Chapter 14

Population Genomics of Fungal Plant Pathogens and the Analyses of Rapidly Evolving Genome Compartments

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Abstract

Genome sequencing of fungal pathogens have documented extensive variation in genome structure and composition between species and in many cases between individuals of the same species. This type of genomic variation can be adaptive for pathogens to rapidly evolve new virulence phenotypes. Analyses of genome-wide variation in fungal pathogen genomes rely on high quality assemblies and methods to detect and quantify structural variation. Population genomic studies in fungi have addressed the underlying mechanisms whereby structural variation can be rapidly generated. Transposable elements, high mutation and recombination rates as well as incorrect chromosome segregation during mitosis and meiosis contribute to extensive variation observed in many species. We here summarize key findings in the field of fungal pathogen genomics and we discuss methods to detect and characterize structural variants including an alignment-based pipeline to study variation in population genomic data.

Key words Fungal pathogens, Genome compartments, Transposable elements, De novo assembly, Multiple genome alignments

1 Introduction

The kingdom Fungi comprises a diverse group of pathogens that infect animals and plants. Understanding the evolution and infection biology of fungal pathogen species is evidently necessary to know how to combat the diseases caused by these organisms. Primary objectives to be addressed in population genomic studies of fungal pathogens relate to the origin of the pathogen, routes of migration, and epidemiology. Moreover, genome data can shed light on the underlying determinants of pathogenicity, which may be new targets in disease control. Finally, as we will outline in this chapter, fungal pathogens provide interesting model systems to study the evolution of genome architecture.

In this chapter, our focus will be on fungi that cause disease on plants. Genome data permitted the reconstruction of the
evolutionary histories of some of the most important fungal plant pathogens. For example, the speciation history of the ascomycete wheat pathogen *Zymoseptoria tritici* has been reconstructed by whole genome coalescence analyses revealing that this pathogen emerged with the onset of wheat domestication in the Middle East during the Neolithic Revolution 10–12,000 years ago [1, 2]. Population genetic analyses of isolates representing a world-wide collection of *Z. tritici* was applied to infer the migration history of the pathogen and showed a subsequent dispersal of the pathogen with the spread of wheat cultivation to Europe, Asia and later to New World countries [3]. Another important and recently emerged wheat pathogen is the wheat blast fungus *Magnaporthe oryzae*. The wheat blast disease first emerged in South America and strict quarantine strategies were employed to contain the pathogen within one region and avoid dispersal to other continents. However, the disease was recently reported in Bangladesh. Islam and colleagues were able to track the origin of the wheat blast outbreak in Bangladesh to South America using a genome-wide SNP dataset from 20 isolates collected from different host species in Brazil and Bangladesh [4]. This type of phylogenomic studies and “genomic surveillance” has proven of great relevance to monitor plant disease outbreaks and support the design of improved disease management strategies.

Genome data from fungal plant pathogens has also been a resource for the discovery of genes encoding virulence determinants. In particular quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) have proven powerful in this field. QTL mapping, based on phenotypic analyses and marker segregation in progeny populations, have been applied to identify the avirulence gene *AvrStb6* in *Z. tritici* [5, 6]. However, QTL analysis has several drawbacks: it relies on the analyses of crosses between two strains. This limits the resolution of the study, depending on the amount of variation between the two strains. Moreover, many fungi propagate primarily by asexual reproduction and many sexual species cannot be crossed under laboratory conditions excluding the possibility of QTL analysis. GWAS on the other hand uses outbred population and polymorphisms that represent the standing genetic variation in a population, providing a higher resolution along the genome [7]. GWAS analyses have been used to identify polymorphisms associated with fungicide sensitivity, mycotoxin production and aggressiveness of the wheat pathogen *Fusarium graminearum* [8], virulence determinants of the pine tree pathogen *Heterobasidion annosum* [9], and toxin production of another wheat pathogen *Parastagnospora nodorum* [10].

