring changes in allele frequencies or registering the emergence and spread of new mutations over a known time frame, it is possible to observe directly how rapidly pathogens can respond to changed selective environments. For example, temporal sampling was used recently to analyze the emergence and spread of fungicide resistance across European populations of the wheat pathogen *Mycosphaerella graminicola* (Brunner et al. 2008; Fraaije et al. 2007; Torriani et al. 2009). These studies showed how mutations in genes conferring resistance toward demethylation inhibitors (DMI) or strobilurin fungicides emerged independently several times, spread rapidly, and increased in frequency over the course of a few years. Similarly, advantageous mutations in effector genes may emerge and spread rapidly to cover large areas in agroecosystems. When population changes cannot be assessed directly through temporal sampling, population genetic and evolutionary analyses can be used to estimate the time of emergence of a new mutation and also to reconstruct how the mutation spread among populations (Brunner et al. 2007).

Diversity in pathogen effector genes.

Phenotypic differences in virulence patterns are well known for plant pathogens and have been the subject of genetic analysis for more than 100 years (Biffen 1905). In some cases, the interaction between an *Avr* gene and its cognate *R* gene is a good predictor of pathogen success (i.e., disease or no disease). However, the genetics of host–pathogen interactions is usually much more complex and consists of a range of interaction mechanisms that involve a number of genes in both partners. Details of these mechanisms are well described elsewhere (Bent and Mackey 2007; Dangl and Jones 2001; Friesen et al.

2008; Jones and Takemoto 2004; Joosten and De Wit 1999). Our focus here will be on the evolutionary processes that affect the diversity of the involved genes, with special emphasis on genes encoding pathogen effectors.

The basal immune system of plants detects broadly conserved molecules associated with a wide range of microbes. These molecules have been named pathogen-associated molecular patterns (PAMPs) and microbe-associated molecular patterns (MAMPs) (Bent and Mackey 2007). Examples of PAMPs that readily elicit a resistance response in plants include fungal chitin and bacterial flagellins (Gómez-Gómez and Boller 2000; Zhang et al. 2002). Because PAMPs are essential for the normal function of the pathogen, the conventional wisdom is that they cannot be lost or significantly altered without affecting pathogen fitness. However, the recent finding of increased diversity in flagellin domains that enabled flagellated pathogenic bacteria to escape host recognition suggests that even PAMPs may be able to evolve to escape host recognition (Andersen-Nissen et al. 2005; Sun et al. 2006). Another defense-avoiding mechanism in bacteria is to shed flagella upon host infection (Drake and Montie 1988). Fungal and oomycete pathogens may also have evolved strategies to avoid host recognition by alteration or loss of their PAMPs and MAMPs.

Although resistance induced by PAMPs is nonspecific, the interaction between the products of *R* genes and their cognate *Avr* genes specify resistance between a particular host genotype and its adapted pathogen. *R* genes are abundant in plant genomes and the majority of characterized *R* genes share similar structures even among distantly related plant genera (Meyer et al. 2005). The common nucleotide-binding leucine-rich repeat (NB-LRR) *R* gene family contains highly conserved NB regions as well as highly variable LRR involved in recognition and specificity. There are several reports of diversifying selection in *R* genes consistent with a model of antagonistic coevolution (Chisholm et al. 2006; Hulbert et al. 2001). *R* genes are often found in rapidly evolving gene clusters and it appears that divergent selection as well as intragenic recombination and gene conversion are important contributors to the diversity that generates new pathogen recognition specificities (Michelmore and Meyers 1998).

Pathogen proteins encoded by *Avr* genes are recognized by proteins encoded by the plant *R* genes. Escape from host recognition is possible through alterations at the protein level due to nonsynonymous codon substitutions, nonsense mutations, or complete or partial deletion of *Avr* genes. All of these mutations have been observed in studies of pathogen effector genes drawn from natural populations (Dodds et al. 2006; Mandel et al. 1997; Schürch et al. 2004; Stergiopoulos et al. 2007; Stukenbrock and McDonald 2007). The functional role as well as the molecular structure of proteins encoded by *Avr* genes appears to be more diverse than the conserved function and structure of proteins encoded by host *R* genes. In some cases the products of *Avr* genes are not only targets of *R*-gene recognition but also play an important functional role for the pathogen. For example, the *Avr4* protein of *Cladosporium fulvum* binds to chitin to protect it from plant chitinases (van den Burg et al. 2003). In other cases, the *Avr* genes make a quantitative contribution to virulence in susceptible hosts. For example, *Avr_{k1}* and *Avr_{a10}* in *Blumeria graminis* enhance infection in susceptible barley cultivars (Ridout et al. 2006), while *ToxA* in the wheat pathogens *Pyrenophora tritici-repentis* and *Phaeosphaeria nodorum* (Ciuffetti et al. 1997; Liu et al. 2006) and *NIP1* in the barley pathogen *Rhynchosporium secalis* enhance infection in their respective hosts (Rohe et al. 1995). *ToxA* is traditionally classified as a host specific toxin (HST) but it appears to be recognized in the same way as an *Avr* factor by the toxin sensitivity gene *Tsn1* (Faris et al. 1996; Friesen et al.

2006) which, based on initial validation experiments, appears to be a member of the NB-LRR class (J. Faris, *personal communication*).

Recent studies have shed more light on the structure and mode of action of HST and on their possible dual role as both Avr factors and toxins. This group of effector proteins is produced by necrotrophic pathogens (Friesen et al. 2008). HST not only play a role in pathogenicity but are also determinants of host specificity because an HST is active only against a specific host that carries a corresponding toxin-sensitivity gene. The GFG interaction between HST and their corresponding toxin-sensitivity genes resembles that of Avr and R genes. However, whereas the product of an Avr gene must avoid recognition by the product of the corresponding R gene to confer virulence in the host (i.e., the increase in pathogen virulence follows from a loss of function and is generally inherited as a recessive trait), the HST must interact with the product of the cognate toxin-sensitivity gene to induce host necrosis (i.e., the increase in pathogen virulence follows from a gain of function and is generally inherited as a dominant trait) (Fig. 1). Differences between the outcome of Avr and HST GFG interactions (inhibition of pathogen infection versus successful pathogen proliferation) are probably linked to the different strategies pathogens use to obtain nutrients (Glazebrook 2005; Kliebenstein and Rowe 2008). Biotrophic pathogens such as the rust fungi (e.g., *Puccinia graminis*) and powdery mildew fungi (e.g., *B. graminis*) feed on living host tissue whereas necrotrophic pathogens such as the wheat glume blotch pathogen *Phaeosphaeria nodorum* and the tan spot pathogen *Pyrenophora tritici-repentis* derive their nutrients from dead or dying plant cells. An Avr-induced resistance response that leads to a hypersensitive response (HR) and local cell death through apoptosis restricts access of biotrophic pathogens to nutrients and water. For necrotrophic pathogens, HR and apoptosis provide easily acquired nutrients for further proliferation in the host. Thus, an HST produced by a necrotroph can operate by activating an R gene (and the corresponding HR pathway) that protects plants from biotrophs but allow the further proliferation of necrotrophic pathogens (Sweat and Wolpert 2008; Wolpert et al. 2002; J. D. Faris, *unpublished data*) (Fig. 1). If a primary function of HST is to activate programmed cell death, then plants face an evolutionary dilemma regarding their use of R genes. As the number of R genes in the genome increases, the ability to detect biotrophic pathogens and activate a defense response leading to apoptosis will also increase. However, an increase in R genes will also increase the number of possible targets for HST produced by necrotrophs. This scenario could lead to a trade-off where the optimum number of R genes in a plant genome will be a function of the relative abundance of necrotrophic and biotrophic pathogens in the plant's environment.

