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Tansley insight

Fundamental wheat stripe rust research in the 21st century

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Summary

In the 21st century, the wheat stripe rust fungus has evolved to be the largest biotic limitation to global wheat production. New pathogen genotypes are more aggressive and able to infect previously resistant wheat varieties, leading to rapid pathogen migration across and between continents. We now know the full life cycle, microevolutionary relationships and past migration routes on a global scale. Current sequencing technologies have provided the first fungal draft genomes and simplified plant resistance gene cloning. Yet, we know nothing about the molecular and microevolutionary mechanisms that facilitate the infection process and cause new devastating pathogen races. These are the questions that need to be addressed by exploiting the synergies between novel 21st century biology tools and decades of dedicated pathology work.

I. Introduction

In the last 15 yr, the disease known as wheat stripe rust has become the largest biotic limitation to wheat production and threatens global food supply. Currently, 88% of the world's wheat production is susceptible to wheat stripe rust, leading to global losses of over 5 million tons of wheat with an estimated market value of $USD 1 billion annually (Wellings, 2011; Beddow *et al.*, 2015).

Wheat stripe rust is also known as wheat yellow rust because of its spore color during its asexual infection cycle on wheat. The disease is caused by the obligate biotrophic fungus *Puccinia striformis* f. sp. *tritici* (*Pst*). The threat of this fungus to agriculture is rooted in its tremendous genetic diversity as a result of sexual recombination occurring predominantly in the Himalayan region, its long-distance dispersal across continents by natural and human means, and its rapid local adaptation via stepwise evolution, overcoming a single resistance gene at a time (Hovmøller *et al.*, 2011). Genetic control of wheat stripe rust is achieved by over 50 formally named *Yellow rust* (*Yr*) *Resistance* (*R*) genes identified by the continuous efforts of plant breeders and pathologists over the last 100 yr (McIntosh *et al.*, 2013). Historically, pathologists have focused on

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the isolation of *Pst* from wheat fields and have determined the ability of these isolates to infect a defined set of wheat lines carrying different *Yr R* genes. The resulting infection phenotypes determine the virulence profiles and pathogen race nomenclature of the *Pst* isolates, enabling a comparison between spatially and temporally distinct collection events (Chen *et al.*, 2014). In the last 10 yr, modern DNA-based tools have expanded our ability to study this fungus. This has led to the cloning of the first *Yr R* genes, the identification of the full life cycle of *Pst*, its center of genetic diversity and past global migration patterns, and the provision of draft *Pst* genomes. In this Tansley Insight, I describe these recent milestones and provide my perspective of what is to come. These are truly exciting times for wheat stripe rust research arising from synergies between novel 21st century biology tools and decades of dedicated pathology work (Hovmøller *et al.*, 2011; Wellings, 2011; Saunders, 2015).

II. Wheat stripe rust can be controlled with genetics

Genetic control via *R* genes is the most economical and preferred containment strategy for wheat stripe rust. *R* genes in wheat are historically divided into two phenotypically, mechanistically and genetically distinct categories. 'Seedling resistance' genes are characterized by a strong to moderate immune response that fully curtails fungal infection and sporulation at all developmental stages. To date, most identified genes conferring 'seedling resistance' against any wheat rust encode classic nucleotide-binding site leucine-rich repeat (NBS-LRR) R proteins that recognize fungal proteins, also known as effectors, inside the plant cytoplasm and trigger a defense response that halts pathogen reproduction (Ellis *et al.*, 2014; Steuernagel *et al.*, 2016). This makes the recognized effector an avirulence factor for a specific wheat cultivar–*Pst* isolate interaction (Ellis *et al.*, 2014). To date, *Yr10* is the only cloned classic stripe rust *R* gene. *Yr10* confers resistance to many stripe rust isolates world-wide, yet several *Pst* isolates virulent on *Yr10* have emerged (Liu *et al.*, 2014). The rapid local stepwise evolution of newly virulent *Pst* isolates is a characteristic of classic *R* genes and is known as the 'boom and bust cycle'. The high selection pressure posed in large-scale monocultures leads to rapid fixation of newly virulent pathogen genotypes. These genotypes arise frequently in the asexual stage of *Pst* because recognition by a single classic *R* gene is overcome easily via a single genetic variation of an erstwhile avirulence gene (Wellings, 2011; Ellis *et al.*, 2014).

