ABSTRACT

Obligate fungal pathogens (ascomycetes and basidiomycetes) and oomycetes are known to cause diseases in cereal crop plants. They feed on living cells and most of them have learned to bypass the host immune machinery. This paper discusses some of the factors that are associated with pathogenicity drawing examples from ascomycetes, basidiomycetes and oomycetes, with respect to their manifestation in crop plants. The comparisons have revealed a striking similarity in the three groups suggesting convergent pathways that have arisen from three lineages independently leading to an obligate lifestyle. This review has been written with the intent, that new information on adaptation strategies of biotrophs, modifications in pathogenicity strategies and population dynamics will improve current strategies for breeding with stable resistance.

INTRODUCTION

Obligate parasites, including filamentous eukaryotes and certain oomycetes, are known to infect plants where they interact and co-evolve. The main characteristic of obligate parasites that differentiates them from other parasites is their inability to survive without a host. As an adaptive measure for survival, they are known to grow asymptomatically. Symptoms are seen during reproduction either when there is rupture of spores through the epidermis or when conidiophores on the leaves of the host plant make entry through stomatal openings (Fig. 1A). These obligate pathogens have modified themselves to reproduce asexually and/or through a sexual cycle, either on the same or different host plants, as seen in some rust fungi.

Obligate parasites influence host behavior and fitness. Usually, the modifications are mostly to the advantage of the parasite, but sometimes the modifications do not have consequences on the host or the parasite. However, infection by a parasite on a host can alter trophic interactions, biodiversity, and wood webs. Hence, these parasites play an important role in shaping the community and the ecological structure. This paper discusses
the evolutionary modifications of these pathogens, and it explains how they acquire and maintain virulence. Our literature review has concluded that not all obligate pathogens are equal. Some obligate pathogens have evolved to be irreversibly specialized pathogens while there are others that are reversibly pathogenic because of transposable elements. The knowledge of this evolutionary pattern of development can help lead to the production of novel strategies to combat some of these pathogens that have impacted our ecosystem.

Characteristic features of obligate parasites are:

1. Need for a living plant tissue as a host. Several unknown factors of growth may contribute to why obligate biotrophic pathogens are unable to grow on artificial medium (Kemen, Agler & Kemen, 2015). To better demonstrate, let us use the example of dimorphic smut fungi of the order Ustilaginales. This fungus exhibits yeast-like patterns of growth on artificial media and has a biotrophic filamentous lifestyle which does not adversely affect the host (Spanu, 2012). Rust fungi were once categorized as obligate parasites, but they have undergone modifications with several species grown in axenic culture (Littlefield & Heath, 1979). Rust fungi exhibit this complicated lifestyle only on living host plants.

2. Haustoria play a necessary role in the pathogenic entry of obligate parasites through the host’s cell wall (Bozkurt, Kamoun & Lennon-Duménil, 2020). Haustoria (Figs. 1A; 1B) are formed when specialized fungal hyphae penetrate the cell wall and expand inside the host cell (Szabo & Bushnell, 2001). Haustoria formation is characterized by invagination and alteration of the host’s plasma membrane. This leads to the formation of the extrahaustorial
membrane, a membrane-like structure which surrounds the haustorial body ([Kemen, Agler & Kemen, 2015](#)). Haustoria allow for transportation between pathogen and host and are associated with the uptake of nutrients like sugars and amino acids. They also possess extrahaustorial matrix which assists in the transportation of mainly effector proteins in the cytoplasm of the host ([Kemen, Agler & Kemen, 2015](#)). The neck ring is a unique feature of the haustoria in obligate pathogens (Fig. 1B). This ring acts as a doorkeeper between the pathogen and the host, creating biotroph-specific compartments within the fungal apoplast ([Kemen, Agler & Kemen, 2015](#)).

### 3. Impaired ability to secrete cell wall-degrading enzymes and inefficient toxin production.

Obligate biotrophs escape the host immune system and establish a smooth host viability to complete their life cycle. They can also recognize signals of altered host cell status as well as from the pathogen ([Jones & Dangl, 2006](#)). Rusts, which are obligate parasites, have a very limited number of genes encoding for secreted proteins and carbohydrate active enzymes ([McDowell, 2011](#)). These genes are known to kill the host cell and produce harmful signals. Low gene copy number for these traits is an evolutionary adaptation allowing for survival inside the host (Figs. 2 and 3).

The immune systems of host plants generally function by using pattern recognition receptors (PRRs) which recognize the microbe-associated molecular patterns (MAMPs) of potential pathogens. This serves as the plant’s primary immune system, establishing a basal defense response in the plant which may be suppressed by effectors from plant pathogens. Pathogen growth may then be impeded by the plant’s secondary immune system which functions by using resistance protein (RPs). Systemic resistance may be induced in the plant after the local primary and secondary immune responses are activated, allowing for the host plant to acquire resistance towards future attacks by the pathogen ([de Wit, 2007](#)). Separate pathways of defense may be activated within the plant by different infiltrating pathogens. For example, necrotrophs may activate immune responses involving ethylene and Jasmonic acid (JA), while biotrophs may activate immune responses involving salicylic acid (SA). Crosstalk between these pathways may allow for the differential recognition and response of host plants to different pathogens ([Garcia-Brugger et al., 2006](#)).

Chitin, a major component of the fungal cell wall, is known to serve as a signal for invasion. A high number of gene families encoding chitin deacetylases are found in Rusts and are thought to interfere with chitin surveillance ([McDowell, 2011](#)). Prior to pre-penetration in rusts there is an upregulation of serine esterases known to exhibit cutinase activity. Cutinase is the enzyme that helps in the attachment of uredospores of *Uromyces viciae-fabae* to the cuticle of the plant. The entry of the pathogen through the stomatal opening enhances chitin deacetylase activity. This enzyme protects fungal machinery inside the host from degradation and also probably helps with chitin surveillance. The formation of haustorial mother cells is associated with the synthesis of polygalacturonate lyase (PL) (Fig. 4). Using differential hybridization, cDNA was obtained for genes that were activated during later stages of infection. These genes were associated with structure differentiation of *Uromyces viciae-fabae*. The transcripts for genes *rif16* and *rif21* were observed during haustorial mother cell formation and their corresponding gene products were anticipated
Figure 2  Predicted pattern of gene families gain and loss in representative fungal genomes. The figure represents the total number of protein families in each species or node estimated by the Dollo parsimony principle. The numbers on the branches of the phylogenetic tree correspond to expanded (left, black), contracted (right, red), or inferred ancestral (oval) protein families along each lineage by comparison with the putative pan-proteome. For each species, the number of gene families, orphan genes, and the total gene number are indicated on the right. Image used from Duplessis et al. (2011).

Full-size DOI: 10.7717/peerj.13794/fig-2

to be useful during infection (Deising et al., 1995). These are some of the modifications that have been employed to evade the host immune system.

4. Impaired uptake of mineral nutrients by obligate biotrophs (Duplessis et al., 2011; Tisserant et al., 2013). It has been observed that rusts’ demand for nitrogen uptake has been met by proteases which are associated with the digestion of extracellular plant proteins (McDowell, 2011). Direct nutrient uptake/transfer from the host evolved over time, allowing the pathogen to escape from competing microorganisms (Fig. 3). Millions of years of evolution led to adaptive measures to combat competition, predation, and mutualism, with respect to other microorganisms (Kemen, Agler & Kemen, 2015). The microbiome also played an important role on the host environment. As a result of new adaptations, the phyllosphere niche is affected in a number of ways. To understand how the biology of obligate biotrophic plant parasites is influenced by the microbiome, it is necessary to first understand how biotrophs evolved together and formed communities.

SURVEY METHODOLOGY

We systematically searched literature databases, included the following: PubMed Advanced Search, Scopus, Institute of Scientific Information (ISI), Web of Science, and Google Scholar. A broad range of keywords and phrases were searched, including: (1) Obligate pathogens (2) Oomycetes evolution (3) Host microbe interaction (4) Parallel and Collateral
**Figure 3**  Origin and stepwise diversification of filamentous obligate biotrophic plant pathogens. Pink boxes indicate significant lifestyle changes of the pathogen during its evolution towards biotrophy. Red and blue arrows indicate movement towards genetic consequences leading to the lifestyle changes. Circles indicate other key features of biotrophy. Transparent green areas highlight the parallel evolution of the traits. The intimate association with the plant tissue is reflected in a loss of genes for pathways highlighted with blue arrows. Plant host signals transferred to the pathogen (purple circle) can drive further diversification and specialization in obligate biotrophic pathogens and effector-triggered immunity. This is an example of collateral evolution.

Full-size DOI: 10.7717/peerj.13794/fig-3

**Figure 4**  Expression profiles of selected genes during the infection process. Red curves represent coordinated waves of expression for secreted protein genes; blue curves represent the main expression profile for CAZyme, protease, and lipase (CPL) encoding genes; green curves represent expression for transporter encoding genes. Figure based on a time-course transcriptomics in Duplessis et al. (2011) and Dobon et al. (2016). Figure adapted and modified from Lorrain et al. (2019).

