Using Population and Comparative Genomics to Understand the Genetic Basis of Effector-Driven Fungal Pathogen Evolution

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Epidemics caused by fungal plant pathogens pose a major threat to agro-ecosystems and impact global food security. High-throughput sequencing enabled major advances in understanding how pathogens cause disease on crops. Hundreds of fungal genomes are now available and analyzing these genomes highlighted the key role of effector genes in disease. Effectors are small secreted proteins that enhance infection by manipulating host metabolism. Fungal genomes carry 100s of putative effector genes, but the lack of homology among effector genes, even for closely related species, challenges evolutionary and functional analyses. Furthermore, effector genes are often found in rapidly evolving chromosome compartments which are difficult to assemble.

We review how population and comparative genomics toolsets can be combined to address these challenges. We highlight studies that associated genome-scale polymorphisms with pathogen lifestyles and adaptation to different environments. We show how genome-wide association studies can be used to identify effectors and other pathogenicity-related genes underlying rapid adaptation. We also discuss how the compartmentalization of fungal genomes into core and accessory regions shapes the evolution of effector genes. We argue that an understanding of genome evolution provides important insight into the trajectory of host-pathogen co-evolution.

Keywords: plant pathogens, genome, population genomics, evolution, molecular, fungi, agricultural ecosystems, association mapping studies

INTRODUCTION

Fungal plant pathogens are major threats to modern agricultural ecosystems and global food security (Strange and Scott, 2005). Many pathogen populations have a high evolutionary potential that enables a rapid response to selection pressure. A better understanding of the processes driving rapid pathogen evolution may be useful for designing more efficient and durable disease management strategies. A dramatic consequence of pathogen evolution is the rapid breakdown of host resistance observed repeatedly in many crops and over many decades (Brown, 2015).

Pathogens surmount plant resistance by secreting effector proteins that manipulate the immune
system and host metabolism. These effector genes evolve quickly and usually lack homologs in closely related species. Most effector genes encode small, cysteine-rich proteins that are secreted and highly expressed in planta (Lo Presti et al., 2015). In response, the host evolved an immune system based primarily on R genes encoding proteins that can directly or indirectly recognize effectors, and trigger defense mechanisms (Jones and Dangl, 2006). This genetic interaction between an avirulence effector (Avr) and its cognate resistance protein is known as the gene-for-gene model (GFG) (Flor, 1971).

The recent broad accessibility of genome sequencing technologies enabled genome-scale analyses of a rapidly growing number of plant pathogen species. These analyses revealed major factors contributing to disease emergence and made plant pathogenic fungi some of the best-studied models of pathogen evolution. Highlights of studies on plant pathogen genomes include the highly plastic genome structure, repertoires of 100s of rapidly evolving candidate effector genes and the non-random distribution of effector genes in the genome. Intriguingly, effector genes are preferentially located in repeat-rich and gene-poor regions of the genome (Raffaele and Kamoun, 2012). The compartmentalization of the genome (i.e., the “two-speed” genome) and the preferential location of effector genes in rapidly evolving compartments emerged independently several times in different fungal lineages and likely plays a key role in the ability of plant pathogens to rapidly respond to selection (Dong et al., 2015).

The genomic revolution also provides a major opportunity to bridge the gaps between molecular biology, evolutionary genetics and epidemiology. Here, we review the major advances made possible by comparative and population genomics to better understand the biology and evolution of effectors. We focus particularly on the assembly of multiple reference genomes and association mapping, which have the potential to significantly accelerate our understanding of pathogen biology (Figure 1). We also discuss the fascinating role accessory genomic regions play in the evolution of effector genes.

What Makes a Pathogen: Comparative Genomics of Fungal Lineages

The ability to cause disease on plants is a widely shared trait in the fungal kingdom. However, plant pathogenic lineages arose many times independently during fungal evolution (James et al., 2006; Soanes et al., 2007). Hence, different lineages of plant pathogens likely evolved the ability to cause disease through different mechanisms. To shed light on processes governing the evolution of pathogenicity, comparative genomics tools were applied widely across the fungal kingdom. Critical first steps in comparative genomic analyses are generating high-quality genome assemblies (Gibriel et al., 2016). A finished genome assembly (i.e., a gapless assembly from telomere to telomere) enables analyses of synteny breakpoints between homologous chromosomes of closely related species and ensures that the gene content of a genome is accurately reflected in genome annotations. Accurate gene predictions are particularly important to identify evolutionary novelty such as recently emerged effector genes.

Saprophytic and necrotrophic fungi feed on dead plant tissues, while biotrophic and endophytic species require living plant cells as a nutrient source. Hence, different fungal species evolved distinct sets of genes to successfully complete their infection lifestyle. Comparative genomics is a powerful tool to decipher specific gene complements associated with fungal lifestyles. Lo Presti et al. (2015) analyzed the genomes of 89 fungal species exhibiting different lifestyles and found that genomes of saprotrophs, necrotrophs, and hemibiotrophs were significantly enriched in plant cell wall degrading enzymes (PCWDE). In contrast, genomes of obligate biotrophs and symbionts were enriched in small secreted proteins (Figure 2). Among plant pathogens, biotrophic fungi have a higher proportion of species-specific predicted effectors than hemibiotrophic and necrotrophic fungi (Kim et al., 2016). Furthermore, biotrophic fungi tend to have fewer carbohydrate-active enzymes (CAZymes) than necrotrophic and hemibiotrophic fungi (Zhao et al., 2014; Kim et al., 2016). Duplessis et al. (2011) compared the genomes of two ascomycetes and 10 basidiomycetes, with a focus on the two obligate biotroph rust species Melampsora larici-populina and Puccinia graminis f. sp. tritici, the causing agents of poplar leaf rust and wheat and barley stem rust, respectively. The genomes of these obligate pathogens are among the largest fungal genomes. Genome expansions were caused by the proliferation of transposable elements and large gene families including oligopeptide and amino acid transporters, copper/zinc superoxide dismutase, helicases, kinases and transcription factors. Lineage-specific expansions included genes encoding small secreted proteins. Theseputative effectors were highly up-regulated in planta and may play a role in host specialization. The obligate biotrophic lifestyle of the two analyzed rust species was associated with a reduction in polysaccharide degrading enzymes and deficiencies in nitrate and sulfate assimilation pathways.

The classification of fungal species into a clear infection lifestyle category can be challenging. For example, Zymoseptoria tritici was long considered to have both biotrophic and necrotrophic lifestyles (hemibiotrophic). Recently, Z. triticici was proposed to be primarily a necroph with an early latency period (Sánchez-Vallet et al., 2015a). Similarly, Ramularia colo-cygni was long described as an asymptomatic endophyte of barley, but recent studies showed that the fungus can induce necrotic lesions and leaf senescence. Hence, R. colo-cygni is now considered to be an emerging pathogen of barley (Walters et al., 2008).

Comparing the gene inventory of fungal plant pathogen genomes can also provide insights into conserved pathogenicity mechanisms and lineage-specific innovations (Figure 2). Soanes et al. (2008) compared the sets of predicted proteins in the genomes of 34 pathogenic and non-pathogenic fungi and oomycetes. The study found that plant pathogenic species shared no unique sets of genes compared to non-pathogens. This suggests that universal pathogenicity factors may be rare. Nevertheless, protein families potentially involved in pathogenic processes, such as plant cell wall degradation, toxin biosynthesis, formation of reactive oxygen species and detoxification were expanded in the genomes of pathogens (Soanes et al., 2008).
Many genes involved in host–pathogen interactions are under continuous selection pressure which leaves footprints of selection in both the pathogen and host genomes (Aguileta et al., 2009). A classical test for the detection of positive selection is based on the ratio of non-synonymous (dN) to synonymous (dS) substitution rates. The dN/dS ratio indicates the direction and magnitude of selection (purifying and positive selection). Since plant pathogenic fungi are involved in an “arms race” with their host plant, genes involved in virulence are likely to show marks of positive selection. For example, in *Puccinia graminis*, selection analyses showed that pathogen-associated gene families were highly polymorphic and almost the only families under positive selection (Sperschneider et al., 2014; Upadhyaya et al., 2015). In *Z. tritici*, genes involved in adaptation to wheat were identified based on comparisons against two closely related sister species infecting wild grasses (Stukenbrock et al., 2011). A total of 27 positively selected genes were identified, none of them having a predicted function. However, two of those genes were shown to play a role in virulence (Poppe et al., 2015). Analyses of selection among different species often fail to identify signatures of recent, strong selection (e.g., selection imposed by a new host or a new fungicide). Intra-specific analyses of selection (i.e., based on populations) require different methodologies and are discussed below.