Another way to detect genes relevant for pathogenicity in fungi, is to apply evolutionary predictions to identify signatures of recent or past selection. Genes involved in host–pathogen
interactions are expected to evolve by antagonistic selection, either following an “arms-race” or a “trench-warfare” scenario of coevolution [11, 12]. The “arms-race” scenario refers to positive selection that repeatedly fixes new advantageous alleles at the locus under selection. The trench-warfare scenario on the other hand refers to the continuous maintenance of different alleles in the population by balancing and diversifying selection. Thus, identifying genes with signatures of positive or balancing selection in pathogen genome will likely uncover genes playing a role in host–pathogen interaction. Evolutionary predictions have been used to identify a number of virulence determinants in fungal plant pathogens and confirm the prediction that virulence determinants indeed often exhibit a signature of positive selection and accelerated evolution [13, 14].

Genome sequencing of hundreds of pathogenic fungal species has revealed extensive variation in genome structure and size [12]. Sequenced genomes range in size from 2 Mb in the Microsporidia to 2 Gb in Pucciniales species, and comprise different levels of ploidy and in some species even aneuploidy [15]. A consistent finding from comparative studies of pathogenic fungi is an extreme extent of genome plasticity whereby closely related species or individuals of the same species can have highly different genome structure and size, and vary in gene content and gene organization [12]. There is evidence that this genome plasticity is crucial for the pathogenic lifestyle. Indeed, variation is essential for pathogens to rapidly adapt to changes in their environment, in particular changes in host resistances, and a highly flexible genome composition appears to be an adaptive mechanism for pathogens to rapidly generate new genetic variation.

The field of fungal pathogen genomics has focused on the sources and patterns of genomic variation, and the contribution of this variation to gene evolution, in particular the evolution of virulence related genes, so called effectors. Effector genes encode secreted proteins that are involved in the suppression of host defenses and these genes are located in genomic segments exhibiting structural variation, including accessory chromosomes and islands of repetitive DNA (e.g., [16–19]). The challenge of studying patterns of evolution in these regions lies in the difficulty of assembling and comparing structurally different sequences.

Population genomic analyses, taking structural variation into account, have been instrumental in determining the underlying drivers of rapid evolution and genome variation in most pathogenic fungal species. This chapter will summarize some of the key discoveries from population genomics analyses of fungal pathogens.
2 Key Discoveries from Population Genomics in Plant Fungal Pathogens

2.1 High Recombination Rates and Population Admixture Contribute to Rapid Adaptation of Fungal Plant Pathogen Genomes

Population genomic data has been applied in a few studies to address the rapid evolution of fungal plant pathogens (reviewed in [12, 20]). Mechanisms that generate genetic variation in a population include mutational processes, recombination and gene flow. The fate of this variation is then determined by selection, genetic drift and the effective population size of the organism. Many aspects make it difficult to study the population genetics and demography of fungi and to assess the contribution of different evolutionary mechanisms to evolution. Most population genetic analyses rely on evolutionary models that make assumptions about the underlying genetic structure of the population (e.g., random mating, infinite site model, a low and constant recombination rate, clonality, skewed offspring, and constant population size).

In fungi, many species reproduce both asexually and sexually. More generally, the reproductive mode of fungi can be considered as a continuum ranging from predominantly clonal to strictly out-crossing. Furthermore, the reproductive mode of a particular taxa may change over time. For example, a species may propagate asexually for a certain time followed by a time of more frequent sexual reproduction. Extensive differences in the content of transposable elements between closely related species may support the occurrence of prolonged periods of asexual reproduction in many individual lineages [21, 22].

In population genetic analyses, it is often necessary to have a clear definition of generation time, in order to convert relative time to actual years. However, the generation time of a fungal individual that produces both by asexual and sexual reproduction is difficult to define. In these organisms, not only sexual generations can contribute to novel genetic variation but also asexual generations where high mutations rates generate clonal variation. Furthermore, little is known about the variation in sexual or asexual generations per year. However, the frequency of sexual mating and spore formation may vary from year to year according to environmental conditions and the availability of compatible sexual partners. In summary, analyses of fungal population genomic data, based on existing population models involve many uncertainties caused by our limited understanding of the population biology of fungal pathogen species and by the inadequacy of classic population genetic assumptions to the life history traits of these organisms.