By contrast, 'adult plant resistance' is seen as more durable because, in the asexual stage of *Pst*, a single genetic variation appears insufficient to overcome this type of resistance (Ellis *et al.*, 2014). Novel genetically diverse *Pst* incursions, however, can reduce the effectiveness of 'adult plant resistance' (Sørensen *et al.*, 2014). In general, 'adult plant resistance' is not complete immunity, but delays infection and spore production, leading to slow rusting phenotypes. The underlying nonclassical *R* genes are involved in general plant physiology and encode resistance allele-specific protein variants that are molecularly unrelated to NBS-LRR proteins (Ellis *et al.*, 2014). In the case of *Pst, Yr18* and *Yr46* encode two distinct transporters (Ellis *et al.*, 2014; Moore *et al.*, 2015) and *Yr36*, a chloroplast-localized kinase regulating reactive oxygen species production (Ellis *et al.*, 2014; Gou *et al.*, 2015). The combination of these nonclassical *R* genes with classic *R* genes is currently seen as the most effective strategy to combat wheat stripe rust. By slowing the fungal life cycle with 'adult plant resistance' *R* genes, spore production and fungal population size are decreased. This reduces the genetic diversity and the potential to evade recognition mediated by classic *R* genes via a single genetic variation, making the latter more durable. Of course, the durability of *R* genes is also defined by the global genetic diversity of the pathogen, which is generated during the sexual and asexual stages of the life cycle of *Pst*.

III. The *Puccinia striformis* f. sp. *tritici* life cycle enables genetic diversity and rapid adaptation

The full life cycle of *Pst* includes five different spore types on two phylogenetically distinct plant hosts (Fig. 1; Boxes 1, 2; Chen *et al.*, 2014). During the economically important asexual infection cycle on wheat, *Pst* produces re-infecting dihaploid dikaryotic urediniospores ($N+N'$), which contain one haploid genome copy (N) in each separate nucleus. In the absence of sexual recombination, each *Pst* nucleus evolves independently via mutagenesis, leading to high heterozygosity (Hovmøller *et al.*, 2011). Somatic hybridization has been suggested to generate genetic diversity during the asexual stage (Park & Wellings, 2012), yet clear molecular evidence for such a phenomenon is lacking. On the contrary, sexual recombination is probably very important for the generation of refreshed genetic diversity during the infection of the evergreen shrub barberry (*Berberis* spp.; see Section IV), commonly referred to as the 'alternate host', which completes the full sexual cycle of *Pst* (Jin *et al.*, 2010; Zhao *et al.*, 2013; Ali *et al.*, 2014a,b; Hovmøller *et al.*, 2016).

To illustrate the contribution of both sexual and asexual reproduction to the evolution of new virulence profiles, one has to appreciate the huge numbers of spores produced at each stage. Based on observations made in the closely related wheat stem rust fungus (*Puccinia graminis* f. sp. *tritici*), a heavily infected barberry shrub at the sexual stage can give rise to up to 70 billion wheat-infecting spores that are genetically diverse (Boxes 1, 2; Littlefield, 1981). In turn, a moderately infected wheat field can produce over 25 million asexual urediniospores per square meter per generation with an estimated mutation frequency of 1×10^{-6} per genetic locus (Littlefield, 1981; Hovmøller & Justesen, 2007). This leads to millions of potential variations at each genetic locus within one growing season when moderately susceptible wheat varieties are grown over billions of square meters. It comes as no surprise that novel virulent isolates that arise by sexual recombination or asexual cumulative genetic variation sweep whole continents within one or two growing sessions, as recently observed in Australia and Europe (Wellings, 2011; Hovmøller *et al.*, 2016).

