Genomics-Driven Surveillance of Brown Wheat Rust (*Puccinia triticina*)

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Abstract

Brown rust, also known as leaf rust, is caused by the obligate biotroph basidiomycete *Puccinia triticina* and is the most common rust disease of wheat worldwide. Whilst the genetic diversity of *P. triticina* has been monitored across the world through the use of Simple Sequence Repeat (SSR) and Random Amplified Polymorphism DNA (RAPD) markers, genomic data has only recently become available, opening up new avenues of research. A novel pathogen surveillance technique, termed 'field pathogenomics', has been developed for the closely related plant pathogen *Puccinia striiformis*, which allows rapid detection of pathogen variants directly from infected wheat samples taken from the field through transcriptomic sequencing of infected material. In this study, a genomic approach was used in combination with the field pathogenomics technique to characterise the European brown rust population and genetic diversity. This involved genomic sequencing of 32 UK brown rust isolates from the years 2006-2015 to characterise the UK brown rust population in the years prior to this project, accompanied by transcriptomic sequencing using the field pathogenomics technique of 73 European field isolates from the 2017 and 2018 growing seasons. Phylogenetic and population genetic analysis of 105 brown rust isolates illustrated that they are all closely related with very little genetic diversity. This study does not find separate populations of *P. triticina*, as have been previously categorised through the use of SSR markers, but demonstrates that the genetic information used to assign samples to population groups must be considered when looking at population genetics of a clonal, highly genetically similar, organism.

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And special thanks to my long suffering partner. Let's get a dog.

Introduction

Wheat is a huge contributor to global food security

An increasing population, demand for a better diet, and the prospect of global warming means that food security is a great challenge the world is facing in the years to come (Godfray *et al.*, 2010).

Wheat is the third most produced global cereal and grown on more land area than any other commercial crop in the world (O'Driscoll *et al*., 2014). This crop considered the most important cereal crop in the Northern Hemisphere, Australia and New Zealand, and is grown on all continents (Oerke and Dehne, 2004).

Worldwide, around 215 million hectares of land annually produce over 8700 million tons of wheat, and by 2050 we will need to grow 60% more wheat than today to meet growing consumer demands (Randhawa *et al*., 2019). Wheat production is also particularly important in the UK, with 16.4 million tonnes of wheat harvested from plants grown across 1.8 million hectares in 2015, giving a provisional economic output of £2.8 million, which is higher than any other cereal crop (DEFRA, 2015).

Wheat rusts threaten global food security

Pre-harvest pests result in significant crop losses, with a global average of 35% of crop yields lost, and up to 70% in some developing countries (Popp and Hantos, 2011). The total potential global loss due to pests has been estimated at up to 50% for wheat (Oerke, 2006), with a loss potential of 16% estimated for fungal and bacterial pathogens alone (Oerke and Dehne, 2004). As wheat crop productivity increases, the occurrence and impact of pathogenic microorganisms increases, meaning their relative importance increases with higher productivity (Oerke and Dehne, 2004). This means that pathogenic microorganisms remain of particular importance to crops here in the UK, as the UK is the 5th highest country for reported wheat yield in recent years (Figure 1).

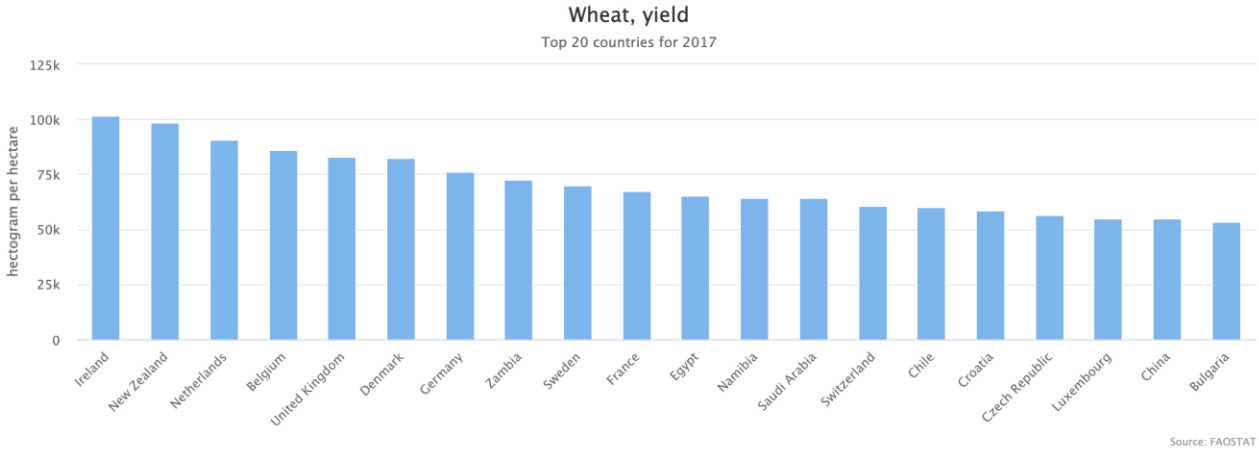

Figure 1 – Top 20 wheat yielding countries in 2017. Figure reproduced from factfish.com.

Crop protection strategies can help to reduce the overall loss potential, with a reduction of total potential losses from 50% down to 29%, and a reduction from a 16% loss due to pathogens down to 10% (Oerke and Dehne, 2004), however the more we understand these pathogens, the more we can aim to reduce losses to a minimum.

The wheat rusts, comprising of the species *Puccinia triticina*, *P. striiformis* f.sp. *tritici* and *P. graminis* f.sp. *tritici*, pose a significant constraint to increased wheat production (McIntosh *et al.*, 1995) and are considered among the most important economically damaging diseases of cereal crops (Hodson, 2011). The impact of these crop pathogens has been highlighted by the development of the Borlaug Global Rust Initiative (BGRI). This consortium was set up in 2008 to co-ordinate efforts to combat these rust pathogens following the outbreak of the Ug99 race of stem rust in Uganda in 1999 (McIntosh and Pretorius, 2011).
Additionally, in 2012 these three plant pathogens were voted third place in a 'Top 10' list of fungal pathogens in molecular plant pathology, with plant mycologists particularly accentuates the importance of the emergence of the stem rust race Ug99, and epidemics of stripe rust attributed to new, more aggressive races adapted to warmer temperatures (Dean *et al.*, 2012).
The main methods of wheat rust control are through the use of fungicides, and through breeding wheat varieties with durable resistance genes (McIntosh *et al.*, 1995). Wheat rusts can be difficult to culture in the lab due to their obligate biotroph nature (Cuomo *et al*., 2011), requiring time-consuming culturing methods on the host plant. This has slowed research progress that aim to apply genomic technologies to surveillance and diagnostic techniques.

Brown rust – An economically important pathogen

Brown Rust, also known as Leaf Rust (*Puccinia triticina*, abbreviated to PTT) is the most common rust disease of wheat (Huerta-Espino *et al*., 2011) and is present in all wheat production areas in the world (Aoun *et al*., 2019). This biotrophic basidiomycete pathogen results in about 10% annual losses globally (Dean *et al*., 2012), although losses can be more severe (30% or more), particularly when the flag leaf is infected before anthesis (Roelfs, 1992). This pathogen generally infects crops late in the season, causing losses in yield due to lower kernel weights and a decrease in the number of kernels per head (Huerta-Espino *et al*., 2011). This can result in economic losses of over £20 million in epidemic years (Bolton *et al*., 2008). Severity of brown rust is mostly reported in wheat-growing areas with warmer and hot climates such as the steppes of Central Asia and the Great Plains in North America, as well as warm and humid climates such as South America, the coastlines of North America and the Mediterranean (Liu *et al*., 2014). In the UK, warmer summers have led to the presence of brown rust later in the season, which can be serious on susceptible wheat varieties (Hubbard *et al*., 2015b). This means that the threat of brown rust in the UK could increase in future with the impact of global warming. Predicting and combatting the spread of brown rust is a prospective target to ameliorate crop losses to pre-harvest pests.

Life History of a Cereal Killer

Brown rust is an obligate biotroph of hexaploid common wheat (*Triticum aestivum* L.,), tetraploid durum wheat (*T. turgidum* L.), and wild emmer wheat (*T. dicoccoides* L.), characterised by brown uredinia seen on susceptible leaf surfaces, and chlorosis on more resistant wheat varieties (Bolton *et al*., 2008). Like other rust species, it has a heteroecious life cycle, with two taxonomically unrelated hosts. The life cycle of PTT is macrocyclic, with five distinct stages of teliospores, basidiospores and urediniospores on the wheat host, and pycniospores and aeciospores on the alternate host (Figure 2) (Bolton *et al*., 2008, Huerta-Espino *et al*., 2011). Sexual pycniospores are spread by insects attracted by nectar surrounding the pyncium, (Kolmer, 2013) and asexual urediniospores can be spread thousands of kilometres by wind (Brown and Hovmoller, 2002). Wheat is the asexual host, upon which dikaryotic urediniospores can reproduce clonally and cycle continuously. Brown rust has two known alternate hosts, required for completion of the sexual cycle. In contrast to the other two wheat rust species, which share common berberry (*Berberis vulgaris*) as an alternate host (Jin *et al*., 2010, Fetch and McCallum, 2014), the sexual cycle has of brown rust has been reported on two *Ranunculaceae* alternate hosts . *Thalictrum speciosissimum* is found in southern Europe and southwest Asia, (although other *Thalictrum* species can be found worldwide and many have been introduced to new areas outside their natural range) and *Isopyrum fumaroides,* can be found in East Asia and the Russian Far East (Bolton *et al*., 2008, Hassler, 2017). The centre of origin for brown rust is thought to be in the Fertile Crescent region of the middle east, where the natural range of primary and alternative hosts coincide (Bolton *et al*., 2008).

While sexual reproduction of PTT has been reported on *Thalictrum* spp. in Spain, Portugal, Italy and the US (Liu *et al*., 2014), and on *Isopyrum fumaroides* in Siberia (Chester 1946), overall there is little evidence of sexual recombination in populations worldwide. Clonal reproduction via dikaryotic urediniospores is predominant, based on data from molecular markers in several population genetic studies (Goyeau *et al*., 2007, Kolmer and Ordoñez, 2007, Kolmer *et al*., 2011, Ordoñez and Kolmer, 2009b) and the lack of susceptible alternate hosts in many areas of the world (Aoun *et al*.,2019).

Despite the lack of sexual recombination, *P. triticina* populations continue to evolve asexually and have a high diversity for virulence to leaf rust resistance (Lr) genes in wheat (Kolmer 2013; McCallum *et al*. 2007; Roelfs *et al*. 1992).

Mutation has generally thought to be the major cause of the emergence of new races of PTT (Ordoñez and Kolmer 2007a). In addition, evidence of hybridisation has been observed in brown rust (Park *et al*., 1999) and could play a role in generation of new genotypes. Furthermore, parasexual recombination, via anastomosis of germ tubes, has been reported in Australia (Park *et al*., 1999) which could also contribute to the advent of new virulence phenotypes arising from clonal lineages of *Puccinia* species (Kolmer, 1996). Genetic drift of large clonal populations, migration and host selection also contribute to the genetic diversity of *P. triticina* populations (Aoun *et al*., 2019).

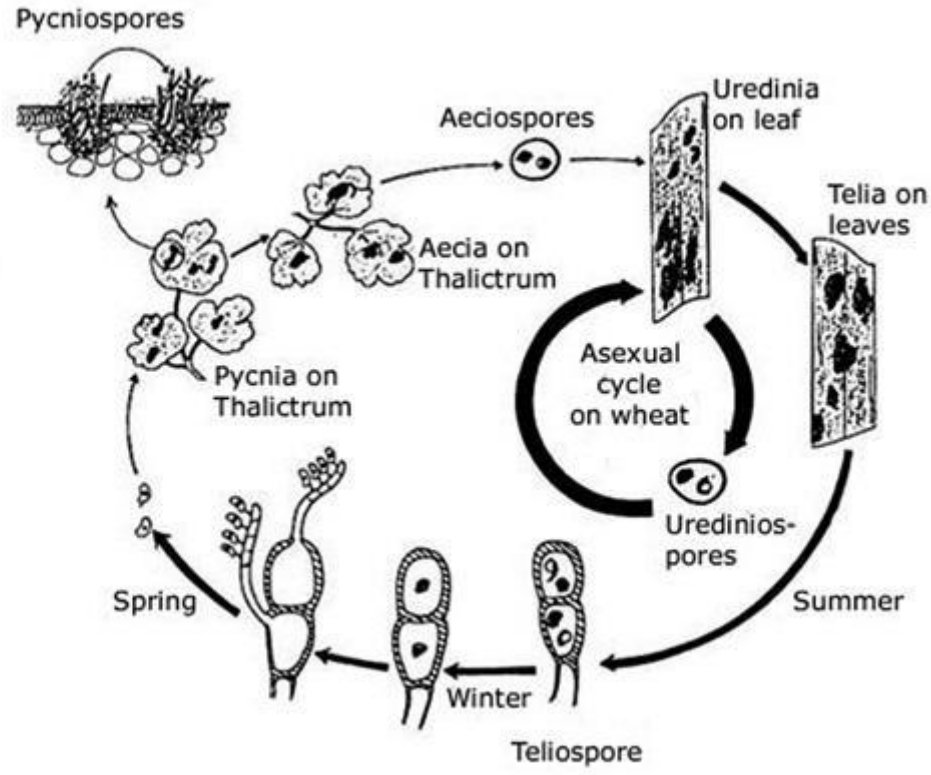

Figure 2 – Life cycle of *Puccinia triticina*, feature the asexual clonal cycle on wheat, and the sexual cycle on the alternate host *Thalictrum*. Figure from Huerta-Espino *et al*., 2011.

Pathogen Resistance is Genetically Controlled

Currently, the most effective and economical method of controlling brown rust is through the deployment of resistant wheat cultivars. To date, there are 77 named and characterised leaf rust resistance genes (Lr genes) (Randhawa *et al*., 2019). Wheat pathogen resistance genes are found in two forms.

With major resistance genes, also known as *R* genes, race specific or seedling resistance, races can be easily be determined and there is an obvious differential reaction (Dubin and Brennan, 2009). Major gene resistance works by a gene-for-gene interaction, comprising of effectors released from the pathogen, and plant cell receptors recognising certain effectors (known as *Avr* genes) to trigger a defence response, often in the form of cell death of the infected cell to prevent pathogen spread (McDonald and Linde, 2002). Most major resistance genes code for intracellular immune receptor proteins of the nucleotide-binding leucine-rich-repeat (NB-LRR) class of proteins (Dodds and Rathjen, 2010). This form of protection is only effective against members of the pathogen population that have the recognised effector (Burdon *et al*., 2014).

Partial resistance can take the form of adult plant resistant genes, minor resistance genes, or partially expressed *R* genes. Incompletely expressed *R* genes are still race-specific, but adult plant resistance genes and minor resistance genes are not race specific (Burdon *et al*., 2014). Partial resistance tends to have a smaller effect and may not provide strong resistance alone, and can allow some rust sporulation, although it takes more inoculum, a longer time and more

favourable conditions for rust to develop (Dubin and Brennan, 2009). These genes of small, additive effects can be built up to higher levels of resistance and can remain effective decades after initial deployment (Pasam *et al*., 2017) although they can be difficult to detect (Dubin and Brennan, 2009) and need to be used in combination with other major resistance genes or at least 3-4 partial resistance genes to retain acceptable levels of crop protection (Pasam *et al*., 2017). Partial resistance has been seen as 'durable', primarily as it does not exhibit the 'boom and bust' cycles of specific resistance genes, and it applies different selection pressures than specific resistance genes, however there is evidence for increased aggressiveness and adaptation by pathogens to partially resistant hosts (Burdon *et al*., 2014), highlighting the need for pyramiding multiple genes into new varieties to maintain durable resistance to disease.

These control measures place strong selection pressures on crop pathogens, which often result in 'boom and bust' cycles of repeated losses of effective control measures as these are overcome by the pathogen (Burdon *et al*., 2016, McDonald and Linde, 2002). This is due to the fact that avirulence genes in *P. triticina* and the corresponding resistance genes in wheat interact in a gene-for-gene relationship (Samborski and Dyck, 1976). Therefore, when a single major resistance gene becomes widespread across an area, (the 'boom' part of the cycle), then the resultant selection pressure on the pathogen encourages it to overcome the resistance gene (causing the 'bust'). This has been demonstrated to occur to large scales in recent years in closely related rust species, including the emergence of the yellow rust 'Warrior' race in the UK 2011 (Hubbard *et al*., 2015a) and the stem rust Ug99 race in Uganda in 1998 (Singh *et al*., 2011). This has the potential to result in large economic losses - annual losses of $3 billion have been predicted if the Ug99 race and its derivatives become established in North Africa, the Middle East and South Asia (Dubin and Brennan, 2009).

In the case of brown wheat rust, the use of cultivars with single resistance genes to *P. triticina* in Australia between 1938-1964 was followed by higher incidences of pathotypes with corresponding virulence (Park *et al*., 2001), and here in the UK use of brown rust resistance genes in the wheat variety Glasgow led to the emergence of the 'Glasgow' race of brown rust in 2005 which carried virulence for that resistance gene (Hubbard *et al*., 2018).

One of the main ways to deploy gene resistances to alter the selection processes on pathogen populations is to pyramid several resistance genes in one cultivar so that any pathogens would have to undergo a series of mutations to overcome the multiple resistance genes, rather than just one (McDonald and Linde, 2002). This has proved successful on experimental lines of rice against the rice blast pathogen , *Mangaporthe oryzae*, using four minor resistance genes (Fukuoka *et al*., 2015).

However, mechanisms still remain for pathogens to overcome even multiple pyramided resistance genes. Multiple virulence could arise through a series of independent mutations including deletion mutations (Timmis *et al*., 1990), loss of Avr-genes particularly those recognised by several R genes (Petit-Houdenot and Fudal, 2017), somatic recombination, transposable elements and suppressor genes able to affect multiple virulence loci (Mundt, 2018). Additionally, many major resistance genes can allow for low levels of pathogen

reproduction, offering the possibility for a pathogen to sequentially develop virulence instead of via independent simultaneous mutations (Brown, 2015)

Therefore, it is imperative that plant pathogens are closely monitored to ensure we have current knowledge on their virulence profiles and can reliably inform food producers on suitable wheat varieties to grow.

Surveillance of Wheat Rust

The fact that novel pathogens and pathotypes can diversify and spread so expeditiously in agro-ecosystems means a rapid, simple diagnostic technique can be highly useful to identify and track new outbreaks.

Wheat rusts have grown in concern in recent years as new races have evolved to overcome host resistance. Stem rust (*Puccinia graminis* f. sp. *tritici*) has had a particular focus due to the devastating outbreaks in Africa of the stem rust race Ug99 (Dubin and Brennan, 2009), and has also been noted to have the potential for re-emergence in the UK following a series of recent outbreaks in Europe and the identification of a single stem rust isolate in the UK for the first time in nearly 60 years (Lewis *et al*., 2018). Yellow rust (*Puccinia striiformis*) has also become an increasing concern in recent years, due to new races emerging that are adapted to warmer temperatures, are more aggressive and with virulence to more resistance genes than previously

Year	Variety	Key Resistance Gene Combination
1973*	Sappo	*Lr20* (WBR3)
1973*	Maris Halberd	*Lr20* (WBR4)
1974*	Maris Fundin	*Lr17b* (WBR2)
1976	Maris Huntsman	WBR5 (APR)
1977	Clement	*Lr26* (WBR1)
1977	Sterna	*Lr3a* (WBR7)
1978	Maris Ranger	WBR8
1980	Avalon	WBR9
1982	Gamin	WBR6
1991	Slejpner	*Lr26* + APR
1993	Spark	Not specified
1994	Flame	Not specified
1995	Chablis	*Lr3a* + ?
1999	Rialto	*Lr17b, Lr26* + APR
2005	Glasgow	*Lr1*
2005	Claire	*Lr3a, Lr17b, Lr20, Lr26*, APR
2006	Robigus	*Lr28*
2006	Multiple *Lr37* varieties	*Lr1,Lr3a,Lr17b,Lr26,Lr37*
2011	Stigg	*Lr24*
2014	Crusoe	*Under investigation*

* Tested for the first time, virulence may have been present in previous years.
APR = Adult plant resistance

Table 1 – Notable *Puccinia triticina* changes seen by the UKCPVS since the survey started in 1967. From Hubbard *et al*., 2018.

characterised races (Hovmøller *et al*., 2010). Despite documented breakdowns of genetic resistance to leaf rust (Bariana *et al*., 2007, Hubbard *et al*., 2018, Table 1) PTT remains understudied compared to the other wheat rusts, with race surveys relying on phenotypic and genetic marker data.