A number of studies have shown that effector proteins can play dual roles as both toxins and inducers of host resistance. Examples include victorin of *Cochliobolus victoriae*, NIP1 of *R. secalis*, and AAL toxin of *Alternaria alternata* (Lorang et al. 2007; Navarre and Wolpert 1999; Rohe et al. 1995; Spassieva et al. 2002; van't Slot and Knogge 2002; Wang et al. 1996; Wolpert et al. 2002). The recently reported examples of diversifying selection in *NIP1* and *ToxA* suggest that interactions with corresponding host-resistance or toxin-sensitivity genes can lead HST genes to evolve in the same way as Avr genes (Schürch et al. 2004; Stukenbrock and McDonald 2007). In the case of HST, however, selection favors mutations that allow the HST to recognize the host-toxin sensitivity gene while mutations in the host gene will be selected to avoid a compatible interaction with the HST (i.e., the pathogen gene will evolve to track changes in the host gene) (Fig. 1).

Several studies have demonstrated that host-pathogen GFG interactions can involve more than two genes. The guard model proposes that the R protein is not the direct target of the Avr factor produced by the pathogen (Van der Biezen and Jones 1998). Instead, the R gene protein acts as a "guard" of the actual pathogen target (the "gardee"). The guard model implies that the pathogen effector plays a significant role in pathogenicity by binding to a specific target in susceptible hosts. A compatible binding between effector and guard suppresses host defenses or leads to a release of nutrients, increasing pathogen fitness. In a resistant host, innate immunity is induced when the pathogen effector binds to the guard and manipulates the guarding R protein. A dilemma associated with the guard model is that it implies opposing selection pressures on the guard and, thus, an evolutionarily unstable situation. When the R protein is absent, diversifying selection should drive evolution of the guard to avoid binding with the pathogen effector protein. In the presence of an R protein, selection will favor binding between guard and effector because only an interaction between two compatible molecules will induce the innate immunity mediated by the guard R protein.

The decoy model was recently proposed as an alternative to the guard model (van der Hoorn and Kamoun 2008). This model suggests the presence of host decoy proteins that mimic effector targets and are specialized in recognizing pathogen effectors. In contrast to the "real" host target, the decoys do not increase pathogen fitness by binding the effector. Van der Hoorn and Kamoun (2008) suggest that decoys may originate through gene duplication of actual host targets, with the duplicated genes subsequently evolving through selection for higher effector perception and target mimicking. Several recent studies provide support for the decoy model (Shabab et al. 2008; Zhou and Chai 2008), but no population genetic studies have analyzed diversity of proposed decoys. Depending on the evolutionary scenario, decoys may be under diversifying selection to maintain the ability to recognize variable pathogen effectors or decoys could be under purifying selection to recognize invariable pathogen effectors. Decoys could also function as selective agents to maintain or increase variability in pathogen effectors in order to prevent binding to "false" target proteins in the host. A thorough analysis of sequence diversity in genes thought to encode decoy or guard proteins as well as the corresponding pathogen effector genes will be needed to determine the specific evolutionary processes operating under the decoy and guard models.

Whereas the GFG, guard, and decoy models describe interactions between individual plants and their associated pathogen strains, other models were developed to describe interactions at the population level. The two most popular models are the arms race model and the trench warfare model. The major difference between these two models is the expected coevolutionary pattern of effectors and R genes. In the arms race model, diversifying selection leads to a rapid evolution and turnover of R genes and corresponding Avr genes. Although diversifying selection may act on both host and pathogen genes, recurrent selective sweeps will decrease variation at the population level. In a particular selective environment (e.g., a field planted to a cultivar carrying a new R gene), one allele may be favored over other alleles and be swept to fixation by selection (e.g., only pathogen strains carrying the mutation from Avr to virulence would survive in the presence of the R gene) (Brown 1994; Brown and Wolfe 1990). This will make the selected population monomorphic at the selected locus. The evidence of such selective sweeps is neighboring genomic regions with reduced levels of polymorphism and elevated levels of linkage disequilibrium compared with neutrally evolving genomic regions. Examples of selective sweeps in plant pathogens include

Fig. 1. Gene-for-gene interactions based on avirulence proteins and host-specific toxins are inversely related, but both can lead to diversifying selection as a result of protein–protein interactions.

the mitochondrial genome of *M. graminicola* (Torriani et al. 2008; Zhan et al. 2004) and the *AvrLm1* gene in *Leptosphaeria maculans* (Gout et al. 2007). The *ToxA* locus in *Phaeosphaeria nodorum* and *Pyrenophora tritici-repentis* and the *NIP1* locus in *R. secalis* also display evidence of selective sweeps because some populations are monomorphic for the *ToxA* or *NIP1* gene while others display much more variation. The presence of only one toxin or virulence variant in some populations may reflect a selective advantage conferred by specific alleles of the cognate host genes *Tsn1* or *Rrs1* that may be more frequent in some host populations than in others. Finally, selective sweeps in pathogen populations are not exclusive to agroecosystems with strong directional selection. Laine and Hanski (2006) found evidence for recurrent selective sweeps in populations of *Podospora plantaginis* infecting natural populations of *Plantago lanceolata*, suggesting a coevolutionary turnover of *R* and *Avr* alleles consistent with an arms race model.

In the trench warfare model, variation within both host and pathogen populations is maintained by balancing selection. Coevolution leads to stable balanced polymorphisms and cycling of alleles in both host and parasite populations (May and Anderson 1983). Patterns of diversity in the *RPP13* resistance gene conferring resistance against the oomycete *Peronospora parasitica* in *Arabidopsis thaliana* are consistent with the trench warfare model, with high levels of protein variability maintained in geographic populations of *A. thaliana* (Rose et al. 2004). Under the trench warfare model, it is expected that populations of *P. parasitica* would similarly maintain a high degree of polymorphism in effectors recognized by *RPP13* but a population survey of *P. parasitica* has not been conducted to test this hypothesis. Elevated levels of polymorphism in pathogen effectors consistent with the trench warfare model have been found in several pathosystems, including the *AvrL567* genes of the rust pathogen *Melampsora lini* (Dodds et al. 2006) and the RXLR effectors of oomycete pathogens (Win et al. 2007).

Whether a coevolutionary dynamic between a host and its pathogen can be better explained by the arms race model or the trench warfare model depends on the ecology and epidemiology specific to the host-parasite system (Tellier and Brown 2007). More population genetic and evolutionary studies of both host-resistance and pathogen-effector genes across a wide array of pathosystems are needed to further develop and extend these models and to place them into a framework that encompasses other involved genes such as decoys and guardreels.

Detecting selection in pathogen effector genes.