A series of recent studies showed that hybridization can lead to the emergence of new plant pathogens (Figure 2; Stukenbrock, 2016). Menardo et al. (2016) sequenced and analyzed the genomes of 46 isolates of *Blumeria graminis* from four different *formae speciales* causing powdery mildew diseases on wheat (*B.g. tritici*), durum wheat (*B.g. diccoci*), rye (*B.g. secalis*), and triticale (*B.g. triticale*) which is a hybrid between wheat and rye. The authors showed that a hybridization event between the two *formae speciales B.g. tritici* and *B.g. secalis* gave rise to the *B.g. triticale* adapted to triticale. Genome-wide scans for nucleotide diversity among *B.g. triticale* isolates showed that large chromosomal tracts were either nearly identical or highly diverse. In contrast, nucleotide diversity was monotonic among isolates of other *formae speciales*. The study by Menardo et al. (2016) provides a fascinating example of “mirrored” evolution in the host (which originated from hybridization between wheat and rye) and the pathogen (which originated from hybridization between *B.g. tritici* and *B.g. secalis*). An hybridization event between two distinct lineages of *Z. tritici* (Stukenbrock et al., 2012) is also responsible for the emergence...
of the species *Z. pseudotritici*. The hybridization event occurred approximately 380 sexual generations ago. These examples highlight the role hybridization events can play in the emergence of pathogens.

**Catching Evolution in Action: The Population Genomics of Plant Pathogens**

While comparative genomics provides powerful insight into the evolution of pathogen lineages, findings from comparative genomics studies are often limited to evolutionary events from the distant past. Agricultural pathogens evolve major phenotypes over much shorter time periods. In modern agricultural ecosystems, the breakdown of host resistance often happens within only a few years (Brown, 2015). For example, *Leptosphaeria maculans*, the causal agent of stem canker on oilseed rape, overcame resistance conferred by the gene *Rlm1* within 5 years after its introduction into new cultivars (Roussel et al., 2003). The evolutionary history and demography of pathogen populations play key roles in the ability of a pathogen to rapidly respond to selection. For an example, population genomics analyses identified major changes in the population structure of *Puccinia striiformis* f. sp. *tritici* over recent years. The population structure changes were accompanied by changes in virulence (Hubbard et al., 2015). Hence, investigating the population structure is an important step in identifying the pathogen’s evolutionary potential.

The ability of the pathogen to disperse varies among plant pathogens and is reflected in the population structure and levels of gene flow (McDonald and Linde, 2002; Zhan et al., 2009). In *Fusarium graminearum*, the causal agent of Fusarium head blight on wheat, barley and maize, natural field populations from different regions in Germany showed low differentiation, suggesting a high gene flow at the regional scale (Talas and McDonald, 2015). In contrast, a study found high differentiation among *Phytophthora infestans* populations infecting two different hosts (cultivated potatoes and wild *Solanum* spp.) despite being collected in the same valley in Mexico. Incipient host specialization among pathogen populations and genetic drift could explain such population subdivision (Flier et al., 2003).

The mode of reproduction also has an impact on the population structure and genotypic diversity (McDonald and Linde, 2002). High recombination rates reduce linkage disequilibrium in the genome. Populations of the sexually reproducing wheat pathogen *Z. tritici* were shown to be genetically diverse and exhibit strong signatures of recombination (Linde et al., 2002; Croll et al., 2015). In contrast, asexual reproduction leads to clonal populations with low genetic diversity. *Fusarium oxysporum* formae speciales are
distinct asexual clonal lineages that are often host specialized. *F. oxysporum* genomes harbor both highly conserved genomic regions and lineage-specific genomic regions that lack evidence of recombination (Ma et al., 2010). The existence of asexual fungal lineages recently led to debates about the evidence of sex and how fungal lineages could cope without it (Seidl and Thomma, 2014). One recent example is *Verticillium dahliae*, which causes vascular wilt on a variety of different hosts and may have evolved in the absence of sex. Population genomic studies provided evidence for cryptic sexual reproduction among clonal lineages despite strong indications for long-term clonality (de Jonge et al., 2013; Milgroom et al., 2014).

**Using Genome Scans to Identify Adaptive Loci in Pathogen Genomes**

Among the most powerful applications of population genomics are genome-wide scans of selection. Until now, most intra-specific analyses of selection were restricted to analyses of non-synonymous substitutions in effector genes (e.g., Schürch et al., 2004; Gout et al., 2007; Stukenbrock and Mcdonald, 2007, Stukenbrock and McDonal, 2009). These types of selection analyses assume absence of intra-genic recombination and are most suitable for genes with substantial divergence (see above). In contrast, genome-wide selection scans search for shifts in allele frequencies consistent with a selective sweep, which are much earlier signatures of selection than non-synonymous substitutions. It is important to note that genome-wide selection scans are only as powerful as signatures of selection can be robustly distinguished from genetic drift (Terauchi and Yoshida, 2010; Grünwald et al., 2016). Genome scans aim to identify signatures of selection contributing to ecological adaptation without relying on phenotypes (Figure 3). When genome scans are performed across populations spanning an ecological gradient the genome scans can be utilized for “reverse ecology,” ecological genomics or landscape genomics (Figure 3). Genome scans test individual loci across the genome for significant departures from neutrality (Oleksyk et al., 2010; Terauchi and Yoshida, 2010; Savolainen et al., 2013). Environmental association studies are increasingly performed in plant species (Weigel and Nordborg, 2015). Striking findings include allele frequencies correlated with climate factors and the finding of candidate genes for climate adaptation in *Arabidopsis thaliana* (Hancock et al., 2011; Fischer et al., 2013). To know our environment, environmental association studies have not yet been applied to any fungal plant pathogens. A frequently used method for genome scans is to calculate fixation indices (e.g., Wright's *F*<sub>St</sub>) to identify regions of unusual differentiation among populations. *F*<sub>St</sub> outliers are likely to result from divergent selection due to local adaptation. In the model species *Neurospora crassa*, Ellison et al. (2011) used this approach to identify genomic islands of divergence between two subtropical populations. These islands of divergence were hypothesized to stem from adaptation to different temperature and daylight exposures. Functional characterization of candidate genes located in the genomic islands confirmed the role of these genes in temperature adaptation (Ellison et al., 2011). Branco et al. (2015) recently used a similar approach to compare a coastal and a mountain population of the ectomycorrhizal fungus *Suillus brevipes* to investigate the genetic basis of adaptation to saline environments. Population divergence was found to be restricted to a few genomic regions and one of these regions contained a gene known to enhance salt tolerance in plants and yeast (Branco et al., 2015). Ideally, a continuous sampling of natural populations should be made across geographical or ecological gradients. For studies on plant pathogens, gradients could include, e.g., temperature, precipitation, and latitude. However, categorical differences in environments can also be used (e.g., different host genotypes).

Positive selection causes a beneficial allele to increase in frequency in a population. Because most alleles are in linkage disequilibrium with alleles at nearby loci, selection on one allele can affect an entire genomic region, an effect called hitchhiking. Strong selection can lead to a selective sweep that depletes genetic diversity in the nearby genomic region. Sweeps are called hard (i.e., a single haplotype rises to high frequency) or soft (i.e., multiple haplotypes are selected simultaneously) depending on the strength of selection and the amount of standing genetic variation. Site frequency spectrum-based tests, including the commonly used Tajima’s D test, aim to quantify the skew toward rare alleles. Linkage disequilibrium-based methods are used to...
Research over the past decades showed that GFG interactions are more suitable for largely clonal organisms (Shapiro et al., 2000). Hence, genome scans could be used to investigate the signatures of adaptation to different hosts or climatic conditions. The ability to hierarchically sample pathogen populations across fields, regions and continents should facilitate such analyses.

Despite their obvious potential, genome scans may not be applicable to all species or may be constrained by confounding factors (Figure 3; Levy and Borenstein, 2013; Savolainen et al., 2013). For example, to ascertain the role of specific loci in adaptation, genome scans must be combined with trait mapping or functional characterization (Stinchcombe and Hoekstra, 2008). In addition, strong population differentiation and significant clonal reproduction can significantly affect the power of genome scans. This is because the degree of association among alleles in a population (i.e., linkage disequilibrium) has a strong impact on the ability to detect signatures of selection (Terauchi and Yoshida, 2010; Jensen et al., 2016). High recombination rates may impede the detection of selective sweeps by eroding haplotype block structures in regions under selection. At the same time, high recombination rates may increase the resolution of genome scans by narrowing candidate regions while low recombination rates increase linkage disequilibrium in populations and weaken the ability of selection to favor particular alleles. Hence, highly clonal populations (e.g., of the rice blast pathogen Pyricularia oryzae or the potato late blight pathogen P. infestans) are unlikely to show strong locus-specific signatures of selection. Selection in mainly asexual pathogens is more likely to act at the level of individual clones than individual alleles. Selection detection methods based on divergence (e.g., dN/dS ratios) are more suitable for largely clonal organisms (Shapiro et al., 2009).