Despite these limitations, population genomic data has, for a few model species, provided new insight into genome the evolution and population biology of the plant pathogens. For example, the impact of recombination on genome evolution has been studied in both ascomycete and basidiomycete pathogens. Badouin and coworkers used population genomic data to infer linkage
disequilibrium (LD) along the genome of two closely related species of the anther smut fungus *Microbotryum* [23]. Using information about the extent of LD and the site frequency spectrum (SFS), the authors could determine the distribution and frequency of selective sweeps along the genomes and thereby demonstrate the recent impact of natural selection on gene and genome evolution in the two species. While recombination in *Microbotryum* has been crucial to fix adaptive mutations, suppression of recombination in other parts of the genome has shaped evolution of mating type chromosomes. On these chromosomes recombination suppression has contributed to the generation and maintenance of “super genes” comprising the genes responsible for pre- and postmating compatibility [24].

The impact of recombination has also been studied in *Z. tritici* and its close relative *Zymoseptoria ardabiliae* using population genomic data. These analyses revealed exceptionally high rates of recombination, including recombination hotspots localizing in protein coding genes [25]. Furthermore a strong correlation of recombination with both positive and negative selection was recently demonstrated [26]. Thereby, a negative correlation of recombination and $pN/pS$, the proportion of nonsynonymous to synonymous polymorphisms, demonstrates an important role of recombination in removing nonadaptive mutations. On the other hand, a positive correlation of recombination with the rate of adaptive nonsynonymous mutations, $\omega_A$, was reported, showing that recombination likewise contributes to the efficient fixation of advantageous mutations in this species.

The impact of intra-specific gene flow on the population genetic structure and dynamic was elegantly demonstrated by a transcriptome sequencing of wheat leaves infected with the yellow stripe rust pathogen *Puccinia striiformis* [27]. *P. striiformis* is an obligate pathogen and difficult to culture on artificial media. Direct sequencing of infected leaf material thus provides a powerful approach to capture the genetic diversity of isolates in the field. Bueano-Sancho and colleagues used data from 246 infected leaves of wheat, triticale, and rye collected in 2 years and at different geographical locations. They used population genetic analyses to infer the population structure and recent patterns of gene flow and admixture of the European rust population and demonstrate extremely diverse populations and rapid seasonal shifts of the rust populations [27]. A significant impact of gene flow on the population genetic structure of fungal pathogens has been demonstrated in other studies also using population genomic data, for example, in the rice blast pathogen *Magnaporthe oryzae* [28] and the ash dieback pathogen *Hymenoscyphus fraxineus* [29].

The impact of new mutations has also been extensively studied in fungal plant pathogen genomes. This is because many species show exceptionally high rates of mutational changes in some
segments of their genomes, and the ability to rapidly generate new genetic variation by mutations likely represents an adaptive trait. In the next section we outline the peculiarity of many plant pathogen genomes with respect to genome architecture of the distribution of mutation-prone genome regions.

3 Fungal Plant Pathogen Genomes Are Often Compartmentalized, A Trait Driven by Transposable Elements

The origin of genome compartments in fungal pathogens is still poorly understood, but can only be studied with well-assembled and aligned genome sequences that allow us to study patterns of nucleotide variation within and around these particular genomic regions. Improved genome assemblies have provided insight into the repetitive fraction of fungal pathogen genomes. Repeat contents can vary from less than 1% in *Fusarium graminearum* to more than 80% in some rust and mildew species [30, 31]. The factors determining repeat accumulation are poorly understood, but can include sexual versus asexual reproduction and different genome defense mechanisms such as DNA methylation and Repeat Induced Point mutations (RIP). Transposable elements may accumulate during prolonged asexual reproduction in the absence of recombination; however, some of the sequenced species with the highest repeat content, such as many rust fungi, are sexual, suggesting that other factors likewise are important determinants of transposable element activities.

In some fungal pathogen species a large portion of the repetitive elements are found in particular accessory segments or entire chromosomes that are nonessential but in some species important for virulence. The genome of the asexual fungus *Verticillium dahliae* comprises particular islands enriched with transposable element and encoding effector genes [16, 32]. These islands are present in different lineages of the pathogen and contribute to variation in virulence. Interestingly, these genomic islands harbor little nucleotide variation among individuals that share a particular island, possibly reflecting the strong impact of natural selection on the genes encoded by these regions. Variation in virulence phenotypes is thus given by the presence–absence polymorphism of an entire genomic fragment.