Natural selection may operate in different ways at both the population and the gene level to increase or eliminate polymorphism at a selected locus. Much of the natural selection acting on genomes is thought to be negative selection acting to remove new deleterious mutations. Positive selection occurs when a new or previously rare mutation confers a fitness advantage to individuals carrying it (e.g., the mutation in a pathogen from avirulent [*Avr*] to virulent [*avr*] in the presence of the corresponding host *R* gene). Diversifying selection operating at the level of genes favors the emergence of new alleles and, thus, leads to an increase in gene diversity. Diversifying selection is an important component of both the arms race and the trench warfare models and has been demonstrated in both *R* and *Avr* genes as well as in HST as an elevated level of replacement mutations (Bakker et al. 2006; Dodds et al. 2006; Liu et al. 2005; McDowell et al. 1998; Rowe and Kliebenstein, 2007; Schürch et al. 2004; Staats et al. 2007; Stukenbrock and McDonald 2007).

Balancing selection operates at the population level to maintain several different alleles at a locus within any population. The evolutionary footprints of balancing selection include ele-

vated levels of polymorphism and a skew of the allele frequencies toward an excess of intermediate frequency alleles. Frequency-dependent selection is often seen under balancing selection. In this case, the fitness of a phenotype is dependent on its frequency relative to the frequencies of other phenotypes in the population. An example of balancing selection in plant-pathogenic fungi is the maintenance of the two mating type idiomorphs at equal frequencies due to regular cycles of recombination (Zhan et al. 2002, 2004).

Statistical tests of selection can be classified into three categories based upon the type of data used: i) within-species tests, ii) within- and between-species tests, and iii) between-species tests. The most common class of within-species tests compares summary statistics of allele frequency distributions with values expected under neutral evolution; it includes Tajima's *D*, *F_u* and Li's *D* and *F*, and *F_u*'s *F_s* (Fu 1997; Fu and Li 1993; Tajima 1989). An advantage of these tests is that they do not require a priori information about coding or noncoding sites and, thus, are suitable for both protein-coding and noncoding loci. Disadvantages of these tests are that they are very sensitive to demographic events such as population growth or population subdivision and they can only detect diversifying selection if it has operated for a long time. More powerful tests for within-species selection use maximum likelihood-based methods applying complete data rather than only summary statistics (Nielsen and Yang 1998; Yang 1997). The computer program PAML uses maximum likelihood analyses to estimate likelihood ratios of different selection models for the data set and can identify specifically selected codons (Yang 2007). Popular methods for within- and between-species analyses are the Hudson-Kreitman-Aguade (HKA) (Hudson et al. 1987) and the McDonald-Kreitman (MK) (McDonald and Kreitman 1991) tests. The HKA test compares levels of diversity within and between species to expected values under neutral evolution while the MK test compares ratios of nonsynonymous (*dN*) and synonymous (*dS*) mutations within and between species. The most common tests between species also compare *dN/dS* ratios to provide evidence of adaptive protein evolution between different species and detect variation in *dN/dS* ratios among lineages (Nielsen and Yang 1998).

Demographic processes will also contribute to patterns of gene diversity and these processes may confound inference of natural selection (Ronald and Akey 2005). For example, the effect of strong population expansion observed for several agricultural plant pathogens (Couch et al. 2005; Stukenbrock et al. 2007; Zaffarano et al. 2008) can mimic the effect of natural selection by increasing genetic variation and generating an excess of low-frequency alleles. Therefore, patterns of diversity in selected genes should be compared with overall diversity in neutrally evolving genes to determine the effect of population dynamics on gene evolution. Demographic changes will affect the entire genome while selection leaves footprints only in the genes subjected to selection.

It should be noted that a survey of selection ideally should include the functional aspect of gene evolution. Elevated levels of replacement mutations may be found in many genes involved in host-pathogen interactions; however, whether different protein variants behave differently can only be tested experimentally. Thus, experimental studies that test the behavior of different host and pathogen haplotypes are needed to attain a complete understanding of selection and evolution in the involved genes (MacCallum and Hill 2006).

Coevolution at the population scale.

Laine (2006) studied coevolution of resistance and virulence in natural populations of *Plantago lanceolata* infected by the mildew pathogen *Podospora lanceolata*. Disease patterns were

assessed across different spatial and temporal scales to characterize disease dynamics. An additional experiment was conducted to infer differences in coadaptation among sympatric versus allopatric combinations of plants and pathogens. The results showed a mosaic of variation for resistance over a spatial scale of a few square meters. Hosts growing in areas with higher pathogen encounter rates had evolved a higher level of resistance and hosts were more resistant toward sympatric than allopatric strains of *P. lanceolata* (i.e., exposure to a pathogen genotype increased resistance toward that particular pathogen through local coevolution at small spatial scales). Laine (2006) concluded that disease dynamics influenced the diversity and distribution of resistant host genotypes through adaptive microevolution, suggesting that a large diversity of *R* genes is maintained by balancing selection to readily respond to new pathogens.

Spatial variation in resistance and virulence in natural ecosystems is also found in the well-studied *Linum-Melampsora* pathosystem. Thrall et al. (2002) showed that local adaptation plays an important role in coevolutionary interactions between flax and the flax rust pathogen. Spatial and temporal patterns of disease dynamics were influenced by complex patterns of coevolutionary interaction determined by factors such as the diversity and structure of host resistance, the tradeoff between pathogen virulence and aggressiveness, and the dispersal range of the pathogen (Thrall and Burdon 2003). The complexity of host-pathogen interactions complicates studies of coevolution in natural ecosystems and Thrall and Burdon (2003) suggest that a combination of large-scale sampling, replicated experimental tests, and complex statistical analyses will be required to decipher coevolutionary processes in natural ecosystems (Thrall and Burdon 2003).

Crop plants harbor much less variation for resistance compared with plants in natural ecosystems. However, different cultivars may have different combinations of *R* genes and toxin-sensitivity genes that can affect the distribution of the corresponding effector genes in pathogen populations. This was shown in a population genetic analysis of the *Avr* gene *NIP1* in the barley scald pathogen *R. secalis* (Schürch et al. 2004). The product of *NIP1* has a dual role as both toxin and elicitor of plant defense. The gene product is a necrosis-inducing peptide that stimulates an H^+ -ATPase in the host (Wevelsiep et al. 1991). The same peptide is also an elicitor of host defense when recognized by the host *R* gene *Rrs1*. Strains of *R. secalis* lacking *NIP1* or with mutated *NIP1* genes were able to overcome *Rrs1*-mediated resistance, suggesting that diversifying selection may act to create new, unrecognizable *NIP1* alleles. Indeed, Schürch and associates (2004) found that diversifying selection was affecting some regions in the gene while other regions were conserved. A large proportion of *R. secalis* strains (45%) were missing the *NIP1* gene, indicating that deletion of the gene is another important way to overcome *Rrs1* resistance. Gene diversities and deletion frequencies differed significantly among geographical populations, suggesting a heterogeneous use of *Rrs1* by breeders in different countries. Further population genetic analyses of *NIP1* revealed that the gene most likely originated in northern Europe and, from there, diversified and spread to other parts of the world during the last 2,500 to 5,000 years (Brunner et al. 2007). This recent diversification of *NIP1* may have been associated with a host shift to barley (Zaffarano et al. 2008). It is possible that *NIP1* was not recognized as an *Avr* gene in the original host of *R. secalis* and that it has evolved under diversifying selection only since beginning its interaction with the barley *Rrs1* resistance gene. Another possibility is that the host shift involved a selective sweep in which only one or a few *NIP1* haplotypes founded the barley-infecting *R. secalis* population.