**Association Mapping Techniques to Identify Fungal Virulence Genes**

Research over the past decades showed that GFG interactions govern many host–plant pathogen interactions, however, identifying the effector genes involved in the interaction is still challenging. Computational approaches can be used to rapidly screen genomes for candidate effectors. However, such screens lack discriminatory power due to our incomplete understanding of the fungal effector delivery system (Sperschneider et al., 2015, 2016). Furthermore, many candidate effectors were found to have no discernible impact on the host physiology and, hence, may either not be interacting with the host, have a redundant function or their impact is too weak to be quantifiable. Association mapping techniques based on natural variation in virulence are promising to provide a powerful complement to *in silico* predictions of effectors.

While selection scans aim to identify loci under selection without prior knowledge of associated phenotypes, quantitative trait locus mapping (QTL) and genome-wide association studies (GWAS) are designed to identify loci that mediate heritable phenotypic variation. GWAS and QTL differ primarily in the selection of the mapping populations (Figure 4). QTL mapping and GWAS can be complementary approaches with complementary strengths. QTL mapping analyzes genetic variants segregating among progenies of a cross, whereas GWAS utilizes genetic variation among unrelated individuals from a natural population. Both GWAS and QTL mapping aim to associate polymorphism at loci with variation for a measured phenotypic trait. QTL mapping is only possible in pathogens for which crosses can be performed. While GWAS does not have this limitation, the power to map a phenotypic trait to a single locus is directly correlated with the level of recombination in a population. Extensive clonal reproduction leads to linkage disequilibrium across the genome and requires correction prior to association mapping analyses. QTL mapping is more reductionist as only phenotypic and genotypic variation existing between the parents can be mapped. GWAS populations generally exhibit a broader range of phenotypes and genotypes that can be screened. Furthermore, genotypes from field populations of sexual pathogens are generally much more recombined than progeny in a cross, which can dramatically increase the physical resolution of associations (Figure 4; Korte and Farlow, 2013; Grünwald et al., 2016).

The genotyping of a mapping population can be based on whole-genome sequencing or reduced-representation sequencing such as restriction-site associated DNA sequencing (RADseq) and genotyping-by-sequencing (GBS). RADseq and GBS offer excellent QTL mapping resolution at low cost (Davey and Blaxter, 2010; Elshire et al., 2011). RADseq and GBS methods reduce the number of loci to be sequenced in the genome by sequencing only at predefined restriction sites. Hence, the choice of restriction enzymes should be carefully evaluated to optimize marker density and sequencing costs (Leboldus et al., 2015). Both RADseq and GBS were successfully used for QTL mapping and GWAS analyses in fungi (Lendenmann et al., 2014, 2015, 2016; Leboldus et al., 2015; Gao et al., 2016; Talas et al., 2016). Nevertheless, the use of reduced-representation sequencing for GWAS should be carefully evaluated. The key parameter to consider is the distance at which linkage disequilibrium decays in the population. If the average distance between markers exceeds the average distance at which linkage disequilibrium decays to low levels, there is a significant risk of false negatives (i.e., missing a region in the genome associated with a phenotype).
Quantitative trait locus mapping has been widely used to identify chromosomal regions linked to phenotypic variation between parents. However, the limited amount of recombination in a single cross retains large linkage blocks that can limit mapping resolution (Figure 4; Hall et al., 2010; Dalman et al., 2013; Korte and Farlow, 2013). Therefore, loci identified by QTL mapping are typically large genomic regions with a high number of candidate genes. Another disadvantage of QTL mapping is the limitation to polymorphism segregating between parental isolates (Hall et al., 2010). Hence, additional sets of crosses or generating multi-parental mapping populations are desirable (Kover et al., 2009; Korte and Farlow, 2013).

Quantitative trait locus mapping has rarely been performed in fungi compared to plants. The few fungal QTL studies were nevertheless highly successful. Virulence loci in several fungal species including *Heterobasidion annosum sensu lato*, *F. graminearum* and *Z. tritici* were mapped using RAPD and AFLP markers (Kema et al., 2002; Cumagun et al., 2004; Lind et al., 2007). More recently, Christians et al. (2011) used whole-genome sequencing to genotype progenies of an *Aspergillus nidulans* cross and mapped three QTLs affecting in vivo virulence. Stewart et al. (2016) identified a major locus in *Z. tritici* governing virulence on a specific wheat cultivar by performing QTL mapping of virulence phenotypes measured with automated image analysis. Further QTL mapping studies in *Z. tritici* identified QTLs affecting several important life history traits including the degree of colony melanisation, thermal adaptation, and fungicide sensitivity (Lendenmann et al., 2014, 2015, 2016).

In contrast to the challenges associated with QTL mapping, the main challenge for GWAS is to identify one (or several) suitable natural field populations. The population should exhibit heritable variation in the phenotypic traits of interest and little sub-structure due to limited gene flow or recombination. A well-chosen GWAS population is expected to have a significantly higher mapping resolution than a population used for QTL mapping (Hall et al., 2010). However, the power of GWAS can be significantly reduced by the inclusion of related individuals and population substructure, both of which can lead to spurious associations of phenotypes and genotypes and require corrections to reduce false positive associations (Connelly and Akey, 2012). Moreover, GWAS performs poorly in detecting associations caused by rare variants and when loci have only small effects on phenotypes (Korte and Farlow, 2013).

Since the establishment of GWAS (Klein et al., 2005), applications in fungi are in their infancy compared to model plant and animal systems. One of the first GWAS in fungi was in the model yeast *Saccharomyces cerevisiae* (Muller et al., 2011). High-density genotypes of 44 clinical and non-clinical strains were used to identify loci associated with virulence. In plant pathogens, GWAS has been applied to identify virulence loci in three species. Dalman et al. (2013) found significant associations for 12 SNPs distributed across seven different contigs of the *H. annosum* genome for virulence on Scots pine and Norway spruce. Gao et al. (2016) applied reduced representation

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**FIGURE 4** Identifying genes underlying phenotypic traits using quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS). The first row illustrates QTL mapping and the second row illustrates GWAS. Genotypes in QTL mapping populations are fundamentally different from genotypes in GWAS populations. The horizontal blocks represent chromosomes from different strains. Chromosomes in QTL mapping are composed of large blocks inherited from the different parents. The block length is determined by the amount of recombination between the parental chromosomes. In contrast, chromosomes in field populations underwent numerous rounds of recombination. Due to this, linkage disequilibrium decays rapidly in GWAS mapping populations. Due to this, GWAS has generally a much stronger ability to resolve associations to small chromosomal regions. The second row shows the result of a QTL mapping with a QTL with a high logarithm of the odds (LOD) ratio located on chromosome 7. The red dotted line shows the significance threshold. The Manhattan plot below shows a significant association on chromosome 8 above the Bonferroni significance threshold. The third row shows the association of phenotypes and genotypes at a significant QTL and a GWAS SNP. The GWAS included populations from four different countries. The phenotype shown is the number of asexual fruiting bodies (pycnidia) per leaf area. Datasets shown here are from Hartmann et al. (2016) and Stewart et al. (2016).
sequencing for a GWAS of Parastagonospora nodorum. Known effector genes (SnToxA and SnTox3) could be used as positive controls to determine the mapping power of the assay (Gao et al., 2016). This study showed that RAD-GBS approaches can be used as an alternative to whole-genome sequencing. However, the marker density and the expected decay of linkage disequilibrium in the mapping population need to be carefully considered to avoid false negatives due to insufficient marker density. In a study on F. graminearum, Talas et al. (2016) reported GWAS associations for aggressiveness and deoxynivalenol production in a mapping population collected across Germany.

A large GWAS of the wheat pathogen Z. tritici identified the first avirulence effector gene associated with aggressiveness on a wheat cultivar (Hartmann et al., 2016). The GWAS included 106 sequenced strains across four populations and identified 25 distinct loci. The major locus encoded a single, small secreted protein, which was highly expressed. Population genomic analyses showed that higher aggressiveness was strongly associated with deletions of the gene, suggesting that the gene product was recognized by a cognate host protein. Hence, the gene loss was likely adaptive for the pathogen. The gene deletion was driven by a highly polymorphic cluster of transposable elements. Interestingly, the gene was not present in any of the closely related sister species suggesting that the gene has been either acquired horizontally or has evolved de novo (see below for a more detailed discussion of such processes).