The genome of the fungus *Leptosphaeria maculans* infecting oil seed rape also comprises repeat rich compartments that encode effector proteins [17]. These regions show a particular mutation pattern conferred by RIP. RIP acts to inactivate transposable elements by introducing mutations in repetitive sequence. RIP produces cytosine to thymine (C to T) mutations and can thereby locally impacts the GC content of the sequence [33]. This is the
case for *L. maculans* where the repeat-rich islands have become AT isochores with highly distinct GC content compared to the remaining genome.

Genome compartments can also be contained in the genome as accessory chromosomes. The wheat pathogen *Zymoseptoria tritici* has a large number of such accessory chromosomes, eight of them have been sequenced in the reference isolate. These chromosomes can be lost and rearranged during mitosis as well as meiosis [34, 35]. Beside this large complement of accessory chromosomes, *Z. tritici* also exhibits a considerable amount of chromosome length polymorphisms of the core chromosomes as demonstrated by electrophoretic separation of chromosomes and PacBio sequencing [36, 37]. In the soil-borne pathogen *Fusarium oxysporum* lineage-specific chromosomes encode virulence determinants that enable the fungus to be pathogenic on specific host species by the defeat of host defenses [22].

How are accessory chromosomes lost and how are they maintained in populations? A few studies mainly focusing on *F. oxysporum* and *Z. tritici* have started to address these questions. These studies have demonstrated the exceptionally fast rate of accessory chromosomes loss during mitosis [38, 39]. In *F. oxysporum* amplification and maintenance of the chromosomes likely depend on the horizontal exchange of these chromosomes by vegetative fusion of hyphae. In *Z. tritici* however, the accessory chromosomes can be amplified during meiosis by a meiotic drive mechanism [40]. In both species, mechanisms that allow the loss of chromosomes as well as mechanisms that reamplify the chromosomes may have evolved to rapidly generate new genetic variation in the populations of pathogens.

### 4 Interspecific Hybridization Contributes to Genome Evolution of Fungal Plant Pathogens

Reproductive barriers between fungal species are in many cases poor predictors of species boundaries. Sexual mating and fusion of hyphae between nonconspecific individuals have been frequently described and demonstrate a pathway of gene exchange across species boundaries in the kingdom Fungi. We have recently reviewed the literature on fungal hybridization [41] and will here only mention a few prominent examples of hybridization and gene exchange between fungal species.

Hybridization has been shown to be responsible for the rapid emergence of new virulent lineages of different fungal plant pathogens, including *Ophiostoma nova-ulmi*, the causal agent of Dutch Elm disease and the powdery mildew pathogen *Blumeria graminis-triticide* on crop species Triticale [42, 43]. For the Dutch Elm
disease fungus, occasional hybridization events have played a role in the exchange of virulence determinants between otherwise distinct lineages. *B. graminis-triticale*, on the other hand, is the product of few hybridization events between powdery mildew species infecting wheat and rye, respectively. The evidence for a hybridization event is a particular mosaic distribution of genetic variation that clearly reflects the two parental genomes recombined in one genome [43]. The two examples demonstrate very different outcome of hybridization ranging from a few signatures of introgression to entirely mixed parental genomes and hybrid speciation.

The exchange of genetic material can also occur as horizontal gene transfer where only a fragment of DNA is integrated into the genome of one species from another organism. The wheat pathogens *Parastagonospora nodorum* and *Pyrenophora tritici-repentis* are two distantly related ascomycete pathogens. However, their genomes comprise one region of exceptionally high sequence identity [44]. This region that is flanked by transposable elements includes a gene that encodes a proteinaceous toxin, ToxA. ToxA is a virulence factor that confers necrosis in susceptible wheat cultivars and the acquisition of the *ToxA* gene by *P. tritici-repentis* from *P. nodorum* by horizontal gene transfer, allowed the emergence a new virulent lineage of *P. tritici-repentis* infecting wheat. Interestingly, genome sequencing revealed that the *ToxA* gene also is present in another wheat pathogen *Bipolaris sorokiniana* suggesting that this gene may be carried by a bacterial or viral vector frequently associated with wheat [45].