The HST ToxA in the wheat pathogen *Phaeosphaeria nodorum* shows a surprisingly similar pattern of population diversification and local selection (Stukenbrock and McDonald 2007). The *ToxA* gene is involved in a GFG interaction with the toxin-sensitivity gene *Tsn1*. *ToxA* gene diversity, deletion frequencies, and patterns of selection were assessed in eight geographical populations of *P. nodorum*. *P. nodorum* is a cosmopolitan pathogen with a high level of genetic diversity. Earlier studies of the *P. nodorum* global population structure identified Europe, China, and North America as the most diverse populations, consistent with a long history of intensive wheat cultivation (Stukenbrock et al. 2006). However, a different pattern emerged when comparing genetic diversity of neutral simple-sequence repeat (SSR) markers to *ToxA* diversity. The lowest diversity for *ToxA* alleles was found in North America, Europe, and China whereas populations in South Africa and Central Asia were the most diverse, with several unique alleles of the *ToxA* gene. In Australia, all pathogen isolates carried the *ToxA* gene although only two different alleles were identified. These findings were interpreted as evidence of local selection imposed by the host *Tsn1* gene and a heterogeneous distribution of *Tsn1* genotypes. A survey of Australian wheat cultivars grown since 1914 showed that the majority of cultivars were sensitive to ToxA, consistent with the high frequency of the *ToxA* gene in the Australian *P. nodorum* population (Oliver et al. 2008). The high ToxA frequency in Australia might suggest a low fitness cost associated with production of ToxA; however, the finding that the *ToxA* deletion occurs at a high frequency in most populations indicates that production of ToxA likely carries a fitness cost in the absence of *Tsn1* (Stukenbrock and McDonald 2007). *P. nodorum* is considered a medium- to high-risk pathogen in terms of evolutionary potential due to its mixed reproductive system and high dispersal potential. Although gene flow acts to reduce subdivision between populations, as observed for the neutral SSR markers, selection can fix particular *ToxA* alleles in different geographical populations. Different *ToxA* alleles may respond differently to different *Tsn1* alleles, consistent with a tightly coupled antagonistic coevolution between *ToxA* and *Tsn1* and reinforcing the importance of the host gene as a determinant of *ToxA* diversity (J. Faris, R. Oliver, and T. Friesen, unpublished data). The example of *P. nodorum* demonstrates the value of combining neutral and selected markers in analyses of population structure and host-pathogen coevolution to distinguish among different evolutionary and demographic processes such as local selection and global gene flow. Both the *ToxA* locus in *P. nodorum* and *Pyrenophora tritici-repentis* and the *NIP1* locus in *R. secalis* display evidence of selective sweeps because some populations are monomorphic for the *ToxA* or *NIP1* gene while others display much more variation, consistent with a coevolutionary arms race model.

Although gene deletions were proposed as a mutational mechanism to escape host recognition in *R. secalis* (Schürch et al. 2004) and *Phaeosphaeria nodorum* (Stukenbrock and McDonald 2007) the molecular basis of these deletions was not investigated. However, deletions of an effector gene were investigated in the stem canker pathogen of oil seed rape, *L. maculans*. *L. maculans* carries a series of *Avr* genes that define different races of the pathogen. The *AvrLm1* gene is recognized by the resistance gene *Rlm1*, which became widely used in oil seed cultivars until *L. maculans* evolved virulence against this *R* gene. Although population subdivision in *L. maculans* is low, the frequency of *AvrLm1* and other *Avr* genes differs among geographic populations (Gout et al. 2007; Stachowiak et al. 2006). The geographic variation in *Avr* allele frequencies probably reflects the heterogeneous distribution of *R* genes in host cultivars as proposed in the *R. secalis* and *P. nodorum* pathosystems. Gout and associates (2007) analyzed *AvrLm1* diversity

in 460 field isolates from three different continents and found that 170 isolates were avirulent on *Rlm1* cultivars while 290 were virulent. Complete deletion of the *AvrLml* gene was responsible for virulence in 98% of the 290 virulent isolates. Only five virulent isolates carried mutated or partially deleted *avr* alleles. Only a few mutations were identified among the 170 *Avr* isolates and there was no signal of diversifying selection. Thus, deletion of the *Avr* factor seemed to be the dominant mechanism for gaining virulence. The authors further investigated the genomic organization of the *AvrLml* locus to characterize deletion breakpoints. *AvrLml* is a solo gene located in a 270-kb heterochromatin-like chromosomal segment composed of mosaics of truncated retrotransposons (Gout et al. 2006). These findings suggested that transposon activity could have played a major role in the evolution of *AvrLml* genomic regions and also could be involved in deletion of the gene. Interestingly, the HST *ToxA* in *P. nodorum* was also located adjacent to a transposase-like sequence that could also play a role in deletions or rearrangements at the locus (Friesen et al. 2006). There were no polymorphisms at deletion breakpoints for *AvrLml* in *L. maculans*. The breakpoints occurred at exactly the same base on both the left and the right border of the deletion site in all sequenced isolates. These findings suggest that the deletion occurred only once. The *L. maculans* example suggests that genomic rearrangements and gene deletions can be very important for pathogen adaptation to new host resistance genes. The function of *AvrLml* during host infection is not known but it appears that deletion of the gene did not impose a fitness penalty on the pathogen. The lack of polymorphisms in both coding and noncoding regions of the chromosomal fragment investigated by Gout and associates (2007) may reflect a recent selective sweep and the rapid spread of virulent *L. maculans* strains on a global scale. Analyses of the *L. maculans* genome will likely provide further knowledge about genomic localization and structure of other *Avr* genes in the pathogen as well as allow further testing of the hypothesis of selective sweeps occurring in genomic regions containing *Avr* genes.

Diversifying selection and coevolution in other pathogen genes.

Evidence for antagonistic coevolution can also be found in other pathogen genes involved in interactions with host proteins. Patterns of diversifying selection were found in phytotoxins of *Phytophthora* and *Botrytis* spp. (Liu et al. 2005; Staats et al. 2007). The *scr74* gene family of *Phytophthora infestans* shows evidence of gene duplication and increased levels of nonsynonymous mutations as well as intragenic recombination. This increased level of variability has resulted in a high level of protein diversity and suggests that the gene product may be recognized and interact with a host gene product. *Scr74* encodes a predicted protein with significant similarity to a phytotoxic necrosis-inducing protein in *P. cactorum*, suggesting that it may act as an HST by inducing necrosis through activation of a resistance response in the host.

Positive selection of particular amino acids was also shown in homologs of the necrosis and ethylene-inducing protein NEP1 in species of *Botrytis* (Staats et al. 2007). This class of genes is found in a wide variety of microorganisms and is thought to be important for necrotrophic plant pathogens. NEP-like homologs are highly diverse among *Botrytis* spp. and this diversity could reflect its wide host range. To our knowledge, the actual targets of the phytotoxins in *Botrytis* and *Phytophthora* spp. have not yet been identified; however, it is possible that the toxin targets are also resistance genes that recognize the toxins as *Avr* factors and induce a resistance response leading to apoptosis.