Although GWAS applications are still scarce for fungi, the GWAS performed to date indicate significant promise. Compared to plant and animal GWAS, even relatively small mapping populations of 50–100 unrelated individuals were sufficient to robustly identify associations. Furthermore, the high-quality genomes now available for many fungi are an excellent resource for downstream validation of GWAS associations.

The Distinction of Core and Accessory Regions in the Genome: Is it Black and White?

Population genomics is a powerful tool to study pathogen evolution. Many population genomic analyses are restricted to variation at SNPs, yet fungal populations often harbor high degrees of plasticity in their genomes (Figure 5). Genes involved in host-pathogen interaction are frequently located in rapidly evolving genomic compartments. The heterogeneity across the genome was called the “two-speed” genome and most often refers to compartments distinguished by uneven mutation rates, GC-content and gene density (Raffaele and Kamoun, 2012). For a comprehensive understanding of the factors governing effector gene evolution, considering the mechanisms that govern genome plasticity is a key step.

Genome size varies extensively among eukaryotes and is only weakly correlated with organismal complexity or the number of genes in the genome (Lynch, 2007). All but the most compact eukaryotic genomes contain genomic regions enriched in non-coding sequences. A key question on how genome structure evolves is why non-coding DNA accumulates in genomes despite the potentially high costs of mutational burden. Lynch (2007) hypothesized that the mutational cost of genome expansion is dependent on the efficiency of selection and, hence, the effective population size. Large, repeat-rich genomes are predominantly found in species with low effective population sizes. Uneven evolutionary rate across the genome is a universal feature. However, whether repeat-rich regions contribute to adaptive evolution in taxa outside of the realm of plant pathogenic fungi remains unclear.

Filamentous pathogens exhibit great flexibility in genome size. This is largely explained by the accumulation of TEs in various genomes (Raffaele and Kamoun, 2012). Some fungal pathogens such as L. maculans (Rouxel et al., 2011) have a genome that has expanded as a result of a massive invasion by TEs, while Sclerotina sclerotiorum (Amselem et al., 2011) has a smaller genome virtually devoid of TEs. Mobile elements predominantly have negative effects on their hosts and increase mutation rates. Defense mechanisms such as RIP (repeat-induced point mutation) (Selker, 1990), MIP (methylation induced pre-miRNA) (Goyon and Faugeron, 1989) and quelling (Romano and Macino, 1992) have evolved to counteract TEs expression and mobility. TEs have been shown to play a prominent role in the evolution of genome structure and pathogenicity in several fungi (Fudal et al., 2009; Manning et al., 2013; Faino et al., 2016).

As many plant pathogenic fungi have genomes expanded by TE invasions, the hypothesis that “bigger can be better” was proposed when faced with antagonistic co-evolution with a host (Raffaele and Kamoun, 2012). The “two-speed” genome concept highlights the compartmentalization into repeat-dense regions with higher mutation rates and regions with a high gene density that remains fairly conserved over evolutionary time (Raffaele and Kamoun, 2012). The “two-speed” genome was hypothesized to shelter genes encoding essential, housekeeping functions in the core genome, while allowing novel genes to evolve in the accessory genome (Raffaele et al., 2010; Croll and McDonald, 2012). In plant pathogenic fungi, the two-speed genome is thought to contribute to the potential to rapidly evolve virulence.

“Two-speed” genome compartments identified by distinct sets of chromosomes (e.g., core and accessory chromosomes) are the simplest to identify structurally and functionally. For example, the secreted in xylem (SIX) virulence genes of F. oxysporum f. sp. lycopersici are located on accessory chromosomes and the loss of these chromosomes leads to a loss of virulence toward tomato (Ma et al., 2010). In many fungi, the origin of accessory chromosomes is uncertain and may be species-specific. In some species accessory chromosomes are hypothesized to have originated via horizontal transfer, while in other species an origin via the degeneration of essential chromosomes is the most likely explanation. Interestingly, accessory chromosomes are not unique to fungi. These chromosomes, referred to as B chromosomes, have also been identified in mammals (Hayman and Martin, 1965), insects (Wilson, 1907) and plants (Longley, 1927). In several instances B chromosomes exhibit sequence homology with “core” (or A) chromosomes and are hypothesized to have a multi-chromosomal origin with sequences originating...
from several of the A chromosomes (Camacho et al., 2000). B chromosomes do not pair with A chromosomes during meiosis and are considered to be autonomously replicating selfish genetic elements with no apparent function.

While virulence factors are frequently found in repeat rich regions, not all accessory compartments have a clear association with the ability to cause disease. *Z. tritici* has the largest accessory chromosome complement identified thus far, consisting of eight chromosomes of various sizes (Goodwin et al., 2011). These chromosomes encode a large array of genes with largely unknown functions and low expression levels during infection (Rudd et al., 2015). Despite this, there is a correlation between the presence of certain accessory chromosomes and increased virulence (Stewart et al., 2016). During sexual crosses the accessory chromosomes were inherited more frequently than expected, suggesting that segregation distortion may serve as a mechanism to prevent loss in populations through genetic drift (Croll et al., 2013). The accessory chromosomes frequently underwent rearrangements and were present in more than one copy in some cases (Wittenberg et al., 2009; Croll et al., 2013).

Accessory chromosomes can accelerate the evolution of pathogenicity. This may happen through horizontal transfer, as has been shown for *F. solani* (Temporini and VanEtten, 2004), *F. oxysporum* (Ma et al., 2010) and *Alternaria alternata* (Akagi et al., 2009). In *F. oxysporum*, host specificity is encoded by genes on accessory chromosomes, as the transfer of chromosome 14 into a non-pathogenic isolate confers virulence on tomato (Ma et al., 2010). In *Colletotrichum* species, the causal agent of anthracnose on different hosts, evidence was found for HGT from bacteria and plants (Jaramillo et al., 2015). In a recent study, Vlaardingerbroek et al. (2016) showed that “core” regions of the genome could be transferred horizontally when accompanied by the transfer of accessory chromosomes. Although HGT is believed to have played a role in the origin of some accessory regions, the ability to be transferred horizontally does not clearly distinguish between core and accessory genomic compartments in many lineages. Overall, horizontal transfer seems to be a major contributor to the emergence of new pathogens (van der Does and Rep, 2007).

Within species, chromosome length polymorphisms are frequent among homologous “core” chromosomes, suggesting that chromosomes harbor highly polymorphic compartments (Zolan, 1995). Hence, the distinction between the regions of the genome that are evolving rapidly and regions that are more static is more blurred than previously thought. For example, both core and accessory chromosomes of *Z. tritici* are highly polymorphic and segregate segmental deletions in field populations (Figure 5).

*L. maculans* effector genes are located in AT-rich, repetitive...
isochores that are arbitrarily distributed between the gene-rich, high GC content regions of the genome (Rouxel et al., 2011). Similarly, in P. infestans effector genes are located in gene-sparse regions that have a seemingly random distribution in the genome (Tyler et al., 2006; Haas et al., 2009; Raffaele et al., 2010).

Whether pathogens have a low-repeat one-speed genome such as Leptosphaeria biglobosa or Ustilago maydis (Kämper et al., 2006; Grandaubert et al., 2014), or a “two-speed” genome with core, and accessory regions, the general trend is that virulence genes were rarely found in conserved compartments of the genome. However, the structural distinction between the genome compartments of the “two-speed” genome is rarely a clear dichotomy: accessory and core, fast and slow. The distinction between the slowly and rapidly evolving genome compartments is likely a gradual transition in, e.g., gene density, accompanied by changes in transposons and repetitive elements content.

There is no experimental evidence that pathogens benefit from a “two-speed” genome (needless to say that such experiments may not be feasible in the first place). However, the evolutionary trade-offs involved in carrying a “two-speed” genome may well be investigated. The rapidly evolving compartment in pathogen genomes is largely controlled by transposable elements. While the activity of such selfish elements can clearly produce beneficial mutations in virulence loci, the overall spectrum of deleterious changes plays an important role in determining the selective advantage. If the rare beneficial mutation is accompanied by an overwhelming number of deleterious mutations, then evolution of such a “two-speed” genome should be counter-selected.

However, if deleterious mutations can largely be prevented, the “two-speed” genome should be strongly favored. The rapidly evolving genome compartment may be best seen as a largely selfishly governed compartment that accidentally produces benefits to the pathogen genome in which it is hosted. Such a largely non-adaptive view of the “two-speed” genome would reconcile views on the organization of genome structure across eukaryotic kingdoms.

How Do Effector Genes Emerge in Fungal Genomes?