Full-size DOI: 10.7717/peerj.13794/fig-4
Evolution (5) Loss and gain of gene function in evolution (6) Convergent evolution (7) Haustoria and pathogenicity (8) Genome Wide Association (GWA) studies and pathogenicity (9) Effector proteins and virulence (10) Arms race (11) Obligate parasitism.

We heavily relied on publications in the last 15 years in our study, although we referenced a few of the older publications for fundamental concepts. We also searched (Google images) for diagrams and for any schematic figures to help our understanding of fundamental concepts in the area. We did not include studies that had only abstracts available with no full text information. Our literature search did not screen papers based on date of publication, the impact factor of the journal, name of the journal, or author affiliation.

**Evolution of pathogenicity in obligate pathogens**

A characteristic feature of obligate pathogens is the ability to inhibit recognition by the host and suppress host defense mechanisms. This characteristic feature developed independently in distantly related clades of fungi and oomycetes (Thines & Kamoun, 2010; Kemen & Jones, 2012). Characteristic features like host entry and haustoria formation are brought about through similar changes within the genome and the proteome. Another characteristic is a lack of lytic enzymes found in obligate biotrophic filamentous fungi and oomycetes (Kemen, Agler & Kemen, 2015). Similar characteristics could be the result of identical genetic changes caused by mutations that are identical in independent decedents from a common ancestor (parallel evolution) or by introducing an allele from a line into another related line by hybridization (Stern, 2013) or horizontal gene transfer (HGT) (collateral evolution) (Fig. 5). With the availability of genomic sequences, it may be argued that all of them contribute to observed convergence. Characteristic biotrophic adaptations of obligate parasites for existence inside plant cells are as follows.

**Parallel evolution**

Parallel evolution is the independent evolution, from a common ancestor, of similar characteristics between two different species due to similar environments or other evolutionary pressures. Parallel evolution is commonly seen in more closely related lineages where several species handle similar challenges in the same way. In the case of obligate parasites, similar genetic changes can be the result of similar or identical mutations in independent lineages. In summary, parallel evolution emerges after strong selection and suggests limited avenues for mutational adaptation to a specific environmental condition (Bailey et al., 2017).

Here are some examples that explain parallel evolution in obligate pathogens not due to a gain in gene function but due to gene loss. Although this is debatable if gene loss is responsible for evolution in obligate pathogens, there are examples to validate the same. Of interest is the obligate human pathogen *Pneumocystis jirovecii*, known to have acquired obligate biotrophy through gene loss. This was validated by comparative genome analysis (Cissé, Pagni & Hauser, 2014). Gene loss can result from insertion of transposable elements, gene loss via deletion (sometimes after gene disruption), and is also known to drive the evolution of obligate pathogenic bacteria (Bryant, Chewapreecha & Bentley, 2012). Host microbe interaction is associated with effector proteins that alter the host’s metabolism and...
Figure 5  Common types of evolution in obligate pathogens. Parallel evolution (highlighted in green) refer to mutations (X) that arise and evolve in different lineages, resulting in similar/identical phenotypes. In collateral evolution (highlighted in orange), through hybridization (Hyb.), a mutation arises in one lineage and spreads via hybridization to other related species. Another possible mechanism of collateral evolution is horizontal gene transfer (HGT) (indicated as black arrow) that enables the transfer of genes across boundaries of phylogenetic domains. Figure adapted from Kemen, Agler & Kemen (2015).

Full-size DOI: 10.7717/peerj.13794/fig-5

suppress the host defense mechanisms (Panstruga & Dodds, 2009). Gain of species-specific variability in effector proteins increases pathogenicity (Kemen & Jones, 2012). The Effector P 2.0 program (Effector P2.0, is a machine learning classifier for fungal effector prediction) works on a large set of effectors and is based on an ensemble of classifiers trained on different subsets of negative data, providing different viewpoints on classification. Effector P2.0, available at http://effectorp.csiro.au/, has made a prediction that 12% of the proteins secreted by saprophytes are effector-like (Sperschneider et al., 2018). Comparative genomic analysis
in obligate pathogens and in saprophytes has unveiled a fascinating role of the collections of effector molecules. Genome-wide studies on how pathogenic lifestyles were achieved by these obligate pathogens validated more microorganism groups that were subspecies (SSPs) specific than groups that were non-pathogenic (Seidl et al., 2015). There were a class of effectors that were associated with the pathogenic group of microorganisms and were probably associated with interaction with the host. However, there was another class that was conserved within the saprophytes (Zhang et al., 2018). Hence, it can be concluded that some effectors are specific to host microbe interaction in pathogenic species, however there are others designated as “effector-like” proteins that were seen in the saprotrophic pathogens and their function with obligate pathogens have yet to be understood.

Because the salicylic acid (SA) pathway is a common immune strategy utilized by hosts to thwart biotrophic plant pathogens, these pathogens have adapted effectors to prevent the accumulation of SA. Ustilago maydis and Hyaloperonospora arabidopsisdis are two examples of unrelated pathogens whose effectors Cmu1 (a chorismite mutase) and HaRxL44 (a nuclear-localized effector) work to regulate the SA pathway within host plants. Synthesis of the host hormone, salicylic acid (SA), is prevented by Cmu1 via the effector’s action of re-channeling host chorismite metabolism. The effector HaRxL44 works by shifting the plant’s immune response from the SA pathway to ethylene and Jasmonic acid (JA) immune defense pathways, making the host plant more susceptible to biotrophic pathogens (Mukhtar et al., 2011; Ökmen & Doehlemann, 2014). It has been proposed that parallel evolution in obligate pathogens is the result of gene duplication and diversification because of a gain in gene function. However, it has also been suggested that evolution in obligate biotrophs is not due to gene loss, rather it is dependent on haustorium development. The essential step required for biotrophy is probably a defense suppression mechanism to facilitate efficient functioning of haustoria; subsequent loss of biosynthetic pathways is likely to be secondary (Kemen et al., 2011) (Fig. 2).

In both fungi and oomycetes, the evolutionary pathway which allows for survival in a living host cell is because of the loss of metabolic pathways (Kemen et al., 2011). This may be seen through the way in which metabolic enzyme production decreased (e.g., for thiamine and molybdopterin biosynthesis) and carbohydrate-active enzyme production reduced (Tisserant et al., 2013). Nitrate and nitrite reductases, a nitrate transporter, or sulfite reductase were also reduced (Fig. 3). The absence of certain pathways in obligate biotrophs indicates that convergent loss was linked to selective advantage (Kemen, Agler & Kemen, 2015). A possible explanation for this could be that when the same function or metabolite was associated with the host plant, the energy cost was significantly reduced (e.g., thiamine, molybdopterin, sulfite oxidase, nitrate oxidase) (Morris, Lenski & Zinser, 2012). Comparative genomic studies between closely related dicot-infecting smut fungi Melanopsichium pennsylvanicum and U. maydis and other monocot-infecting smuts show that they possess similar core eukaryotic genes, but M. Pennsylvanicum also lacks some secreted proteins (Kemen, Agler & Kemen, 2015). Based on the information, to adapt to the dicot host, gene loss was more likely than gene gain (Sharma et al., 2014) only if it is assumed that the fungus was associated with a monocot.
In yet another example, *Pneumocystis jirovecii* (human pathogen) evolved to an obligate biotroph through gene loss. Inorganic nitrogen and sulfur assimilation, thiamine biosynthesis, and purine catabolism accounted for the majority of the functions associated with the genes lost (*Cissé, Pagni & Hauser, 2014*). This example shows that gene loss is not exclusive to plant pathogens but is also present in animal pathogens that are obligate biotrophs.

**Collateral evolution**

HGT is an example of collateral evolution, and it causes a transfer of genes from fungi to oomycete genomes. Although the exact mechanism by which this HGT occurs is unclear, HGT has been demonstrated to be an important part of collateral evolution between fungi and oomycetes (*Savory, Leonard & Richards, 2015*). In obligate pathogens, the genes transferred encode proteins that attack or feed on plants (*Richards et al., 2011*). Hemibiotrophic oomycetes are more likely to exhibit HGT than obligate biotrophic oomycetes such as *H. arabidopsidis* and *Albugo laibachii*. *H. arabidopsidis* has been reported to have 21 putative HGTs (representing 3.6% of the secretome); computational validation was possible with only one HGT in *Albugo* (*Richards et al., 2011*). The HGTs transferred from fungi to oomycetes encode for proteins that assist in sugar degradation, transportation, and reorganization. HGTs from fungi to oomycetes involve genes coding for proteins that function as catalysts in the metabolism, transport, and structural changes in sugars. However, obligate biotrophs do not possess many plant degrading enzymes to avoid defense activation. Because lytic enzymes are harmful for obligate pathogen sustenance, it will not be in the best interest of the pathogen if these genes are transferred. Effectors with no known function and origin are race-specific and usually are not functional in other organisms.