Signals of diversifying selection were also found in cell-wall-degrading enzymes in both *Botrytis cinerea* (polygalacturonases) (Rowe and Kliebenstein 2007) and *Mycosphaerella graminicola* (β -xylosidase) (P. Brunner, unpublished data). Selection in the *B. cinerea* polygalacturonase genes was proposed to be an adaptation to different host species and an escape from host recognition if the enzyme acts as a PAMP to induce host defenses. Similarly, strong evidence for diversifying selection was found for the β -xylosidase gene of *M. graminicola* and we hypothesize that the product of this gene also acts as a PAMP that stimulates host resistance responses. The rate of evolution of β -xylosidase accelerated following the domestication of wheat 10,000 years ago, suggesting that this coevolution was coincident with the domestication of wheat and development of the wheat agroecosystem. Although further experimental studies as well as protein characterization will be needed to gain more insight into the evolutionary forces that selected for increased diversity in these enzymes, we hypothesize that cell-wall-degrading enzymes involved in bacterial or fungal metabolism may also act as MAMPs that can be recognized by corresponding plant *R* genes.

Glucanase inhibitor proteins (GIPs) in *Phytophthora* spp. also showed an elevated level of polymorphisms and evidence of diversifying selection (Damasceno et al. 2008). These pathogen proteins inhibit plant endoglucanases (EGases) that digest fungal and oomycete cell walls. Analyses of the three-dimensional structure of EGases and GIPs revealed that amino acid substitutions in both proteins were located at sites involved in the protein-protein interaction. Thus, it appears that EGases and GIPs also evolved antagonistically in response to each other in the same way as *R* genes and toxin-sensitivity genes and their cognate effector genes. The GIPs-EGases interaction presents a compelling example of a molecular GFG arms race between host and pathogen in surface residues shown to be in close proximity to each other in the protein complex (Bishop et al. 2005; Damasceno et al. 2008). Other plant proteins that interact with pathogen proteins also exhibit diversifying selection. For example, class 1 chitinases are produced to degrade fungal cell wall components. Mapping replacement sites onto crystal structure of the class 1 chitinase showed that amino acid replacements occurred mainly in the active site cleft of the enzyme. Variability in pathogen cell wall architecture and enzymatic inhibitors have been proposed as agents of positive selection in the active site of chitinases (Bishop et al. 2000). Under this scenario, the host enzyme will be under positive selection for both increased binding affinity to the fungal target and selection to escape from fungal inhibitors.

Host resistance genes are not the only factors that impose strong selection on pathogens in agroecosystems. Pesticides also act as strong selective agents. The sterol 14 α -demethylase (*CYP51*) gene is a major checkpoint in membrane sterol biosynthesis and also the primary target of DMI fungicides widely used against a broad range of fungal plant pathogens. Reduced sensitivity to DMI fungicides in *M. graminicola* was caused by selective changes in the *CYP51* gene (Brunner et al. 2008). Evidence for selective replacement of wild-type sensitive alleles by mutant resistance alleles and for intragenic recombination bringing together mutations in different parts of the gene demonstrate the rapid selective response following the intensive use of DMI in Europe. Although *CYP51* is an important house-keeping gene in *M. graminicola*, protein changes evolved rapidly to maintain appropriate protein function while avoiding inhibition by the DMI fungicide.

Population genomics.

Comparisons of entire genomes of several individuals from the same pathogen species will likely make a significant con-

tribution to our understanding of the population genetics and evolution of pathogenicity-related genes. As an example, the *ToxA* gene was initially identified and characterized in the wheat pathogen *Pyrenophora tritici-repentis* and was coincidentally discovered in *Phaeosphaeria nodorum* through a search for homologous genes in the newly sequenced genome (Friesen et al. 2006). We now know that the frequency of *ToxA* differs significantly among *P. nodorum* populations, with *ToxA* at a high frequency in Australian populations and a low frequency in North American populations. If a North American strain had been sequenced, the *P. nodorum ToxA* gene probably would not have been found. Deletions of genes encoding effector molecules seem to be common in plant pathogens; thus, it is possible that many genes encoding important effector molecules will be missed in analyses focused on a single genome. Another reason to sequence several strains is that some fungal genome sequences are from intensively studied laboratory strains that have been kept in culture for many years. After many transfers in lab media, some interesting genes may have been lost. If 10 pathogen strains from different populations were completely sequenced, we consider it likely that all of the important pathogenicity genes would be found among the pooled genomes.

Sequencing of multiple genomes from the same species will also significantly increase our understanding of pathogen genome structure and plasticity. Many electrophoretic karyotype studies have detected differences in chromosome number and size among strains. In *M. graminicola*, 7 of the 21 described chromosomes are dispensable, suggesting a very high level of genome plasticity (Mehrabi et al. 2007; S. B. Goodwin, unpublished data). The gene content of the seven dispensable chromosomes has not yet been described but it is possible that some pathogenicity-related genes are located on these smaller, dispensable chromosomes. Thus, a representative sample of *M. graminicola* strains will need to be sequenced to truly understand the pathogen's genome complexity and the processes affecting the evolution of its genome.

The acquisition of whole genome sequences from several strains of the same species is already possible through next-generation sequencing technologies that provide rapid and inexpensive large-scale sequencing. Although population genomics is still in its infancy, it will provide new opportunities for understanding pathogen evolution at the genome level (Begun et al. 2007).

Conclusions

Plant-pathogen interactions are characterized by a complex interplay of attack, defense, counter-attack, and counter-defense. These interactions are the result of long periods of antagonistic coevolution. Plants evolved NB-LRR recognition systems as early warning devices to detect pathogens while pathogens evolved effectors to turn off plant defenses and HST to activate programmed cell death in the host. Selection led to alteration or deletion of resistance or compatibility genes in the host and corresponding changes in *Avr* or HST genes in the pathogen. Signatures of coevolution can be found through careful analysis of patterns of selection at both the fine-scale molecular genetic level and large-scale population genetic level.

Genes involved in key processes of plant infection are naturally of great interest as potential targets for the development of sustainable disease management strategies. To gain a comprehensive understanding of how coevolutionary processes led to the observed spatial and temporal variation in resistance and virulence, it will be necessary to combine methods from different fields of research (e.g., molecular analyses of protein structure should be coupled with statistical analyses of population

genetics and evolution). Although most studies to date have focused on molecular biology of the protein products of single genes, population studies have revealed significant heterogeneity in the distribution and diversity of effector genes in nature. This suggests that local adaptation plays an important role in governing the distribution of particular effector variants in pathogen populations in both natural and agricultural ecosystems. Thus, samples should be drawn from many geographically distinct pathogen populations to be confident that the most common effector variants have been considered while conducting detailed molecular analyses. Another important finding to emerge from population genetic studies is that population demography can influence genetic diversity and may bias signals of selection. It is likely that agricultural practices speed up the emergence of new *Avr* or HST alleles by allowing many mutations to arise in large pathogen populations and by increasing the selection pressure from the host side (McDonald and Linde 2002; Stukenbrock and McDonald 2008). Thus, large-scale sampling is needed to be confident that all allelic variants have been sampled in studies of selection and coevolution in plant-pathogen systems.

Given the steady increase in pathogen and host sequence data, the near-term goal should be to see individual genes not in selective isolation but in the context of genome-wide patterns of diversity to assess signals of selection (MacCallum and Hill 2006). Comparisons of complete genome sequences will allow the discovery of many more genes evolving under natural selection in response to host or environmental factors. This can be accomplished through systematic intraspecific surveys of a number of genomes to identify outlier loci in which levels of gene diversity exceed the background level of diversity.