IV. *Puccinia striformis* f. sp. *tritici* evolves and migrates rapidly on a global scale

To limit such rapid spread of virulent *Pst* isolates, it is vital to understand the underlying global population structures and

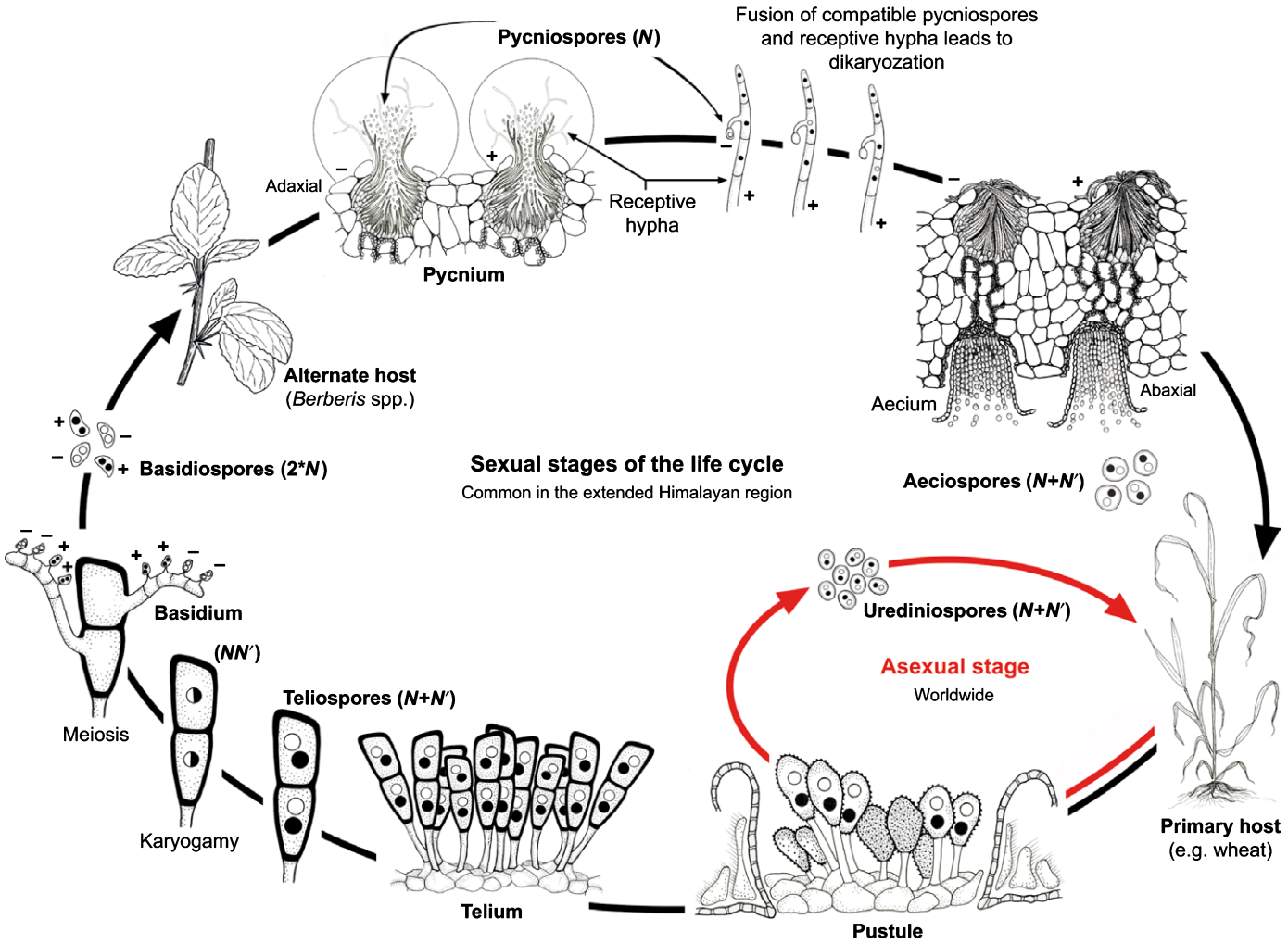

Fig. 1 Life cycle of *Puccinia striformis* f. sp. *tritici*. Adapted from the *Puccinia graminis* f. sp. *tritici* life cycle (Kolmer, 2013). Original illustration from Jacolyn A. Morrison at the USDA-ARS Cereal Disease Laboratory, St Paul, MN, USA.