Oomycetes that possess diverse families of Nep1-like proteins (NLPs) which are species-specific have undergone one HGT event and experienced considerable divergent selection (*Soanes & Richards, 2014*). As well as causing necrosis in certain host species, NLPs can contribute to virulence and disease (*Gijzen & Nürnberger, 2006*). A comparison between downy mildews and *Phytophthora* revealed NLPs are reduced in downy mildews, with regards to *Phytophthora* (*Baxter et al., 2010*). The 12 NLP-coding genes found in downy mildew are not responsible for necrosis (*Cabral et al., 2012*). In contrast to its hemibiotrophic relative, the biotrophic pathogen’s proteins appear to have evolved over time (*Baxter et al., 2010*). This also suggests a possible role in microbe–microbe interaction. In gnotobiotic systems, the function of NLPs as environmental factors can be determined by using downy mildew NLP-knockout mutants. By HGT, a virulence factor (e.g., NLP) introduced between populations can give rise to identical phenotypes (collateral evolution), however over time the development of these new functions may cause confusion regarding the original similarity (*Fig. 5*).

**Host jumps to escape environmental pressure**

Host jumps are essential for pathogen survival in a host. The phenomenon is brought about by effector molecules which allow for infection of and survival within another host. This
is seen when the phylogenetic distance is short among the two hosts with similar effector targets. This can also be seen in the hemibiotrophic lineage *Proteus mirabilis* (*Dong et al., 2014*).

A drastic change, such as gene loss, is required if the jump is made from a monocot plant host to a dicot plant host as in the case of *Melanopsichium pennisylvanicum* (*Sharma et al., 2014*). A bigger jump to a host that is very distantly related happens when the defense mechanism of the new host is suppressed in relation to the original host. When susceptibility does not occur, natural infection can be observed in a nontraditional host. This occurs when the pathogen silences its own defense mechanism (*Cooper et al., 2008*). Host jumps happen when the immunity of the host is in question and when induced susceptibility is a natural phenomenon. An example is spore dispersal to a dead nearby host (*Kemen, Agler & Kemen, 2015*). This is an example of an environmental change that leads to temporary infection abilities which help in host jumps by the pathogen (*Antia et al., 2003*).

Compatibility of pathogens leads to susceptibility of the isolates that were not compatible at first (*Ouchi, Oku & Hibino, 1976; Heath, 1980*). Several studies have confirmed this in rust and powdery mildew fungi. If plants are co-infected, their susceptibility is limited to a few cells away from the primary infection site. Under such conditions, reproduction may not be possible (*Kemen, Agler & Kemen, 2015*). The induced susceptibility of oomycetes between *A. candida* and *H. arabidopsis* has been studied as well. *A.candida* and *H. arabidopsis* co-infect Brassica sp. naturally. When appropriately pre-infected, *A. candida* greatly enhanced the disease-causing ability of compatible *H. arabidopsis*, but *Albugo* showed a lower multiplication rate (*Singh et al., 2002*). An infection with a non-sporulating *H. Arabidopsis* was caused by rapid spore formation caused by virulent *A. candida* (*Kaur et al., 2011*). Isolates of the same species that are incompatible cannot cause susceptibility to *A. candida* (*Singh et al., 1999*). Thus, susceptibility that is induced and not natural is efficient among microbes that utilize the same target effectors or other resources. There has also been evolution to limit resource competition among obligate biotrophs, perhaps through effector mediated relationships.

**Role of transposable elements (TEs)**

TEs have a role in evolutionary changes that lead to pathogenicity and survival ability (*Manning et al., 2013*). They are also responsible for gain and loss of gene function, chromosomal rearrangements, and complete inactivation of genes (*Biémont, 2010*). Expansion in genome size, alternative splicing and exonization, alteration of gene expression, alteration of a regulatory network, epigenetic control, and TEs all contribute to genome plasticity. Genome plasticity enables organisms to adapt to environmental changes. Adaptive evolution mediated by TEs is facilitated by recombination events resulting in genomic diversification. This is achieved through genomic changes which persist under positive selection in obligate fungal pathogens.

TEs induce pathogenicity by their proximity with avirulence/pathogenicity associated genes. TEs are known to gain virulence through deletion of avirulence genes, and they promote pathogenicity by inducing nucleotide diversity. Mutations from TE insertions
can lead to genetic variability that generates many new pathogenic variants with conferred ability to invade previously resistant host plants (overcome host plant resistance) and hence expand on the host range. TEs are known to alter host fitness through deleterious insertions, driving speciation and adaptive evolution.

The occurrence of many TEs in wheat biotrophs had led to a rapid evolution of the genome (Amselem, Lebrun & Quesneville, 2015). As an example, we have seen an expansion of the genome size of rust fungi, often between 100 and 200 Mb (Cuomo et al., 2017). Most genome sizes of other basidiomycete fungi are less than 50 Mb (Duplessis, Bakkeren & Hamelin, 2014). The larger size is mainly contributed to from high amounts of repetitive DNA referred to as TEs. A Pst race PST-130 has TEs which contribute to 17.8% of the genome. The genome of the Chinese isolate of CYR32 has 50% TEs, hence the genome size almost twice as large (Zheng et al., 2013; Cuomo et al., 2017). A member of the Puccinia genus, Pucciniatriticina (Pt), had a higher repeat content, with an average of more than 51% (Cuomo et al., 2017). The Bgt genome had even more TEs, reaching 90% (Wicker et al., 2013).

Role of secreted proteins

Obligate biotrophic pathogens are associated with secreted proteins (SPs) that help with escaping host immune responses (Fig. 4). Species-specific genes coding for SPs were found in the genomes of rust and powdery mildew. The Pst genome contains 2092 SPs, which account for 8.3% of the total number of predicted protein genes (Zheng et al., 2013). Two Pt races produced 660 SPs (Kiran et al., 2016), while a member of the Puccinia genus, Pgraminis f.sp. tritici (Pgt), produces 1459 SPs (Zheng et al., 2013). A virulent Pt isolate showed more SPs than race-specific isolates with a narrow virulence scope. These SPs are unique to each species of the pathogen because of the fast-evolutionary modification of the protein. The rapid evolution of some effectors indicates their individual pathogen specificity. As discussed previously, of the three rust fungi, 62% of SPs are unique to that species. It has been shown that 5% of SPs are found only exclusively in the rust and powdery mildew fungi (Spanu et al., 2010; Dean et al., 2005), indicating that each pathogen evolved differently.

Role of haustoria

Haustoria (Fig. 1B) are associated with effector delivery and nutrient uptake (Voegele et al., 2001). RNA transcriptomic studies of Pgt haustoria and germinated urediniospores showed genes that were upregulated in germinated urediospores. These genes were associated with cell proliferation, cell wall synthesis (Upadhyaya et al., 2014), and DNA replication. The haustorium is crucial for biotrophic colonization of Pst as it enhances expression of genes involved in ATP and TCA synthesis (Garnica et al., 2013). A total of 520 secreted proteins (HSPs), 430 upregulated secreted proteins in haustoria, and 90 genes were identified for Pgt (Cuomo et al., 2017). To identify specific avirulence alleles whose transcripts bind resistance genes, the effectors must interact with their corresponding targets (Flor, 1959; Moseman, 1959). Rust and powdery mildew produce effectors in haustoria, which are then transferred to host cells. Novel effector haustorial proteins are described here (Elmore et
al., 2020; Garnica et al., 2014; Link et al., 2014; Lorrain et al., 2019; Polonio et al., 2019; Tao et al., 2017). *U. fabae’s* haustorium expressed the rust transferred protein 1 (RTP1), which translocated into the cytoplasm of the host during the interaction (Lawrence, Dodds & Ellis, 2010). Stripe Rust CYR31 race haustoriums contained 1,197 secreted proteins, 69 of which inhibited tobacco cell death and 49 of which suppressed wheat callose deposition. Transcriptomic studies were used to identify these proteins. Infection processes are associated with these proteins in *P. striiformis* (Xu et al., 2020). It is possible to further screen these effector proteins identified by haustorial studies for features associated with avirulence.

### Role of effectors

There have been no definitive studies determining whether rust fungi effectors play a role in pathogenicity. In rust pathogens, there were no knockout mutants available. Small interfering RNAs (siRNAs) obtained from pathogen dsRNA can be expressed in plants. These siRNAs were able to enter the pathogen and silence their transcripts (Jaswal et al., 2020), which is referred to as host-induced gene silencing (HIGS). Fungi, oomycetes, and insects have been shown to exhibit this phenomenon. Based on this information, a Barley stripe mosaic virus (BSMV)-mediated HIGS system was created to silence any *Pst* genes, by expressing dsRNAs derived from Puccinia (Yin, Jurgenson & Hulbert, 2011). It has been demonstrated that BSMV-HIGS inhibited the expression of haustoria-specific genes. In plants, compromising *Pst* infection also led to silencing effectors (PEC6 and PSTha5a23) (Yin, Jurgenson & Hulbert, 2011; Cheng et al., 2017). This model system helps evaluate rust pathogen effectors via transient silencing.