LITERATURE CITED

- Andersen-Nissen, E., Smith, K. D., Strobe, S. L. R., Cookson, B. T., Logan, S. M., and Aderem, A. 2005. Evasion of Toll-like receptor 5 by flagellated bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 102:9247-9252.
- Bakker, E. G., Toomajian, C., Kreitman, M., and Bergelson, J. 2006. A genome-wide survey of *R* gene polymorphisms in *Arabidopsis*. *Plant Cell* 18:1803-1818.
- Begun, D. J., Holloway, A. K., Stevens, K., Hillier, L. W., Poh, Y. P., Hahn, M. W., Nista, P. M., Jones, C. D., Kern, A. D., Dewey, C. N., Pachter, L., Myers, E., and Langley, C. H. 2007. Population genomics: Whole-genome analysis of polymorphism and divergence in *Drosophila simulans*. *PLoS Biol.* 5:e310.
- Bent A. F., and Mackey, D. 2007. Elicitors, effectors, and *R* genes: The new paradigm and a lifetime supply of questions. *Annu. Rev. Phytopathol.* 45:399-436.
- Biffen, R. H. 1905. Mendel's laws of inheritance and wheat breeding. *J. Agric. Sci.* 1:4-48.
- Bishop, J. G., Dean, A. M., and Mitchell-Olds, T. 2000. Rapid evolution in plant chitinases: Molecular targets of selection in plant-pathogen coevolution. *Proc. Natl. Acad. Sci. U.S.A.* 97:5322-5327.
- Bishop, J. G., Ripoll, D. R., Bashir, S., Damasceno, C. M. B., Seeds, J. D., and Rose, J. K. C. 2005. Selection on glycine {beta}-1,3-endoglucanase genes differentially inhibited by a *Phytophthora* glucanase inhibitor protein. *Genetics* 169(2):1009-1019.
- Bos, J. I. B., Armstrong, M., Whisson, S. C., Torto, T. A., Ochwo, M., Birch, P. R. J., and Kamoun, S. 2003. Intraspecific comparative genomics to identify avirulence genes from *Phytophthora*. *New Phytol.* 159:63-72.
- Brown, J. K. M. 1994. Chance and selection in the evolution of barley mildew. *Trends Microbiol.* 2:470-475.
- Brown, J. K. M. and Wolfe, M. S. 1990. Structure and evolution of populations of *Erysiphe graminis* f. sp. *hordei*. *Plant Pathol.* 39:376-390.
- Brunner, P., Stefanato, F. L., and McDonald, B. A. 2008. Evolution of the *CYP51* gene in *Mycosphaerella graminicola*: Evidence for intragenic recombination and selective replacement. *Mol. Plant Pathol.* 9:305-316.
- Brunner, P. A., Schürch, S., and McDonald, B. A. 2007. The origin and colonization history of the barley scald pathogen *Rhynchosporium secalis*. *J. Evol. Biol.* 20:1311-1321.
- Chisholm, S. T., Coaker, G., Day, B., and Staskawicz, B. J. 2006. Host-microbe interactions: Shaping the evolution of the plant immune response.