Multiple signatures of hybridization and interspecific gene exchange supports a high extent of flexibility in terms of genome content and structure in fungal plant pathogens. The finding that introgression and horizontal gene transfer in some cases involve virulence determinants underlines the importance of studying not only these regions, but also the processes whereby they occur. However, hybridization events between more distantly related species may be challenging to identify with population genomic data. This is because outlier loci in the genome that comprise highly diverged haplotypes can be difficult to assemble by reference-based assembly approaches. Below we discuss how to circumvent this issue by alignment of de novo assembled genomes.

5 Discovering Variation in Population Genomic Data

5.1 Variant Calling Through Short-Read Mapping: Methods and Limits

Most population genomics approaches are based on the mapping of short sequencing reads, using software such as bwa or bowtie to a well-assembled reference genome [46–48]. Tools such as GATK, SAMtools mpileup, or FreeBayes can be used to call single nucleotide variants and small indels from the mapping file and output this information in a Variant Call Format (VCF) file [49–52]. Here,
we will not go further into details about these methods for SNP discovery as these have been extensively reviewed elsewhere (e.g., [53–55]).

Variant discovery through mapping of short reads to a reference is supported by a large number of well-documented tools. However, these methods have drawbacks, some of them especially relevant in nonmodel organisms such as most fungal pathogens. As mentioned above, many pathogenicity-related genes locate in repeat rich compartments of fungal pathogen genomes, and mapping based approaches may not be ideal for the characterization of genetic variation in these regions. Alignment in low-complexity or repetitive regions, although facilitated by paired or mated reads, is often challenging due to the difficulty of correctly mapping the sequence to the reference [55]. Dependence on a reference genome can also be an issue in nonmodel organisms for which a complete reference genome is not always available. Indeed, any misassembly or single nucleotide error in the reference genome could be reflected in the final variants. Poor assembly quality would also lead to structural variation being impossible to discover. Finally, mapping of short reads will not perform efficiently in presence of high genetic variability. Such high variability may be found locally in genomes that have experienced introgression or in some regions have a higher mutation rate. In either case, reads containing multiple alternative alleles might not map correctly, resulting in the under-representation of the diverging haplotypes [55].

Another limitation to mapping-based approaches is the detection of structural variation. To detect translocations or inversions, genomes can be de novo assembled and compared in a multiple genome alignment (Fig. 1). Fungal genomes are convenient for this approach as they often are relatively small and can be sequenced in the haploid phase, therefore preventing issues with heterozygosity and phasing.

Sequencing technologies based on longer reads (e.g., PacBio SMRT or Nanopore sequencing) provide improved resources for de novo assembly. These technologies have proven valuable in the improved detection of structural variation in plant pathogen genomes, including repeat-rich accessory segments on core chromosomes [56, 57]. Below, we describe methods to use de novo genome assembly based on both long and short read sequencing and give the details of a pipeline which allows variants calling from these assembled genomes.

6 De Novo Assembly and the Rise of Long-Read Sequencing

A number of assemblers are available for the different types of sequencing reads available including short reads produced by Illumina sequencing and long reads produced, for example, by SMRT
sequencing. For de novo assembly of short read data programs like SPAdes [58], SOAPdenovo2 [59] or IDBA-UD [60] based on de Bruijn graph assemblies are available [61]. De Bruijn graph-based assemblers work by splitting the short reads into even shorter units of uniform size, the so-called k-mers. These k-mers provide the basis for the reconstruction of the genome sequence based on overlap of different k-mers while information about the local connectivity of each k’mer is preserved by a De Bruijn graph structure (see, e.g., ref. 62, 63). To properly handle repetitive regions the De
Bruijn graph assembler masks repetitive and low-complexity regions and assemble the remaining genome into many contigs and scaffolds.