As many fungal effector genes lack homologs in closely related species, such effector genes may best be described as orphan genes. Orphan genes are genes exclusively found in a species or in a taxonomic lineage (Tautz and Domazet-Lošo, 2011). The identification of orphan genes, or “taxonomically restricted genes,” is a major interest in many eukaryotic lineages, as orphan genes are likely playing a key role in speciation and specialization (Long et al., 2003). Two major hypotheses have been proposed to explain the emergence of orphan genes in genomes (Tautz and Domazet-Lošo, 2011). The first scenario for orphan gene evolution is called “duplication-divergence” (Figure 6): following a gene duplication event, one copy diverges to the extent that homology is no longer detectable and the gene simultaneously evolves a distinct function. This mechanism was originally considered as the only source of de novo gene evolution in genomes (Jacob, 1977). However, genes can also evolve de novo from non-coding DNA by fixing mutations required for a functional gene (Figure 6; McLysaght and Guerzoni, 2015). This mechanism requires both the evolution of an open reading frame (ORF) from non-coding DNA and functional cis-regulatory elements. Analysis of de novo gene birth from proto-genes in Saccharomyces cerevisiae suggested that de novo gene birth is likely a widespread mechanism in eukaryotic genomes (Carvunis et al., 2012), though, most of the de novo genes are short-lived and only a small fraction is likely to become fixed in a species (Palmieri et al., 2014).

Plant pathogenic fungi can harbor repertoires of 100s of putative effector genes (Lo Presti et al., 2015), how effector genes emerge in fungal populations remains unknown. Though, as effectors can confer a significant fitness benefit, orphan genes evolving to encode an effector protein function should be under strong selection pressure. The hypothesis of effectors being orphan de novo genes is challenged by the fact that effector genes are often highly up-regulated in planta. In contrast, de novo genes are characterized by their low expression as compared to core genes (Carvunis et al., 2012). Transcription of effector genes is tightly regulated and synchronized during plant colonization (Kleemann et al., 2012; Soyer et al., 2014; Palma-Guerrero et al., 2016). Hence, regulatory evolution is required to properly express a de novo effector gene at the critical time point of plant colonization. The transcriptional synchronization is especially important for the switch between biotrophic and necrotrophic phases in hemibiotrophic fungi.

The evolutionary trajectory of orphan effector genes needs to be put into the perspective of the co-evolution driving host-pathogen interactions. Genes encoding effector proteins that are recognized by host receptor proteins (i.e., avirulence effectors) are often rapidly lost or mutated. When this occurs, frequently lost “core” effector genes could then be mistaken as orphan de novo genes. The preferential localization of effector genes in gene-poor and repeat-rich regions may hold strong clues about the emergence and rapid evolution of effector genes. The evolution of effector genes is tightly linked to the activity of transposable elements (Rouxel et al., 2011; Castanera et al., 2016; Dutheil et al., 2016; Faino et al., 2016; Yoshida et al., 2016).

![FIGURE 6](image-url) Two models explaining the evolution of orphan genes. Orphan genes can originate from duplication-divergence events. Following a duplication event, one copy of the ancestral gene diversified to the extent that homology is no longer detectable (yellow vs. blue gene). Red circles show transcribed but non-coding sequences that can lead to the emergence of an open reading frame (ORF).
It is important to note that not all effector genes are orphans and some are highly conserved among fungal species. The LysM effector protein family is present in most fungal species, independently of their lifestyle (Akcapanar et al., 2015). LysM effectors interact with chitin compounds to protect fungi from plant chitinases or inhibit the recognition of chitin oligomers by plant receptors (Sánchez-Vallet et al., 2015b). The avirulence effector Ave1 in V. dahliae, which triggers Vel-mediated resistance in tomato, shares homologs across a broad range of species, including bacteria. Ave1 is likely to have been acquired from plants through horizontal transfer (de Jonge et al., 2012). The Ustilago maydis avirulence effector Pep1 is another example of a widely conserved effector, as it is found in most smut species (Hemetsberger et al., 2015). Moreover, in some cases, despite a complete lack of sequence homology, effector proteins share strong similarities in their 3D structure. Avr1Co39, AvrPi-A, AvrPhTZ in Magnaporthe oryzae and ToxB in Pyrenophora tritici-repentis completely lack sequence homology but have very similar 3D structures, which suggests that these proteins share a common function (Guillen et al., 2015).

Is the Role of Effectors in Virulence Reflected in the Evolutionary History of Effector Genes?
Gene-for-gene model interactions trigger a strong immune response in the host, which prevents colonization by the pathogen. The deployment of major R genes (i.e., qualitative resistances) in crop cultivars is very effective to prevent pathogen epidemics. However, qualitative resistances exert strong selection pressures on avirulence effector loci. As a result, qualitative resistances can be rapidly overcome by pathogen populations. Hence, increasing the durability of resistance genes is a major challenge in agro-ecosystems. The cost of virulence associated with the loss of an avirulence effector is an opportunity to infer the potential durability of a qualitative resistance in fields (Leach et al., 2001), but only few fungal effectors have been functionally characterized and their exact role in virulence can be difficult to assess. First, the lack of homologs of effector genes in closely related species makes the identification of effector genes in silico very challenging. Second, effector genes can be functionally redundant, which leads to false negative outcomes in single-gene knockout studies.

Diverse evolutionary mechanisms allow fungal populations to escape R gene mediated recognition. Deletion of avirulence effector genes is a common mechanism that has been observed in many plant pathogenic fungi (Schürch et al., 2004; Gout et al., 2007). The inactivation of an avirulence gene can also result from its disruption by the insertion of a transposable element (Inami et al., 2012). The modification of the avirulence protein is another mechanism to escape host recognition. In these cases, effector gene sequences are likely to show hallmarks of positive selection (Iida et al., 2015; Poppe et al., 2015). In Melampsora lini, changes in the amino acid sequence of the avirulence protein AvrL567 allows to escape recognition by three distinct resistance proteins (L5, L6, and L7) (Dodds et al., 2006).

Moreover, fungi evolved complex mechanisms to escape effector-triggered immunity without any modification of the corresponding avirulence gene. In the tomato fungal pathogen F. oxysporum, the presence of the avirulence gene Avr1 suppresses the recognition of both avirulence genes Avr2 and Avr3 (Houterman et al., 2008). Similarly, in L. maculans, the avirulence gene AvrLm4-7 allows to escape the recognition of the avirulence gene AvrLm3 (Plissonneau et al., 2016a). In both cases, the “masked” avirulence genes are expressed and the virulence function of the encoded proteins is likely to be maintained. This suggests that the loss of the “hidden” avirulence genes would impose an important fitness cost.

Future Directions
Over the past decade, the outstanding progress in fungal genomics revolutionized our knowledge of plant pathogens. However, the progress inevitably opened numerous new questions. Despite the prediction of extensive effector gene repertoires in many fungal genomes, only a few effectors were experimentally shown to play a role in virulence. Moreover, most effector genes lack homologs in closely related species, which limits both our understanding of effector gene evolution and our ability to reliably identify effector functions in silico. Plant pathogen population genomics offers an excellent opportunity to determine the genetic basis of many fungal phenotypes, including virulence. In particular, techniques such as GWAS, QTL mapping and genome scans were already shown to be powerful tools to identify effector genes involved in host-specific interactions.

Most population genomics analyses are currently limited to variation at SNPs, though structural variations are also likely to play an important role in fungal genome evolution. The emergence of third-generation sequencing technologies, providing sequencing reads in excess of 10 kb at reasonable costs, is a milestone to capture the full complexity of polymorphism in fungal genomes. Complete genome assemblies revealed the important roles transposable elements play in genomic plasticity and effector gene evolution (Faino et al., 2016; Plissonneau et al., 2016b). However, our knowledge of structural variation in fungal populations is still limited. Expanding the analyses of complete genomes to the population and species scale will provide a particularly powerful approach to understand the emergence of evolutionary novelty in pathogens.

Population genomics of plant pathogens offers also an opportunity to indirectly assess the durability of major resistance genes in agricultural fields. For example, effector genes showing mostly presence/absence polymorphism in populations are likely to be dispensable for host colonization. These genes will impose either no or low costs of virulence for the pathogen. On the contrary, effector genes under strong positive selection for protein modifications or epistasis with other effectors, the fitness cost associated with virulence should be high.