migration patterns. Extensive global population genetic studies identified the extended Himalaya region (Nepal, Pakistan and China) as a hotspot of sexual recombination and genetic diversity, and as the putative center of origin (Duan *et al.*, 2010; Ali *et al.*, 2014a). The Middle East and the Mediterranean also show slight signatures of sexual reproduction, but are most likely dominated by the asexual cycle (Ali *et al.*, 2014a; Thach *et al.*, 2016). In all other regions, *Pst* reproduces completely asexually only infecting wheat leading to clonal population structures. These regions include the Americas, North West Europe, East Africa and Australia, where *Pst* adapts via stepwise evolution as it evades recognition by classic *R* genes (Wellings, 2011; Ali *et al.*, 2014a; Thach *et al.*, 2016). The knowledge of global population structures, combined with *Pst* virulence profiles and sample dates, comprises a powerful tool to trace past global pathogen migrations (Fig. 2). For example, the first *Pst* incursion into Australia in 1979 originated from Europe, probably as a result of human activities (Wellings, 2011). The second most recent wave of colonization and displacement originated from *Pst* populations (*PstS1*) in East Africa, first detected in the early 1980s, from where they spread to the Americas and Australia in 2000 and 2002 (Walter *et al.*, 2016). During the same time period, the derivative race *PstS2* became prevalent in the Middle East and Central Asia (Ali *et al.*, 2014a; Walter *et al.*, 2016). This race group (*PstS1* and *PstS2*) was more aggressive, producing more urediniospores, was better adapted to higher temperatures and displayed a novel virulence profile (Hovmøller *et al.*, 2008; Milus *et al.*, 2008). These phenotypes facilitated their rapid global spread, readily replacing many existing *Pst* populations, and made *Pst* the largest threat to global wheat production (Fig. 2; Beddow *et al.*, 2015). Only Europe was spared because the majority of cultivated wheat varieties carried *R* genes providing adequate resistance to this race group (Hovmøller *et al.*, 2008; Milus *et al.*, 2008). Many of these *R* genes became ineffective during the most recent devastating *Pst* incursion into Europe, which originated from the Himalaya region and was probably caused by long-distance wind dispersal (Fig. 2; Hovmøller *et al.*, 2016). This new race group was first identified on the wheat variety 'Warrior' in 2011 and fully replaced the existing population by 2013 (Hubbard *et al.*, 2015; Hovmøller *et al.*, 2016). The *Pst* 'Warrior' race group is genetically more heterogeneous than previous *Pst* populations in Europe,

Box 1 The complete life cycle of the wheat stripe rust fungus *Puccinia striformis* f. sp. *tritici* (*Pst*)

In spring, short-lived, binucleated, double-haploid basidiospores (2*N*) infect barberry at the start of the sexual infection cycle (Fig. 1). During successful infections, *Pst* forms pycnia on the adaxial side of the leaf and produces mating type-specific mononucleated haploid pycniospores (*N*). The fusion of pycniospores with receptive hyphae of a mating type-compatible pycnia initiates dikaryozation and the development of an aecium (*N*+*N*′) on the abaxial side of the leaf. Multiple distinct dikaryozation events may happen within a single pycnium, giving rise to genetically diverse aecia. The vegetative aeciospores (*N*+*N*′) are only able to infect the 'primary host', such as wheat, and initiate the asexual infection cycle. Successful infection of wheat leads to the formation of yellow pustules on both sides of the leaf, with each pustule ejecting thousands of dikaryotic urediniospores (*N*+*N*′). Urediniospores can lead, on average, to 15 re-infection cycles of wheat within one growing season. To complete its sexual life cycle, *Pst* switches to the formation of telia at the end of the wheat growing season and produces thick-walled, long-lived, initially dikaryotic teliospores (*N*+*N*′). In spring, these spores undergo nuclear fusion (karyogamy, *NN*′) and successive meiosis with sexual recombination generating fresh genetic diversity. Spores then germinate immediately initiating basidium development and the production of four binucleated double-haploid basidiospores (2*N*) ready to infect the 'alternate host' barberry (Fig. 1; Littlefield, 1981; Hovmøller & Justesen, 2007; Chen *et al.*, 2014; Rodriguez-Algaba *et al.*, 2014).