De novo genome assemblies of long read data is based on other algorithms and build on the alignment of overlapping reads \cite{64,65}. Long read sequencing with SMRT technology provides an average read length of 10 kb that can be assembled with assemblers like Canu \cite{64}, Falcon \cite{65}, or SMRTAssembly (©Pacific Biosciences). Nanopore sequencing is producing even longer reads, mostly dependent on the length of the extracted DNA fragment, by a MinION instrument. Methods to assemble genomes based on this technology partially overlap with the ones used with SMRT technology, for example, with the Canu assembler \cite{64} and are reviewed in de Lannoy et al. \cite{66}. Nanopore reads have been used to improve the N50 of the maize pathogen Rhizoctonia solani by an order of magnitude compared to previous efforts \cite{67}. This improvement is even more pronounced in genomes with high repetitive content (e.g., \cite{56,68}). In the oat crown rust fungus Puccinia coronata f. sp. avenae with a genome-wide repeat content of more than 50%, long read sequencing has enabled detailed characterization of structural variants \cite{69}. Moreover, assembly of long read data provided a map of SNPs not only between individuals but also between nuclei in the dikaryotic hyphae of P. coronata f. sp. avenae, a level of variation so far poorly studied in fungi.

The main inconvenience with long-read sequencing methods so far is the high error rate. To circumvent this issue, it is necessary to either increase the sequencing depth or to combine the advantages of short and long reads. Indeed, assemblies of long and short read data from the same genome is also possible with “hybrid assemblers” like hybridSPAdes whereby the long read data ensures the assembly of long scaffolds and the short read data provides high coverage of individual nucleotides in the assembly \cite{70}. Instead of using both types of reads during the assembly process, it is also possible to correct long-read de novo assembly with short-read data, using software like Pilon \cite{71}. Such an approach was recently used to assemble genomes of the species Leptosphaeria and Zymoseptoria \cite{37,72}.

It is important to note that different assemblers (for short-read data as well as long-read data) may perform differently with different genome datasets depending on the repeat content and sequence complexity. Moreover, the long-read technologies are improving at a very rapid rate and new tools and methods are constantly developed. We therefore advise reviewing the latest methods, testing different assemblers with a given dataset and comparing the resulting assemblies with tools such as Quast to determine the best performance \cite{73}. To evaluate the quality of the assemblies, key parameters to compare are the total length of the assembly, the
number of contigs and the overall size of the assembled fragments which can be summarized by the N50 value (defined as the largest contig length, \( L \), whereby contigs of length superior or equal to \( L \) accounts for at least 50\% of the bases of the assembly).

For population genomics analyses of the fungal wheat pathogen *Z. tritici* we have developed a pipeline based on de novo genome assembly and multi genome alignments (Fig. 1). This method has allowed us to quantify and characterize accessory regions in the genome of *Z. tritici* and to identify hitherto overseen signatures of introgression along the genome of the pathogen [74]. Following de novo assembly of either short or long read sequencing, the next step in our pipeline is the generation of a multiple genome alignment with a multiple genome aligner such as a TBA [75], Mugsy [76], or progressiveMauve [77]. These aligners first generate pairwise alignments of all genomes and next combine these into a multiple genome alignment. The resulting alignment, for example, in “multiple alignment format” maf file consists of a large number of local alignment blocks, that differ in their length and the number of sequences included in the block (see Chapter 2). The variation in sequence numbers per block along the genome may reflect actual presence/absence variation in genome segments, but can also reflect the parts of the genome that is prone to assembly and/or alignment errors. A thorough filtering and realignment of the alignment blocks is therefore necessary to ensure that the observed patterns are biologically relevant. Programs like Mafft or T-Coffee are available for realignment of alignment blocks to ensure the optimal comparison of sequences [78, 79].

Filtering and variant detection from a multiple genome alignment can be done with programs like Maffilter [80] (see also Chapter 2). Maffilter allows the list of variant sites identified across the aligned genomes to be outputted as a VCF file. This format is identical to the one used by classic variant calling following a mapping approach. This is especially convenient, as it will allow for these variants to be used as input by any population genomics programs designed to work on this well-known format. Another advantage of this pipeline is that it allows to detect variants simultaneously using sequencing data produced by different technologies, for example, in the case here some genomes are obtained by Illumina sequencing and other by PacBio SMRT sequencing (Feurtley et al. unpublished).