Traditional surveillance methods are pathology based. This involves infecting wheat plants with collected isolates, which is time-consuming, can only process low numbers of isolates (<25 per year), and phenotypic information is reported up to a year after samples are taken. This means that new pathotypes arising through mutation, recombination and exotic incursions may not be detected soon after they have arisen, allowing them time to spread across susceptible host varieties (Hubbard *et al*., 2015a). Several organisations around the world monitor wheat rust populations, including the United States Department of Agriculture Research Service, the Australian Cereal Rust Survey at the Plant Breeding Institute, and the Global Rust Reference Centre. Here in the UK, the United Kingdom Cereal Pathogen Virulence Survey (UKCPVS) was established in 1967 following an unexpected breakdown of resistance in the variety Rothwell Perdix and continues to monitor the status of brown wheat rust in the UK (Hubbard *et al*., 2015b). Therefore, it provides a good resource for materials for this project.

Molecular markers have been used for the past 20 years to assess genetic diversity of *P. triticina* populations (Aoun *et al*., 2019). The first markers to be used to genotype brown rust populations were random amplified polymorphism DNA (RAPD) markers (Kolmer and Liu 2000; Park *et al*. 2000), followed by Amplified Fragment Length Polymorphism (AFLP) markers (Kolmer 2001). In addition, Simple Sequence Repeat (SSR) markers have been used to characterise populations around the world. The use of a set of 23 SSRs has resulted in the observation of a moderate correlation between virulence phenotype and SSR genotype (Kolmer 2015; Kolmer and Acevedo 2016; Kolmer *et al*. 2011, 2013; Ordoñez *et al*. 2010; Ordoñez and Kolmer 2007b, 2009). This correlation between phenotype and genotype has also been seen in multilocus genotypes of *P. triticina* isolates from across the world, and through SNP markers obtained by Restriction-Associated DNA Genotyping-by-Sequencing (RAD-GBS) (Kolmer *et al*., 2019, Aoun *et al*., 2019).

Advances in Genetic Sequencing Offer Genomic Resources for Rust Research

With the advent of more affordable genome sequencing in recent years, the genomes of the three cereal rusts have been sequenced, allowing estimation of genome sizes and revealing genomic features such as the annotation of proteins and prediction of candidate effector proteins, and opening new avenues of research, such as comparative studies. In 2011 the *P. graminis* genome was sequenced, revealing genomic features including effector-like small secreted proteins, lineage-specific gene families, and expanded families of amino acid and oligopeptide membrane transporters, all relating to the obligate biotrophic nature of *P. graminis* (Duplessis *et al*., 2011). Shortly afterward in the same year the first *P. striiformis*

genome was released and compared to the stem rust genome. This revealed a relatively high degree of identity between annotated proteins between these two species and extended regions of micro-synteny between the genomes, allowing identification of orthologous genes and early comparisons between two different wheat rust species. In addition, a draft genome of brown rust was completed using 454 sequencing by the Broad Institute, revealing the large size of the genome in line with other rust species, and a large number of repetitive elements (Kiran *et al*., 2016).

Following this, a comparative study between the genomes of all three wheat rusts highlighted the fact that all three genomes were highly heterozygous, and had expanded gene families associated with a biotrophic lifestyle (Cuomo *et al*., 2017).

Having a sequenced and assembled genome can then offer avenues for further research useful to understanding the molecular basis of the plant-pathogen interactions, which are imperative to assist development of more effecting resistance breeding strategies to achieve long-term resistance to crop pathogen. Effector proteins are useful research targets, as understanding their function and the evolutionary processes acting upon them would greatly aid our understanding of the evolution of rust fungi and their pathogenicity mechanisms. Work has been done to understand the wheat-PTT pathosystem at the molecular level, including the production of a haustorial cDNA library (Xu *et al*., 2011), the secretome prediction of six races of PTT (Bruce *et al*., 2014) and subsequent infection of these races on the host plant to investigate host expression (Neugebaeur eta l., 2018), investigation of small RNAs that may interfere with host processes (Dubey *et al*., 2019), and two proteins have been identified that induce a reduction in ß-glucoronidase expression in wheat lines with corresponding resistance genes (Segovia *et al*., 2016). However, we are yet to formally validate any predicted PTT effectors (Segovia *et al*., 2016).

In crop pathogens, the field pathogenomics technique has proved successful as a rapid diagnostic and surveillance method, using a transcriptomics approach to categorise field-collected isolates into genetic groups, helping to guide crop protection and breeding strategies. The transcriptomic data produced also has the potential to be used in further studies. This technique was pioneered in yellow rust and unveiled a shift in the UK and European population of *P. striiformis* due to multiple exotic incursions (Hubbard *et al*., 2015a). It also proved useful in monitoring a new disease outbreak of wheat blast in Bangladesh, sourcing this outbreak to a wheat-infecting South American lineage of the blast fungus *Magnaporthe oryzae* (Islam *et al*., 2016), and elucidating the population structure and differential gene expression of candidate pathogenesis-related genes of *Fusarium graminearum* in Illinois (Fall *et al*., 2019). The field pathogenomics technique has also been further expanded upon through the development of the 'PenSeq' (Pathogen Enrichment Sequencing) method by using complexity reduction methods to sequence *Albugo candida* isolates, previously very difficult to sequence due to the obstacle in obtaining sufficient DNA of the pathogen compared to the host plant due to its biotrophic nature, and the fact it cannot be cultured axenically (Jouet *et al*., 2019).

The field pathogenomics technique has been proven to allow rapid identification of new populations, allowing timely disease prevention measures that can be applied to fungal pathogens (Hubbard *et al*., 2015a, Islam *et al*., 2016, Fall *et al*., 2019). Therefore, it represents a technique readily applicable for a project that aims to both characterise and monitor populations of brown wheat rust.

Project Aims

Basis for Project

The basis for this project is the successful application of the field pathogenomics technique to several plant pathogens, including yellow rust and powdery mildew (Hubbard *et al*., 2015a, Islam *et al*., 2016, Fall *et al*., 2019, Jouet *et al*., 2019). Brown rust presents a major threat to wheat crops worldwide and the field pathogenomics technique could be a useful tool in the surveillance and population analysis of this plant pathogen. Through a collaboration with the UK Cereal Pathogen Virulence Survey and the company BASF, we have access to brown rust samples from the years prior to this project and the growing seasons throughout the duration of this project. This allows the genotyping of multiple samples representing different growing seasons, and from different geographic locations, allowing the investigation of UK and European brown rust populations both spatially and temporally.

Aims and Objectives

The long-term objective of this research project is to explore the diversity and population structure of brown rust in the UK and Europe. Using DNA extracted from samples collected in the years prior to this project, and RNA extracted from isolates collected during the growing seasons of the course of the PhD using the field pathogenomics technique, we can compare genetic data of isolates within and between each other to elucidate evolutionary similarities and differences. Ultimately the aim is to investigate the past and current population structure of UK and European brown rust, if the changes seen in the virulence profiles of isolates collected in the UK by the UKCPVS in the years 2006-16 represent a shift in the genotypes of brown rust, and if so, if we are able to identify these potential genotypes.

Methods

Historical Isolates

45 isolates from across the UK were chosen from samples originally collected in the field in the years 2006-2015 for the UKCPVS. Infected wheat leaves were received from wheat growers, agronomists and trials operators for the wheat Recommended List trials. The majority of samples are from Cambridgeshire (17 samples) and Lincolnshire (7 samples) (Figure 3, Supplemental Table 1). Samples originate from 26 different host varieties, plus two samples with hosts of an unknown wheat variety.

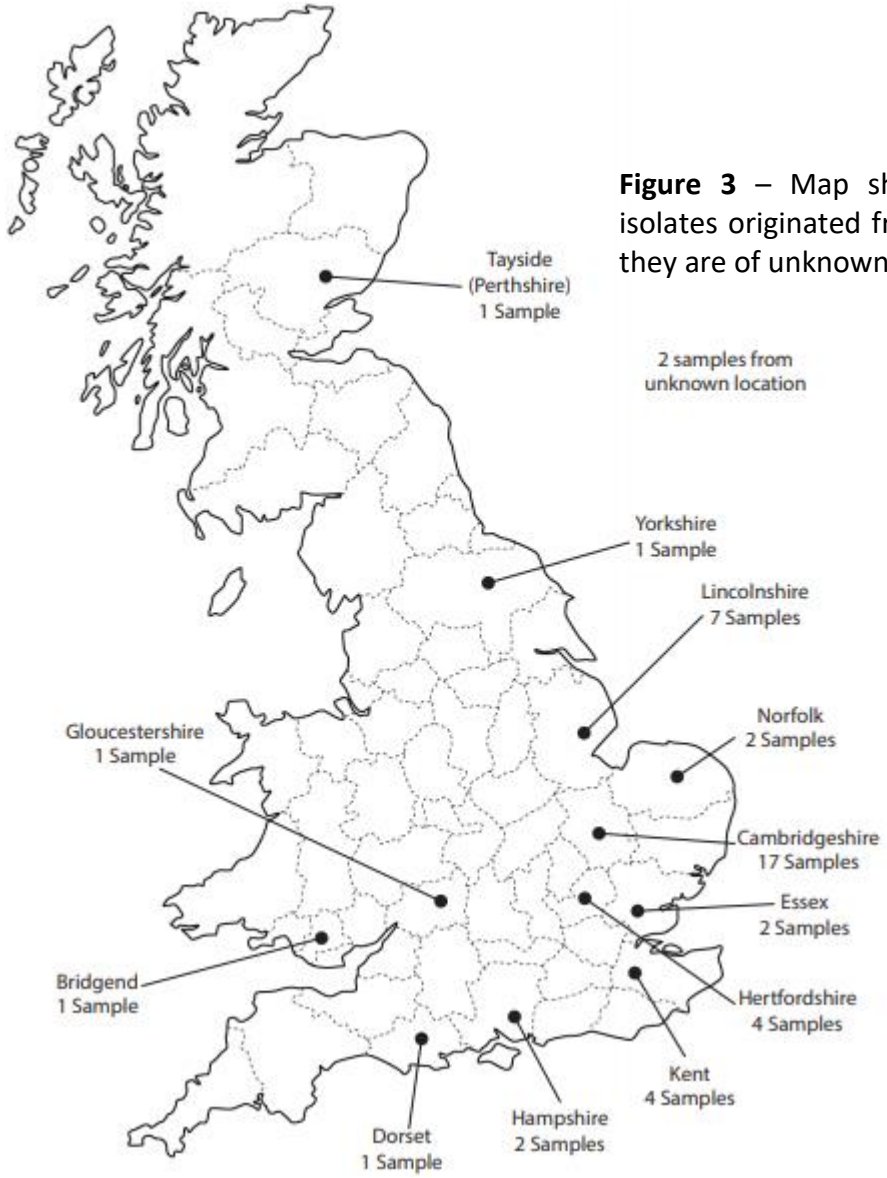

Figure 3 – Map showing counties the historical isolates originated from. 2 isolates are not listed as they are of unknown providence.

Obtaining Spore Material of Historical Brown Rust Isolates

The 45 historical isolates were received from NIAB as dried spore samples stored at -80°C. It was unknown whether they consist of a single genotype, so it was necessary to obtain single pustule isolates in order to be sure that a single genotype is sequenced. Subsequently isolates then needed to be multiplied until sufficient spore quantities were available for DNA extraction (~1000mg). Spores stored at -80°C were heat shocked at by placing sealed Eppendorfs containing the spores used for inoculation in a water bath at 40°C for five minutes.

Infection was achieved by suspending a quantity of spores (~0.5mg spores per isolate) in a small amount of Novec 7100 (Sigma-Aldrich) and applying the spore suspension to the plant using a hobby airbrush and air compressor. Newly inoculated plants were placed in a dark room in seal zip-tied autoclave bags with 100% humidity at 10-12°C for 48 hours. After incubation, plants were isolated by enclosing the plant pot with cellulose bags that allow gas exchange. Plants were then grown in glasshouse conditions at the John Innes Centre, Norwich, UK. To obtain a single genotype of rust, at first pustule emergence 5-8 days after inoculation, one single infection lesion was cut from the leaf and physically rubbed onto the leaves of an

approximately two-week old wheat seedling (variety Armada). This is to maximise the amount of inoculum, as typically there were low amounts of spores from single early pustules. The plant was then incubated in a dark room at 100% humidity at 8-10°C as above. Following single pustule isolation, spores were multiplied by collecting fresh spores from previous infections (approximately 2-4 weeks after inoculation) and then sprayed onto armada variety wheat seedlings using a suspension of Novec 7100 and spores as above. When sporulation occurs, cellulose bags were folded over to ensure that when spores drop off from lesions they are collected at the bottom of the bag. After 2-4 weeks after inoculation, spores were harvested by using gravity to funnel them into a corner of the bag, which is then cut off using scissors and spores were collected in a 1.8ml Eppendorf tube. Fresh spores for inoculation of new plants were stored for up to five days at 4°C. Spores not used for new inoculations were dried by placing in a desiccator filled with silica gel beads for 48 hours, then stored at -80°C (Figure 4).

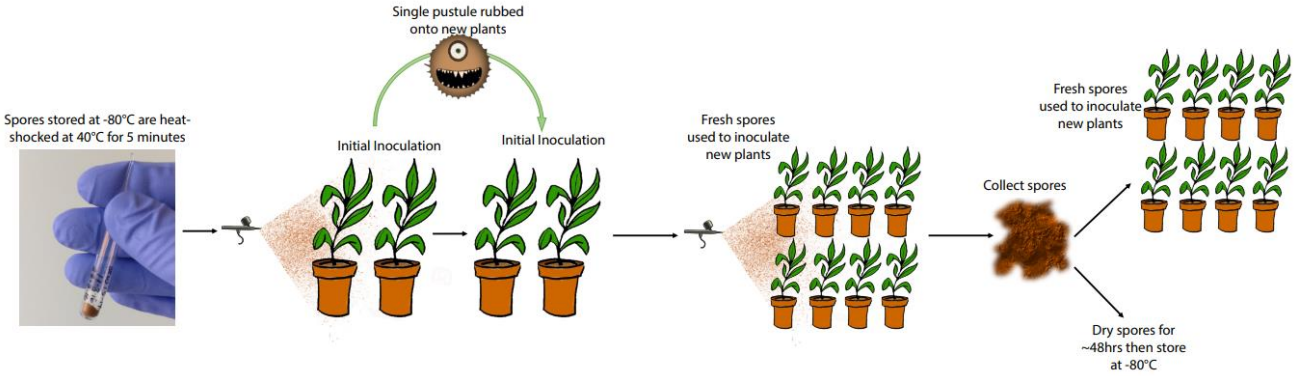

Figure 4 – Schematic showing the process of multiplying a historical brown rust isolate from stock spores.

Overall, a total of 32 isolates were genome sequenced. One isolate (08-09) was bulked and DNA extracted, but failed to yield high quality sequences. Two isolates (06-094 and 08-015) were inoculated twice but failed to grow and are assumed to be unviable, and so were excluded from the study. The remaining 10 isolates were not multiplied to a sufficient spore quantity for extraction in the time course of this study, but single pustule isolates have been obtained and can be used for future studies.

Whole-Genome Sequencing of Historical Brown Rust Isolates

DNA was extracted from the 32 isolates with sufficient spore material using a modified CTAB method as described by (Chen *et al*., 1993), and quality checked and quantified on the Qubit 2.0 fluorometer (Thermo Fisher Scientific, Paisley, UK). DNA was then sent to Genewiz (South Plainfield, New Jersey, US) for sequencing, using their standard library preparation (Illumina TruSeq DNA kit) and subsequently sequenced on the Illumina Hiseq (Illumina, San Diego, California, US).

Transcriptome Sequencing of Brown-Rust Infected UK and European Field Isolates

Brown-rust infected samples were collected by the UKCPVS and the partner company BASF during the growing seasons of 2017 and 2018. In 2018, 57 samples were received from eight countries (UK, France, Belgium, Germany, Poland, Denmark, Switzerland, Netherlands). 37 of these were RNA sequenced, representing all countries sampled. In addition to this, the isolate 17-028, which failed to result in good sequencing reads in 2017, was re-extracted and sequenced in 2018. Supplemental Table 3 contains a full list of all isolates sequenced.

All isolates consisted of leaf samples of brown rust infected wheat, collected in the field and stored in the nucleic acid stabilisation solution RNA-later® (Thermo Fisher Scientific, Paisley, UK), transported at room temperature and stored at 4°C on reaching the lab at the John Innes Centre in the UK.

RNA was extracted from all samples using the Qiagen RNeasy plant mini kit (Qiagen, Manchester, UK) according the manufacturer's protocol and assessed on the Agilent 2100 Biolanalyzer (Agilent Technologies, CA, USA) to examine the quantity and quality of RNA extracted. Extracted RNA was sent to Genewiz (South Plainfield, New Jersey, US) for sequencing, where complementary DNA libraries were prepared with polyA selection using the Illumina TruSeq RNA Sample Preparation Kit (Illumina, CA, USA) and subsequently sequenced on the Illumina Hiseq 2500 (Illumina, CA, USA).

Turkish Samples

In addition to this, 12 samples from Turkey were received. As the alternate host of brown rust has been reported in Turkey (Kolmer *et al*., 2011), it was important to include these in order to check if we could detect any signs of sexual reproduction. These samples were sent as dried leaves in paper envelopes and transferred to storage in RNA-later at 4°C on arrival at the John Innes Centre in the UK. Samples had RNA extracted and nine were sequenced according to the method stated above (Table 2). These nine were chosen as they had the best quality RNA out of the samples extracted as assessed on the Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA).

New Zealand Samples

After initial analyses indicated high levels of genetic similarity in the European population of brown rust, we obtained 12 samples from New Zealand as spores in order to explore the genetic diversity of brown rusts outside of Europe (Table 3). These samples were inoculated onto the susceptible wheat variety Armada. Two samples failed to grow, but the other 10 successfully grew to sporulation. Samples were collected from these 10 and stored in RNA-later solution as for the UK and European field isolates. These 10 were subject to RNA extraction using the same method as the UK and European field isolates. Of these, four were RNA-sequenced to the same methods as the UK and European field isolates, chosen as they had the best quality RNA out of the 10 samples processed as measured on the Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA.

Isolate	Infective on:	Location	Province	Sequenced?
SN:1	Lr8	Foça	Izmir	Yes
SN:2	Lr28	Bergama	Izmir	Yes
SN:3	Lr58	Biga	Çanakkale	Yes
SN:4	Lr62	Manyas	Balikesir	Yes
SN:5	Lr64	Manyas	Balikesir	No
SN:6	Lr67	Gönen	Balikesir	Yes
SN:7	Lr69	Biga	Çanakkale	No
SN:8	Lr70	Gönen	Balikesir	Yes
SN:9	Lr73	Bigadiç	Balikesir	Yes
SN:10	Lr77	Biga	Çanakkale	Yes
SN:11	Lr81	Honaz	Denizli	No
SN:12	Lr83	Honaz	Honaz	No
SN:13	Lr85	Menderes	Izmir	Yes
SN:14	?		Izmir	Yes

Table 2 – Table showing list of Turkish isolates in this study, the Lr gene they are able to infect (pers. Comm), and the location and province they were collected from where known. All were collected in 2018.