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REFERENCES

Agüileta, G., Refregier, G., Yockteng, R., Fournier, E., and Giraud, T. (2009). Rapidly evolving genes in pathogens: methods for detecting positive selection and examples among fungi, bacteria, viruses and protists. Infect. Genet. Evol. 9, 656–670. doi: 10.1016/j.meegid.2009.03.010

Akagi, Y., Akamatsu, H., Otani, H., and Kodama, M. (2009). Horizontal chromosome transfer, a mechanism for the evolution and differentiation of a plant-pathogenic fungus. Eukaryot. Cell 8, 1732–1738. doi: 10.1128/EC.00135-09

Akcapinar, G. B., Kappel, L., Scezman, O. U., and Seidl-Seiboth, V. (2015). Molecular diversity of LysM carbohydrate-binding motifs in fungi. Curr. Genet. 61, 103–113. doi: 10.1007/s00294-014-0471-9

Amselem, J., Cuomo, C. A., van Kan, J. A. L., Viala, M., Benito, E. P., Cough, X., and Giraud, T. (2011). Genomic analysis of the necrotrophic fungal pathogens Sclerotinia sclerotiorum and Botrytis cinerea. PLoS Genet. 7:e1002230. doi: 10.1371/journal.pgen.1002230

Baute, G. J., Kane, N. C., Grassa, C. J., Lai, Z., and Rieseberg, L. H. (2015). Genome scans reveal candidate domestication and improvement genes in cultivated sunflower, as well as post-domestication introgression with wild relatives. New Phytol. 206, 830–838. doi: 10.1111/nph.13255

Bigham, A. W., and Lee, F. S. (2014). Human high-altitude adaptation: forward genetics meets the HIF pathway. Genes Dev. 28, 2189–2204. doi: 10.1101/gad.250167.114

Bonhomme, M., Boitard, S., Clemente, H. S., Dumas, B., Young, N., and Jacquet, C. (2015). Genetic mapping of pathogenicity and aggressiveness of Medicago truncatula to root-associated microorganisms. Mol. Biol. Evol. 32, 2097–2110. doi: 10.1093/molbev/msv092

Branco, S., Gladieux, P., Ellison, C. E., Kuo, A., LaButti, K., Lipsen, A., et al. (2015). Genetic isolation between two recently diverged populations of a symbiotic fungus. Mol. Ecol. 24, 2747–2758. doi: 10.1111/mec.13132

Brown, J. K. M. (2015). Durable resistance of crops to disease: a Darwinian perspective. Annu. Rev. Phytopathol. 53, 513–539. doi: 10.1146/annurev-phytop-010213-045914

Camacho, J. P., Kane, T. F., and Beukeboom, L. W. (2000). B-chromosome chromosome transfer, a mechanism for the evolution and differentiation of a plant-pathogenic fungus. Eukaryot. Cell 8, 1732–1738. doi: 10.1128/EC.00135-09

Croll, D., and McDonald, B. A. (2012). The accessory genome as a cradle for adaptive evolution in pathogens. PLOS Pathog. 8:e1002608. doi: 10.1371/journal.ppat.1002608

Croll, D., Zala, M., and McDonald, B. A. (2013). Breakage-fusion-bridge cycles and large insertions contribute to the rapid evolution of accessory chromosomes in a fungal pathogen. PLoS Genet. 9:e1003567. doi: 10.1371/journal.pgen.1003567

Cumagun, C. J. R., Bowden, R. L., Jurgenson, J. E., Leslie, J. F., and Miedaner, T. (2004). Genetic mapping of pathogenicity and aggressiveness of Gibberella zeae (Fusarium graminearum) toward wheat. Phytopathology 94, 520–526. doi: 10.1094/PHYTO.2004.94.5.520

Dalman, K., Himmelstrand, K., Olson, Å., Lind, M., Brandström-Durling, M., and Stenlid, J. (2013). A genome-wide association study identifies genomic regions for virulence in the non-model organism Heterobasidion annosum s.s. PLOS ONE 8:e53525. doi: 10.1371/journal.pone.0053525

Davey, J. W., and Blaxter, M. L. (2010). RADSeq: next-generation population genetics. Brief. Funct. Genom. 9, 416–423. doi: 10.1093/bfgp/elq031

de Jonge, R., Bolton, M. D., Kombrink, A., van den Berg, G. C. M., Yadeta, K. A., and Thomma, B. P. H. J. (2013). Extensive chromosomal reshuffling drives evolution of virulence in an asexual pathogen. Genome Res. 23, 1271–1282. doi: 10.1101/gr.152660.112

Dong, J., Rong, B., Ede, H. P., van Maruthachalam, K., Bolton, M. D., Santhanam, P., Saber, M. K., et al. (2012). Tomato immune receptor Vr1 recognizes effector of multiple fungal pathogens uncovered by genome and RNA sequencing. PNAS 109, 5110–5115. doi: 10.1073/pnas.1119623109

Dods, P. N., Lawrence, G. J., Catanizariti, A.-M., Teh, T., Wang, C.-I. A., Ayliffe, M. L., et al. (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. doi: 10.1073/pnas.0602577103

Duplessis, S., Cuomo, C. A., Lin, Y.-C., Aerts, A., Tisserant, E., Veneault-Fourrey, C., et al. (2011). Obligate biotrophy features unraveled by the genomic analysis of rust fungi. Proc. Natl. Acad. Sci. U.S.A. 108, 9166–9171. doi: 10.1073/pnas.1019315108

Eshel, I., Mannhaupt, G., Schweizer, G., Sieber, C. M. K., Münsterkötter, M., and Güldener, U., et al. (2016). A tale of genome compartmentalization: the evolution of virulence clusters in smut fungi. Genome Biol. Evol. 8, 681–704. doi: 10.1093/gbe/evw626

Evans, L. M., et al. (2016). Transposons passively and actively contribute to evolution of the two-speed genome of a fungal pathogen. Genome Res 26, 1091–1100. doi: 10.1101/gr.204971.116

Fischer, M. C., Rellstab, C., Tedder, A., Zoller, S., Gugerli, F., Shimizu, K. K., et al. (2013). Population genomics of LysM carbohydrate-binding motifs in fungi. Mol. Ecol. 22, 5594–5607. doi: 10.1111/mec.12521
Plissonneau et al. Genomic Basis of Pathogen Evolution

Fler, W. G., Grünwald, N. J., Kroon, L. P. N. M., Sturbaum, A. K., van den Bosch, T. B. M., Garay-Serrano, E., et al. (2003). The population structure of *Phytophthora infestans* from the Toluca valley of central Mexico suggests genetic differentiation between populations from cultivated potato and wild *Solanum* spp. *Phytopathology* 93, 382–390. doi: 10.1094/PHYTO.2003.93.4.382

Fler, H. H. (1971). Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9, 275–296. doi: 10.1146/annurev-phyto-072910-095339

Fu, W., and Akey, J. M. (2013). Selection and adaptation in the human genome. *Annu. Rev. Genomics Hum. Genet.* 14, 467–489. doi: 10.1146/annurev-genom-091212-153509

Fudal, I., Ross, S., Brun, H., Besnard, A.-L., Ermel, M., Kuhn, M.-L., et al. (2009). Repeat-Induced Point mutation (RIP) as an alternative mechanism of evolution toward virulence in *Leptosphaeria maculans*. *Mut. Plant Microbe Interact.* 22, 932–941. doi: 10.1094/MPMI-22-8-0932

Gao, Y., Liu, Z., Faris, J. D., Richards, J., Brueggeman, R. S., Li, X., et al. (2016). Valuation of genome-wide association studies as a tool to identify virulence factors in *Parastagonospora nodorum*. *Phytopathology* 106, 1177–1185. doi: 10.1094/PHYTO-02-16-0113-FI

Gribel, H. A. Y., Thomma, B. P. H. J., and Seidl, M. F. (2016). The age of effectors: a wide analysis of fungal small secreted proteins (SSPs) reveals their potential role in host association. *Front. Plant Sci.* 7:186. doi: 10.3389/fpls.2016.00186

Goyal, C., and Faugeron, G. (1989). Targeted transformation of *Arabidopsis thaliana* local: evidence for positive selection in Swedish *Cladosporium fulvum*. *PLoS ONE* 10:e0123271. doi: 10.1371/journal.pone.0123271

Hancock, A. M., Brachi, B., Faure, N., Horton, M. W., Jarymowycz, L. B., Sperone, M. D., et al. (2009). A multiparent advanced generation inter-cross to fine-map of a diverse wheat yellow rust population. *Genome Biol.* 16, 23. doi: 10.1186/gb-2015-16-11-23

Huber, C. D., Nordborg, M., Hermisson, J., and Hellmann, I. (2014). Keeping it local: evidence for positive selection in Swedish *Arabidopsis thaliana*. *Mol. Biol. Evol.* 31, 3026–3039. doi: 10.1093/molbev/msu247