The rust fungal pathogens are known to contain eight effector proteins, including RTp1 which was transferred from rust pathogens and obtained from *Uromyces fabae*, four effector proteins from *M. lini*, AvrP4, AvrM, AvrL567 and AvrP123, and three effectors PGTAUSPE-10-1, Avr35 and Avr50 from *P. graminis* (Cheng et al., 2017; Petre, Joly & Duplessis, 2014; Prasad et al., 2019; Salcedo et al., 2017). Two additional AVRs were seen in *P. graminis*: Avr35 and Avr50. *P. graminis* mutants and non-mutant isolates were analyzed using comparative genetics in order to identify AVRSr35 associates with Sr35 (wheat resistance gene). A mobile element inserted into the AvrSr35 gene altered functional characteristics which also included susceptibility (Salcedo et al., 2017). Another study was able to demonstrate the interaction between the avirulence gene-encoding protein AvrSr50 secreted by haustoria cells and the immune receptor Sr50. AvrSr50 originated from a naturally occurring mutant of *P. graminis* with a 2.5 megabase pair deletion in its genome. As a result of these groundbreaking studies, susceptibility factors of pathogens have been identified (Cheng et al., 2017). Genome-wide association studies (GWAS) and map-based cloning have identified nonvirulent genes that have structural resemblance to RNase-like proteins seen in wheat and rye powdery mildew pathogens (Praz et al., 2017).

Studies on effector molecules in powdery mildew detected an association between (*Mla*) genes and barley mildew resistance. These genes have the functional capability to identify effectors from a wide variety of gene families (Jaswal et al., 2020).
Role of small RNA
It is believed that small noncoding RNA molecules, such as microRNA, regulate vital functions in the host (Dubey et al., 2019; Kusch et al., 2018; Mueth, Ramachandran & Hulbert, 2015; Wang et al., 2017a; Weiberg & Jin, 2015; Jaswal et al., 2020). They regulate genes associated with defense mechanisms and immunity at different developmental stages with a functional role similar to effector proteins. It has been shown that sRNAs can move in the cytoplasm and silence the host defense genes of *P. striiformis*. This movement happens because of the presence of extracellular vesicles (Wang et al., 2017a; Wang et al., 2017b; Jaswal et al., 2020). Genome-wide sRNA association studies in the host and pathogen indicated that sRNA may inhibit the host’s own effector genes and target any genes associated with immunity, such as RLKs (receptor-like kinase) and NBS-LRR (nucleotide binding site-leucine-rich repeat) proteins. As a result, they can interact with the host similarly to how effector molecules do (Dubey et al., 2019; Sperschneider et al., 2018; Jaswal et al., 2020). The presence of these molecules in wheat leaf rust is seen at different developmental stages (resting spores, germinated spores at 16 and 24 h, and highly infected wheat leaf) demonstrated the presence of multiple defense-related genes, like reactive oxygen species (ROS), transcription factors (RLKs), and any resistant genes associated with diseases (Dubey et al., 2019). In *P. striiformis* (Pstr) there are a wide range of sRNAs that silence any endogenous genes in the host and genes related to defense and immunity (Mueth, Ramachandran & Hulbert, 2015).

Role of secondary metabolites
The secondary metabolites (SMs) act as non-proteinaceous effectors that manipulate the host with toxins (Castro-Moretti et al., 2020; Collemare, O’Connell & Lebrun, 2019; Pusztahelyi, Holb & Pocsi, 2015). SMs also function as nonvirulent factors and suppressing host defense mechanisms and strengthening cell wall factors (Collemare, O’Connell & Lebrun, 2019; Lo Presti et al., 2015; Pusztahelyi, Holb & Pocsi, 2015). By inducing penetration of the fungal cell, SMs are primarily engaged in biotrophic infection, causing infection to take place without killing the host. There has been an increase of SM production reported in genome and transcriptomic studies during different developmental stages of pathogen development in the host (Collemare, O’Connell & Lebrun, 2019; Keller, 2019; Rokas, Wisecaver & Lind, 2018).

Role of transporters
During infection, the transporter gene family was upregulated (Fig. 4). When rust pathogenesis is triggered, hyphae from haustoria which feed off the plant’s carbohydrates and amino acids at their active functional state (Ellis et al., 2009; El Gueddari et al., 2002; Voegele et al., 2001). Membrane transporters of *M. larici-populina* and *P. graminis* f. sp. *tritici* have homologs of the HXT1, AAT1, AAT2, and AAT3 transporters and H + ATPases from the bean rust pathogen *U. fabae* (Duplessis et al., 2011). Whenever a pathogen interacts with a host, all these transporters are upregulated. *M. larici-populina* and *P. graminis* f. sp. *tritici* exhibit higher levels of peptide uptake due to the presence of 22 and 21 oligopeptide membrane transporter (OPT) genes, respectively. Only 5-16 OPT genes
were found in other basidiomycete fungi (Duplessis et al., 2011). OPT genes upregulated in planta (Duplessis et al., 2011) transport peptides released by inducible proteases (aspartic peptidase, subtilisin) once the leaf tissues are infected (Fig. 4). A reduction of the Major Facilitator Superfamily (MFS) is observed in M. larici-populina and P. graminis f. sp. tritici genomes when compared with other basidiomycetes (Duplessis et al., 2011). However, many MFS transcripts are upregulated in planta, such as HXT1 homologues. In the plant host expression of M. larici-populina and P. graminis f. sp. tritici, invertase genes are upregulated (Duplessis et al., 2011). Host hexoses, such as sucrose transporter Srt1, are typically used by invading rust pathogen hyphae (Voegele et al., 2001). There is no homologue for this sucrose transporter. During the invasion of rust fungi, membrane transporters play a critical role in providing the necessary fuel due to the high metabolic activity (Duplessis et al., 2011). It was also noticed that auxin efflux gene expression was much higher in rust fungi compared to other basidiomycetes (Duplessis et al., 2011). In U. maydis, auxin synthesis gene homologs are upregulated during infection of the host (Turian & Hamilton, 1960; Basse et al., 1996; Reineke et al., 2008). The growth of plants depends on auxin synthesis while pathogen auxins are crucial for host signaling, defense strategies, or plant cell wall integrity during rust infections.

**Role of carbohydrate-active enzymes**

In addition to proteases, lipases, and sugar-cleaving enzymes, a variety of carbohydrate-active enzymes (CAZymes) are up-regulated (Fig. 4) in rust pathogen plants (Cantarel et al., 2009; Duplessis et al., 2011). This suggests that the pathogen uses degradative enzymes and fungal hyphae to penetrate and enter the host cell. Upon comparing 21 sequenced fungi, it was found that glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), and carbohydrate esterases (CE) (Duplessis et al., 2011) are similar to those found in the basidiomycete symbiont, L. bicolor (Martin et al., 2008), but are less numerous than hemibiotrophs and necrotrophs (e.g., Magnaporthe oryzae), and saprotrophs (including Neurospora crassa; Coprinopsis cinerea; Schizophyllum commune) (Ohm et al., 2010). The biotroph U. maydis does not contain many CAZymes (100 members) (Kämper et al., 2006). The evolution of a biotrophic lifestyle in rust fungi resulted in the loss of secreted hydrolytic GH and PL enzymes known to interact with plant cell wall polysaccharides (Duplessis et al., 2011). Evolution to the biotrophic lifestyle also led to the loss of cellulose binding carbohydrate-binding module 1 (CBM1) (Fig. 4). The GHs that cleave plant cellulososes and hemicelluloses are moderately upregulated (e.g., GH7, GH10, GH12, GH26, and GH27) in comparison with biotrophs U. maydis or M. oryzae (which is the hemibiotroph). Plants with these upregulated enzymes have also shown that presence of α-mannosidase (GH47) and β-1,3-glucanase (GH5) transcripts (Duplessis et al., 2011) are involved with colonization or penetration of parenchymal cells. The cell wall of a fungus is reconstructed and altered to disguise invading hypha from the host when there is an infection (El Gueddari et al., 2002) by chitin deacetylases (CE4), something which is also seen in P. graminis f. sp. tritici, M. laricipopulina, and the symbiont L. bicolor (Martin et al., 2008).
Nitrate and sulfate assimilation pathway deficiencies in rust fungi

Rust fungi, like other obligate pathogens, cannot grow in vitro. Hence, *M. larici-populina* and *P. graminis* f. sp. *tritici* are unlikely to carry genes normally found in saprotrophic basidiomycetes. Several anabolic pathways have been scrutinized for possible deficiencies. NH4+ assimilation enzymes are present. However, nitrate assimilation genes were not present in either rust pathogen genome. Nitrate/nitrite porters and nitrite reductases were not found in other fungis' gene clusters associated with nitrate assimilation (*Slot & Hibbett, 2007*). Sulfate assimilation genes were found in *M. larici-populina*, but not in *P. graminis* f. sp. *tritici*. SiR subunits, α- and β of sulfite reductase, were absent in the latter fungus, whereas the *M. larici-populina* β-subunit of SiR has no transketolase domain similar to the SiRs found in other fungal systems. Both rust fungi have dysfunctional nitrate and sulfate assimilation pathways that can be related to obligate biotrophs. This is because they need minimal nitrogen sources (different amino acids and ammonium ion) and there is no uptake of sulfur from the plant system (*Duplessis et al., 2011*). The same metabolic deficiencies (Fig. 3) have been discovered in different plant pathogens from different evolutionary lineages, one belonging to the oomycete (*H. arabidopsis*) and the other to the ascomycete (*B. graminis*) (*Spanu et al., 2010; Baxter et al., 2010*).