Box 2 Glossary

Alternate host	The host of lesser economic importance and, in the case of *Puccinia striformis* f. sp. *tritici* (*Pst*), the host that produces pycnia and aecia
Dikaryon	A dihaploid organism that contains two nuclei per cell with each holding one haploid genome copy (*N*)
Isolate	A culture of microorganisms isolated for study
Primary host	The host of the largest economic importance and, in the case of *Pst,* the host that produces telia
Race group	A group of genetically related isolates with similar virulence profiles
Stepwise evolution	In its asexual stage, *Pst* is believed to rapidly evolve to overcome one resistance gene via one single genetic variation
Virulence profile	Defined by the ability of a given *Pst* genotype to complete its infection cycle on a set of wheat lines differing for resistance genes
Wheat stripe rust	The disease on wheat that is caused by the fungus *Pst*

suggesting multiple source populations (Hovmøller *et al.*, 2016). This high genetic diversity might increase the potential for local adaptation and asexual stepwise evolution to virulence in years to come.

The knowledge of past *Pst* evolution and migration will inform future containment strategies; yet, to date, most population studies lack detailed sequence information regarding the underlying genetic changes. New approaches are aiming to tackle this important biological question by incorporating whole-genomic and transcriptomic data into field pathogen surveys (Hubbard *et al.*, 2015). These genomic-guided approaches have the potential to directly link DNA sequence variations to novel virulence profiles and changes in pathogen fitness, identifying causative alleles.

V. *Puccinia striformis* . sp. *tritici* genomes are highly heterozygous and encode over 1000 candidate effectors

Annotation-rich genomic data are the foundation for the identification of causative genetic variation underlying phenotypic changes. Considerable efforts have been made to sequence and assemble the *Pst* genome in the urediniospore stage (*N*+*N*′). Draft genome assemblies of six distinct isolates are publicly available. All are based largely on short-read sequencing data and come with limited functional annotation (Cantu *et al.*, 2011, 2013; Zheng *et al.*, 2013). Approximately 50% of the *Pst* genome is predicted to consist of repetitive sequences and transposable elements. In addition, the dihaploid dikaryotic genome is highly heterozygous, making contiguous fully haplotype-phased genome assemblies challenging using short-read sequencing data (Zheng *et al.*, 2013). As a result, all six draft genomes are highly fragmented and split into well over 4000 scaffolds. In comparison, the genome of *P. graminis* f. sp. *tritici* is much more connected and consists of only 392 scaffolds spanning over 90% of the estimated genome (88 Mbp) (Duplessis *et al.*, 2011). Consistent with the high fragmentation of the individual *Pst* genome assemblies, the predicted haploid genome size (*N*) varies considerably, ranging from 50 to 110 Mbp. The number of predicted protein coding genes varies between 18 000 and 25 000 genes, whereby over 50% have no functional homolog and lack any predicted function (Cantu *et al.*, 2011, 2013; Zheng *et al.*, 2013). It is unclear whether these disparities in genome size and gene number represent real biological differences between the different *Pst* isolates or whether they are rooted in the applied methodologies. Clearly, the community will greatly benefit from the resolution of these issues and from full open-access contiguous haplotype-phased reference genomes with information-rich annotations.

Nonetheless, these draft genome assemblies and associated transcriptomic studies provide the first insight into the molecular biology of *Pst.* To complete its life cycle, *Pst* needs to avoid plant immune responses and to absorb nutrients directly from the colonized plant tissue, whilst keeping its host alive (Garnica *et al.*, 2013, 2014). To achieve these goals, the fungus must actively manipulate the host plant by secreting metabolites and effector proteins (Saunders, 2015). Computational prediction pipelines estimate that *Pst* genomes encode for over 1000 candidate effectors (Cantu *et al.*, 2013; Zheng *et al.*, 2013). It is currently unknown what role any predicted candidate effector plays during the infection process of wheat or barberry. We know nothing about the molecular mechanisms mediating susceptibility and pathogenicity. We do not know the identity of any effector that is recognized by classic *R* genes, betraying the invader and triggering plant immunity. In the future, we need to understand the frequencies and identities of genetic variation of recognized effectors within *Pst* populations to contain wheat stripe rust durably (Ellis *et al.*, 2014).