Iida, Y., van ’t Hof, P., Beenen, H., Messirth, C., Kubota, M., Stergiopoulou, I., et al. (2015). Novel mutations detected in avirulence genes overcomes tomato Cf resistance genes in isolates of a Japanese population of *Cladosporium fulvum*. *PLoS ONE* 10:e0123271. doi: 10.1371/journal.pone.0123271

James, T. Y., Kauff, F., Schoch, C. L., Matheny, P. B., Hofstetter, V., Cox, C. J., et al. (2006). Reconstructing the early evolution of Fungi using a six-genome phylogeny. *Nature* 443, 818–822. doi: 10.1038/nature05110

Jaramillo, V. D. A., Sukno, S. A., and Thon, M. R. (2015). Identification of horizontally transferred genes in the genus *Colletotrichum* reveals a steady tempo of bacterial to fungal gene transfer. * BMC Genomics* 16:2. doi: 10.1186/1471-2164-16-2

Jensen, D. J., Foll, M., and Bernatczek, L. (2016). The past, present and future of genomic scans for selection. *Mol. Ecol.* 25, 1–4. doi: 10.1111/mec.13493

Jones, J. D. G., and Dangl, J. L. (2006). The plant immune system. *Nature* 444, 323–329. doi: 10.1038/nature05286

Kämper, J., Kuhmann, R., Bölker, M., Ma, L.-J., Brefort, T., Saville, B. J., et al. (2006). Insights from the genome of the bistrophic fungal plant pathogen *Ustilago maydis*. *Nature* 444, 97–101. doi: 10.1038/nature05248

Kema, G. H. J., Goodwin, S. B., Hamza, S., Verstappen, E. C. P., Cavaletto, J. R., Van der Lee, T. A. J., et al. (2002). A combined amplified fragment length polymorphism and randomly amplified polymorphism DNA genetic kinkage map of *Mycosphaerella graminicola*, the septoria tritici leaf blotch pathogen of wheat. *Genetics* 161, 1497–1505.

Kim, K.-T., Jeon, J., Choi, J., Cheong, K., Song, H., Choi, G., et al. (2016). Kingdom-wide analysis of fungal small secreted proteins (SSPs) reveals their potential role in host association. *Front. Plant Sci.* 7:186. doi: 10.3389/fpls.2016.00186

Kleemann, J., Rincon-Rivera, L. J., Takahara, H., Neumann, U., van Themaat, E. V. L., van der Does, H. C., et al. (2012). Sequential delivery of host-induced virulence effectors by appressoria and intracellular hyphae of the phytopathogen *Colletotrichum higginsianum*. *PLoS Pathog.* 8:e1002643. doi: 10.1371/journal.ppat.1002643

Klein, R. J., Zeiss, C., Chew, E. Y., Tsai, J.-Y., Sackler, R. S., Haynes, C., et al. (2005). Complement factor H polymorphism in age-related macular degeneration. *Science* 308, 385–389. doi: 10.1126/science.1109557

Korte, A., and Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods* 9, 29. doi: 10.1186/1746-4811-9-29

Kover, P. X., Valdar, W., Trakalo, J., Scarcelli, N., Ehrenreich, I. M., Purugganan, M. D., et al. (2009). A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. *PLoS Genet.* 5:e1000551. doi: 10.1371/journal.pgen.1000551

Leach, J. E., Vera Cruz, C. M., Bai, J., and Leung, H. (2001). Pathogen fitness penalty as a predictor of durability of disease resistance genes. *Annu. Rev. Phytopathol.* 39, 187–224. doi: 10.1146/annurev.phyto.39.1.187

Leboldus, J. M., Kinzer, K., Richards, J., Ya, Z., Yan, C., Sun, J., et al. (2015). Genotype-by-sequencing of the plant-pathogenic fungi *Pyrenophora teres* and *Sphaerulina musiva* using Ion Torrent sequence technology. *Mol. Plant Pathol.* 16, 623–632. doi: 10.1111/mpp.12214

Lendenmann, M. H., Croll, D., and McDonald, B. A. (2015). QTL mapping of fungicide sensitivity reveals novel genes and pleiotropy with melanization in the pathogen *Zymoseptoria tritici*. *Fungal Genet. Biol.* 80, 53–67. doi: 10.1016/j.fgb.2015.05.001

Lendenmann, M. H., Croll, D., Palma-Guerrero, J., Stewart, E. L., and McDonald, B. A. (2016). QTL mapping of temperature sensitivity reveals candidate genes for thermal adaptation and growth morphology in the plant pathogenic fungus *Zymoseptoria tritici*. *Heredity* 116, 384–394. doi: 10.1038/hdy.2015.111
Plissonneau, C., Stürchler, A., and Croll, D. (2016b). The evolution of orphan regions in genomes of a fungal pathogen of wheat. bioRxiv 7, e12231-e12216. doi: 10.1128/bioRxiv.01231-16

Popp, S., Dorshheimer, L., Happel, P., and Stukenbrock, E. H. (2015). Rapidly evolving genes are key players in host specialization and virulence of the fungal wheat pathogen Zymoseptoria tritici (Mycosphaerella graminicola). PLoS Pathog. 11, e1005055. doi: 10.1371/journal.ppat.1005055

Raffaele, S., and Kamoun, S. (2012). Genome evolution in filamentous plant pathogens: why bigger can be better. Nat. Rev. Microbiol. 10, 417-430. doi: 10.1038/nrmicro2790

Raffaele, S., Win, J., Cano, L. M., and Kamoun, S. (2010). Analyses of genome architecture and gene expression reveal novel candidate virulence factors in the secretome of Phytophthora infestans. BMC Genomics 11:637. doi: 10.1186/1471-2164-11-637

Romano, N., and Macino, G. (1992). Quelling: transient inactivation of gene expression in Neurospora crassa by transformation with homologous sequences. Mol. Microbiol. 6, 3343-3353. doi: 10.1111/j.1365-2958.1992.tb02202.x

Rouxel, T., Grandaubert, J., Hane, J. K., Hoebe, C., van de Wouw, A. P., Couloux, A., et al. (2011). Effector diversification within compartments of the Leptosphaeria maculans genome affected by repeat-induced point mutations. Nat. Commun. 2, 202. doi: 10.1038/ncomms1189

Rouxel, T., Penaud, A., Pinochet, X., Brun, H., Gout, L., Delourme, R., et al. (2003). A 10-year survey of populations of Leptosphaeria maculans in France indicates a rapid adaptation towards the Rlm1 resistance gene of oilseed rape. Eur. J. Plant Pathol. 109, 871-881. doi: 10.1023/A:1026819225466

Rudd, J. J., Kanaya, K., Hassani-Pak, K., Derbyshire, M., Andongabo, A., Devonsire, J., et al. (2015). Transcriptome and metabolite profiling of the infection cycle of Zymoseptoria tritici on wheat reveals a biphasic interaction with plant immunity involving differential pathogen chromosomal contributions and a variation in the hemibiotrophic lifestyle definition. Plant Physiol. 167, 1158-1185. doi: 10.1104/pp.114.255927

Sánchez-Vallet, A., McDonald, M. C., Solomon, P. S., and McDonald, B. A. (2015a). Is Zymoseptoria tritici a hemibiotroph? Fungal Genet. Biol. 79, 29-32. doi: 10.1016/j.fgb.2015.04.001

Sánchez-Vallet, A., Mesters, J. R., and Thomma, B. P. H. J. (2015b). The battle for chitin recognition in plant-microbe interactions. FEMS Microbiol. Rev. 39, 171-183. doi: 10.1093/femsme/fuu003

Savolainen, O., Lascoux, M., and Merilä, J. (2013). Ecological genomics of local adaptation. Nat. Rev. Genet. 14, 807-820. doi: 10.1038/nrg3522

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. doi: 10.1094/MPMI.2004.17.10.1114

Seidl, M. F., and Thomma, B. P. H. J. (2014). Sex or no sex: evolutionary adaptation occurs regardless. Bioessays 36, 335–345. doi: 10.1002/bies.201300155

Selker, U. (1990). Premiotic instability of repeated sequences in Neurospora crassa. Annu. Rev. Genet. 24, 579-613. doi: 10.1146/annurev.ge.24.020190.003051

Shapiro, B. J., David, L. A., Friedman, J., and Alm, E. J. (2009). Looking for Darwin’s footprints in the microbial world. Trends Microbiol. 17, 196-204. doi: 10.1016/j.tim.2009.02.002

Skrede, I., and Brändström Durling, M. (2015). Population genomic analyses reveal possible drivers of population divergence. Mol. Ecol. 24, 2598-2600. doi: 10.1111/mec.13209