## 7 Detection of Structural Variation in Genomes

Structural variation is increasingly being recognized as an important level of genetic variation to study. In a study of a single human genome, Pang and colleagues found that the genome differed from the reference human genome by only 0.1% when considering SNPs
but by approximately 1.2% when considering other source of genetic variation such as insertions, deletions, or copy number variations [81]. In fungal plant pathogens, structural variation is recognized as an important type of variation as highlighted in some of the examples summarized above.

Methods based on read mapping can be applied to characterize structural variation along genomes [82]. These methods rely on several types of information to detect structural variants including read-depth, and the distribution of paired-end and split reads. Read depth in a mapping, that is, the number of sequencing reads aligning to a specific locus, can give information about copy number variations and deletions. For example, a locus with a higher depth than expected could indicate a duplication and a lower depth (close to 0 in a haploid genome, half the expected depth in a diploid genome) a deletion [83].

Deletions in the resequenced genome compared to the reference genome will cause the insert size of paired-end reads (the DNA fragment including the sequenced reads and the gap sequence between the reads) to appear larger than expected, while an insertion will make the insert look smaller than expected. Furthermore, pairs in which one read aligns to the genome while the other does not may reflect an insertion of a TE if the second read aligns to a repeated element somewhere else in the genome. Likewise translocations, inversions, and other kinds of structural variants can be inferred from pairs of reads. Aligning DNA genomic sequencing reads using an aligner created for RNAseq and thus able to split a read sequence (usually, due to intronic sequences being spliced out of the read) would allow detecting deletions since the deleted sequence will look like a splicing junction site. Software that can detect such structural variants include *Pindel*, *Delly*, or *LUMPY* [84–86]. More details about these methods and software can be found for instance in [83, 87, 88].

Although these methods can uncover many structural variants from short and long reads, they do have their limits. Some of these methods make strict assumptions about the sequencing data, which are not always met in real data. Methods based on read depth assume that the sequencing depth is uniform across the genome and that variation mainly is explained by structural variants. However, variation in GC content and sequence composition along the genome can also cause variation in sequencing efficiency and thereby sequencing depth [83, 89]. Moreover, genomic segments such as accessory chromosomes or large insertions which do not always exist in a reference genome cannot be detected by mapping of short reads to a reference [88].

Whole genome assembly is able to uncover all types of structural variation, including large DNA fragments, which are not present in the reference genome. Another advantage of whole genome assembly is that, if the quality of the assembly is good, it
provides strong evidence that no structural variant has gone undetected [88]. When the number of genomes is low, structural variants can be identified visually using, for instance, Symap or circos, which provide easily interpretable visualization of genome alignments [90, 91]. Specific software able to detect structural variants from de novo assemblies have also been developed such as Assemblytics and AsmVar, an automatization step that accounts for structural variants a population level [92, 93]. In summary several tools are available to detect and characterize structural variants in population genomic data. In organisms, like fungal pathogens, with highly variable genomes, accounting for structural variants is essential in order to understand genome evolution and the impact of mutation and recombination along the genome.

8 Conclusion

Analyses of genetic variation in fungal plant pathogen genomes have to a large extent focused on highly variable regions, on species-specific traits and presence–absence variation. More detailed analyses in a few species point to these regions being of particular interest as they can encode important pathogenicity factors. Variation in these regions is therefore considered to be adaptive in accordance with rapid host–pathogen coevolution. Population genomic studies that aim to characterize genetic variation in highly variable regions rely on high quality assemblies and alignments. De novo assemblies of long read sequence data provide an important new resource to capture variation in these regions, including variation in transposable element sequences.

The processes that drive genome evolution in fungal pathogen genomes is still poorly understood. We have demonstrated exceptionally high rates of recombination and particular mechanisms that introduce new mutations at high a rate. Furthermore, we know that fungal pathogens can exchange genes with other species either by sexual mating or fusion of asexual structures. However, the underlying mechanism of these processes, as well as the impact of natural selection on genetic variation generated by these is still to be unraveled.

With their small genome size and in many cases particular genome architecture, fungal pathogens, however, provide excellent models organisms for fundamental studies of genome evolution. Moreover, a better understanding of evolutionary processes occurring in pathogen populations is crucial for the development of agricultural ecosystems with higher disease resistance [94].
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