Isolate	Pathotype	Year of first record	Sequenced?
00/1A	104-1, 3, 4, 6, 7, 10, 12 + Lr37	2000	Yes
14/17A	53-1, (6), (7), 9, 10, 12	2015	Yes
14/18A	76-1, 3, 5, 7, 9, 10, 12, 13 +Lr37	2014	Yes
14/27A	??-3, 4, 7, 9, 10, 12	2014	Yes
00/2A	??-7, 10	2000	No
12/01A (Mackellar type)	10-1, 3, (6), (7), 9, 10, 11, 12	2012	No
12/01B (Mackellar standard)	10-1, 3, (7), 9, 10, 11, 12	2012	No
12/01C	104-1, 3, 4, 6, 7, 9, 10, 12 +Lr37	2012	No
14/13	??-1, 3, (7), 9, 10, 12	2014	No
14/41A	76-3, 5, 7, 9, 10, 12, 13 +Lr37	2014	No
WLR-2	76-1, 3, 10, 12	1986	Failed to grow
14/12	104-1, 3, 4, 6, 7, 8, 9, 10, 12 +Lr37	2014	Failed to grow

Table 3 – Table showing pathotype and year of first record for 12 isolates obtained from collaborators in New Zealand.

Australasian Samples

We further expanded our set of isolates by including publicly available isolates from Australasian from Wu *et al*., 2017 (Table 4). These are useful to include as they were originally collected in years prior to this study (ranging from 1974-1991) and can represent an older, more geographically distant dataset compared to the UK and European isolates sequenced in this study.

Sequencing No	Isolate No	no of Bases (G)	Size (Gb)	Date	Location	Territory
SRR4254441	**760285**	5.2	3.5	1976	Coorangy	Queensland
SRR4254442	**630846**	7.1	4.7	1980	Gurley	Fairall
SRR4254443	**750299**	7.3	4.9	1976	Wandoan	Queensland
SRR4254444	**700201**	7.1	4.7	1974	QWRI	Queensland
SRR4254445	**740408**	7.6	5	1975	Biloela	Queensland
SRR4254446	**740606**	7.5	5	1975	Narrabi	New South Wales
SRR4254447	**700575**	6	4	1974	Wellington	New Zealand
SRR4254448	**QWRI**	6.3	4.2	1990	QWRI	Queensland
SRR4254449	**900084**	5.4	3.6	1991	PBI Cobbitty	Lansdowne
SRR4254450	**89-L-1**	7	4.7	1989	Castle Hill Glasshouse	
SRR4254451	**890155**	6.9	4.6	1990	Tamworth	
SRR4254452	**900273**	6.6	4.4	1991	Mt Ridley	
SRR4254453	**790197**	7.2	4.8	1979	Bookpurnong	South Australia
SRR4254454	**670028**	7.2	4.8	1980	Takaka	New Zealand
SRR4254455	**730003**	7.5	5	1974	University of New England	New South Wales
SRR4254456	**66-L-3**	7	4.6	1974	Castle Hill Glasshouse	
SRR4254457	**60-L-2**	4.8	3.2	1978	Castle Hill Glasshouse	
SRR4254458	**64-L-3**	8.9	5.9	1976	Castle Hill Glasshouse	
SRR4254459	**630550**	6.8	4.5	1974	Ravensworth	New South Wales
SRR4254460	**BCL 75**	6.2	4.1	1974	University of Sydney	

Table 4 – Table showing Size, no of Bases, date collected and location collected of the Australasian isolates included in this study from Wu *et al*, (2017).

Quality Checking Data

Both RNA and DNA data was quality checked using the fastqc package (Andrews, 2010). The ILLUMINACLIP option of the programme Trimmomatic (Bolger *et al*., 2014) was used to trim adapter and barcode sequences from all reads, and low quality base scores were filtered using a LEADING and TRAILING option of 5, SLIDINGWINDOW with a threshold value of 4 per 10 bases, and a minimum length of 40bp. Paired-end reads were then aligned to the Race 1-1 *P. triticina* genome generated by the Broad Institute (Cuomo *et al*., 2011) using bowtie2 (version 2-2.2.1) with default parameters (Langmead and Salzberg, 2012) for RNA data, and BWA with default parameters (Li and Durbin, 2009) for DNA data.

Calling Single Nucleotide Polymorphisms

Pileup from SAMtools was used to sort and index BAM files, and to identify SNPs using raw allele counts for each site (Li *et al*., 2009). For genomic data, a minimum depth of coverage of 10x was necessary for a site to be recorded, and for RNA_seq data, a minimum coverage of 20x. Sites with allelic frequencies below 0.2 or above 0.8 were counted as homokaryotic, those with allelic frequencies between 0.2 – 0.8 were classified as heterokaryotic. Allele frequencies for biallelic heterokaryotic SNP sites were calculated and then plotted using the ggplot2 package in R, which could then be used to determine whether the sample consisted of a single brown rust genotype. As brown rust is a dikaryon with two haploid nuclei per cell, the mean read counts at heterokaryotic sites should have a mode at 0.5, with each haploid nuclei being represented by one of two alternative alleles (Hubbard *et al*., 2015a). Proportions of reads per SNP different to this could be explained by either polyploidy or by mixed infections. In this study, shifted read proportions are assumed to be indicative of mixed infections due to the fact that samples are originally collected in the field, and therefore have the potential to comprise of multiple infections on a single leaf sample. Therefore, field isolates that displayed a heterokaryotic site SNP profile without a peak at 0.5 were excluded from the study. Heterokaryotic and homokaryotic SNP sites that resulted in synonymous and non-synonymous substitutions were determined using version 3.6 of SnpEff (Cingolani *et al*., 2012).

Phylogenetic Analysis

Phylogenetic analysis on 105 PTT isolates (comprising of all successfully sequenced genomic isolates RNA-sequenced isolates determined to be single-sourced from this study, and 20 Australian phenotype paired isolates from Wu *et al*., (2017) using the third-codon position of 9449 genes with ≥60% breadth of coverage was performed using a maximum likelihood model. For both DNA and RNA sequenced isolates, nucleotides residues different to the Race 1-1 BBBD reference genome were identified and recorded if they had a minimum of 10x depth of coverage for DNA isolates, and 20x depth of coverage for RNA isolates. Sites identical to the reference genome were recorded if they had a minimum of 2x depth of coverage. These sites were used to create a synthetic gene set for each individual isolate. Genes with a minimum of ≥60% breadth of coverage were selected, and the third codon position of these genes was used

to build the maximum likelihood tree using RaxML 8.0.2 (Stamatakis *et al*., 2014). Phylogenetic trees were visualised in Dendroscrope (Huson and Scornavacca, 2012)

Population Structure Analysis

Genetic variation of the single-sourced *P. triticina* field isolates, all successfully sequenced historical isolates and the 20 phenotype-paired isolates from Wu *et al* (2017) were assessed using the multivariate DAPC contained within the adegenet R package (Jombart, 2008). 51001 bialleleic SNP sites that result in a synonymous change in at least one isolate were identified, and used to summarise genetic variation within the isolates through principal component analysis. The lowest Bayesian Information Criterion (BIC) is used to divide isolates into population clusters. On this subset of data, 9-10 or 12-13 population groups were suggested (Fig 5). Due to the genetic similarity of brown rust seen in the phylogenetic analysis, DAPC was performed cautiously in case of overfitting data, and ran several times on differing numbers of population groups. DAPC was ran 12 times, separating the isolates into 2 to 13 population groups and assigning them to each potential population group.

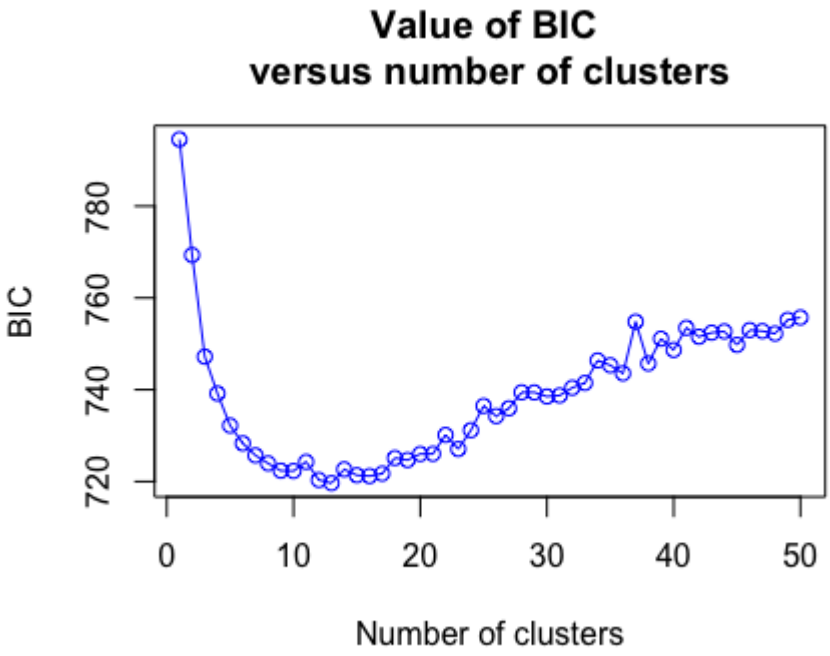

Figure 5 - Plot showing Bayesian Information Criterion value (BIC) fitted against different potential population clusters.

Assessing Diversity Between and Within Potential Population Clusters

To assess genetic diversity within all isolates, all homokaryotic and heterokaryotic SNPs determined from the above individual alignment of each isolate to the Race 1-1 BBBD reference genome were combined into a synthetic gene set for that isolate. During the DAPC analysis, it seemed that three population clusters were most logical with the resultant data (see results), and so these synthetic genes were combined for all PTT isolates within each of these three

potential population groups. Genes with ≥80% breadth of coverage for all isolates were selected, and used to calculate the nucleotide diversity between isolates within a single potential genetic group using the software EggLib, version 2.1.2 (De Mita *et al*., 2012).

To investigate genetic variance attributable to differences between populations, Wright's F_{ST} statistic, the net pairwise genetic distance between groups (D_A distance, Nei *et al*., 1983), and the absolute divergence (D_{XY}; Nei and Kumar 2000) was calculated using the synthetic gene sets produced above for each population group, using the software EggLib, version 2.1.2 (De Mita *et al*., 2012). The excess of heterozygotes, F_{IS}, was calculated using Genepop (Rousset, 2008) in order to assess the level of inbreeding in the overall PTT population.

Exploring the Effector Complement of *P. triticina*

The effector complement of *P. triticina* was investigated by obtaining the effector complement of the race 1-1 BBBD reference genome. The proteome was downloaded from Genbank, then secreted proteins were predicted using SignalP2 with default parameters (Nielsen *et al*., 1997). Proteins predicted to be secreted were further filtered by discarding proteins with a length of <50 amino acids, and inputting them to the programme TargetP (Emanuelsson *et al*., 2000), where proteins predicted to localise to the mitochondria were removed from the dataset. Proteins containing transmembrane domains were identified using the programme TMHMM (Krogh *et al*., 2001) and discarded from the dataset. The number of secreted proteins was also calculated in this way for a draft genome of *Puccinia graminis* (race SCCL, (Duplessis *et al*., 2011)) and *Puccinia striiformis* (Race 130, (Cantu *et al*., 2011)) to facilitate comparisons in the number of secreted proteins. Further exploration of the *P. triticina* putative effector complement was conducted, with cysteine content calculated as a %age from the amino acid sequence of each remaining predicted proteins (a total of 1039 proteins). Known effector motifs were also searched for in the amino acid sequence of the whole proteome using pattern matching in an in-house perl script. A list of the motifs searched for and examples of these motifs are presented in Table 5.

Results

RNA-Seq of Field Collected Infected Wheat Samples

In order to characterise the genotypic diversity of UK and European brown rust in the field, we collected a total of 132 samples of *P. triticina*-infected wheat from 9 countries. This included 75 samples collected during the Spring/Summer growing season of 2017, and 57 collected during the growing season in 2018 (Supplemental Table 1).

From these, 93 samples had total RNA extracted and were subject to RNA-seq analysis using the field pathogenomics method (Supplemental Table 2). 13 isolates were discounted from further analysis due to low read alignments to the race 1-1 BBBD reference genome (<8%). The remaining isolates had an average mapping percentage of 18.2%, with a standard deviation of 8.1% (Supplemental Table 2). This is a lower percentage of mapped reads than that found for the closely related plant pathogen yellow rust, which had an average of 36.87% (standard deviation 19.73%) of reads align to the PST-130 reference genome (Hubbard *et al*., 2015a). However, it is a higher percentage of mapped reads for the wheat blast fungus *Magnaporthe oryzae*, which ranged from 0.5-18.5% of the total reads. This proved sufficient for analyses revealing the origin of an outbreak of wheat blast in Bangladesh, including phylogenomic and population analyses (Islam *et al*., 2016), meaning the overall number of transcripts recovered in this study should be sufficient for sample analysis.

To ensure that each sample remaining was composed of a single PTT genotype without bias in allele-specific expression between the two haploid nuclei, the distribution of read counts of biallelec single nucleotide polymorphisms was calculated based on alignment to the race 1-1 BBBD reference genome. As brown rust is dikaryotic, the mean of read counts at heterokaryotic sites is expected to have a mode of 0.5, with each of the two haploid nuclei represented by two alternative alleles, with little bias in allele expression (Hubbard *et al*., 2015a). 10 RNA-sequenced isolates did not present a single peak at 0.5, so were excluded from further analysis on the assumption that these represent samples containing more than one genotype (Supplemental Figure 1, Supplemental Table 2).

Genomic sequencing of UK PTT isolates

In order to determine the genetic relationships between the field collected isolates from 2017/18 and any earlier PTT populations, the genomes of 34 UK isolates collected in the years prior to this study (2006-2015) were sequenced using an Illumina whole-genome shotgun approach. One isolate (15-014, the only isolate sequenced from the year 2015) was not sequenced to sufficient standard (mean read depth of 0.37, breadth of coverage of 18.71) and so was excluded from further analysis. To ensure that the genomic sequenced isolates were composed of a single genotype, the distribution of read counts at biallelec SNP sties was plotted, as above (Supplemental Figure 2). Following filtering, reads were independently aligned to the Race 1-1 BBBD reference genome (Supplemental Table 3).

PTT Isolates are Highly Related

To elucidate relationships between all PTT isolates included in this study, phylogenetic analysis was conducted using the third codon positions of 9449 genes (1846765 sites) with ≥60% breadth of coverage for all isolates using a maximum likelihood approach.

The most notable aspect of the phylogenetic tree produced from the maximum likelihood analysis is that the scale is very small (Figure 6), meaning that all isolates are very closely related. Brown rust is not known to undergo the sexual stage in the UK (Huerta-Espino *et al*., 2011), and has been shown to have high levels of heterozygosity, indicating clonal reproduction (Kolmer *et al*., 2017). The very low genetic diversity of the isolates indicates that these isolates are likely asexual, agreeing with the current hypothesis that brown rust largely consists of clonal populations.

The 8 samples from Turkey were obtained and sequenced after early analysis of the 2017 field isolates revealed these close genetic relationships between sequenced isolates. *P. triticina* is thought to have a centre of origin around the Fertile Crescent, and its alternate host has been reported in Turkey (Kolmer *et al*., 2011) so we obtained some isolates from that region to see if we could find some isolates with a higher genetic diversity, with the possibility of this being due to sexual recombination. The phylogenetic analysis reveals that the Turkish isolates sampled are also highly related to the other sequenced isolates, and so also imply a lack of sexual reproduction.

Isolates do seem to loosely group based around geographic location, for example, all the Australian isolates are found largely across one clade. The RNA-sequenced European isolates are found grouped together along with the Turkey isolates, which were sampled at a similar timepoint in 2018. The three sequenced New Zealand isolates are also found grouped with the European isolates, indicating a closer relationship to those isolates than those found geographically closer in Australia. The New Zealand isolates were collected in 2000, 2014 and 2015. The presence of the isolate collected in the year 2000 indicates that this might not be an overall shift in the genetic structure over time however, otherwise it would be expected to be found situated more closely to the historical UK and Australian isolates. The reference genome is found grouped with the 2017/18 field isolates, which is interesting as this sample is from America and originally collected in 1954 (Cuomo *et al*., 2017), and is therefore temporally and geographically distinct compared to the other isolates it is grouped closely alongside. The historical UK isolates are all found separate to the 2017/18 field isolates and New Zealand samples, grouping more closely to the Australian samples. Overall, the phylogenetic tree shows very close genetic relationships in the data obtained from expressed genes shared between all isolates.

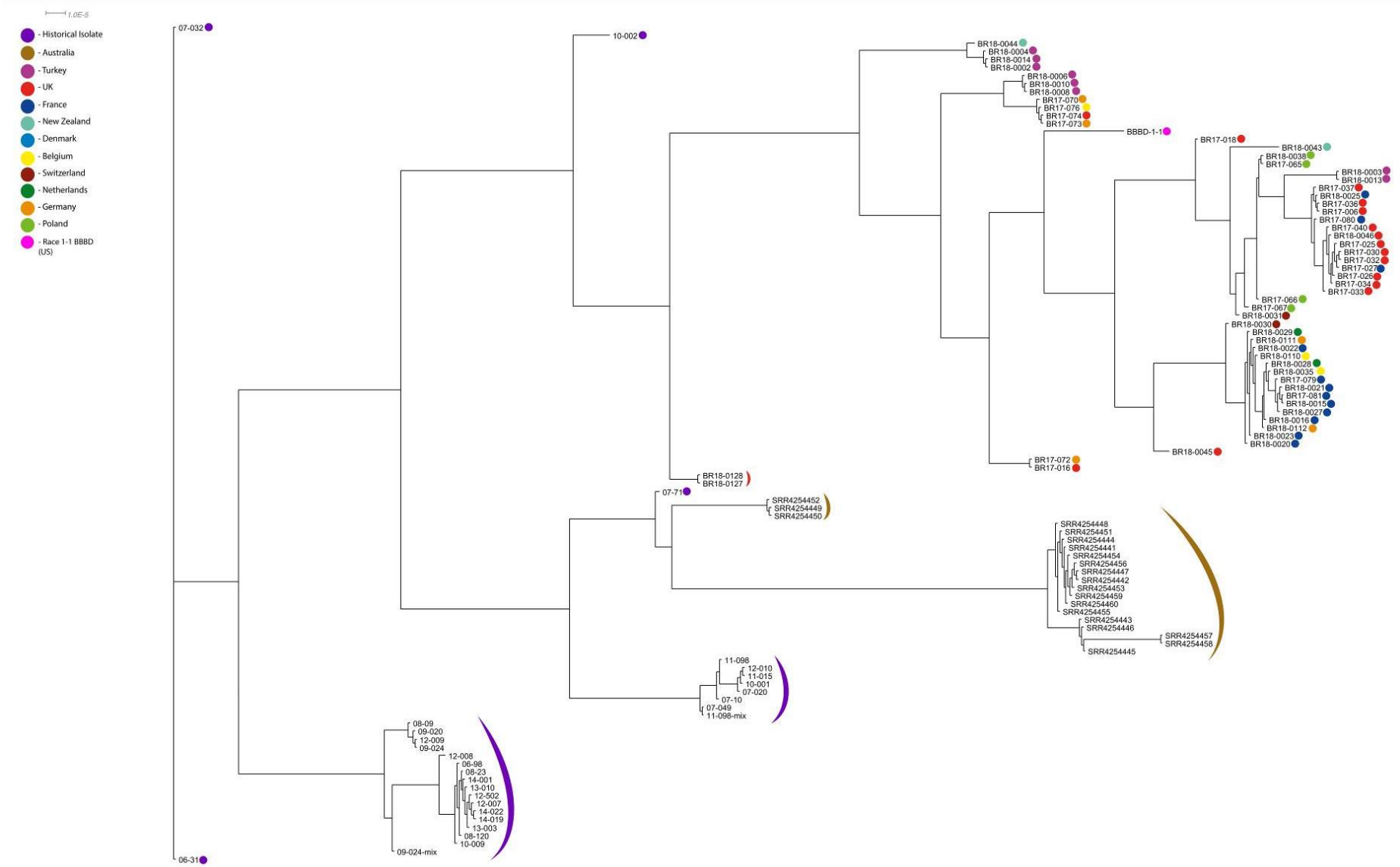

Figure 6 – Maximum Likelihood Phylogenetic Tree constructed using the third codon positions of 9449 genes (1846765 sites) with ≥60% breadth of coverage for 105 *Puccinia triticina* isolates from Europe, New Zealand, and Australia.