- Cell 124:803-814.
- Ciuffetti, L. M., Tuori, R. P., and Gaventa, J. M. 1997. A single gene encodes a selective toxin causal to the development of tan spot of wheat. *Plant Cell* 9:135-144.
- Couch, B. C., Fudal, I., Lebrun, M.-H., Tharreau, D., Valent, B., van Kim, P., Notteghem, J. L., and Kohn, L. M. 2005. Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. *Genetics* 170:613-630.
- Damasceno, C. M. B., Bishop, J. G., Ripoll, D. R., Win, J., Kamoun, S., and Rose, J. K. C. 2008. Structure of the glucanase inhibitor protein (GIP) family from *Phytophthora* species suggests coevolution with plant endo-beta-1,3 glucanases. *Mol. Plant-Microbe Interact.* 21:820-830.
- Dangl, J. L., and Jones, J. D. 2001. Plant pathogens and integrated defense responses to infection. *Nature* 411:826-833.
- Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T.K., Orbach, M. J., Thon, M., Kulkarni, R., Xu, J. R., Pan, H., Read, N. D., Lee, Y. H., Carbone, I., Brown, D., Oh, Y. Y., Donofrio, N., Jeong, J. S., Soanes, D. M., Djonovic, S., Kolomiets, E., Rehmeier, C., Li, W., Harding, M., Kim, S., Lebrun, M. H., Bohnert, H., Coughlan, S., Butler, J., Calvo, S., Ma, L. J., Nicol, R., Purcell, S., Nusbaum, C., Galagan, J. E., and Birren, B. W. 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434:980-986.
- De Wit, P. J. G. M. 1992. Molecular characterization of gene-for-gene systems in plant-fungus interactions and the application of avirulence genes in control of plant pathogens. *Annu. Rev. Phytopathol.* 30:391-418.
- Dodds, P. N., Lawrence, G. J., Catanzariti, A.-M., Teh, T., Wang, C.-I. A., Ayliffe, M. A., Kobe, B., and Ellis, J. G. 2006. Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proc. Natl. Acad. Sci. U.S.A.* 103:8888-8893.
- Drake, D., and Montie, T. C. 1988. Flagella, motility and invasive virulence of *Pseudomonas aeruginosa*. *J. Gen. Microbiol.* 134:43-52.
- Faris, J. D., Anderson, J. A., Francel, L. J., and Jordahl, J. G. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. *Phytopathology* 86:459-463.
- Fraaije, B. A., Cools, H. J., Kim, S.-H., Motteram, J., Clark, W. S., and Lucas, J. A. 2007. A novel substitution I381V in the sterol 14 α -methylase (CYP51) of *Mycosphaerella graminicola* is differentially selected by azole fungicides. *Mol. Plant Pathol.* 8:245-254.
- Friesen, T. L., Stukenbrock, E. H., Liu, Z., Meinhardt, S., Ling, H. J., Faris, J. D., Rasmussen, J. B., Solomon, P. S., McDonald, B. A., and Oliver, R. P. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. *Nat. Genet.* 38:953-956.
- Friesen, T. L., Faris, J. D., Solomon, P. S., and Oliver, R. P. 2008. Host-specific toxins: Effectors of necrotrophic pathogenicity. *Cell. Microbiol.* 10:1421-1428.
- Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:915-925.
- Fu, Y. X., and Li, W. H. 1993. Statistical tests of neutrality of mutations. *Genetics* 133:693-709.
- Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43:205-227.
- Gómez-Gómez, L., and Boller, T. 2000. FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Mol. Cell* 5:1003-1011.
- Gout, L., Fudal, I., Kuhn, M. L., Vincenot, L., Blaise, F., Eckert, M., Cattolico, L., Balesdent, M.-H., and Rouxel, T. 2006. Lost in the middle of nowhere: The *AvrLm1* avirulence gene of the Dothideomycete *Leptosphaeria maculans*. *Mol. Microbiol.* 60:67-80.
- Gout, L., Kuhn, M. L., Vincenot, L., Bernard-Samain, S., Cattolico, L., Barbetti, M., Moreno-Rico, O., Balesdent, M.-H., and Rouxel, T. 2007. Genome structure impacts molecular evolution at the *AvrLm1* avirulence locus of the plant pathogen *Leptosphaeria maculans*. *Environ. Microbiol.* 9:2978-2992.
- Huang, S., van der Vossen, E. A. G., Kuang, H., Vleeshouwers, V. G. A. A., Zhang, N., Borm, T. J. A., Van Eck, H. J., Baker, B., Jacobsen, E., and Visser, R. G. F. 2005. Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *Plant J.* 42:251-261.
- Hudson, R. R., Kreitman, M., and Aguade, M. 1987. A test of neutral molecular evolution based on nucleotide data. *Genetics* 116:153-159.
- Hulbert, S. H., Webb, C. A., Smith, S. M., and Sun, Q. 2001. Resistance gene complexes: Evolution and utilization. *Annu. Rev. Phytopathol.* 39:285-312.
- Jones, D. A., and Takemoto, D. 2004. Plant innate immunity—direct and indirect recognition of general and specific pathogen-associated molecules. *Curr. Opin. Immunol.* 16:48-62.
- Joosten, M. H. A. J., and De Wit, P. J. G. M. 1999. The tomato-*Cladosporium fulvum* interaction: A versatile experimental system to study plant-pathogen interactions. *Annu. Rev. Phytopathol.* 37:335-367.
- Kämper, J., Kahmann, R., Bölker, M., Ma, L.-J., and Brefort, T. 2006. Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature* 444:97-101.
- Kliebenstein, D. J., and Rowe, H. C. 2008. Ecological costs of biotrophic versus necrotrophic pathogen resistance, the hypersensitive response and signal transduction. *Plant Sci.* 174:551-556.
- Laine, A.-L. 2006. Evolution of host resistance: Looking for coevolutionary hotspots at small spatial scales. *Proc. R. Soc. B Biol. Sci.* 273:267-273.
- Laine, A.-L., and Hanski, I. 2006. Large-scale spatial dynamics of a specialist plant pathogen in fragmented landscape. *J. Ecol.* 94:217-226.
- Liu, Z., Bos, J. I. B., Armstrong, M., Whisson, S. C., da Cunha, L., Torto-Alalibo, T., Win, J., Avrova, A. O., Wright, F., Birch, P. R. J., and Kamoun, S. 2005. Patterns of diversifying selection in the phytoalexin-like scr74 gene family of *Phytophthora infestans*. *Mol. Biol. Evol.* 22:659-672.
- Liu, Z., Friesen, T., Ling, H., Meinhardt, S. W., Oliver, R. P., Rasmussen, J. B., and Faris, J. D. 2006. The *Tsn1*-ToxA interaction in the wheat-*Stagonospora nodorum* pathosystem parallels that of the wheat-tan spot system. *Genome* 49:1265-1273.
- Lorang, J. M., Sweat, T. A., and Wolpert, T. J. 2007. Plant disease susceptibility conferred by a "resistance" gene. *Proc. Natl. Acad. Sci. U.S.A.* 104:14861-14866.
- MacCallum, C., and Hill, E. 2006. Being positive about selection. *Plos Biol.* 4:e87.
- Mandel, M. A., Crouch, V. W., Gunawardena, U. P., Harper, T. M., and Orbach, M. J. 1997. Physical mapping of the *Magnaporthe grisea* *AVR1-MARA* locus reveals the virulent allele contains two deletions. *Mol. Plant-Microbe Interact.* 10:1102-1105.
- May, R. M., and Anderson, R. M. 1983. Epidemiology and genetics in the coevolution of parasites and hosts. *Proc. R. Soc. London B* 219:281-313.
- McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential and durable resistance. *Annu. Rev. Phytopathol.* 40:349-379.
- McDonald, J. H., and Kreitman, M. 1991. Adaptive protein evolution at the *Adh* locus in *Drosophila*. *Nature* 351:652-654.
- McDowell, J. M., Dhandaydham, M., Long, T. A., Aarts, M. G. M., Goff, S., Holub, E. B., and Dangl, J. L. 1998. Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the *RPP8* locus of *Arabidopsis*. *Plant Cell* 10:1861-1874.
- Mehrabi, R., Taga, M., and Kema, G. H. J. 2007. Electrophoretic and cytological karyotyping of the foliar wheat pathogen *Mycosphaerella graminicola* reveals many chromosomes with a large size range. *Mycologia* 99:868-876.
- Meyer, B. C., Kaushik, S., and Nandety, R. S. 2005. Evolving disease resistance genes. *Curr. Opin. Plant Biol.* 8:129-134.
- Michelmores, R. W., and Meyers, B. C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-death process. *Genome Res.* 8:1113-1130.
- Navarre, D. A., and Wolpert, T. J. 1999. Victorin induction of an apoptotic/senescence-like response in oats. *Plant Cell* 11:237-250.
- Nielsen, R., and Yang, Z. 1998. Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene. *Genetics* 148:929-936.
- Oliver, R. P., Lord, M., Rybak, K., Faris, J. D., and Solomon, P. 2008. Emergence of tan spot disease caused by toxigenic *Pyrenophora tritici-repentis* in Australia is not associated with increased deployment of toxin-sensitive cultivars. *Phytopathology* 98:488-491.
- Ridout, C. J., Skamnioti, P., Porritt, O., Sacristan, S., Jones, J. G. D., and Brown, J. K. 2006. Multiple avirulence paralogues in cereal powdery mildew fungi may contribute to parasite fitness and defeat of plant resistance. *Plant Cell* 18:2402-2414.
- Rohe, M., Gierlich, A., Hermann, H., Hahn, M., Schmidt, B., Rosahl, S., and Knogge, W. 1995. The race-specific elicitor, NIPI, from the barley pathogen, *Rhynchosporium secalis*, determines avirulence on host plants of the *Rrs1* resistance genotype. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:4168-4177.
- Ronald, J., and Akey, J. M. 2005. Genome-wide scans for loci under selection in humans. *Hum. Genomics* 2:113-125.
- Rose, L. E., Bittner-Eddy, P. D., Langley, C. H., Holub, E. B., Michelmores, R., and Beynon, J. L. 2004. The maintenance of extreme amino acid diversity at the disease resistance gene, RPP13, in *Arabidopsis thaliana*. *Genetics* 166:1517-1527.
- Rowe, H. C., and Kliebenstein, D. J. 2007. Elevated genetic variation within virulence-associated *Botrytis cinerea* polygalacturonase loci. *Mol. Plant-Microbe Interact.* 20:1126-1137.