Role of signal molecules in obligate parasitism

A new hypothesis suggests signal molecules coming from the host plant helps in an obligate biotrophic lifestyle through the regulation of metabolic gene expressions. (Fig. 3). Infection specific organs, like haustoria and appressoria (specialized structures for nutrient uptake and entry respectively), develop in response to plant signals (*Hamer & Talbot, 1998; Tucker & Talbot, 2001*). In plants, for example, rusts recognize the surface of the cell wall, prompting hyphae to form (*Staples, 2000*). In *Magnaporthe*, hydrophobic surfaces cause appressoria to develop (*Talbot et al., 1996; Hamer & Talbot, 1998*). Products known to degrade cutin cause appressoria formation in Blumeria (*Both & Spanu, 2004*). It is possible that this essential factor controls the essential metabolic machinery of biotrophs involved in nutrient uptake and utilization. The fungus must reveal its transporters and pumps at the exact moment, at the right locations, and at the correct intensity to survive. Plant derived stimuli, rather than feeding structures, are essential to survival. Furthermore, during their life cycle, it is these stimuli that catalyze the catabolic and anabolic reactions necessary for growth and nutrition of biotrophs. At the moment, the alternative hypothesis is supported by some direct evidence. A variety of metabolic genes are expressed during both development and pathogen attack in barley powdery mildew (*Both et al., 2005*), as well as functional characteristics associated with uptake in rust haustoria (*Sohn et al., 2000; Voegele et al., 2001; Jakupovic et al., 2006*). In arbuscular mycorrhizal fungi, intraradical mycelium was found to contain fatty acid synthase activity (*Trepanier et al., 2005*), and mycelium associated with intraradical and extraradical growth exhibit differential expression of genes related to nitrogenous compound metabolism (*Govindarajulu et al., 2005*). Based on this new hypothesis, the expression pattern displayed during pathogen germination should be disrupted when fungi are observed to partially grow in vitro. Another test will determine if there are deficiencies or mutations in the regulatory elements (e.g., promoters) that control...
metabolic genes. The whole genomes of obligate and non-obligate related fungi can be compared to unravel this information. There may be a connection between evolutionary radiation and the spontaneous development and modifications in regulatory mechanisms of genes which bring about a major change in the expression pattern (Gilad et al., 2006).

**DISCUSSION**

In summary, it can be concluded from the literature that there are adaptative features in both fungi and oomycetes which are essential for maintaining adaptation strategies and pathogenicity of obligate biotrophs. These include:

(i) A very large genome size which is crucial to rapid evolution. For example, two genomes of rust fungi, M. larici-populina (89 Mbp, 16,339 proteins) and P. graminis (101 Mbp, 17,773 proteins), exhibit minimal conservation of gene order. It is possible that recombination between transposable elements (TEs) and TE proliferation is what causes the dynamicity of rust genomes. It has been seen that within rust pathogen genomes there are an increased number of gene families which encode DNA repair enzymes. Possessing a fluid genome could allow for a pathogen which lives solely within a host to rapidly adapt, allowing for the continuation of the pathogen’s survival within the host. A total of 65% of $P. graminis$ predicted proteins, and 59% of $M. larici-populina$ predicted proteins are not available in the Genbank database, showing that rust genomes possess unique genes. Furthermore, lineage-specific expansions of the gene families abundantly transcribed during infection show gene families that are specific to rusts. Future studies should examine these rust-specific gene families to develop an deeper understanding of the adaptations which may enhance a host-specific obligate lifestyle (McDowell, 2011).

(ii) Uptake and assimilation of organic nitrogen and sulfur from host sources. In the case of downy mildews, these pathogens have lost enzymes which allow them to assimilate inorganic nitrogen and sulfate. Additionally, powdery mildews are also unable to assimilate inorganic nitrogen due to a loss of enzymes. Compared to related pathogenic ascomycetes, the genomes of powdery mildews are larger by over fourfold. Among pathogenic oomycetes, the genomes of downy mildews are some of the largest. Both the genomes of powdery mildews and downy mildews have a high percentage of transposable and repeated elements, as well as a high number of lineage-specific genes (McDowell, 2011).

(iii) Loss of genes to suppress host immune responses. When compared to other necrotrophic species, downy and powdery mildews have a substantial reduction in activators of host defense, such as reduced secreted degradative enzymes. Mechanisms which allow the pathogen to avoid the host’s defense are pervasive, and likely necessary among obligate pathogens. It is furthermore necessary for obligate biotrophs to maintain the viability of their host cell throughout the pathogen’s life. The host’s defenses may be activated not only due to signals released from the pathogen but may also be activated due to an altered host cell status (McDowell, 2011). In rusts, genes encoding proteins which may harm the host cell are reduced, such as genes encoding carbohydrate active enzymes and secreted toxins. However, in rusts there is an expansion of chitin deacetylases which help interfere with the fungal cell wall’s structural component, chitin, as chitin may activate
the host’s defense mechanisms. These adaptations of rusts allow for their ability to remain undetected within the tissue of their host plants (McDowell, 2011).

(iv) An array of secreted effector proteins functioning inside and outside of host cells to build immunity and initiate survival within a host. Evidence indicates that host immunity may be interfered with by oomycete effectors, such as can be seen in the case of downy and powdery mildews which contain genomes encoding for a large number of candidate secreted effector proteins. The effectorome of *P. graminis* encodes for 1,106 small secreted proteins (SSPs), and the effectorome of *M. larici-populina* encodes for 884 SSPs. Interestingly, over one-half of these SSPs are transcribed during infection of the host plant, 16% of which are conserved between *P. graminis* and *M. larici-populina*. This may be an indication of rapid turnover, allowing these pathogens to either evade the host’s immune surveillance or to interact with new host targets (McDowell, 2011).

These findings can improve current strategies for plant breeding with stable resistance.

**CONCLUSIONS**

Genomic and transcriptomic studies have allowed us to conclude that the evolution of biotrophy is a multistep process (Duplessis et al., 2011). Characteristic features in the evolutionary pathway include (1) progressive development of effectors to assist in defense, (2) attenuated activation of defense by decreasing cell wall hydrolyzing enzymes, resulting in, (3) certain biosynthetic pathways functioning poorly if their reactants are obtained from the host. Eventually, this process leads to irreversible biotrophy due to progressive auxotrophy.

Information related to the obligate biotrophic lifestyle can be obtained by sequencing the genomes of wheat stem rust fungi and poplar leaf. Scientists are unsure exactly how nonbiotrophic progenitors evolved into obligate biotrophs. Comparisons of obligate biotrophs *M. larici-populina* and *P. graminis* f. sp. *tritici*, to other saprotrophic, pathogenic, and symbiotic basidiomycetes did not show any alteration in the conserved regions of the proteins of the rust fungi with different lineages. However, changes in oligopeptide membrane transporters, auxin efflux carriers, copper/zinc superoxide dismutase, and signaling elements may have resulted from modifications of this pathogen to a more parasitic lifestyle (Duplessis et al., 2011). Also, the zinc finger proteins in the two fungi, seen during plant-pathogen interaction and contributed by transcription factors, do not follow the traditional transcriptional functioning seen in other cases (Duplessis et al., 2011).

Modifications of gene content in these pathogens largely revolves around sets of wider gene families that have a very specific lineage. These gene families assist in structural and functional adaptation.

Lineage-specific proteins enable accumulation of the pathogen in the host leaf, cellular differentiation of pathogenic structures, and plant immune system regulation (Duplessis et al., 2011). These obligate pathogens have evolved and adapted to the plant’s immune system by producing candidate effectors like the SSPs. Gene loss and genome compaction result in the development of bacterial biotrophs and microsporidium fungal parasites (Haas et al., 2009; Levesque et al., 2010), but with rust pathogen genomes, it is
different. Because of the large gene families and abundance of TEs, rusts possess one of the largest fungal genomes. There have been no significant gene losses in *M. larici-populina* and *P. graminis* f. sp. *Tritici*. However, gene losses with no major impact have been noticed, such as in the case of nitrate and sulfur assimilation.

In rust fungi and all biotrophic pathogens, gene losses with major impact were observed, such as a reduction in enzymes that degrade polysaccharides (*Thines & Kamoun, 2010; Holub & Beynon, 1997*). It might be beneficial to understand how factors like SSPs (that are effector like) could affect coevolution and host-pathogen interactions in agricultural and forest ecosystems in order to have efficient parasite-control methods.