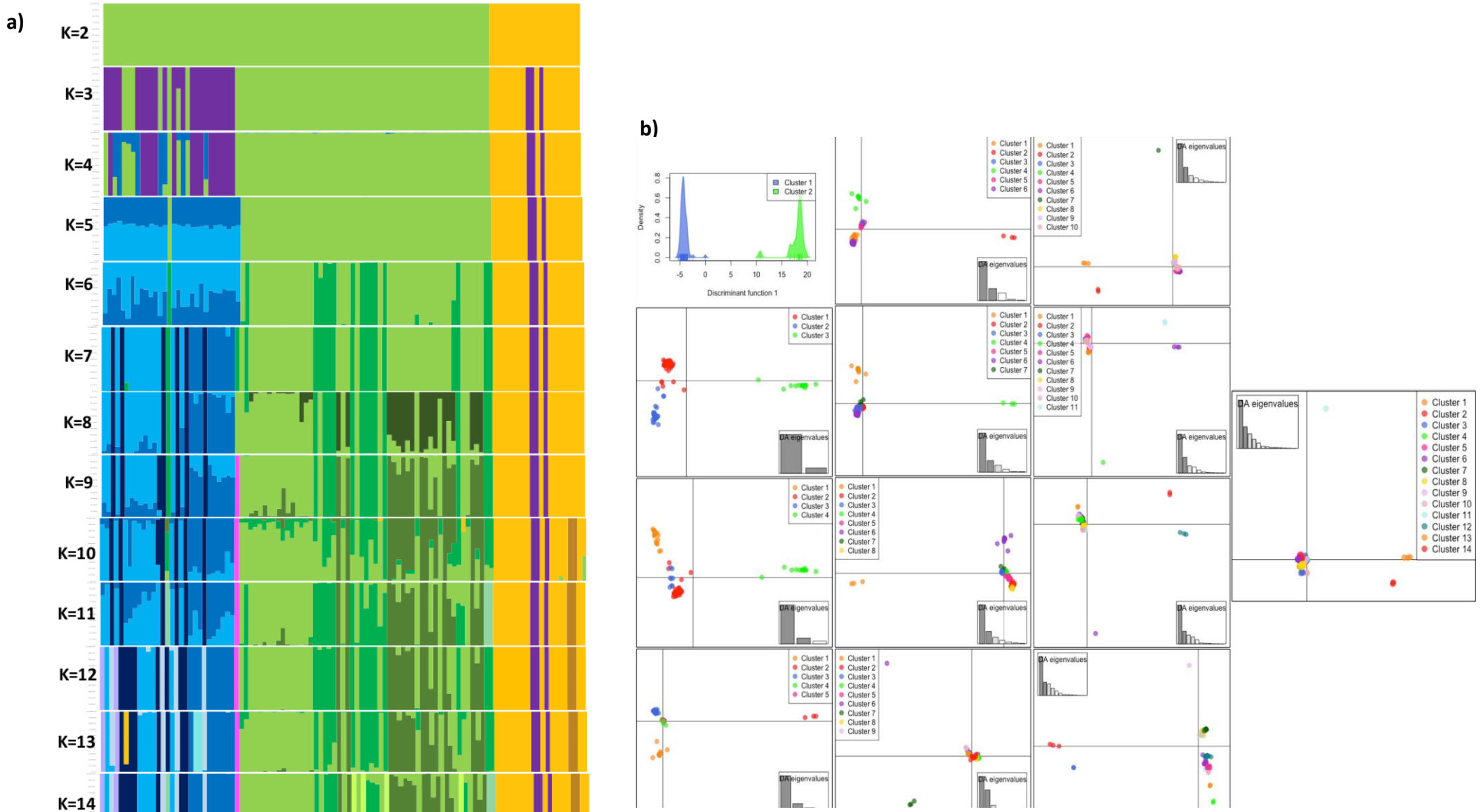

Figure 7 – DAPC results of an analysis on 105 *Puccinia triticina* isolates. 51001 bialleleic SNP sites were used to define population subdivisions at a range of K values (2-13) using multivariate discriminant analysis of principal components (DAPC). a) K values graph – bars represent estimated membership fractions for each individual. b) Scatterplots showing the first two principal components (X axis and Y axis respectively) of 105 isolates using 51001 SNP sites. Each plot shows isolates grouped into different numbers of potential population clusters. Principal component analysis shows three potential separated populations at k=3, however at other values population structure is not clearly defined.

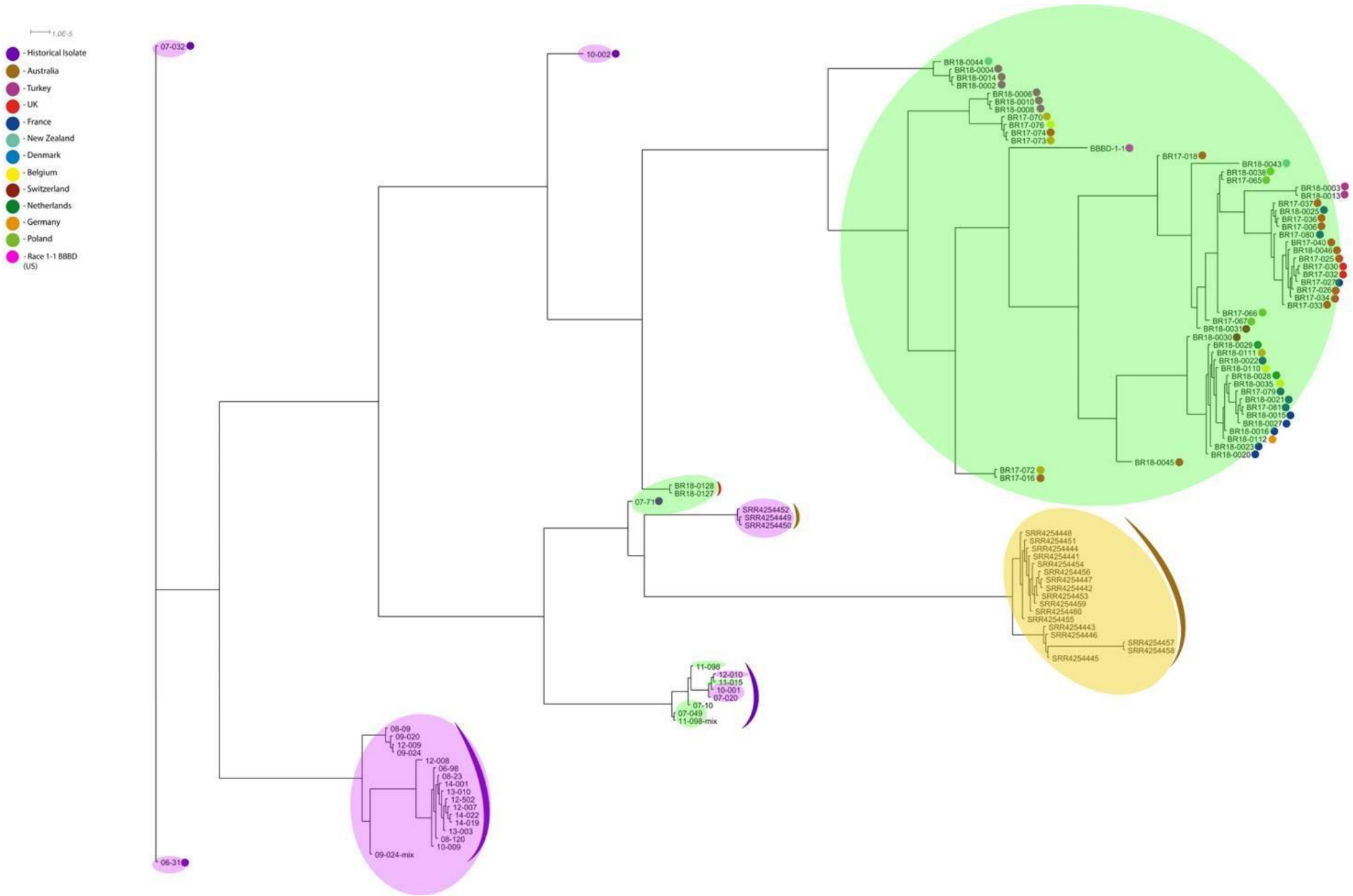

Figure 8 – Maximum Likelihood Phylogenetic Tree constructed using the third codon positions of 9449 genes (1846765 sites) with ≥60% breadth of coverage for 105 *Puccinia triticina* isolates from Europe, New Zealand, and Australia, with colours overlapped corresponding to which potential population group an isolate is assigned to when k=3.

PTT Populations are Genetically Similar

To investigate the population structure across all PTT isolates included in this study, a list of 51001 bialleleic SNP sites was produced and used in a multivariate discriminant analysis of principal components (DAPC) to define the population structure. The Bayesian Information Criterion (BIC) indicated the division of the PTT isolates into 9-10 or 12-13 population groups as the optimum clustering solution (Figure 5) However, to avoid overfitting data, DAPC was ran 12 times, on 2-13 potential population groups. When the population clustering was visualised in both scatterplot and bar form, however, it revealed that three population clusters were most clearly defined (Figure 8). These genetic groups were formed of: 1) a large group consisting of all 2017/2018 field isolates, plus the race 1-1 BBBD reference genome and 7 historical isolates from 2007, 2009 and 2011, 2) a group consisting of 17 out of 20 of the Australian phenotype-paired isolates from Wu *et al*., (2017), and 3) a group containing the remaining three phenotypic-paired Australian isolates, and 22 historical isolates from 2006-2014.

Population Statistics Support the Genetic Similarity of Brown Rust

To determine the level of heterozygosity in the PTT isolates examined in this study, the F_{IS} value for the whole PTT population was calculated, resulting in a value of -0.1817 (Table 5). A negative value of F_{IS} indicates an excess of heterozygotes compared to what is expected under hardy-weinberg equilibrium, which has been associated with asexuality and clonal reproduction (Balloux *et al*., 2003). The nucleotide diversity was calculated for each potential population group, and the population as a whole to assess genetic similarity of these groups (Table 5). The overall nucleotide diversity was very low, with a value of 5.22E-05. Similarly, the value for the green putative population group was 4.56E-05. Both of these calculations relied on a low number of genes used to calculate the statistic (Table 5), and so these values are likely to be unreliable. However, the values obtained for the orange and purple potential population groups are more in line with what is expected for this organism, with values comparable to that seen in worlwide populations of PTT, which have ranged from 0.002 to -0.0567, (Table 5, Kolmer and Ordoñez, 2007b, Kolmer *et al*., 2019).

Potential Population Group	No of Isolates in Population	Nucleotide Diversity	No of Genes	F_{IS}
Green	63	4.56E-05	273	
Orange	17	0.00326	14347	
Purple	25	0.00995	13288	
All Isolates	105	5.22E-05	352	-0.1817

Table 5 - Nucleotide Diversity for three potential population groups of *Puccinia triticina*, and for all 105 isolates included in this study.

To understand the differences between the potential population groups, the net pairwise genetic distance (D_A), F_{St} and absolute divergence (D_{XY}) were calculated (Table 6). The low D_A value indicates small differences between these populations since they theoretically split, and a

low D_{XY} suggests that there is a small average number of pairwise differences between the sequences from these populations (Cruickshank and Hahn, 2014) . These population statistics therefore indicate these potential populations are highly related with little variation between them. This backs up the genetic similarity of PTT isolates seen in the phylogenetic tree (Figure 7), and lends credence to the possibility that the three potential population groups are potentially artefacts of over-fitting data. However, values including the green potential population are less likely to be reliable, due to the low number of genes available to conduct the pairwise comparisons (Table 5). This lower number of genes is likely to be due to the fact that the green potential population is the only one that includes the transcriptomic sequenced field isolates, limiting the genetic information available to expressed genes only, whereas the other two potential populations are comprised of genomic sequenced isolates, and therefore have genetic information from non-coding regions in addition to expressed genes. Overall, the differences in genetic information available for transcriptomic data vs genomic data likely has an impact on the observed variation between these potential population groups, reducing the reliability of these results.

F_{st}		Green
	Orange	0.2469
Purple	0.1442	0.2352

D_A		Green
	Orange	0.009461
Purple	0.000349	0.008938

D_{xy}		Green
	Orange	0.02942
Purple	0.005567	0.03246

Table 6 - Table showing interpopulation statistics for the three potential population groups: F_{St}, net pairwise genetic distance (D_A), and absolute divergence (D_{XY}). These pairwise comparisons indicate that all potential groups are highly related.

Phenotype Data of UK 2017 Isolates

60 samples were collected by the UKCPVS in the Spring/Summer of 2018, of which 33 which were pathotyped as part of the cereal pathogen virulence survey. However, RNA extracted from these samples proved too poor for sequencing. The PTT isolates we have with phenotypic data and genotypic data include 17 isolates collected by the UKCPVS in 2016 (Figure 9, Supplemental Tables 5, 6) and the sequenced historical isolates. However, the 2017 field isolates were tested on a different differential set, as the UKCPVS updated their differential set in 2017 to be more in line with other virulence surveys used around the world (Hubbard *et al*., 2017). Therefore, we cannot easily compare virulence profiles between the historical isolates and 2017 field isolates, so the virulence profiles of these two datasets have been plotted separately.

Overall, many of the 2017 UK field isolates show a very similar virulence profile, with little variation between overall virulence phenotypes on the UKCPVS differential set (Figure 9). These variations consist of isolate 17-018 being infective on the wheat variety Sappo, 17-016 being able to infect the wheat variety Stigg and being borderline infective on the variety Warrior (postulated to carry the Lr28 wheat resistance gene), and 17-026 being virulent on wheat lines carrying the Lr26 resistance gene.

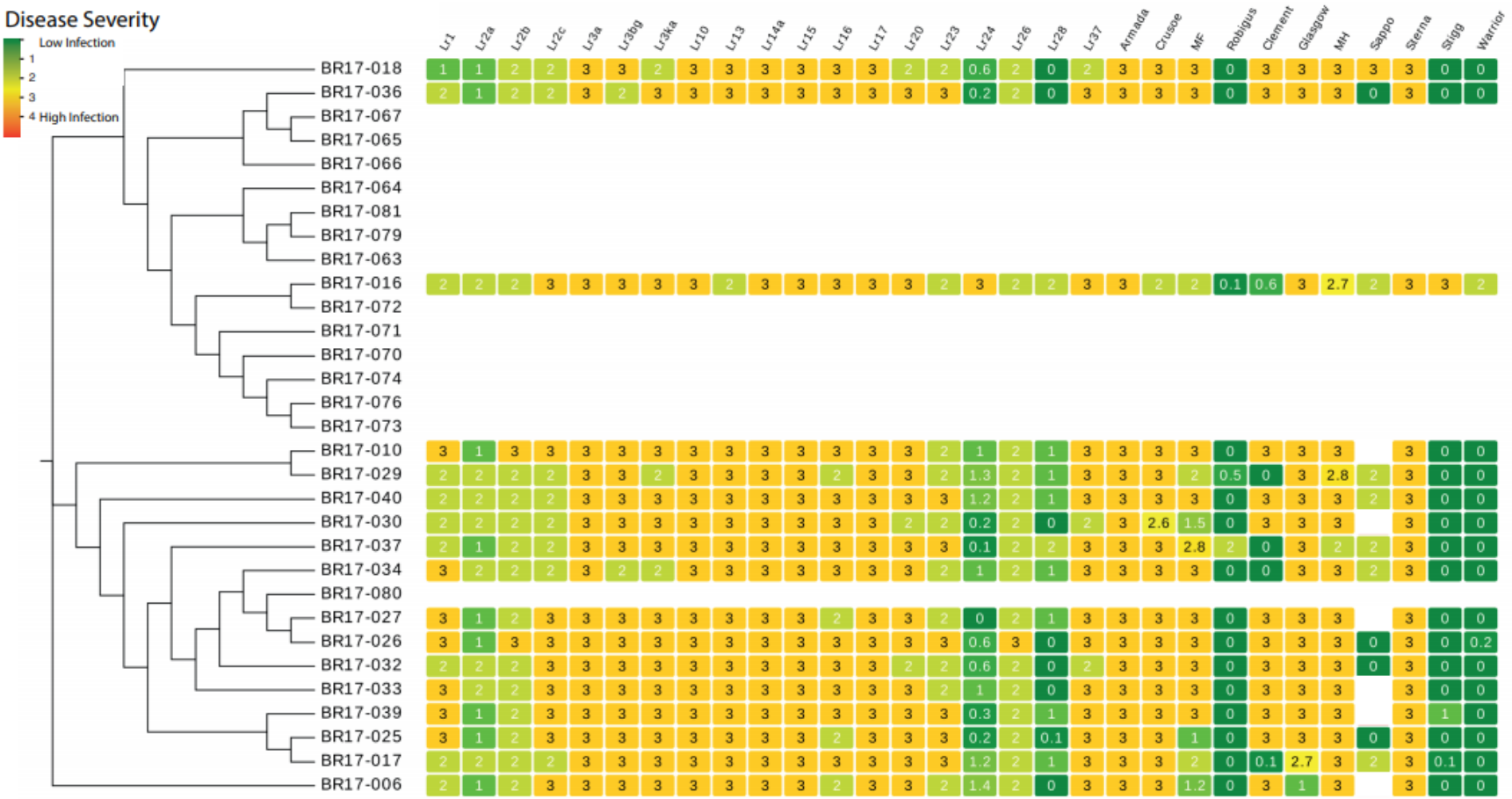

Figure 9 – Cladogram of 2017 transcriptomic sequenced UK *Puccinia triticina* isolates from the UK Cereal Pathogen Virulence Survey, showing Average Infection Scores on 30 wheat differential lines. AIC scores from Hubbard *et al.*, 2018.

Analysis of the *P. triticina* Effector Complement Identifies Small Secreted Proteins

To identify candidate effectors of PTT, the effector pipeline shown in Figure 5 and based on the pipeline of (Saunders *et al*., 2012) was used. Effectors are known to be highly evolutionary diverse and are often dissimilar to characterised proteins (Rep, 2005), so this pipeline uses multiple features to identify and classify potential effector proteins, including the presence of a signal peptide, lack of a transmembrane domain, and cysteine content.

1039 secreted proteins were predicted from the proteome of the reference genome, type 1-1 BBBD isolate. This totals 6.6% of the pathogen's proteome. This is a similar proportion of the proteome to that of the other two wheat rusts, P. striiformis and P. graminis, which had 1187 predicted secreted proteins (6.6% of the proteome) and 1310 predicted secreted proteins (7.2% of the proteome) respectively.

As most identified rust effectors are small, cysteine rich secreted proteins (Catanzariti *et al*., 2006, Ramachandran *et al*., 2016), the cysteine content of all PTT predicted secreted proteins was calculated. Cysteine content of the 1039 predicted secreted proteins varied from 0% - 10.7%, with 283 containing a cysteine content of >3%, and 133 proteins having a cysteine content of >3% and with a length of <150 amino acids.

Effector Motifs Can Assist In The Search For Putative Effector Proteins

Putative motifs in the amino acid sequence of effector genes have been identified for several plant pathogens. The *P. triticina* proteome was searched for known effector motifs in order to identify putative effector genes (Table 7). Of these known motifs, 6 had no matches to the predicted effector proteins of the Race 1-1 brown rust. The motif KRLTG has been seen in the predicted effector ps87 in the closely related plant pathogen *P. striiformis* (Gu *et al*., 2011), yet is not seen in any of the predicted secreted proteins in this study. Overall however, this is not unexpected, as effectors are known to be very diverse and are only rarely similar to characterised proteins (Rep, 2005). Two motifs were found in a single predicted protein, each as the only motif found in that predicted protein – KECxD, which is known from a PcF toxin family found in *Phytophthora* spp., (Nicastro *et al*., 2009), and RkcxxCx12H, a motif known from AVR genes in *Magnaporthe oryzae* (Yoshida *et al*., 2009). Both putative proteins containing just the KECxD motif (OAV96473.1) and RKCxxCx12H motif (OAV95351.1) have a higher length (435 and 308 amino acids respectively) and a lower cysteine content (0.9% and 0.6%) than expected for a cysteine-rich secreted protein, which indicates that they are unlikely to be an effector that remains in the extracellular matrix between the host and plant or is host-translocated as they are less likely to contain intramolecular disulphide bridges theorised to help stabilise tertiary structure in the plant apoplast (Saunders *et al*., 2012). Both of these proteins were predicted to be secreted however, so it is possible that they may be effectors that do not enter the apoplast.