Soanes, D. M., Alam, I., Cornell, M., Wong, H. M., Hedeler, C., Paton, N. W., et al. (2008). Comparative genome analysis of filamentous fungi reveals gene family expansions associated with fungal pathogenesis. PLoS ONE 3:e2300. doi: 10.1371/journal.pone.0002300

Soanes, D. M., Richards, T. A., and Talbot, N. J. (2007). Insights from sequencing fungal and oomycte genomes: what can we learn about plant disease and the evolution of pathogenicity? Plant Cell 19, 3318-3326. doi: 10.1105/tpc.107.056663

Soyer, J. L., El Ghalid, M., Glaser, N., Ollivier, B., Linglin, J., Grandaubert, J., et al. (2014). Epigenetic control of effector gene expression in the plant pathogenic fungus Leptosphaeria maculans. PLoS Genet. 10:e1004227. doi: 10.1371/journal.pgen.1004227

Sperschneider, J., Dodd, P. N., Gardiner, D. M., Manners, J. M., Singh, K. B., and Taylor, J. M. (2015). Advances and challenges in computational prediction of
effectors from plant pathogenic fungi. *PLOS Pathog.* 11:e1004806. doi: 10.1371/journal.ppat.1004806

Sperschneider, J., Gardiner, D. M., Dodds, P. N., Tinn, F., Covarelli, L., Singh, K. B., et al. (2016). EffectorP: predicting fungal effector proteins from secretomes using machine learning. *New Phycol.* 210, 745–761. doi: 10.1111/nph.13794

Sperschneider, J., Ying, H., Dodds, P. N., Gardiner, D. M., Upadhyaya, N. M., Singh, K. B., et al. (2014). Diversifying selection in the wheat stem rust fungus acts predominantly on pathogen-associated gene families and reveals candidate effectors. *Front. Plant Sci.* 5:372. doi: 10.3389/fpls.2014.00372

Stewart, E. L., Croll, D., Lendenmann, M. H., Sanchez-Vallet, A., Hartmann, F. E., Palma-Guerrero, J., et al. (2016). QTL mapping reveals complex genetic architecture of quantitative virulence in the wheat pathogen *Zymoseptoria tritici*. *Mol. Plant Pathol.* doi: 10.1111/mpp.12515 [Epub ahead of print].

Stinchcombe, J. R., and Hoekstra, H. E. (2008). Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Hereditas* (Edinb) 100, 158–170. doi: 10.1038/sj.hdy.6800937

Strange, R. N., and Scott, P. R. (2005). Plant disease: a threat to global food security. *Annu. Rev. Phytopathol.* 43, 83–116. doi: 10.1146/annurev.phyto.43.113004.133839

Stukinbrock, E. H. (2016). Hybridization speeds up the emergence and evolution of a new pathogen species. *Nat. Genet.* 48, 113–115. doi: 10.1038/ng.3494

Stukinbrock, E. H., Bataillon, T., Dutheil, J. Y., Hansen, T. T., Li, R., Zala, M., et al. (2011). The making of a new pathogen: insights from comparative population genomics of the domesticated wheat pathogen *Mycosphaerella graminicola* and its wild sister species. *Genome Res.* 23, 473–485. doi: 10.1101/gr.118851.110

Stukinbrock, E. H., Christiansen, F. B., Hansen, T. T., Dutheil, J. Y., and Schierup, M. H. (2012). Fusion of two divergent fungal individuals led to the recent emergence of a unique widespread pathogen species. *Proc. Natl. Acad. Sci. U.S.A.* 109, 10954–10959. doi: 10.1073/pnas.1201403109

Stukinbrock, E. H., and McDonald, B. A. (2007). Geographical variation and positive diversifying selection in the host-specific toxin StiToxA. *Mol. Plant Pathol.* 8, 321–332. doi: 10.1111/j.1364-3703.2007.00396.x

Stukinbrock, E. H., and McDonald, B. A. (2009). Population genetics of fungal and oomycete effectors involved in gene-for-gene interactions. *Mol. Plant Microbe Interact.* 22, 371–380. doi: 10.1094/MPMI-22-4-0371

Talas, F., Kalih, R., Miedaner, T., and McDonald, B. A. (2016). Genome-wide association study identifies novel candidate genes for aggressiveness, deoxynivalenol production, and azole sensitivity in natural field populations of *Fusarium graminearum*. *Mol. Plant Microbe Interact.* 29, 417–430. doi: 10.1094/MPMI-09-15-0218-R

Talas, F., and McDonald, B. A. (2015). Genome-wide analysis of *Fusarium graminearum* field populations reveals hotspots of recombination. *BMC Genomics* 16:996. doi: 10.1186/s12864-015-2166-0

Tautz, D., and Domazet-Looo, T. (2011). The evolutionary origin of orphan genes. *Nat. Rev. Genet.* 12, 83–116. doi: 10.1038/nrg.2985

Temporini, E. D., and VanEtten, H. D. (2004). An analysis of the phylogenetic distribution of the pea pathogenicity genes of *Nectria haematococca* MPVI supports the hypothesis of their origin by horizontal transfer and uncovers a potentially new pathogen of garden pea: *Neocosmospora boniensis*. *Curr. Genet.* 46, 29–36. doi: 10.1007/s00294-004-0506-8

Terauchi, R., and Yoshida, K. (2010). Towards population genomics of effector-effector target interactions. *New Phycol.* 187, 929–939. doi: 10.1111/j.1469-8137.2010.03408.x

Tyler, B. M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R. H. Y., Aerts, A., et al. (2006). Phytophthora genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* 313, 1261–1266. doi: 10.1126/science.1128796

Upadhyaya, N. M., Garnica, D. P., Karaoglu, H., Sperschneider, J., Nemri, A., Xu, B., et al. (2015). Comparative genomics of Australian isolates of the wheat stem rust pathogen *Puccinia graminis* f. sp. tritici reveals extensive polymorphism in candidate effector genes. *Front. Plant Sci.* 5:759. doi: 10.3389/fpls.2014.00759

van der Does, H. C., and Rep, M. (2007). Virulence genes and the evolution of host specificity in plant-pathogenic fungi. *Mol. Plant Microbe Interact.* 20, 1175–1182. doi: 10.1094/MPMI-20-10-1175

Vitti, J. J., Grossman, S. R., and Sabeti, P. C. (2013). Detecting natural selection in genomic data. *Annu. Rev. Genet.* 47, 97–120. doi: 10.1146/annurev-genet-111212-133526

Vlaardingerbroek, I., Beerens, B., Schmidt, S. M., Cornelissen, B. J. C., and Rep, M. (2016). Dispensable chromosomes in *Fusarium oxysporum* f. sp. lycopersici. *Mol. Plant Pathol.* 17, 1455–1466. doi: 10.1111/mpp.12440

Walters, D. R., Havis, N. D., and Oxley, S. J. P. (2008). Ramularia collo-cygni: the biology of an emerging pathogen of barley. *FEBS Microbiol. Lett.* 279, 1–7. doi: 10.1111/j.1574-6968.2007.00986.x

Weigel, D., and Nordborg, M. (2015). Population genomics for understanding adaptation in wild plant species. *Annu. Rev. Genet.* 49, 315–338. doi: 10.1146/annurev-genet-120213-092110

Wilson, E. (1907). The supernumerary chromosomes of Hemiptera. *Science* 26, 870–871.

Wittenberg, A. H. J., Lee, T. A. I., van der, M., Barek, S. B., Ware, S. B., Goodwin, S. B., et al. (2009). Meiosis drives extraordinary genome plasticity in the haploid fungal plant pathogen *Mycosphaerella graminicola*. *PLoS ONE* 4:e5863. doi: 10.1371/journal.pone.0005863

Yoshida, K., Saunders, D. G. O., Mitsuoka, C., Natsume, S., Kosugi, S., Saitoh, H., et al. (2016). Host specialization of the blast fungus *Magnaporthe oryzae* is associated with dynamic gain and loss of genes linked to transposable elements. *BMC Genomics* 17:370. doi: 10.1186/s12864-016-2690-6

Zahn, A., Hu, J., Hu, X., Zhou, Z., Hui, M., Wang, S., et al. (2009). Fine-scale population genetic structure of Zhikong scallop (*Chlamys farreri*): do local marine currents drive geographical differentiation? *Mar. Biotechnol.* 11, 223–235. doi: 10.1007/s10126-008-9138-1

Zhao, Z., Liu, H., Wang, C., and Xu, J.-R. (2014). Correction: comparative analysis of fungal genomes reveals different plant cell wall degrading capacity in fungi. *BMC Genomics* 15:6. doi: 10.1186/1471-2164-15-6

Zolan, M. E. (1995). Chromosome-length polymorphism in fungi. *Microbiol. Rev.* 59, 686–698.

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