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Funding**
This work was supported by the Science and Technology Innovation and Demonstration Promotion Fund of Hangzhou Academy of Agricultural Sciences, No. 2019HNCT-07, Agricultural and Social Development Project of Hangzhou, No. 20201203B104. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Grant Disclosures**
The following grant information was disclosed by the authors:
Science and Technology Innovation and Demonstration Promotion Fund of Hangzhou Academy of Agricultural Sciences: 2019HNCT-07.
Agricultural and Social Development Project of Hangzhou: 20201203B104.

**Competing Interests**
The authors declare there are no competing interests.

**Author Contributions**
- Moytri RoyChowdhury conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Jake Sternhagen analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Ya Xin analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Binghai Lou conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
- Xiaobai Li performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, arrange the submission, and approved the final draft.
- Chunnan Li analyzed the data, prepared figures and/or tables, and approved the final draft.
Data Availability
The following information was supplied regarding data availability:

This is a literature review article.

REFERENCES

Amselem J, Lebrun MH, Quesneville H. 2015. Whole genome comparative analysis of transposable elements provides new insight into mechanisms of their inactivation in fungal genomes. *BMC Genomics* 16:141 DOI 10.1186/s12864-015-1347-1.

Antia R, Regoes RR, Koella JC, Bergstrom CT. 2003. The role of evolution in the emergence of infectious diseases. *Nature* 426:658–661 DOI 10.1038/nature02104.

Bailey SF, Blanquart F, Bataillon T, Kassen R. 2017. What drives parallel evolution? How population size and mutational variation contribute to repeated evolution. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* 39(1):1–9 DOI 10.1002/bies.201600176.

Basse CW, Lottspeich F, Steglich W, Kahmann R. 1996. Two potential indole-3-acetaldehyde dehydrogenases in the phytopathogenic fungus Ustilago maydis. *European Journal of Biochemistry* 242(3):648–656 DOI 10.1111/j.1432-1033.1996.0648r.x.

Baxter L, Tripathy S, Ishaque N, Boot N, Cabral A, Kemen E, Thines M, Ah-Fong A, Anderson R, Badejoko W, Bittner-Eddy P, Boore J, Chibucos M, Coates M, Dehal P, Delehanty K, Dong S, Downton P, Dumas B, Fabro G, Fronick C, Fuerstenberg S, Fulton L, Gulin E, Govers F, Hughes L, Humphray S, Jiang R, Judelson H, Kamoun S, Kyung K, Meijer H, Minx P, Morris P, Nelson J, Phuntumart V, Qutob D, Rehmany A, Rougon-Cardoso A, Ryden P, Torto-Alalibo T, Studholme D, Wang Y, Win J, Wood J, Clifton S, Rogers J, Van den Ackerveken G, Jones J, McDowell J, Beynon J, Tyler B. 2010. Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsis* genome. *Science* 330:1549–1551 DOI 10.1126/science.1195203.

Biémont C. 2010. A brief history of the status of transposable elements: from junk DNA to major players in evolution. *Genetics* 186:1085–1109 DOI 10.1534/genetics.110.124180.

Both M, Csukai M, Stumpf MPH, Spanu PD. 2005. Gene expression profiles of Blumeria graminis indicate dynamic changes to primary metabolism during development of an obligate biotrophic pathogen. *The Plant Cell* 17:2107–2122 DOI 10.1105/tpc.105.032631.

Both M, Spanu P. 2004. Blumeria graminis f. sp. Hordei, an obligate pathogen of barley. *Plant Pathogen Interactions* 11:202–218.

Bozkurt TO, Kamoun S, Lennon-Duménil A-M. 2020. The plant–pathogen haustorial interface at a glance. *Journal of Cell Science* 133(5):jcs237958 DOI 10.1242/jcs.237958.

Bryant J, Chewapreecha C, Bentley SD. 2012. Developing insights into the mechanisms of evolution of bacterial pathogens from whole-genome sequences. *Future Microbiology* 7(11):1283–1296 DOI 10.2217/fmb.12.108.
Cabral A, Oome S, Sander N, Kufner I, Nurnberger T, Vanden Ackerveken G. 2012. Nontoxic Nep1-like proteins of the downy mildew pathogen *Hyaloperonospora arabidopsidis*: repression of necrosis-inducing activity by a surface-exposed region. *Molecular Plant–Microbe Interactions* 25:697–708 DOI 10.1094/MPMI-10-11-0269.

Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The carbohydrate-active enzymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Research* 37(Database issue):D233–D238 DOI 10.1093/nar/gkn663.

Castro-Moretti FR, Gentzel IN, Mackey D, Alonso AP. 2020. Metabolomics as an emerging tool for the study of plant–pathogen interactions. *Metabolites* 10:52 DOI 10.3390/metabo10020052.

Cheng YL, Wu K, Yao JN, Li SM, Wang XJ, Huang LL, Kang ZS. 2017. PSTha5a23, a candidate effector from the obligate biotrophic pathogen *Puccinia striiformis* f. sp. tritici, is involved in plant defense suppression and rust pathogenicity. *Environmental Microbiology* 19:1717–1729 DOI 10.1111/1462-2920.13610.

Cissé OH, Pagni M, Hauser PM. 2014. Comparative genomics suggests that the human pathogenic fungus *Pneumocystis jirovecii* acquired obligate biotrophy through gene loss. *Genome Biology and Evolution* 6:1938–1948 DOI 10.1093/gbe/evu155.

Collemare J, O’Connell R, Lebrun M. 2019. Nonproteinaceous effectors: the terra incognita of plant–fungal interactions. *New Phytologist* 223:590–596 DOI 10.1111/nph.15785.

Cooper AJ, Latunde-Dada AO, Woods-Tor A, Lynn J, Lucas JA, Crute IR, Holub EB. 2008. Basic compatibility of *Albugo candida* in *Arabidopsis thaliana* and *Brassica juncea* causes broad-spectrum suppression of innate immunity. *Molecular Plant–Microbe Interactions* 21:745–756 DOI 10.1094/MPMI-21-6-0745.

Cuomo CA, Bakkeren G, Khalil HB, Panwar V, Joly D. 2017. Comparative analysis highlights variable genome content of wheat rusts and divergence of the mating loci. *G3-Genes Genomes Genetics* 7:361–376.

de Wit PJ. 2007. How plants recognize pathogens and defend themselves. *Cellular and Molecular Life Sciences: CMLS* 64(21):2726–2732 DOI 10.1007/s00018-007-7284-7.

Dean R, Talbot N, Ebbole D, Farman M, Mitchell T, Orback M, Thon M, Kulkarni R, Xu J, Pan H, Read N, Lee Y, Carbone I, Brown D, Oh Y, Nicole D, Jeong J, Soanes D, Djonovic S, Kolomiets E, Rehmeyer C, Li W, Harding M, Kim S, Lebrun M, Bohnert H, Coughlan S, Butler J, Calvo S, Ma L, Nicol R, Purcell S, Nusbaum C, Galagan J, Birren B. 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434:980–986 DOI 10.1038/nature03449.

Deising H, Frittrang AK, Kunz S, Mendgen K. 1995. Regulation of pectin methylesterase and polygalacturonate lyase activity during differentiation of infection structures in *Uromyces viciae-fabae*. *Microbiology* 141(3):561–571 DOI 10.1099/13500872-141-3-561.

Dobon A, Bunting DCE, Cabrera-Quio LE, Uauy C, Saunders DGO. 2016. The host-pathogen interaction between wheat and yellow rust induces temporally coordinated waves of gene expression. *BMC Genomics* 17:380 DOI 10.1186/s12864-016-2684-4.
Dodds PN. 2009. Terrific protein traffic: the mystery of effector protein delivery by filamentous plant pathogens. *Science* 324(5928):748–750 DOI 10.1126/science.1171652.

Dong S, Stam R, Cano LM, Song J, Sklenar J, Yoshida K, Bozkurt TO, Oliva R, Liu Z, Tian M, Win J, Banfield MJ, Jones AM, van der Hoorn RA, Kamoun S. 2014. Effector specialization in a lineage of the Irish potato famine pathogen. *Science* 343(6170):552–555 DOI 10.1126/science.1246300.

Dubey H, Kiran K, Jaswal R, Jain P, Kayastha AM, Bhardwaj SC, Mondal TK, Sharma TR. 2019. Discovery and profiling of small RNAs from *Puccinia triticina* by deep sequencing and identification of their potential targets in wheat. *Functional & Integrative Genomics* 19:1–17 DOI 10.1007/s10142-018-00652-1.

Duplessis S, Bakkeren G, Hamelin R. 2014. Advancing knowledge on biology of rust fungi through genomics. *Advances in Botanical Research* 70:173–209 DOI 10.1016/B978-0-12-397940-7.00006-9.