The effector motif YxSL[RK] appeared in 7 proteins. All these proteins have a length of higher than 150 amino acids, and two have a cysteine content of >3%. 32 proteins contain the motif G[I/F/Y][A/L/S/T]R. Only two of these had a cysteine content of >3%, however these two both had a protein length of <150 amino acids, and so could be good candidates for small cysteine-

rich effectors. 32 proteins contain the motif RXLR, a common motif in oomycetes shown to present in Avr genes of *Phytophthora infestans* (Whisson *et al*., 2007). 7 of these contain a cysteine content of >3%, however only one of these also has a length of <150 amino acids. The [Li]xAR motif was seen in 65 candidates, and is also shown to be on Avr genes, this time in *Magnaporthe oryzae* (Yoshida *et al*., 2009). The motif found in the most proteins is [Y/F/W]XC, seen in putative effectors in both *Blumeria graminis* and *Melampsora larici-populina* (Yoshida *et al*., 2009), found in 318 proteins in this study. These final three motifs are relatively short and with more variation, and therefore also have a higher chance of finding spurious matches due to the short length and variability. For these, it might be prudent to prioritise certain candidate effectors over others, as in Saunders *et al*., (2012), to identify candidates that are more likely to be effectors for further testing.

Motif	Reference	Examples	No of Proteins
[Y/F/W]XC	(Godfrey *et al*., 2010, Hacquard *et al*., 2012)	*Y/F/WxC*-protein candidates*, Blumeria graminis;* putative effectors*, Melampsora larici-populina*	318
[LI]xAR	(Yoshida *et al*., 2009, Li *et al*., 2009b)	*AVR-Piaa (PEX33), AVR-pi*, *AVR-Pik/km/kp*, *AVR-piz-t, Magnaporthe Oryzae*	158
RXLR	(Whisson *et al*., 2007)	*Avr3a, Phytophthera infestans*	65
G[I/F/Y][A/L/S/T]R	(Catanzariti *et al*., 2006)	*AvrL567, AvrM, AvrP4, AvrP123, Melampsora lini*	32
YxSL[RK]	(Lévesque *et al*., 2010)	*YxSL[RK]* candidate effectors*, Phytophthera ultimum*	7
RkcxxCx12H	(Yoshida *et al*., 2009)	*AVR-Piaa (PEX33), Magnaporthe oyrzae*	1
KECxD	(Nicastro *et al*., 2009)	*PcF toxin family*, *Phytophthera spp.*	1
RQHHKRx9HRRHK	(Kemen *et al*., 2005)	*Uf-Rtp1p, Uromyces fabae*	0
[R/K]VY[L/I]R	(Ridout *et al*., 2006)	AVRk1 family, *Blumeria graminis* f. sp. *hordei*	0
LXLFLAK	(Cheung *et al*., 2008)	CRN-like proteins, *Phytophthera* spp., *Pythium ultimum*	0
RQHHKRx9HRRHK	(Kemen *et al*., 2005)	*Uf-Rtp1p, Uromyces fabae*	0
CX3-7CX4-6CX0-5CX1-4CX4-10C	(Van der Merwe *et al*., 2009)	Toxins and inhibitors of receptors or proteases, *Melampsora* spp.. Spacing is typical of cysteine-knotted peptides.	0
KRLTG	(Gu *et al*., 2011)	*ps87*, *Puccinia striiformis*	0

Table 7 – List of effector motifs searched for in this study, and number of Race 1-1 BBBD proteins containing the assessed motifs found.

Discussion

Genetic Data and Population Data of UK, European and Australasian Isolates Show High Levels of Relatedness

In this study, we have found PTT isolates to be highly related with low differentiation between isolates through the use of phylogenetic analysis (Figure 7), population statistic analyses (Tables 5, 6) and DAPC analysis (Figure 8), . This was found to be true even across continents when the Australian and New Zealand isolates are taken into account, both found grouping with European isolates in both the phylogenetic tree (Figure 7) and the DAPC analysis (Figure 8).

Studies based around the use of SSR markers have successfully found separate population groups (Kolmer 2015; Kolmer and Acevedo 2016; Kolmer *et al*. 2011, 2013; Ordoñez *et al*. 2010; Ordoñez and Kolmer 2007b) including a recent worldwide analysis of 23 SSR loci that revealed regional clonal populations and signs of long-distance dispersal in the form of a wide geographic distribution of identical and highly related multilocus genotypes (Kolmer at al., 2019).

However, a recent study based on 6745 SNPs produced by genotype-by-sequencing could not separate a worldwide collection of PTT isolates into distinct groups via DAPC, with the BIC value declining continuously, resulting in no clear optimum number of population clusters (Kolmer *et al*., 2019b). This agrees with the high levels of genetic relatedness seen in this study. The tentative separation of the isolates in this study into three population groups is potentially not reliable, as the BIC suggested a maximum clustering number of 9-10 or 12-13 populations, yet when this is visualised, the potential population structure breaks down (Figure 8). The high clustering number suggested by the BIC value also results in groups containing very low numbers of isolates – again, an impression of over-fitting the data. It could be that adding more samples to this dataset would also produce a failure of the BIC to separate the population into groups, as it did in the study of Kolmer *et al*., (2019).

PTT is known to have a higher repeat content across the genome when compared to other wheat rusts. Effector genes are known to be preferentially located in repeat-rich, gene-poor genomic regions (Raffaele and Kamoun, 2012), and some small RNAs produced by PTT have been computationally predicted to be targeted to repetitive (Dubey *et al*., 2019). Expressed genes should be captured in the transcriptomic sequencing of this project, however expanded repeat elements have been theorised to affect the expression of nearby genes and hence contribute to phenotypic differences of closely related isolates (Cuomo *et al*., 2017). If repeat regions affecting gene expression are indeed a major source of PTT diversity that result in the varied virulence phenotypes reported across the world (Kolmer *et al*., 2019, Ordoñez and Kolmer 2007b), then this source of diversity could be missed in this study. In addition, the SSR studies that have found separate population groups might be able to pick up the level of diversity necessary to distinguish between potential populations due to the fact that they rely

on repeat elements in order to distinguish between isolates (Kolmer *et al*., 2019). It could be possible that some of the SSR markers used in these SSR-based studies could be responsible for some phenotypic differences, therefore contributing to the virulence-molecular genotype association seen in several molecular marker-based studies in North America (Kolmer 2001, Kolmer *et al*., 1995, Kolmer and Liu 2000).

It is notable that there are two historical isolates that were sequenced before and after bulking, 09-024 and 11-098. Both of these fell into different potential population groups before and after bulking, with 09-024 moving from the green population group to the purple after sequencing, and 11-098 moving from purple to green.
Based on the allele frequency plots (Supplemental Figure 2) these two isolates may consist of mixed spore genotypes. It is possible that the predominant genotype in the stock spores before bulking was different to that after the bulking process. Difficulties in the bulking process have been seen before – possible mixed isolates have been postulated to confound field trials at the UKCPVS for a number of years (Hubbard *et al*., 2017, 2018). If this is the case, it is still notable that spores from two different potential population groups were collected on the same plant at the same time.

The population statistic analysis supports the genetic relatedness of PTT isolates in this study (Table 6), and agrees with close genetic relationships seen in literature (Kolmer *et al*., 2019). However, it must be noted that the overall statistics for all populations in this study, including the F_{is} value, and inter-population statistics involving the green potential population group suffer from a low number of sites used to calculate the statistics (Table 6). This is likely due to the transcriptomic data limiting the number of sites shared between isolates as it contains only expressed genes. The nature of the genetic markers used is likely to affect the observed variability between population groups seen in this study. Conducting population analyses using one type of genetic marker to avoid differences in genetic regions or expression limiting usable information could increase the robustness of these population analyses. The addition of further samples, or further filtering of samples with lower coverage, could also help add reliability to these calculations in future.

Phenotype Data of UK 2017 Isolates Shows Phenotypic Similarity

Overall, many of the 2017 UK field isolates show a very similar virulence profile (Figure 10). These isolates were all collected from the UK in 2017. Therefore, they would be under similar selection pressures due to the fact that they all faced resistance genes carried by wheat varieties grown in the UK in that year.
Previous research has revealed some correlation between genotype and phenotype in *P. triticina*, for example, Kolmer *et al* (2017) used 23 SSR makers to find 27 SSR genotypes, which had significant correlation with virulence phenotype and high levels of observed heterozygosity, indicating clonal reproduction. Brown rust is not known to undergo the sexual stage in the UK (Huerta-Espino *et al*., 2011), and this study shows high levels of genetic relatedness between all

assessed isolates, particularly the field isolates (Figure 7), and so it is possible that they are from a clonal lineage and have similar virulence profiles as they have not diversified enough to overcome more resistance genes.

The Life History of *Puccinia triticina* Could Allow for Highly Similar Genotypes to Persist

The life history of PTT has a potential to allow the highly genetically related isolates seen in this study to persist. PTT is able to spread rapidly in a suitable environment thanks to its clonal reproduction of urediniospores (Brown and Hovmoller, 2002). Therefore, any isolate able to persist has the potential to spread throughout a suitable host population rapidly. This, coupled with the fact that PTT predominantly reproduces via the clonal, asexual cycle (Huerta-Espino *et al*., 2011), and the urediniospores are wind-dispersed with a high potential for long-distance dispersal (Brown and Hovmoller, 2002), results in large clonal populations, which can be seen in SSR marker analysis (Kolmer *et al*., 2019a).

In a landscape of genetically similar hosts, such as that seen in the wheat-PTT pathosystem, there is a strong selection pressure for only isolates able to successfully reproduce to persist. These isolates could be genetically similar in addition to the host, as there is little to no selection pressure for them to diversify. Furthermore, if a plant pathogen race evolved to overcome resistance conferred by two or more genes in a gene-for-gene system, if it went onto to sexually reproduce with another race, allele combinations specifically adapted to that host genotype could be broken up, and therefore the progeny could have a lower fitness compared to the parent on the resistant host genotype. In this case, if there is a monoculture of resistant host genotypes as can be found in many agricultural landscapes, there will be a selection pressure to be asexual as long as there is a stable host population. (Jouet *et al*., 2019). Furthermore, in clonal populations, this can result in what is known as 'frozen heterozygosity' (Schwarz, 2017), where a polymorphism at a genetic loci can become fixed in a clonal, asexual lineage, enabling it to avoid eroding effects associated with genetic drift (Jouet *et al*., 2019). The F_{is} value found in this study, -0.1817, indicates higher than expected heterozygosity, and so clonal reproduction. The F_{is} value calculated in this study may not be wholly reliable due to the low number of sites available to calculate the F_{is} statistic in this study, although it is not far misaligned with F_{is} values calculated for *P. triticina* populations using microsatellite (Kolmer and Ordoñez, 2007b) and genotype-by-sequencing (Komer *et al*., 2019) data (Table 8). Balloux *et al*., (2003) state that expected values of heterozygosity can be found even with very low rates of sexual recombination, so the fact that the F_{is} statistic is not closer to -1 could indicate very low levels of sexual recombination, however isolating the effects of limited sexual recombination in largely clonal organisms is known to be very difficult (Balloux *et al*, 2003).

Species	Population	F_{is}	Reference
Puccinia triticina	Europe, Australasia	-0.1817	This study
Puccinia triticina	Caucausus	-0.371	Kolmer and Ordoñez, 2007b
Puccinia triticina	North Kazakhstan	-0.157	Kolmer and Ordoñez, 2007b
Puccinia triticina	South Kazakhstan	-0.07	Kolmer and Ordoñez, 2007b
Puccinia triticina	Tajikistan + Kyrgystan	-0.563	Kolmer and Ordoñez, 2007b
Puccinia triticina	Uzbekistan	-0.507	Kolmer and Ordoñez, 2007b
Puccinia triticina	North America	-0.516	Kolmer and Ordoñez, 2007b
Puccinia triticina	Central Asia	-0.103	Kolmer *et al*., 2019
Puccinia triticina	China	−0.087	Kolmer *et al*., 2019
Puccinia triticina	East Africa	0.002	Kolmer *et al*., 2019
Puccinia triticina	Europe	−0.028	Kolmer *et al*., 2019
Puccinia triticina	Middle East	−0.104	Kolmer *et al*., 2019
Puccinia triticina	North America	−0.101	Kolmer *et al*., 2019
Puccinia triticina	New Zealand	−0.288	Kolmer *et al*., 2019
Puccinia triticina	Pakistan	-0.129	Kolmer *et al*., 2019
Puccinia triticina	Russia	−0.092	Kolmer *et al*., 2019
Puccinia triticina	South America	−0.070	Kolmer *et al*., 2019
Puccinia triticina	South Africa	−0.306	Kolmer *et al*., 2019

Table 8 - F_{is} values for populations of *Puccinia triticina* from this study and two others.

The host-pathogen association of the PTT-wheat pathosystem also allows potential for genetically similar isolates to persist. As much of wheat pathogen resistance is gene-for-gene (McDonald and Linde, 2002), there is a potential for only one or a few mutations to be necessary to overcome novel host resistances. When there are large populations with rapid reproductive rate, such as with rust species, the adaptive potential can allow for this to occur (Brown and Hovmoller 2002), which could explain how PTT has been able to overcome new resistance gene combinations such as those recorded by the UKCPVS (Hubbard *et al*., 2018, Table 1).

Effector Gene Prediction of the *Puccinia triticina* Race 1-1 BBBD Reference Genome

In-silico effector gene prediction based on the pipeline of Saunders *et al*., 2012 estimated a total of 1039 proteins to be secreted, through the identification of a predicted signal peptide,

no mitochondria-targeted motif, and no transmembrane domain. This represents 6.6% of the PTT proteome, which is in line with the percentage of predicted secreted proteins for *Puccinia graminis* (6.6%) and *Puccinia striiformis* (7.2%).

Obligate biotrophs are also known to be rich in small secreted proteins (Lo Presti *et al*., 2015), and most identified rust effector genes to date are small, cysteine rich secreted proteins (Catanzariti *et al*., 2006, Ramachandran *et al*., 2016). Known small cysteine-rich effectors are typically found to be <150 amino acids in length and have a cysteine content of >3% (Saunders *et al*., 2012). For this reason, the cysteine content of the predicted secreted proteins was investigated, resulting in the identification of 133 proteins with a cysteine content of >3% and with a length of <150 amino acids, demonstrating similarity to existing small cysteine-rich effectors. However, some known avirulence effectors are also cysteine-poor, including AcrL567 and AvrM from *Melampora lini* (Catanzariti *et al*., 2006), and so a low cysteine content does not necessarily exclude a potential secreted protein from being an effector.

The 1039 predicted secreted proteins were also searched for similarity to known effector motifs. Whilst known effector motifs could indicate a higher potential that a protein behaves as an effector, there is currently no known protein motif that characterises effectors of rust fungi (Nemri *et al*., 2014). However, this knowledge might be pertinent if any of these proteins are used in future experiments.

The predicted secreted proteins could be further refined to only candidates with a higher chance of possessing effector functions. The presence of a nuclear localisation signal could be an indicator that they are translocated to the host cell (Nemri *et al*., 2014). Furthermore, the PTT expressed sequence tag database (Xu *et al*., 2011) could be used to further check that a predicted secreted protein is found in the infection structure. BLAST searches may also be conducted to search for similarity to existing known rust effectors, giving further credence to these proteins having functional similarity to known effectors.

Some potential effector genes are likely to have been missed in this pipeline. Two bean rust candidate effector genes are very small (PIG11 and PIG13, 24 and 31 amino acids in length respectively, Hahn and Mendgen, 1997), and would be missed in the 50 amino acid cut-off prediction. In addition, some effector genes such as the barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) genes AVR_{k1} and AVR_{a10} do not have a conventional eukaryotic secretion signal (Ridout *et al*., 2006), and so would be overlooked in this set of predicted effector proteins.

Transcriptomic sequencing has been used to predict *P. triticina* effector genes, with one study using RNA-sequencing of wheat leaves infected with six different rust races. This resulted in 543 predicted secreted proteins, taken at time points where PTT is actively in the infection process (Bruce *et al*., 2014). The transcriptomic data produced in this study could provide a useful dataset for investigating further potential secreted proteins, particularly if they are correlated with differential virulence on wheat races, although it must be taken into account that these samples were sequenced after storage in RNA-later, and so may have differing quantities of transcripts related to the infection process.

Use of Transcriptomic Sequencing for Population Surveillance and Analysis of *Puccinia triticina*

Constant monitoring of plant pathogens can be highly useful to pinpoint and treat outbreaks as they happen to mitigate crop losses and pathogen spread. New emerging technologies including next generation sequencing have been demonstrated to be useful in plant pathogen monitoring and tracking of outbreaks, particularly in plant pathogens that cannot easily be cultured in the lab (Hubbard *et al*., 2015a, Islam *et al*., 2016, Fall *et al*., 2019, Jouet *et al*., 2019). Large-scale analysis of fungal plant pathogen populations have also been hampered by the lengthy processes of purification and multiplication of isolates sufficient for next generation sequencing, which have been encountered in the course of this project. Using a transcriptomics approach that directly sequences from field samples of infected material has been useful for both characterising populations of plant pathogens (Hubbard *et al*., 2015a) and for sourcing emerging outbreaks (Islam *et al*., 2016).

Whilst sufficient pathogen sequences have been recovered for analysis in the samples assessed in this study, it also demonstrated strong similarities between UK and European isolates of PTT. This results in difficulties categorising isolates into population groups and hence distinguishing between isolates with specific properties, including virulence phenotype in field populations. This limits the ability to inform disease management approaches, and so application of the field pathogenomics technology on PTT. In future, the use of targeted sequencing could potentially be used to overcome some of the difficulties seen in this project, and would also overcome limitations and costs due to the size of fungal genomes. Targeted sequencing has been successfully applied to the closely related pathogen *P. striiformis*, (Radhakrishnan *et al*., 2019). However this method relies on the ability to distinguish between individuals with specific properties, such as virulence profile or fungicide resistance. SSR markers have demonstrated a phenotype-genotype correlations (Kolmer and Acevedo 2016; Kolmer 2015; Kolmer *et al*. 2011, 2013; Ordoñez *et al*. 2010; Ordoñez and Kolmer 2007b, 2009) and so it is possible that in the future sequencing technologies could be applied to PTT isolates in this way, or that this data could be analysed using the populations established through SSR markers, as done in Kolmer *et al*., (2019). The next step to facilitate this would be identifying regions associated with phenotypic differences. Comparative genomics has already been proven useful for this in PTT, with a study identifying candidate genes associated with Lr20 resistance (Wu *et al*., 2017), and has also been successfully alongside gene ontology searches in *P. striiformis* to identify variable genes between different populations to facilitate targeted sequencing (Radhakrishnan *et al*., 2019).

Further work examining putative effectors and other phenotype-associated genes could open the doors for diagnostic sequencing of brown rust, and could even be applied to real-time field sequencing using portable devices such as the Oxford Nanopore Technologies MinION sequencer, which has been applied in another rust species (Radhakrishnan *et al*., 2019).

Conclusions

Population genetic analyses of 105 European and Australasian isolates in this study have revealed high levels of genetic relatedness between isolates. A recent study based on genotype-by-sequencing of a worldwide population of *P. triticina* also found high degree of genetic similarity in their Discriminate Analysis of Principal Components (DAPC) analysis (Kolmer *et al*., 2019). However this study failed to distinguish between the different populations seen through SSR-marker based studies (Kolmer and Acevedo 2016; Kolmer 2015; Kolmer *et al*. 2011, 2013; Ordoñez *et al*. 2010; Ordoñez and Kolmer 2007b, 2009). This demonstrates that the particular genetic markers used in analysis of PTT are important to consider when exploring the diversity of *P. triticina*, and this particular use of the field pathogenomics technique is not suitable for the surveillance of new *P. triticina* pathotypes due to the difficulty in assigning isolates to phenotype-shared populations. However, it supplies a useful dataset of genomic and transcriptomic-sequenced *P. triticina* isolates that can be used in future studies to further explore the genetic diversity of brown rust, and also informs future genetic-based surveillance studies that may occur.