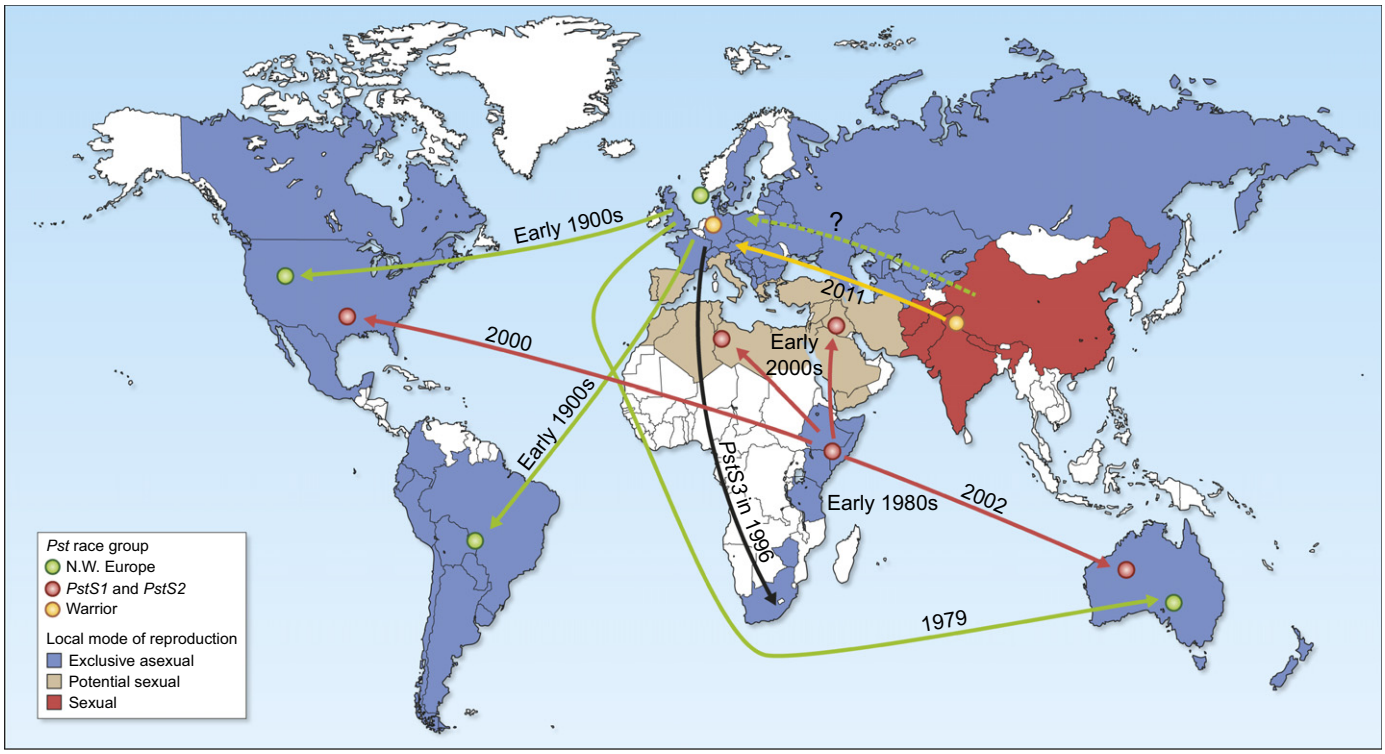

Fig. 2 Primary modes of reproduction and global pathogen migration. Origin and migration routes of globally most important *Puccinia striformis* f. sp. *tritici* (*Pst*) race groups. Predominant local modes of reproduction are depicted using specific colors. Based on, and inferred from, previous work (Hovmøller *et al.*, 2008; Wellings, 2011; Ali *et al.*, 2014a,b; Sørensen *et al.*, 2014; Beddow *et al.*, 2015; Thach *et al.*, 2016; Walter *et al.*, 2016).