Duplessis S, Cuomo C, Lin Y, Aerts A, Tisserant E, Veneault-Fourrey C, Joly D, Hacquard S, Amselem J, Cantarel B, Chiu R, Coutinho P, Feau N, Field M, Frey P, Gelhaye E, Goldberg J, Grubbherr M, Kodira C, Kohler A, Kues U, Lindquist E, Lucas S, Maga R, Maillet E, Morin E, Murat C, Pangelinan J, Park R, Pearson M, Quesnelle H, Rouhier N, Sakthikumar S, Salamov A, Schmutz J, Selles B, Shapiro H, Tanguay P, Tuskan G, Henrissat B, Van de Peer Y, Rouzé P, Ellis J, Dodds P, Schein J, Zhong S, Hamelin R, Grigoriev I, Scabo L, Martin F. 2011. Obligate biotrophy features unraveled by the genomic analysis of rust fungi. *Proceedings of the National Academy of Sciences of the United States of America* 108:9166–9171 DOI 10.1073/pnas.1019315108.

El Gueddari NE, Rauchhaus U, Moerschbacher BM, Deising HB. 2002. Developmentally regulated conversion of surface-exposed chitin to chitosan in cell walls of plant pathogenic fungi. *New Phytologist* 156:103–112 DOI 10.1046/j.1469-8137.2002.00487.x.

Ellis JG, Rafiqi M, Gan P, Chakrabarti A, Dodds PN. 2009. Recent progress in discovery and functional analysis of effector proteins of fungal and oomycete plant pathogens. *Current Opinion in Plant Biology* 12:399–405 DOI 10.1016/j.pbi.2009.05.004.

Elmore MG, Banerjee S, Pedley KF, Ruck A, Whitham SA. 2020. De novo transcriptome of *Phakopsora pachyrhizi* uncovers putative effector repertoire during infection. *Physiological and Molecular Plant Pathology* 110:101464 DOI 10.1016/j.pmpp.2020.101464.

Flor H. 1959. Differential host range of the monocaryon and the dicaryons of a euautoecious rust. *Phytopathology* 794–795.

Garcia-Brugger A, Lamotte O, Vandelle E, Bourque S, Lecourieux D, Poinssot B, Wendehenne D, Pugin A. 2006. Early signaling events induced by elicitors of plant defenses. *Molecular Plant-Microbe Interactions: MPMI* 19(7):711–724 DOI 10.1094/MPMI-19-0711.

Garnica DP, Nemri A, Upadhyaya M, Rathjen JP, Dodds PN. 2014. The ins and outs of rust haustoria. *PLOS Pathogens* 10:e1004329 DOI 10.1371/journal.ppat.1004329.
Garnica DP, Upadhyaya NM, Dodds PN, Rathjen JP. 2013. Strategies for wheat stripe rust pathogenicity identified by transcriptome sequencing. *PLOS ONE* 8:e67150 DOI 10.1371/journal.pone.0067150.

Gijzen M, Nürnberger T. 2006. Nep1-like proteins from plant pathogens: recruitment and diversification of the NPP1 domain across taxa. *Phytochemistry* 67:1800–1807 DOI 10.1016/j.phytochem.2005.12.008.

Gilad Y, Oshlack A, Smyth GK, Speed TP, White KP. 2006. Expression profiling in pri-mates reveals a rapid evolution of human transcription factors. *Nature* 440:242–245 DOI 10.1038/nature04559.

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhiza symbiosis. *Nature* 435:819–823 DOI 10.1038/nature04360.

Haas BJ, Kamoun S, Zody MC, Jiang RH, Handsaker RE, Cano LM, Grabherr M, Kodira CD, Raffaele S, Torto-Al abolio T, Bozkurt TP, Ah-Fong AMV, Alvarado L, Andrson V, Armstrong MR, Avrova A, Baxter L, Beynon J, Boevink PC, Bollmann SR, Bos JJB, Bulone V, Cai G, Cakir C, Carrington JC, Chawner M, Conti L, Costanzo S, Ewan R, Fahlgren N, Fischbach MA, Fugelstad J, Gilroy EM, Gnerre S, Green PJ, Grenville-Briggs LJ, Griffith J, Grünwald NJ, Horn K, Horner NR, Hu C, Huitema E, Jeong DH, Jones AME, Jones JDG, Jones RW, Karlsson EK, Kunjeti SG, Lamour K, Liu Z, Ma L, MacLean D, Chibucos MC, McDonald H, McWalters J, Meijer HJG, Morgan W, Morris PF, Munro CA, O’Neill K, Ospina-Giraldo M, Pinzón A, Pritchard L, Ramsahoye B, Ren Q, Restrepo S, Roy S, Sadanandom A, Savidor A, Schornack S, Schwartz DC, Schumann UD, Schwessinger B, Seyer L, Sharpe T, Silvar C, Song J, Studholme DJ, Sykes S, Thines M, Van de Vondervoort PJJ, Phuntumart V, Wawra S, Weide R, Wind J, Young C, Zhou S, Fry W, Meyers BC, Van West P, Ristaino J, Govers F, Birch PRJ, Whisson SC, Judelson HS, Nusbaum C. 2009. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature* 461:393–398 DOI 10.1038/nature08358.

Hamer JE, Talbot NJ. 1998. Infection-related development in the rice blast fungus *Magnaporthe grisea*. *Current Opinion in Microbiology* 1:693–697 DOI 10.1016/S1369-5274(98)80117-3.

Heath MC. 1980. Effects of infection by compatible species or injection of tissue-extracts on the susceptibility of nonhost plants to rust fungi. *Phytopathology* 70:356–360 DOI 10.1094/Phyto-70-356.

Holub EB, Beynon JL. 1997. Symbiology of mouse-ear cress (*Arabidopsis thaliana*) and oomycetes. *Advances in Botanical Research* 24:227–273 DOI 10.1016/S0065-2296(08)60075-0.

Jakupovic M, Heintz M, Reichmann P, Mendgen K, Hahn M. 2006. Microarray analysis of expressed sequence tags from haustoria of the rust fungus *Uromyces fabae*. *Fungal Genetics and Biology* 43:8–19 DOI 10.1016/j.fgb.2005.09.001.

Jaswal R, Kiran K, Rajarammohan S, Dubey H, Singh PK, Sharma Y, Deshmukh R, Sonah H, Gupta N, Sharma TR. 2020. Effector biology of biotrophic plant fungal
pathogens: current advances and future prospects. *Microbiology Research* **241**:126567 DOI 10.1016/j.micres.2020.126567.

**Jones JD, Dangl JL. 2006.** The plant immune system. *Nature* **444**:323–329 DOI 10.1038/nature05286.

**Kämper J, Kahmann R, Bölker M, Ma LJ, Brefort T, Saville BJ, Banuett F, Kronstad JW, Gold SE, Müller O, Perlin MH, Wösten HAB, de Vries R, Ruiz-Herrera J, Reynaga-Peña CG, Snetselaar K, McCann M, Pérez-Martín J, Feldbrügge M, Basse CW, Steinberg G, Ibeas JJ, Holloman W, Guzman P, Farman M, Stajich JE, Sentandreu R, González-Prieto JM, Kennell JC, Molina L, Schirawski J, Mendoza-Mendoza A, Greiling A, Münch K, Rössel R, Scherer M, Vraně M, Ladendorf O, Vincon B, Fuchs U, Sandrock B, Meng S, Ho ECH, Cahill MJ, Boyce KJ, Klose J, Klosterman SJ, Deelstra HJ, Ortiz-Castellanos L, Li W, Sanchez-Alonso P, Schreier PH, Häuser-Hahn I, Vaupel M, Koopmann E, Friedrich G, Voss H, Schlüter T, Margolis J, Platt D, Swimmer C, Gnírke A, Chen F, Vysotskaia V, Mannhaupt G, Güldener U, Münsterkötter M, Haase D, Oesterheld M, Mewes HW, Mauceli EW, DeCaprio D, Wade CM, Butler J, Young S, Jaffe DB, Calvo S, Nusbaum C, Galagan J, Birren BW. 2006. Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature* **444**:97–101 DOI 10.1038/nature05248.

**Kaur P, Sivasithamparam K, Li H, Barbetti MJ. 2011.** Pre-inoculation with *Hyaloperonospora parasitica* reduces incubation period and increases severity of disease caused by *Albugo candida* in a *Brassica juncea* variety resistant to downy mildew. *Journal of General Plant Pathology* **77**:101–106 DOI 10.1007/s10327-011-0293-2.

**Keller NP. 2019.** Fungal secondary metabolism: regulation, function and drug discovery. *Nature Reviews Microbiology* **17**:167–180 DOI 10.1038/s41579-018-0121-1.

**Kemen AC, Agler MT, Kemen E. 2015.** Hostmicrobe and microbemicrobe interactions in the evolution of obligate plant parasitism. *New Phytologist* **206**:1207–1228 DOI 10.1111/nph.13284.

**Kemen E, Gardiner A, Schultz-Larsen T, Kemen AC, Balmuth AL, Robert-Seilaniantz A, Bailey K, Holub EB, Studholme DJ, MacLean D, Jones JDG. 2011.** Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of *Arabidopsis thaliana*. *PLOS Biology* **9**:1–21 DOI 10.1371/journal.pbio.1001094.