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Collection ID	Isolate Name	Country	Location	Host Variety
BR17.001		UK	Somerset	KWS Kielder
BR17.002		UK	Cambridgeshire	Unknown
BR17.003		UK	Cambridgeshire	Savello
BR17.004		UK	Devon	Bennington
BR17.005		UK	Devon	LG Generation
BR17.006		UK	West Sussex	Crusoe
BR17.007		UK	West Sussex	Crusoe
BR17.008		UK	West Sussex	Crusoe
BR17.009		UK	Lincolnshire	Crusoe
BR17.010		UK	Kent	Solstice
BR17.011		UK	Oxfordshire	Buster
BR17.012		UK	Lincolnshire	Freiston
BR17.013		UK	Lincolnshire	Elation
BR17.014		UK	Lincolnshire	KWS Santiago
BR17.015		UK	Lincolnshire	KWS Crispin
BR17.016		UK	Lincolnshire	Shabras
BR17.017		UK	Berkshire	ORC Wakelyns Population
BR17.018		UK	Dorset	Crusoe
BR17.019	BR17.019	UK	Lincolnshire	Cougar
BR17.020		UK	Kent	KWS Santiago
BR17.021		UK	Kent	Solstice
BR17.022		UK	Unknown	Stigg
BR17.023		UK	Unknown	Crusoe
BR17.024		UK	Norfolk	Costello
BR17.025		UK	Cambridgeshire	Spyder
BR17.026		UK	Cambridgeshire	Evolution
BR17.027		UK	Cambridgeshire	Hardwicke
BR17.028	BR17.028	UK	Cambridgeshire	KWS Silverstone
BR17.029		UK	Cambridgeshire	KWS Trinity
BR17.030		UK	Cambridgeshire	RGT Illustrious
BR17.031		UK	Cambridgeshire	KWS Siskin
BR17.032		UK	Cambridgeshire	KWS Lili
BR17.033		UK	Cambridgeshire	KWS Zyatt
BR17.034		UK	Cambridgeshire	KWS Barrel
BR17.035		UK	Cambridgeshire	Zulu
BR17.036		UK	Cambridgeshire	KWS Basset
BR17.037		UK	Cambridgeshire	Revolution
BR17.038		UK	Cambridgeshire	Leeds
BR17.039		UK	Hereford	Graham
BR17.040		UK	Hereford	Shamrock
BR17.041		UK	Hereford	KWS Siskin
BR17.042		UK	Hereford	KWS Santiago
BR17.043		UK	Kent	Solstice
BR17.0501		UK	Cambridgeshire	Dunston
BR17.0502		UK	Cambridgeshire	Maris Huntsman
BR17.0503		UK	Cambridgeshire	Buster

Collection ID	Isolate Name	Country	Location	Host Variety
BR17.0504		UK	Cambridgeshire	RGT Universe
BR17.0505		UK	Cambridgeshire	LG Generation
BR17.056	BR17.0001	UK	Buxhall, Suffolk	Crusoe
BR17.057	BR17.0002	UK	Buxhall, Suffolk	Crusoe
BR17.058	BR17.0003	UK	Buxhall, Suffolk	Crusoe
BR17.059	BR17.0004	UK	Mickfield, Suffolk	
BR17.060	BR17.0005	UK	Rougham, Suffolk	
BR17.061	BR17.0006	France	Marchelepot	Dinosor
BR17.062	BR17.0007	France	Marchelepot	Beauregard
BR17.063	BR17.0008	France	Marchelepot	Glascow
BR17.064	BR17.0009	Poland	Chrzelive	Unknown
BR17.065	BR17.0010	Poland	Pagow	Princeps
BR17.066	BR17.0011	Poland	Guvcz	Kepler
BR17.067	BR17.0012	Poland	Gova-Jciow	Skagen
BR17.068	BR17.0013	Poland	Jaroseawiec	Astoria
BR17.069	BR17.0014	Germany	Gommershoven	Reform
BR17.070	BR17.0015	Germany	Gommershoven	Tobak
BR17.071	BR17.0016	Germany	Gommershoven	Tobak
BR17.072	BR17.0017	Germany	Altenberge	Laer
BR17.073	BR17.0018	Germany	Iserlohn	Tobak
BR17.074	BR17.0019	Belgium	Mignault	Tobak/Ceriax
BR17.075	BR17.0020	Belgium	Mignault	Benchmark
BR17.076	BR17.0021	Belgium	Mignault	Tobak
BR17.077	BR17.0022	Belgium	Mignault	Henrik
BR17.078	BR17.0023	Poland	Janvszeno	Hyvento
BR17.079	BR17.0024	France	Fresne L'Archeveque	Fructidor
BR17.080	BR17.0025	France	Ecouis	Terroir
BR17.081	BR17.0026	France	Beauval Encaux	Trapez
BR17.082	BR17.0027	France	Le Gios Thiel	Alixau
18-Fr-01	BR18.0015	France	76890 Beauval en Caux	Chevron
18-Fr-02	BR18.0016	France	27370 Le Thuit Signol	Bermude
18-Fr-03		France	Néant sur Yvel	Bermude
18-Fr-04		France	Campeneac	RGT Tekno
18-Fr-05	BR18.019	France	Allouagne 62157	Dinosor
18-Fr-06	BR18.020	France	Neuville-saint-Vaast 62580	Dinosor
18-Fr-07	BR18.021	France	Lihons	Dinosor
18-Fr-08	BR18.022	France	Marchélepot	Kilimanjaro
18-Fr-09	BR18.023	France	Marchélepot	Dinosor
18-Fr-10		France	Marchélepot	Creek
18-Fr-11	BR18.025	France	Marquette-en-Ostervant	Dakota
18-Fr-12		France	Achiet-le-grand	Chevron
18-Fr-13	BR18.027	France	Frévin-Capelle	Chevron
18-NL-01	BR18.028	Netherlands	Nieuw Beerta	Henrik
18-NL-02	BR18.029	Netherlands	Dronten	Henrik

Collection ID	Isolate Name	Country	Location	Host Variety
18-Be-01	BR18.035	Belgium	Corroy-le-Château	Unknown
18-Be-02	BR18.036	Belgium	Mignault	Tobak
18-Be-03	BR18.0110	Belgium	Sombreffre	Unknown
18-Pl-01		Poland	Szwarcenowo	Natula
18-Pl-02	BR18.038	Poland	Pągów	Princeps
18-Pl-03	BR18.039	Poland	Pągów	Turnia
18-Pl-04	BR18.040	Poland	Rolewice	Linus
18-Pl-05		Poland	Jabłonka	Julius
18-Ch-01	BR18.030	Switzerland	Vufflens-la-Ville	Neice
18-Ch-02	BR18.031	Switzerland	Windisch	Claro
18-UK-01	BR18.046	UK	Suffolk	Crusoe
18-UK-02		UK	Suffolk	Crusoe
18-UK-03		UK	Suffolk	Gator
18-UK-04		UK	Cambridgeshire	Santiago
18-UK-05		UK	Gloucester	Crusoe
18-UK-06		UK	Gloucester	Crusoe
18-UK-07		UK		
18-UK-08		UK		
UKCPVS 17-019 (re-sequenced)	BR18-0127	UK		
UKCPVS 17-028 (re-sequenced)	BR18-0128	UK		
BR 18.0061	BR18-009	UK		
BR 18.0064	BR18-012	UK		
BR 18.0067	BR18-015	UK		
BR 18.0071	BR18-019	UK		
BR 18.0073	BR18-021	UK		
BR 18.0074	BR18-022	UK		
BR 18.0078	BR18-026	UK		
BR 18.0080	BR18-028	UK		
BR 18.0088	BR18-036	UK		
BR 18.0093	BR18-041	UK		
BR 18.0097	BR18-045	UK		
BR 18.0098	BR18-046	UK		
BR 18.0102	BR18-051	UK		
18-Dm01	BR18.032	Denmark	Dorthealyst	KWS Lily
18-Dm02		Denmark	Christensen	Torp
18-Dm03	BR18.034	Denmark	Dyrehavegaard	Torp
18De-01	BR18.0111	Germany	Gronae Rödersheim	Ritmo
18De-02	BR18.0112	Germany	Boehl Iggelheim	Ritmo
18De-03		Germany	Hans Disse	Bosporks
18De-04		Germany	Hans Daisk	Kamerad
18De-05		Germany	Lippetal 59510	Kashmir
18De-06	BR18.0116	Germany	Lippetal 59510	Tobak
18De-07		Germany	27318	Tobak
18De-08		Germany	27318	Aflceus
18/001		UK	Warwickshire	KWS Siskin

Collection ID	Isolate Name	Country	Location	Host Variety
18/002		UK	Cambridgeshire	Jaidor
18/003		UK	Norfolk	KWS Siskin
18/004		UK	Hertfordshire	Unknwon
18/005		UK	Lincolnshire	Santiago
18/006		UK	Lincolnshire	KWS Siskin
18/007		UK	Lincolnshire	Dunston
18/008		UK	Lincolnshire	Barrel
18/009		UK	Lincolnshire	Moulton
18/010		UK	Lincolnshire	Graham
18/011		UK	Cambridgeshire	Graham
18/012		UK	Cambridgeshire	Cougar
18/013		UK	Cambridgeshire	KWS Siskin
18/014		UK	Cambridgeshire	LG Firefly
18/015		UK	Cambridgeshire	Freiston
18/016		UK	Cambridgeshire	KWS Kerrin
18/017		UK	Cambridgeshire	Moulton
18/018		UK	Cambridgeshire	Revelation
18/019		UK	Cambridgeshire	KWS Trinity
18/020		UK	Cambridgeshire	LG Motown
18/021		UK	Cambridgeshire	Spyder
18/022		UK	Cambridgeshire	RGT Illustrious
18/023		UK	Cambridgeshire	Evolution
18/024		UK	Cambridgeshire	Crusoe
18/025		UK	Cambridgeshire	KWS Lili
18/026		UK	Cambridgeshire	Bennington
18/027		UK	Cambridgeshire	KWS Siskin
18/028		UK	Cambridgeshire	SY Medea
18/029		UK	Cambridgeshire	Elicit
18/030		UK	Lincolnshire	Buster
18/031		UK	Northamptonshire	RGT Universe
18/032		UK	Northamptonshire	Dunston
18/033		UK	Essex	KWS Lili
18/034		UK	Hampshire	LG Sundance
18/035		UK	Hampshire	Skyfall
18/036		UK	Hampshire	KWS Barrel
18/037		UK	Hampshire	Crusoe
18/038		UK	Kent	Zyatt
18/039		UK	Hampshire	Revelation
18/040		UK	Hampshire	Crusoe
18/041		UK	Hampshire	Santiago
18/042		UK	Cambridgeshire	Robigus
18/043		UK	Cambridgeshire	Robigus
18/044		UK	Lincolnshire	KWS Trinity
18/045		UK	Lincolnshire	LG Motown
18/046		UK	Lincolnshire	Evolution
18/047		UK	Roxburghshire	Myriad
18/048		UK	Gloucestershire	KWS Firefly
18/049		UK	Norfolk	Costello
18/050		UK	Norfolk	KWS Siskin

Collection ID	Isolate Name	Country	Location	Host Variety
18/051		UK	Norfolk	Crusoe
18/052		UK	Cambridgeshire	KWS Lili
18/053		UK	Lincolnshire	KWS Siskin
18/054		UK	Cambridgeshire	Unknown
18/055		UK	Scottish Borders	Hereford
18/501		UK	Cambridgeshire	Sappo
18/502		UK	Cambridgeshire	Buster
18/503		UK	Cambridgeshire	KWS Siskin
18/504		UK	Cambridgeshire	KWS Basset
18/505		UK	Cambridgeshire	Sappo
SN:1	BR 18.0001	Turkey	Izmir	Lr8
SN:2	BR 18.0002	Turkey	Izmir	Lr28
SN:3	BR 18.0003	Turkey	Çanakkale	Lr58
SN:4	BR 18.0004	Turkey	Balikesir	Lr62
SN:5		Turkey	Balikesir	Lr64
SN:6	BR 18.0006	Turkey	Balikesir	Lr67
SN:7		Turkey	Çanakkale	Lr69
SN:8	BR 18.0008	Turkey	Balikesir	Lr70
SN:9		Turkey	Balikesir	Lr73
SN:10	BR 18.0010	Turkey	Çanakkale	Lr77
SN:11		Turkey	Denizli	Lr81
SN:12		Turkey	Honaz	Lr83
SN:13	BR 18.0013	Turkey	Izmir	Lr85
SN:14	BR 18.0014	Turkey	Izmir	?
00/1A	BR 18.0119	New Zealand		
14/17A	BR 18.0043	New Zealand		
14/18A	BR 18.0044	New Zealand		
14/27A	BR 18.0045	New Zealand		
00/2A		New Zealand		
12/01A		New Zealand		
12/01B		New Zealand		
12/01C		New Zealand		
14/13		New Zealand		
14/41A		New Zealand		
WLR-2		New Zealand		
14/12		New Zealand		

Supplemental Table 1 – List of all field isolates collected during the course of this study, location, and where known, host wheat variety it was collected from. Green shaded cells denote that these samples were sequenced.

Sample Name	Country	Overall read mapping rate	Multiple Alignments	Aligned pairs	Aligned Discordant Pairs	Concordant pair alignment rate
BR 18.0001	Turkey	27.8%	2.5%	3940985	0.1%	25.2%
BR 18.0002	Turkey	24.7%	2.3%	3176866	0.2%	22.5%
BR 18.0003	Turkey	31.0%	2.5%	3694169	0.1%	28.2%
BR 18.0004	Turkey	23.3%	2.3%	2930897	0.2%	21.1%
BR 18.0006	Turkey	31.1%	2.3%	3614671	0.1%	28.1%
BR 18.0008	Turkey	18.8%	2.7%	1991155	0.1%	17.0%
BR 18.0010	Turkey	20.7%	2.4%	3103728	0.2%	18.8%
BR 18.0013	Turkey	33.5%	2.4%	4924284	0.1%	30.6%
BR 18.0014	Turkey	31.1%	2.4%	4480515	0.2%	28.0%
BR 18.0015	France	27.3%	2.5%	3845684	0.2%	24.8%
BR 18.0016	France	10.4%	2.1%	1662434	0.1%	9.5%
BR 18.0019	France	5.1%	1.9%	658465	0.3%	4.5%
BR 18.0020	France	8.9%	2.1%	1129258	0.3%	7.9%
BR 18.0021	France	22.9%	2.1%	3532761	0.2%	20.8%
BR 18.0022	France	10.1%	2.3%	1128242	0.2%	9.0%
BR 18.0023	France	17.1%	2.5%	2193343	0.3%	15.4%
BR 18.0025	France	9.4%	2.0%	991752	0.2%	8.4%
BR 18.0027	France	21.0%	2.3%	2361527	0.2%	19.0%
BR 18.0028	Netherlands	13.5%	2.0%	1896295	0.2%	12.3%
BR 18.0029	Netherlands	7.4%	2.0%	819781	0.3%	6.5%
BR 18.0030	Switzerland	14.5%	2.2%	2365194	0.3%	13.0%
BR 18.0031	Switzerland	8.3%	2.1%	1077341	0.3%	7.3%
BR 18.0032	Denmark	2.6%	1.4%	190644	0.3%	2.0%
BR 18.0034	Denmark	5.8%	2.1%	706776	0.5%	4.9%
BR 18.0035	Belgium	16.8%	2.0%	2114782	0.2%	15.2%
BR 18.0036	Belgium	14.3%	2.0%	1947203	0.3%	12.7%
BR 18.0038	Poland	11.2%	2.1%	1502064	0.2%	10.0%
BR 18.0039	Poland	5.2%	1.9%	798054	0.3%	4.5%
BR 18.0040	Poland	4.3%	1.9%	545937	0.3%	3.8%
BR 18.0043	New Zealand	7.1%	2.2%	941746	0.3%	6.3%
BR 18.0044	New Zealand	10.3%	2.0%	1353454	0.2%	9.3%
BR 18.0045	New Zealand	10.5%	2.1%	1409756	0.2%	9.5%
BR 18.0046	UK	9.6%	1.8%	1444210	0.1%	8.8%
BR 18.0061	UK	7.7%	3.4%	1090741	0.0%	6.3%
BR 18.0064	UK	4.3%	3.2%	447504	0.0%	3.6%
BR 18.0067	UK	18.4%	7.2%	2343977	0.1%	15.9%
BR 18.0071	UK	17.0%	5.8%	1874345	0.0%	14.6%
BR 18.0073	UK	17.6%	3.8%	2413284	0.1%	15.5%

Sample Name	Country	Overall read mapping rate	Multiple Alignments	Aligned pairs	Aligned Discordant Pairs	Concordant pair alignment rate
BR 18.0074	UK	20.5%	6.6%	2854351	0.1%	17.9%
BR 18.0078	UK	7.9%	3.5%	901597	0.0%	6.7%
BR 18.0080	UK	45.0%	4.9%	6010387	0.1%	40.6%
BR 18.0088	UK	37.9%	4.9%	5463760	0.1%	33.9%
BR 18.0093	UK	23.2%	9.7%	2819188	0.1%	20.6%
BR 18.0097	UK	12.4%	5.1%	1732060	0.1%	10.7%
BR 18.0098	UK	12.1%	6.0%	1499607	0.0%	10.4%
BR 18.0102	UK	7.8%	7.9%	740470	0.0%	6.9%
BR 18.0110	Belgium	13.0%	2.0%	1662267	0.2%	11.7%
BR 18.0111	Denmark	9.8%	2.3%	1503002	0.3%	8.8%
BR 18.0112	Denmark	18.5%	2.6%	2906067	0.2%	16.8%
BR 18.0116	Denmark	6.9%	2.2%	824430	0.4%	5.9%
BR 18.0119	New Zealand	6.1%	2.0%	629345	0.4%	5.3%
BR 18.0127	UK					
BR 18.0128	UK	23.1%	1.9%	2902792	0.2%	20.9%
BR17.006	UK	9.1%	1.9%	776648	0.1%	8.1%
BR17.010	UK	3.6%	2.2%	372051	0.1%	3.2%
BR17.016	UK	10.2%	2.8%	1394799	0.1%	9.0%
BR17.017	UK	4.2%	2.7%	508355	0.1%	3.7%
BR17.018	UK	9.7%	2.3%	1353994	0.1%	8.6%
BR17.025	UK	22.7%	2.5%	2571719	0.1%	20.2%
BR17.026	UK	22.7%	2.3%	2370216	0.2%	20.0%
BR17.027	UK	25.5%	2.4%	2422226	0.2%	22.4%
BR17.028	UK	1.4%	1.9%	68238	0.1%	1.0%
BR17.029	UK	4.4%	2.1%	475513	0.2%	3.8%
BR17.030	UK	24.5%	2.3%	2624416	0.2%	21.7%
BR17.032	UK	19.6%	1.9%	1943737	0.2%	17.4%
BR17.033	UK	20.2%	2.2%	2145057	0.2%	17.8%
BR17.034	UK	22.4%	2.4%	2400710	0.2%	19.7%
BR17.036	UK	19.1%	2.0%	1540286	0.2%	16.9%
BR17.037	UK	18.4%	2.1%	2250341	0.2%	16.3%
BR17.039	UK	5.7%	2.3%	610112	0.1%	5.1%
BR17.040	UK	13.5%	1.9%	1594388	0.1%	12.0%
BR17.061	France	27.3%	2.3%	2330343	0.2%	24.0%
BR17.062	France	22.8%	2.0%	2458390	0.2%	19.9%
BR17.063	France	2.7%	2.0%	1988631	0.3%	19.2%
BR17.064	Poland	4.8%	1.7%	629645	0.3%	4.0%
BR17.065	Poland	9.4%	2.2%	1511073	0.4%	8.1%
BR17.066	Poland	8.7%	2.1%	1363309	0.4%	7.5%
BR17.067	Poland	9.7%	1.8%	1437950	0.3%	8.4%
BR17.068	Poland	6.7%	2.0%	860132	0.4%	5.8%

Sample Name	Country	Overall read mapping rate	Multiple Alignments	Aligned pairs	Aligned Discordant Pairs	Concordant pair alignment rate
BR17.069	Germany	27.1%	2.2%	2770794	0.2%	23.8%
BR17.070	Germany	24.7%	2.4%	2150765	0.1%	21.4%
BR17.071	Germany	4.4%	1.8%	679562	0.3%	3.7%
BR17.072	Germany	8.2%	2.1%	1416636	0.3%	6.9%
BR17.073	Germany	23.3%	2.2%	4253988	0.2%	20.3%
BR17.074	Belgium	17.8%	1.8%	2924218	0.2%	15.7%
BR17.075	Belgium	20.1%	2.0%	2348062	0.3%	17.7%
BR17.076	Belgium	25.1%	2.0%	2853854	0.2%	22.1%
BR17.077	Belgium	15.6%	2.1%	2674436	0.3%	13.7%
BR17.078	Poland	20.0%	2.0%	2474559	0.2%	17.9%
BR17.079	France	26.6%	1.9%	3012941	0.2%	23.7%
BR17.080	France	11.0%	2.0%	1389948	0.2%	9.5%
BR17.081	France	27.7%	2.3%	4860166	0.2%	24.8%
BR17.082	France	16.4%	2.1%	2542649	0.1%	14.6%

Supplemental Table 2 – Mapping statistics of all isolates RNA-sequenced in this study. Red highlighted cells with white text indicate samples that would not sequenced to sufficient coverage (<7% overall read mapping rate), and so were excluded from this study. Blue highlighted cells with black text indicate isolates that may consist of mixed isolates, based on allele frequency at heterokaryotic SNP sites, and so were excluded from the study.