VI. Novel 21st century tools to provide insight into *Puccinia striformis* f. sp. *tritici* biology

Our understanding of the molecular biology of *Pst* is in its infancy because of the difficulties posed in studying an obligate biotrophic fungus with a highly heterozygous genome. Most research has focused on the identification of the role of effectors during the infection process of wheat using heterologous expression systems in the absence of a scalable stable transformation system for *Pst*.

For example, heterologous delivery of computational predicted candidate effectors is an approach that uses the bacterial type-three delivery system to inject fungal proteins into the wheat cytoplasm to assay for potential functions, such as recognition by R proteins or suppression of plant immunity (Upadhyaya *et al.*, 2013). Similarly, the heterologous expression of candidate effectors in nonhost plants, such as *Nicotiana benthamiana*, can be a tool to study protein localization, immune suppression and the identification of interacting proteins by tandem mass spectrometry (Saunders, 2015; Petre *et al.*, 2016). By contrast, host-induced gene silencing of fungal genes targets fungal genes directly by the expression of an RNAi hairpin construct in the host, which is taken up by the fungus via an unknown mechanism (Panwar *et al.*, 2013). This leads to the temporary manipulation of *Pst* when grown on wheat, but not to stable transformation. A stable transformation system for *Pst* might well be possible, as it has been successfully applied recently to the closely related flax rust fungus (*Melampsora lini*; Lawrence *et al.*, 2010). Such a stable transformation system for *Pst* might well be revolutionary, especially when successfully combined with novel genome editing technologies, such as clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) (Saunders, 2015). This technology will be tremendously useful for the functional characterization of candidate genes identified by current sequence technologies in natural variation screens of *Pst* isolates with distinct virulence profiles (Cantu *et al.*, 2013; Hubbard *et al.*, 2015), in chemical mutagenesis screens or during segregation analysis of genetic crosses (Rodriguez-Algaba *et al.*, 2014).

Current sequencing technologies are also supercharging wheat genetics (Borrill *et al.*, 2015), classic *R* gene cloning (Steuernagel *et al.*, 2016) and concomitant pathogen identification (Hubbard *et al.*, 2015). For example, 'field pathogenomics' deduces fungal genetic variation from RNA expression data of infected wheat leaves. This reduces the time required to identify the *Pst* isolate and the infected wheat variety at the same time. In the future, the combined knowledge of plant and pathogen genotype and traditional *Pst* virulence profiling will accelerate the identification of virulence-causing mutations (Hubbard *et al.*, 2015).

VII. Conclusion

Using 21st century molecular tools, researchers have generated the first blueprints of the *Pst* genome (Cantu *et al.*, 2011, 2013; Zheng

et al., 2013), and have identified past migration routes, population structures, reproduction modes and patterns of evolution (Ali *et al.*, 2014a,b; Hubbard *et al.*, 2015; Hovmøller *et al.*, 2016; Thach *et al.*, 2016; Walter *et al.*, 2016). In addition, current sequencing technologies have accelerated *Yr R* gene cloning from wheat (Ellis *et al.*, 2014; Borrill *et al.*, 2015; Steuernagel *et al.*, 2016) and have laid the foundations for in-field pathogen identification (Hubbard *et al.*, 2015). To build on these foundations and to fulfill their potential, the community needs to collaborate synergistically, whilst playing to individual strengths. We need openly available haplotype-phased contiguous reference genomes and a stable transformation system for *Pst*. Such fundamental advances will enable us to probe and answer important biological questions including the following. What are the *R* gene-recognized effectors? What are the moleclular mechanisms driving *Pst* evolution at nucleotide resolution? What is the contribution of each haploid genome to the evolution of new *Pst* virulence profiles? In summary, we may be able to make genuine progress on understanding the molecular means that makes *Pst* such a successful pathogen.

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