- Schürch, S., Linde, C. C., Knogge, W., Jackson, L. F., and McDonald, B. A. 2004. Molecular population genetic analysis differentiates two virulence mechanisms of the fungal avirulence gene *NIP1*. *Mol. Plant-Microbe Interact.* 17:1114-1125.
- Shabab M., Shindo, T., Gu, C., Kaschani, F., Pansuriya, T., Chintha, R., Harzen, A., Colby, T., Kamoun, S., and van der Hoorn, R. A. L. 2008. Fungal effector protein AVR2 targets diversifying defense-related Cys proteases of tomato. *Plant Cell* 20:1169-1183.
- Spassieva, S. D., Markham, J. E., and Hille, J. 2002. The plant disease resistance gene *Asc-1* prevents disruption of sphingolipid metabolism during AAL-toxin-induced programmed cell death. *Plant J.* 32:561-572.
- Staats, M., van Baarlen, P., Schouten, A., van Kan, J. A. L., and Bakker, F. T. 2007. Positive selection in phytotoxic protein-encoding genes of *Botrytis* species. *Fungal Genet. Biol.* 44:52-63.
- Stachowiak, A., Olechnowicz, J., Jedryczka, M., Rouxel, T., Balesdent, M.-H., Happestadius, I., Gladders, P., Latunde-Dada, A., and Evans, N. 2006. Frequency of avirulence alleles in field populations of *Lepidosphaeria maculans* in Europe. *Eur. J. Plant Pathol.* 114:67-75.
- Stahl, E. A., and Bishop, J. G. 2000. Plant-pathogen arms races at the molecular level. *Curr. Opin. Plant Biol.* 3:299-304.
- Stahl, E. A., Dwyer, G., Mauricio, R., Kreitman, M., and Bergelson, J. 1999. Dynamics of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis*. *Nature* 400:667-671.
- Stergiopoulos, I., De Kock, M. J. D., Lindhout, P., and De Wit, P. J. G. M. 2007. Allelic variation in the effector genes of the tomato pathogen *Cladosporium fulvum* reveals different modes of adaptive evolution. *Mol. Plant-Microbe Interact.* 20:1271-1283.
- Stukenbrock, E. H., and McDonald, B. A. 2007. Geographical variation and positive diversifying selection in the host specific toxin *SnToxA*. *Mol. Plant Pathol.* 8:321-323.
- Stukenbrock, E. H., and McDonald, B. A. 2008. The origins of plant pathogens in agro-ecosystems. *Ann. Rev. Phytopathol.* 46:75-100.
- Stukenbrock, E. H., Banke, S., and McDonald, B. A. 2006. Global migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum*. *Mol. Ecol.* 15:2895-2904.
- Stukenbrock, E. H., Banke, S., Javan-Nikkhah, M., and McDonald, B. A. 2007. Origin and domestication of the fungal wheat pathogen *Mycosphaerella graminicola* via sympatric speciation. *Mol. Biol. Evol.* 24:398-411.
- Sun, W., Dunning, F. M., Pfund, C., Weingarten, R., and Bent, F. A. 2006. Within-species flagellin polymorphism in *Xanthomonas campestris* pv. *campestris* and its impact on elicitation of *Arabidopsis* *Flagellin sensing2*-dependent defenses. *Plant Cell* 18:764-779.
- Sweat, T. A. and Wolpert, T. J. 2008. Thioredoxin *h5* is required for victorin sensitivity mediated by a CC-NBS-LRR gene in *Arabidopsis*. *Plant Cell* 19:673-687.
- Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585-595.
- Tellier, A., and Brown, J. K. M. 2007. Stability of genetic polymorphism in host-parasite interactions. *Proc. R. Soc. B* 274:809-817.
- Thrall, P. H., and Burdon, J. J. 2003. Evolution of virulence in a plant host-pathogen metapopulation. *Science* 299:1735-1737.
- Thrall, P. H., Burdon, J. J., and Bever, J. D. 2002. Local adaptation in the *Linum marginale*-*Melampsora lini* host-pathogen interaction. *Evolution* 56:1340-1351.
- Torriani, S. F. F., Goodwin, S. B., Kema, G. H. J., Pangilinan, J. L., and McDonald, B. A. 2008. Intraspecific comparison and annotation of two complete mitochondrial genome sequences from the plant pathogenic fungus *Mycosphaerella graminicola*. *Fungal Genet. Biol.* 45:628-637.
- Torriani, S. F. F., Brunner, P. C., McDonald, B. A., and Sierotzki, H. 2009. QoI resistance emerged independently at least four times in European populations of *Mycosphaerella graminicola*. *Pest Manage. Sci.* 65:155-162.
- van den Burg, H. A., Westerink, N., Francoijs, K.-J., Woestenenk, R., Roth, R., Woestenenk, E., Boeren, S., De Wit, P. J. G. M., Joosten, M. H. A. J., and Vervoort, J. 2003. Natural disulfide bond-disrupted mutants of AVR4 of the tomato pathogen *Cladosporium fulvum* are sensitive to proteolysis, circumvent Cf-4-mediated resistance, but retain their chitin binding ability. *J. Biol. Chem.* 278:27340-27346.
- van der Biezen, E. A., and Jones, J. D. G. 1998. Plant disease-resistance proteins and the gene-for-gene concept. *Trends Biochem. Sci.* 23:454-456.
- van der Hoorn, R. A. L., and Kamoun, S. 2008. From guard to decoy: A new model for perception of plant pathogen effectors. *Plant Cell* 20:2009-2017.
- Van't Slot, K. A. E., and Knogge, W. 2002. A dual role for microbial pathogen-derived effector proteins in plant disease and resistance. *Crit. Rev. Plant Sci.* 21:229-271.
- Wang, H., Li, J., Bostock, R. M., and Gilchrist, D. G. 1996. Apoptosis: A functional paradigm for programmed plant cell death induced by a host-selective phytotoxin and invoked during development. *Plant Cell* 8:375-391.
- Wevelslep, L., Kogel, K. H., and Knogge, W. 1991. Purification and characterization of peptides from *Rhynchosporium secalis* inducing necrosis in barley. *Plant Dis.* 39:471-482.
- Win, J., Morgan, W., Bos, J., Krasileva, K. V., Cano, L. M., Chaparro-Garcia, A., Ammar, R., Staskawicz, B. J., and Kamoun, S. 2008. Adaptive evolution has targeted the C-terminal domain of RXLR effectors of plant pathogenic oomycetes. *Plant Cell* 19:2349-2369.
- Wolpert, T. J., Dunkle, L. D., and Ciuffetti, L. M. 2002. Host-selective toxin and virulence determinants: What's in a name? *Annu. Rev. Phytopathol.* 40:251-285.
- Yang, Z. 1997. PAML: A program package for phylogenetic analysis by maximum likelihood. *Comput. Appl. Biosci.* 13:555-556.
- Yang, Z. 2007. PAML 4: Phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* 24:1586-1591.
- Zaffarano, P. L., McDonald, B. A., and Linde, C. C. 2008. Rapid speciation following recent host shifts in the plant pathogenic fungus *Rhynchosporium*. *Evolution* 62:1418-1436.
- Zhan, J., Kema, G. H. J., and McDonald, B. A. 2004. Evidence for natural selection in the mitochondrial genome of *Mycosphaerella graminicola*. *Phytopathology* 94:261-267.
- Zhan, J., Kema, G. H. J., Waalwijk, C., and McDonald, B. A. 2002. Distribution of mating type alleles in the wheat pathogen *Mycosphaerella graminicola* over spatial scales from lesions to continents. *Fung. Genet. Biol.* 36:128-136.
- Zhang, B., Ramonell, S., Somerville, S., and Stacey, G. 2002. Characterization of early, chitin-induced gene expression in *Arabidopsis*. *Mol. Plant-Microbe Interact.* 15:936-970.
- Zhou, J.-M. and Chai, J. 2008. Plant pathogenic bacterial type III effectors subdue host responses. *Curr. Opin. Microbiol.* 11:179-185.