Isolate	Mean Read Depth	Breadth of Coverage	% mapped
06/031	19.3798	76.8967	83.42
06/098	18.9038	77.2056	93.38
07_020	11.6763	73.7458	49.23
07/010	9.68948	77.4542	42.91
07/032	24.9984	78.1584	89.43
07/049	17.2013	77.3473	90.03
07/071	14.4995	77.6331	81.36
08/09	17.6284	77.8703	94.68
08/012	19.3394	77.1028	57.34
08/023	19.5844	77.126	51.47
09/020	20.2366	77.7201	92.06
09/024	20.9672	77.6401	81.83
09/024_2	12.7394	76.8464	58.26
10/001	17.6878	77.0169	58.74
10/002	6.80072	68.5347	54.67
10/009	15.4058	76.8635	63.66
11/015	21.0161	77.3128	66.23
11/098	16.2368	77.2464	84.13
11/098_2	13.6281	77.5811	50.42
12/007	32.6525	77.4508	79.41
12/008	32.8412	78.0107	73.9
12/009	26.8992	77.6049	91.78
12/010	31.1998	77.6572	72.73
12/502	38.2778	77.2823	85.33
13/003	38.009	77.2667	80.36
13/010	34.6899	77.2517	84.84
14/001	31.4372	77.19	90.38
14/019	25.6544	77.0787	81.97
14/022	29.7059	77.1392	86.22
07/005	7.59087	76.5752	39.87
07/019	6.08853	75.4995	32.56
11/110	1.37356	24.1271	14.05
14/013	11.0098	73.4139	36.76
15/014	0.372211	18.7133	92.99

Supplemental Table 3 – Depth of coverage, breadth of coverage, and % mapped to the Race 1-1 BBBD Reference Genome for the genomic sequenced isolates in this study. Red highlighted cells include isolates that mapped poorly or were not sequenced to sufficient depth, and so were excluded from the analysis.

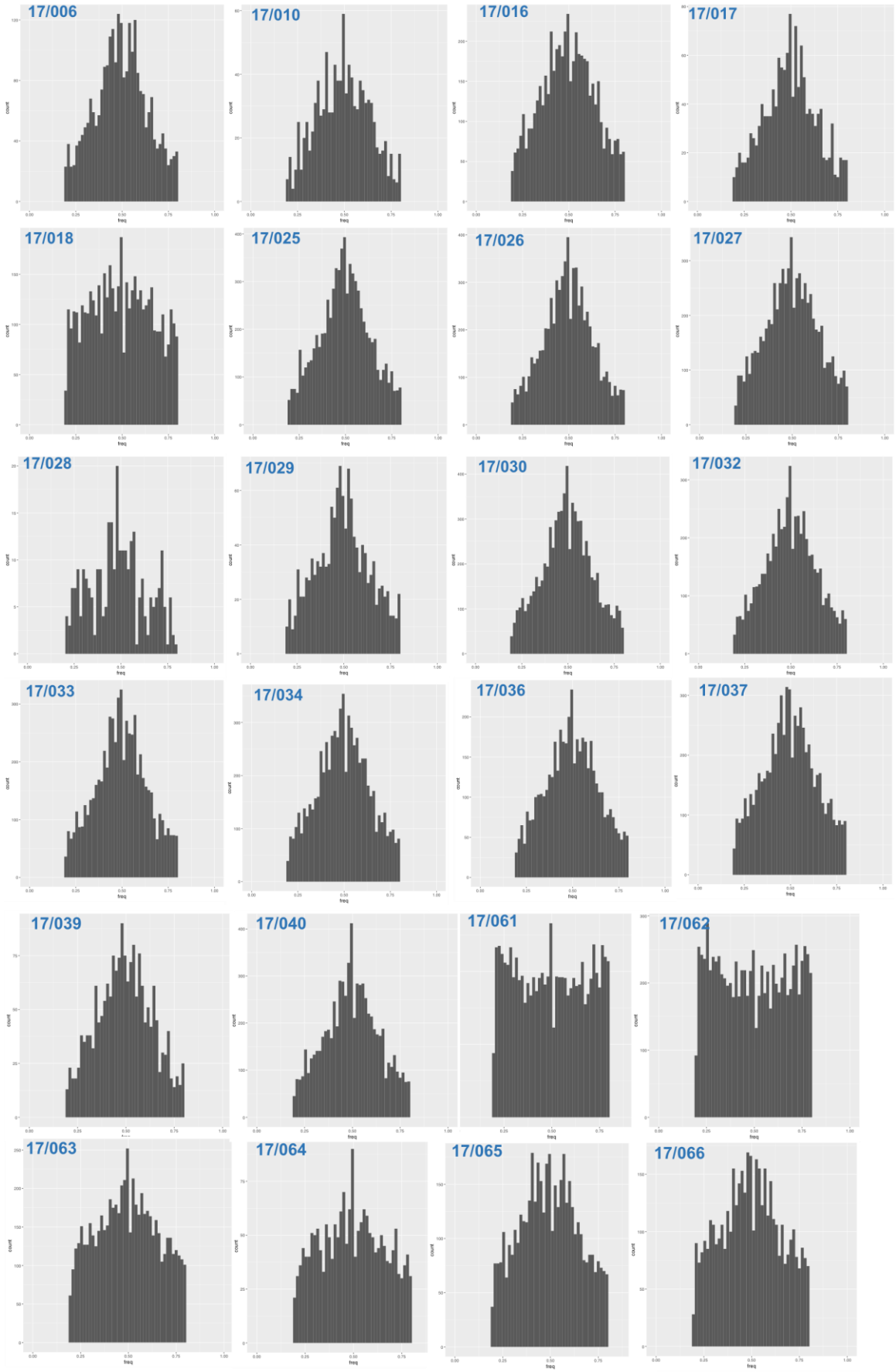
Year collected	Library name	Data type	Country
2018	BR180061	Transcriptomic	UK
2018	BR180064	Transcriptomic	UK
2018	BR180067	Transcriptomic	UK
2018	BR180071	Transcriptomic	UK
2018	BR180073	Transcriptomic	UK
2018	BR180074	Transcriptomic	UK
2018	BR180078	Transcriptomic	UK
2018	BR180080	Transcriptomic	UK
2018	BR180088	Transcriptomic	UK
2018	BR180093	Transcriptomic	UK
2018	BR180097	Transcriptomic	UK
2018	BR180098	Transcriptomic	UK
2018	BR180102	Transcriptomic	UK
2018	BR18-0001	Transcriptomic	Turkey
2018	BR18-0002	Transcriptomic	Turkey
2018	BR18-0003	Transcriptomic	Turkey
2018	BR18-0004	Transcriptomic	Turkey
2018	BR18-0006	Transcriptomic	Turkey
2018	BR18-0008	Transcriptomic	Turkey
2018	BR18-0010	Transcriptomic	Turkey
2018	BR18-0013	Transcriptomic	Turkey
2018	BR18-0014	Transcriptomic	Turkey
2018	BR18-0015	Transcriptomic	France
2018	BR18-0016	Transcriptomic	France
2018	BR18-0019	Transcriptomic	France
2018	BR18-0020	Transcriptomic	France
2018	BR18-0021	Transcriptomic	France
2018	BR18-0022	Transcriptomic	France
2018	BR18-0023	Transcriptomic	France
2018	BR18-0025	Transcriptomic	France
2018	BR18-0027	Transcriptomic	France
2018	BR18-0028	Transcriptomic	Netherlands
2018	BR18-0029	Transcriptomic	Netherlands
2018	BR18-0030	Transcriptomic	Switzerland
2018	BR18-0031	Transcriptomic	Switzerland
2018	BR18-0032	Transcriptomic	Denmark
2018	BR18-0034	Transcriptomic	Denmark
2018	BR18-0035	Transcriptomic	Belgium
2018	BR18-0036	Transcriptomic	Belgium
2018	BR18-0038	Transcriptomic	Poland
2018	BR18-0039	Transcriptomic	Poland

Year collected	Library name	Data type	Country
2018	BR18-0040	Transcriptomic	Poland
2018	BR18-0043	Transcriptomic	New Zealand
2018	BR18-0044	Transcriptomic	New Zealand
2018	BR18-0045	Transcriptomic	New Zealand
2018	BR18-0046	Transcriptomic	UK
2018	BR18-0110	Transcriptomic	Belgium
2018	BR18-0111	Transcriptomic	Germany
2018	BR18-0112	Transcriptomic	Germany
2018	BR18-0116	Transcriptomic	Germany
2018	BR18-0119	Transcriptomic	New Zealand
2018	BR18-0127	Transcriptomic	UK
2018	BR18-0128	Transcriptomic	UK
2017	BR17-006	Transcriptomic	UK
2017	BR17-010	Transcriptomic	UK
2017	BR17-016	Transcriptomic	UK
2017	BR17-017	Transcriptomic	UK
2017	BR17-018	Transcriptomic	UK
2017	BR17-025	Transcriptomic	UK
2017	BR17-026	Transcriptomic	UK
2017	BR17-027	Transcriptomic	UK
2017	BR17-028	Transcriptomic	UK
2017	BR17-029	Transcriptomic	UK
2017	BR17-030	Transcriptomic	UK
2017	BR17-032	Transcriptomic	UK
2017	BR17-033	Transcriptomic	UK
2017	BR17-034	Transcriptomic	UK
2017	BR17-036	Transcriptomic	UK
2017	BR17-037	Transcriptomic	UK
2017	BR17-039	Transcriptomic	UK
2017	BR17-040	Transcriptomic	UK
2017	BR17-061	Transcriptomic	France
2017	BR17-062	Transcriptomic	France
2017	BR17-063	Transcriptomic	France
2017	BR17-064	Transcriptomic	Poland
2017	BR17-065	Transcriptomic	Poland
2017	BR17-066	Transcriptomic	Poland
2017	BR17-067	Transcriptomic	Poland
2017	BR17-068	Transcriptomic	Poland
2017	BR17-069	Transcriptomic	Germany
2017	BR17-070	Transcriptomic	Germany
2017	BR17-071	Transcriptomic	Germany

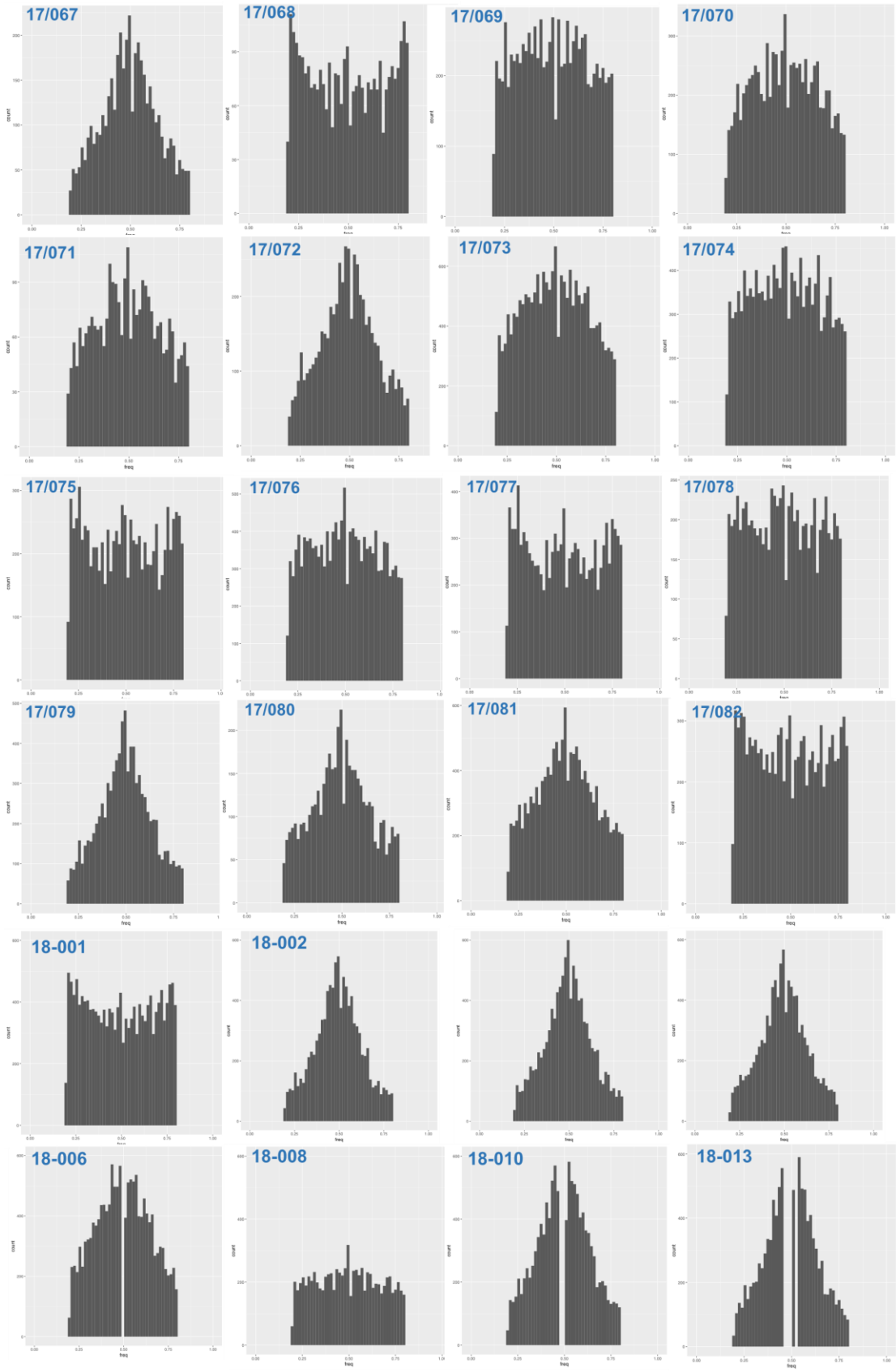
Year collected	Library name	Data type	Country
2017	BR17-072	Transcriptomic	Germany
2017	BR17-073	Transcriptomic	Germany
2017	BR17-074	Transcriptomic	Belgium
2017	BR17-075	Transcriptomic	Belgium
2017	BR17-076	Transcriptomic	Belgium
2017	BR17-077	Transcriptomic	Belgium
2017	BR17-078	Transcriptomic	Poland
2017	BR17-079	Transcriptomic	France
2017	BR17-080	Transcriptomic	France
2017	BR17-081	Transcriptomic	France
2017	BR17-082	Transcriptomic	France
2006	06/31	Genomic	UK
2006	06/98	Genomic	UK
2007	07/05	Genomic	UK
2007	07/10	Genomic	UK
2007	07/19	Genomic	UK
2007	07/71	Genomic	UK
2008	08/09	Genomic	UK
2008	08/023	Genomic	UK
2009	09/020	Genomic	UK
2009	09/024	Genomic	UK
2009	09/024-2	Genomic	UK
2011	11/015	Genomic	UK
2011	11/098	Genomic	UK
2017	17/056	Genomic	UK
2007	07/032	Genomic	UK
2007	07/049	Genomic	UK
2006	06/94	Genomic	UK
2007	07-020	Genomic	UK
2008	08-120	Genomic	UK
2010	10-001	Genomic	UK
2010	10-002	Genomic	UK
2010	10-009	Genomic	UK
2011	11-098	Genomic	UK
2011	11-098-2	Genomic	UK
2011	11-110	Genomic	UK
2012	12-007	Genomic	UK
2012	12-008	Genomic	UK
2012	12-009	Genomic	UK
2012	12-010	Genomic	UK
2012	12-502	Genomic	UK

Year collected	Library name	Data type	Country
2013	13-003	Genomic	UK
2013	13-010	Genomic	UK
2014	14-001	Genomic	UK
2014	14-013	Genomic	UK
2014	14-019	Genomic	UK
2012	12-022	Genomic	UK
2015	15-014	Genomic	UK

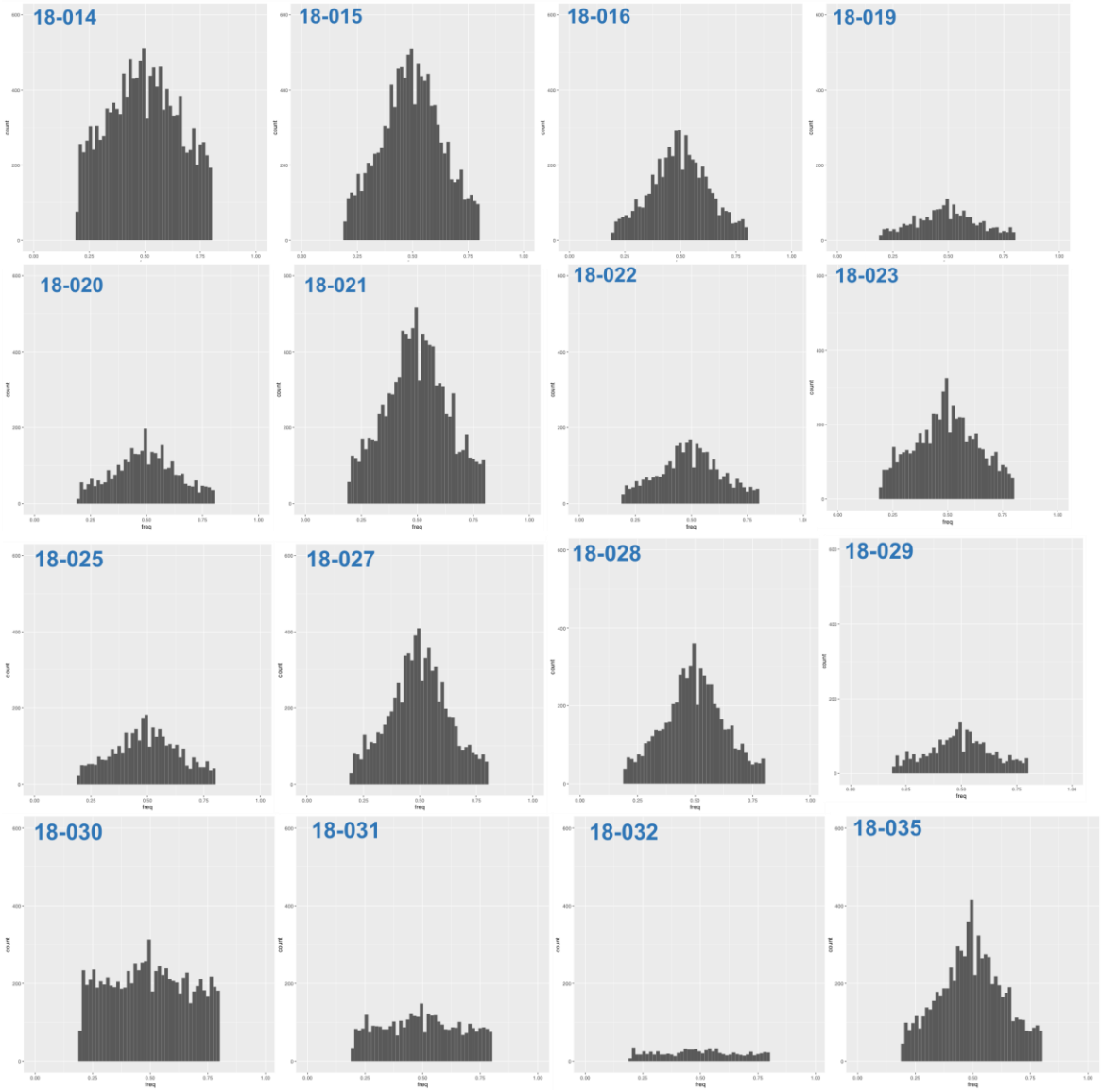
Supplemental Table 4 – List of all isolates sequenced during this study, including date of collection, type of sequencing, and country of origin. Red highlighted cells with white text indicate samples that would not sequenced to sufficient coverage, and so were excluded from this study. Blue highlighted cells with black text indicate isolates that may consist of mixed isolates, based on allele frequency at heterokaryotic SNP sites.



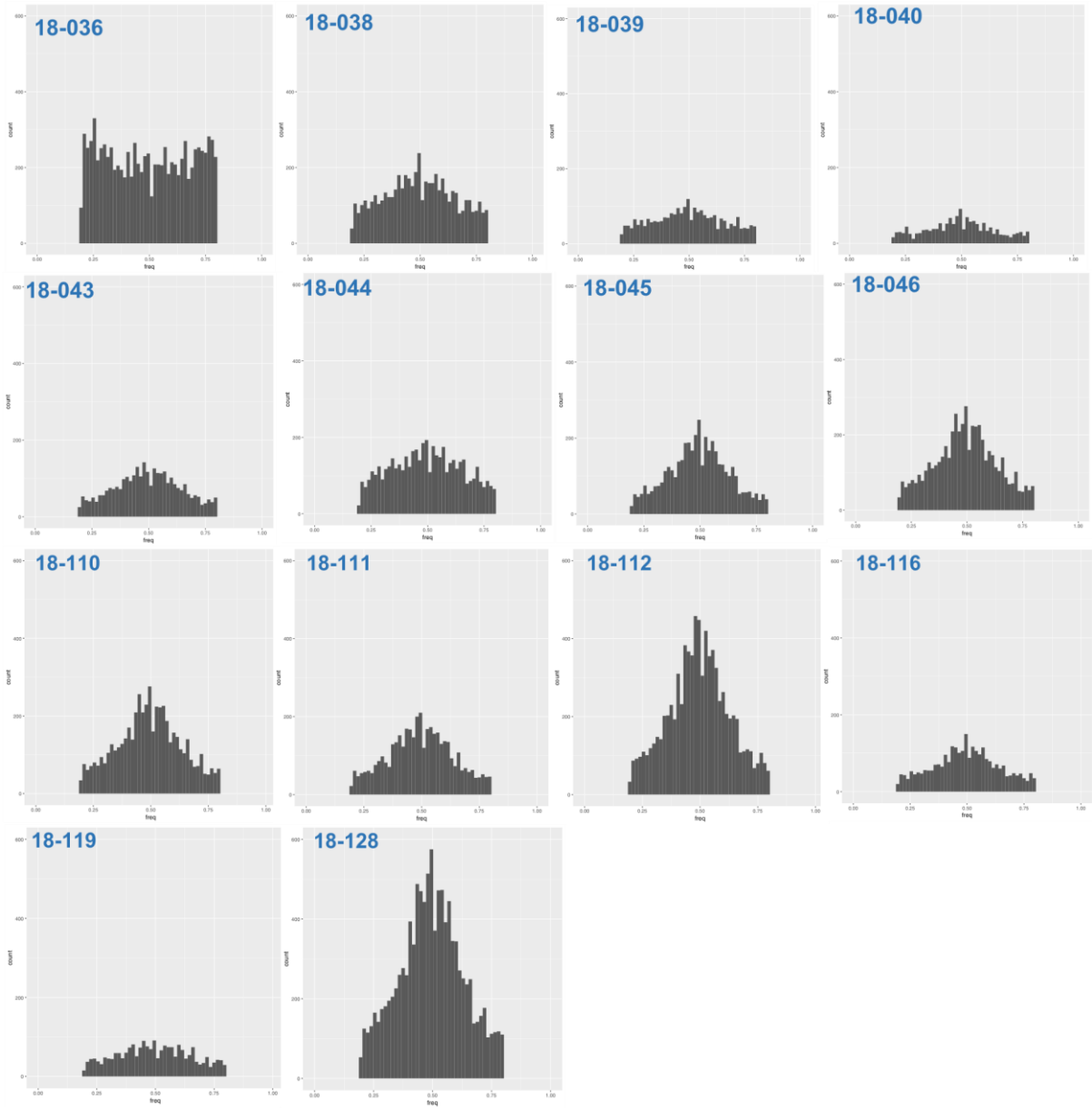
Supplemental Figure 1 – Distribution of bialleleic read counts for transcriptomic sequenced *Puccinia triticina* isolates.



Supplemental Figure 1 – Distribution of bialleleic read counts for transcriptomic sequenced *Puccinia triticina* isolates, cont.



Supplemental Figure 1 – Distribution of bialleleic read counts for transcriptomic sequenced *Puccinia triticina* isolates, cont.



Supplemental Figure 1 – Distribution of bialleleic read counts for transcriptomic sequenced *Puccinia triticina* isolates, cont.

Supplemental Figure 2 – Distribution of bialleleic read counts for genomic sequenced UK *Puccinia triticina* isolates

Isolate	Lr1	Glasgow	Sterna	Maris Fundin	Lr10	Sappo	Maris Halberd	Lr24	Stigg	Warrior	Clement	Lr26	Robigus
06/023	3.3	3.0	3.0	3.2	3.5						3.3	3.0	0.1
06/029	4.0	3.1	3.8	3.7	4.0						3.5	4.0	0.1
06/031	3.5	3.5	4.0	3.5	3.0						3.5	3.0	0.1
06/094	3.0	0.2	3.0	3.0	3.2						0.5	3.0	3.7
06/098	2.1	0.3	3.0	3.2	3.5						0.8	0.7	3.5
07/002	0.1	0.4	2.0	3.0	2.2	1.3	0.2				0.9	0.0	0.0
07/005	1.9	1.4	1.8	4.0	4.0	1.2	1.1				2.7	2.3	4.0
07/010	0.0	0.1	2.0	3.5	3.0	1.0	1.0				0.4	0.1	2.1
07/019	2.0	2.0	1.9	4.0	2.0	4.0	4.0				4.0	2.2	0.2
07/032	2.0	2.0	2.3	3.0	3.0	2.0	2.0				2.3	2.0	0.0
07/049	0.0	0.1	0.0	4.0	3.0	0.0	0.0				0.0	0.0	0.0
07/071	0.0	0.1	1.0	3.0	4.0	0.2	1.0				0.0	0.0	3.8
08/009	4.0	3.0	4.0	3.0		3.0	2.0				3.8	4.0	1.0
08/012	3.0	4.0	2.0	3.0		1.0	1.0				2.0	1.1	0.0
08/015	1.0	1.0	1.0	3.0		0.0	0.0				1.0	1.0	3.0
08/023	0.0	0.0	3.0	4.0		0.0	0.0				0.4	0.0	3.0
09/020	1.0	0.0	0.0	4.0		0.0	0.0				0.0	0.2	4.0
09/024	3.0	3.0	0.0	2.0		0.0	0.0				0.4	0.0	0.0
10/001	0.0	0.0	1.9	2.1		0.0	0.0			0.2	1.9	0.8	0.1
10/002	0.0	0.0	3.0	3.0		0.8	1.0			1.3	3.0	2.0	0.6
10/009	0.0	0.2	2.9	2.9		0.2	0.2			0.0	3.0	2.5	0.1
11/015	0.0	0.0	0.0	3.0		0.0	0.0		3.0	0.9	0.0	0.9	0.0
11/098	0.0	0.0	0.0	4.0		1.5	2.0		0.0	0.2	0.0	0.0	4.0
11/110	0.0	0.0	0.5	4.0		0.4	1.1		0.3	0.1	0.0	0.0	3.0
11/126	0.0	0.0	0.0	0.5		0.0	0.0		2.6	1.0	0.2	0.0	0.0
12/007	0.2	0.0	0.0	3.0		0.0	0.0	3.0	3.0	3.0	0.0	0.3	0.4
12/008	3.0	3.0	3.0	3.0		1.0	3.0	0.0	0.0	0.1	3.0	3.0	0.3

Supplemental Table 5 – Average Infection Type (AIT) scores for the historical isolates on different wheat varieties. A score of 0 – 2.3 corresponds to an avirulent reactions, 2.4-2.6 indicates a borderline reaction, and scores of 2.7-4 indicate a compatible reaction and that the isolates is virulent on that differential wheat variety. Yellow shading shows a compatible reaction, indicating the isolate is virulent against a particular resistance gene or variety. Orange shading indicates a borderline reaction, which must be cautiously interpreted as it is uncertain whether the reaction is one of virulence or avirulence.

Isolate	Lr1	Glasgow	Sterna	Maris Fundin	Lr10	Sappo	Maris Halberd	Lr24	Stigg	Warrior	Clement	Lr26	Robigus
12/010	0.1	0.0	0.2	3.0		1.0	1.0	0.5	0.8	0.7	1.0	1.0	3.0
12/014	0.0	0.0	0.0	3.0		3.0	3.0	3.0	3.0	3.0	0.0	0.3	3.0
12/502													
12/507													
13/003	0.0		2.5	3.0		0.1	0.0	1.0	0.4	1.0	3.0	3.0	0.0
13/010	0.0	0.0	0.0	3.0		3.0	2.0	0.0	0.0	0.0	0.1	0.0	3.0
13/034	2.5	3.0	1.0	3.0		1.0	1.0	3.0	3.0	3.0	3.0	2.0	i
14/001	0.0	0.0	3.0	3.0		3.0	3.0	0.0	0.0	0.0	3.0	3.0	0.0
14/011	0.3	0.3	2.8	3.0		3.0	3.0	0.0	0.0	0.0	3.0	3.0	3.0
14/013	3.0	3.0	3.0	3.0		3.0	3.0	0.0	0.0	0.0	3.0	3.0	3.0
14/019	3.0	3.0	3.0	3.0		3.0	3.0	0.0	0.0	0.0	3.0	3.0	0.0
14/022	3.0	3.0	3.0	3.0		3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.0
15/014	0.0	0.5	3.0	2.8		3.0	3.0	0.0	0.0	0.0	2.8	2.8	0.0
15/017	3.0	3.0	2.8	2.8		2.5	3.0	0.0	0.0	0.0	2.8	2.8	3.0
15/019	3.0		3.0	3.0		3.0	3.0	3.0	3.0	3.0	2.8	3.0	3.0
15/025	3.0	3.0	3.0	3.0		3.0	3.0	1.0	1.0	0.5	3.0	3.0	0.0
15/035	3.0	3.0	3.0	3.0		2.8	2.8	0.0	0.0	1.0	3.0	3.0	1.0

Supplemental Table 5 – Continued, showing AIC results on more brown rust isolates.

Isolate	Scout	Horatio	Leeds	Lr37	Alchemy	Cocoon	Tuxedo	KWS Sterling	Crusoe	Cougar	Dickens	Revelation	Chronicle	Armada
06/023				3.0	3.3									3.5
06/029				3.6	3.3									3.8
06/031				3.2	3.3									3.5
06/094				3.0	3.1									3.5
06/098				3.3	3.5									3.8
07/002				1.3	3.0									3.0
07/005				3.0	2.0									4.0
07/010				2.1	2.3									2.9
07/019				2.0	2.0									2.8
07/032				3.0	4.0									2.8
07/049				2.0	2.8									3.0
07/071				3.0	4.0									3.0
08/009	2.0			3.0	3.0									4.0
08/012	0.0			3.0	4.0									3.0
08/015	3.0			3.0	3.0									3.0
08/023	4.0			4.0	3.0									4.0
09/020	3.0			3.0	4.0									4.0
09/024	0.3			2.0	3.0									3.0
10/001	0.0			3.0	3.0									3.0
10/002	0.3			2.8	3.5									4.0
10/009	0.3			2.7	2.9									3.0
11/015	0.0	0.0		2.5	3.0									3.0
11/098	3.0	2.6		4.0	4.0									3.0
11/110	2.6	2.4		3.0	3.5									3.0
11/126	0.0	0.1		3.6	3.0									2.6
12/007	0.6		0.4	3.0	3.0	3.0	3.0	3.3	3.0	0.7	3.3	3.0	3.3	3.0
12/008	0.2		0.4	3.0	3.0	3.0	3.0	3.0	3.0	0.2	3.0	3.0	3.0	3.0
12/009	4.0		3.0	3.0	4.0	4.0	3.0	3.0	4.0	3.0	3.0	4.0	4.0	4.0

Supplemental Table 5 – Continued, showing AIC results on more differential varieties.

Isolate	Scout	Horatio	Leeds	Lr37	Alchemy	Cocoon	Tuxedo	KWS Sterling	Crusoe	Cougar	Dickens	Revelation	Chronicle	Armada
12/010	3.0		3.0	3.0	3.0	3.0	3.0	1.0	3.0	3.0	3.0	3.0	3.0	3.0
12/502														
12/507														
13/003	i		i	3.0	3.0	3.0	2.0	3.0	3.0	0.0	3.0	3.0	3.0	3.0
13/010	3.0		2.0	3.0	3.0	2.0	2.5	2.0	2.0	2.0	2.0	2.5	3.0	3.0
13/034	0.0		0.0	2.0	2.0	2.5	2.0	2.0	2.0	0.0	2.5	2.5	2.5	2.0
14/001	0.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	3.0
14/011	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
14/013	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
14/019	0.0	0.0	0.0	3.0	3.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	3.0
14/022	3.0	3.0	2.5	3.0	3.0	3.0	3.0	3.0	3.0	2.5	3.0	3.0	3.0	3.0
15/014	0.0			2.8	2.8			2.8	2.8	0.0	2.8	2.8		2.8
15/017	3.0			2.8	2.8			2.8	2.8	2.8	2.8	2.8		2.8
15/019	3.0			3.0	3.0			2.8	2.8	1.0	2.8	2.8		3.0
15/025	0.0			3.0	3.0			3.0	3.0	0.0	3.0	3.0		3.0
15/035	3.0			3.0	3.0			3.0	3.0	2.8	3.0	3.0		3.0

Supplemental Table 5 – Continued, showing AIC results on more *Puccinia triticina* isolates.

		Pathotype	Thatcher Lr1	Thatcher Lr2a	Thatcher Lr2b	Thatcher Lr2c	Thatcher Lr3a	Thatcher Lr3bg	Thatcher Lr3ka	Thatcher Lr10	Thatcher Lr13	Thatcher Lr14a	Thatcher Lr15	Thatcher Lr16	Thatcher Lr17	Thatcher Lr20	Thatcher Lr23	Thatcher Lr24	Thatcher Lr26	Thatcher Lr28	Thatcher Lr37	Armada	Crusoe	Maris Fundin (Lr17b)	Robigus (Lr28)	Clement (Lr26)	Glasgow (Lr1)	Maris Halberd (Lr20)	Sappo (Lr20)	Sterna (Lr3a)	Stigg (Lr24)	Warrior (Lr24)
17/018	Crusoe	1,3a,3bg,10,13,14a,15,16,17,17b,20,26	1.0	1.0	2.0	2.0	3.0	3.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0	0.6	2.0	0.0	2.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	3.0	3.0	0.0	0.0
17/036	KWS Bassett	1,3a,3ka,10,13,14a,15,16,17,17b,20,23,26,37	2.0	1.0	2.0	2.0	3.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.2	2.0	0.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	0.0	3.0	0.0	0.0
17/016	Shabras	1,2c,3a,3bg,3ka,10,14a,15,16,17,20,24,37	2.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0	2.0	3.0	3.0	3.0	3.0	3.0	2.0	3.0	2.0	2.0	3.0	3.0	2.0	2.0	0.1	0.6	3.0	2.7	2.0	3.0	3.0	2.0
17/010	Solstice	1,2b,2c,3a,3bg,3ka,10,13,14a,15,16,17,17b,20,26,37	3.0	1.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	1.0	2.0	1.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	*	3.0	0.0	0.0
17/029	KWS Trinity	1,3a,3bg,10,13,14a,15,17,20,37	2.0	2.0	2.0	2.0	3.0	3.0	2.0	3.0	3.0	3.0	3.0	2.0	3.0	3.0	2.0	1.3	2.0	1.0	3.0	3.0	3.0	2.0	0.5	0.0	3.0	2.8	2.0	3.0	0.0	0.0
17/006	Crusoe	2c,3a,3bg,3ka,10,13,14a,15,17,20,26,37	2.0	1.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	3.0	3.0	2.0	1.4	2.0	0.0	3.0	3.0	3.0	1.2	0.0	3.0	1.0	3.0	*	3.0	0.0	0.0
17/040	Shamrock	1,3a,3bg,3ka,10,13,14a,15,16,17,17b,20,23,26,37	2.0	2.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	1.2	2.0	1.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	2.0	3.0	0.0	0.0
17/030	RGT Illustrious	1,3a,3bg,3ka,10,13,14a,15,16,17,20,26	2.0	2.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0	0.2	2.0	0.0	2.0	3.0	2.6	1.5	0.0	3.0	3.0	3.0	*	3.0	0.0	0.0
17/037	Revelation	1,3a,3bg,3ka,10,13,14a,15,16,17,17b,20,23,37	2.0	1.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.1	2.0	2.0	3.0	3.0	3.0	2.8	2.0	0.0	3.0	2.0	2.0	3.0	0.0	0.0
17/034	KWS Barrel	1,3a,10,13,14a,15,16,17,17b,20,37	3.0	2.0	2.0	2.0	3.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	1.0	2.0	1.0	3.0	3.0	3.0	3.0	0.0	0.0	3.0	3.0	2.0	3.0	0.0	0.0
17/027	Hardwicke	1,2c,3a,3bg,3ka,10,13,14a,15,17,17b,20,26,37	3.0	1.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	3.0	3.0	2.0	0.0	2.0	1.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	*	3.0	0.0	0.0
17/026	Evolution	1,2b,2c,3a,3bg,3ka,10,13,14a,15,16,17,17b,20,23,26,37	3.0	1.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.6	3.0	0.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	0.0	3.0	0.0	0.2
17/032	KWS Lili	1,2c,3a,3bg,3ka,10,13,14a,15,16,17,17b,20,26	2.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0	0.6	2.0	0.0	2.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	0.0	3.0	0.0	0.0
17/033	KWS Zyatt	1,2c,3a,3bg,3ka,10,13,14a,15,16,17,17b,20,26,37	3.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	1.0	2.0	0.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	*	3.0	0.0	0.0
17/039	Graham	1,2c,3a,3bg,3ka,10,13,14a,15,16,17,17b,20,23,26,37	3.0	1.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.3	2.0	1.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	*	3.0	1.0	0.0
17/025	Spyder	1,2c,3a,3bg,3ka,10,13,14a,15,17,20,23,26,37	3.0	1.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	3.0	3.0	3.0	0.2	2.0	0.1	3.0	3.0	3.0	1.0	0.0	3.0	3.0	3.0	0.0	3.0	0.0	0.0
17/017	Breeding/Research Line	1,3a,3bg,3ka,10,13,14a,15,16,17,20,23,37	2.0	2.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	1.2	2.0	1.0	3.0	3.0	3.0	2.0	0.0	0.1	2.7	3.0	2.0	3.0	0.1	0.0

Supplemental Table 6 – Average Infection Types (AIT) of 2017 field isolates, as pathotyped by the UKCPVS on the 2017 differential set (Hubbard et al., 2018).