**Kemen E, Jones JD. 2012.** Obligate biotroph parasitism: can we link genomes to lifestyles? *Trends in Plant Science* **17**:448–457 DOI 10.1016/j.tplants.2012.04.005.

**Kiran K, Rawal HC, Dubey H, Jaswal R, Devanna B. 2016.** Draft genome of the wheat leaf rust pathogen (*Puccinia triticina*) unravels genome-wide structural variations during evolution. *Genome Biology and Evolution* **8**:2702–2721 DOI 10.1093/gbe/evw197.

**Kusch S, Frantzkesakis I, Thieron H, Panstruga R. 2018.** Small RNAs from cereal powdery mildew pathogens may target host plant genes. *Fungal Biology* **122**:1050–1063 DOI 10.1016/j.funbio.2018.08.008.

**Lawrence GJ, Dodds PN, Ellis JG. 2010.** Technical advance: transformation of the flax rust fungus. *Melampsora lini*: selection via silencing of an avirulence gene. *The Plant Journal* **61**:364–369.
Levesque CA, Brouwer H, Cano L, Hamilton JP, Holt P, Huitema EP, Raffaele SP, Robideau GPP, Thines MP, Win JP, Zerillo MMP, Beakes GWP, Boore JLP, Busam DP, Dumas BP, Ferriera SP, Fuerstenberg SIP, Gachon CMMP, Gaulin EP, Govers FP, Grenville-Briggs LP, Hornor NP, Hostetler JP, Jiang RHYP, Johnson JP, Krajaejun TP, Lin HP, Meijer HJGP, Moore BP, Morris PP, Phuntmart VP, Puiu DP, Shetty JP, Stajich JEP, Tripathy SP, Wawra SP, van West PP, Whitty BRP, Coutinho PMP, Henriassat BP, Martin FP, Thomas PDP, Tyler BMP, De Vries RPP, Kamoun SP, Yandell MP, Tisserat NP, Buell CRP. 2010. Genome sequence of the necrotrophic plant pathogen, Pythium ultimum, reveals original pathogenicity mechanisms and effector repertoire. *Genome Biology* 11:R73 DOI 10.1186/gb-2010-11-7-r73.

Link TI, Lang P, Scheffler BE, Duke MV, Graham MV, Cooper B, Tucker ML, Van De Mortel ML, Voegele RT, Mendgen K, Baum TJ, Whitmam SA. 2014. The haustorial transcriptomes of Uromyces appendiculatus and *Phakopsora pachyrhizi* and their candidate effector families. *Molecular Plant Pathology* 15:379–393 DOI 10.1111/mpp.12099.

Littlefield LJ, Heath MC. 1979. *Ultrastructure of rust fungi.* New York: Academic Press.

Lo Presti I, Lanver D, Schweizer G, Tanaka S, Liang L, Tollot M, Zuccaro A, Reissmann S, Kahmann R. 2015. Fungal effectors and plant susceptibility. *Annual Review of Plant Biology* 66:513–545 DOI 10.1146/annurev-arplant-043014-114623.

Lorrain C, Gonçalvesdos Santos KC, Germain H, Hecker A, Duplessis S. 2019. Advances in understanding obligate biotrophy in rust fungi. *New Phytologist* 222(3):1190–1120 DOI 10.1111/nph.15641.

Manning VA, Pandelova I, Dhillon B, Wilhelm LJ, Goodwin SB. 2013. Comparative genomics of a plant-pathogenic fungus, *Pyrenophora tritici-repentis*, reveals transduplication and the impact of repeat elements on pathogenicity and population divergence. *G3-Genes Genomes Genetics* 3:41–63.

Martin F, Ahrén D, Brun A, Danchin EGJ, Duchaussay F, Gibon J, Kohler A, Lindquist E, Pereda V, Salamov A, Shapiro HJ, Wuyts J, Blaudez D, Buée M, Brokstein P, Canbäck B, Cohen D, Courty PE, Coutinho PM, Delaruelle C, Detter JC, Deveau A, DiFazio S, Duplessis S, Fraissinet-Tachet L, Lucic E, Frey-Klett P, Fourrey C, Feussner I, Gay G, Grimwood J, Hoegger PJ, Jain P, Kilaru S, Labbé J, Lin YC, Legué V, Le TF, Marmessire R, Melayah D, Montanini B, Muratet M, Nehls U, Niculita-Hirzel H, Secq MP, Oudot-Le PM, Quesneville H, Rajashekar B, Reich M, Rouhier N, Schmutz J, Yin T, Chalot M, Henriassat B, Kües U, Lucas S, Van de Peer Y, Podila GK, Polle , Pukkila PJ, Richardson PM, Rouzé P, Sanders IR, Stajich JE, Tunlid A, Tuskan G, Grigoriev IV. 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* 452:88–92.15 DOI 10.1038/nature06556.

McDowell JM. 2011. Genomes of obligate plant pathogens reveal adaptations for obligate parasitism. *Proceedings of the National Academy of Sciences of the United States of America* 108(22):8921–8922 DOI 10.1073/pnas.1105802108.

Morris JJ, Lenski RE, Zinser ER. 2012. The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. *mBio* 3:e00036–e00012.
Moseman J. 1959. Host-pathogen interaction of the genes for resistance in hordeum-vulgare and for pathogenicity in *Erysiphe-graminis* f. sp. Hordei. *Phytopathology* 49:469–474.

Mueth NA, Ramachandran SR, Hulbert SH. 2015. Small RNAs from the wheat stripe rust fungus (*Puccinia striiformis* f. sp. tritici). *BMC Genomics* 16:718 DOI 10.1186/s12864-015-1895-4.

Mukhtar MS, Carvunis AR, Dreze M, Epple P, Steinbrenner J, Moore J, Tasan M, Galli M, Hao T, Nishimura M, Pevzner SJ, Donovan SE, Ghamsari L, Santhanam B, Romero V, Poulin MM, Gебreab F, Gutierrez BJ, Tam S, Monachello D, Boxem M, Harbort CJ, McDonald N, Gai L, Chen H, He Y, European Union Effectoromics Consortium, Vandenhau te J, Roth FP, Hill DE, Ecker JR, Vidal M, Beynon J, Braun P, Dangl JL. 2011. Independently evolved virulence effectors converge onto hubs in a plant immune system network. *Science* 333:596–601 DOI 10.1126/science.1203659.

Ohm RA, de Jong JF, Lugones LG, Aerts A, Kothe E, Stajich JE, de Vries RP, Record E, Levasseur A, Baker SE, Bartholomew KA, Coutinho PM, Erdmann S, Fowler TJ, Gathman AC, Lombard V, Henri ssat B, Knabe N, Kues U, Lilly WW, Lindquist E, Lucas S, Magnuson JK, Piumi F, Raudaskoski M, Salamov A, Schmutz J, Schwarze FWMR, van Kuyk PA, Horton JS, Grigoriev IV, Wosten HAB. 2010. Genome sequence of the model mushroom Schizophyllum commune. *Nature Biotechnology* 28:957–963 DOI 10.1038/nbt.1643.

Ökmen B, Doehlemann G. 2014. Inside plant: biotrophic strategies to modulate host immunity and metabolism. *Current Opinion in Plant Biology* 20C:19–25.

Ouchi S, Oku H, Hibino C. 1976. Localization of induced resistance and susceptibility in barley leaves inoculated with powdery mildew fungus. *Phytopathology* 66:901–905 DOI 10.1094/Phyto-66-901.

Panstruga R, Dodds PN. 2009. Terrific protein traffic: the mystery of effector protein delivery by filamentous plant pathogens. *Science* 324:748–750 DOI 10.1126/science.1171652.

Petre B, Joly DL, Duplessis S. 2014. Effector proteins of rust fungi. *Frontiers in Plant Science* 5:416.

Polonio A, Seoane P, Claros MG, Perez-Garcia A. 2019. The haustorial transcriptome of the cucurbit pathogen *Podosphaera xanthii* reveals new insights into the biotrophy and pathogenesis of powdery mildew fungi. *BMC Genomics* 20:543 DOI 10.1186/s12864-018-5938-0.

Prasad P, Savadi S, Bhardwaj SC, Gangwar OP, Kumar S. 2019. Rust pathogen effectors: perspectives in resistance breeding. *Planta* 250:1–22 DOI 10.1007/s00425-019-03167-6.

Praz CR, Bourras S, Zeng F, Sanchez-Martin J, Menardo F, Xue M, Yang L, Roffler S, Boni R, Herren G. 2017. *AvrPm2* encodes an RNase-like avirulence effector which is conserved in the two different specialized forms of wheat and rye powdery mildew fungus. *New Phytologist* 213:1301–1314 DOI 10.1111/nph.14372.

Pusztahelyi T, Holb IJ, Pocsi I. 2015. Secondary metabolites in fungus-plant interactions. *Frontiers in Plant Science* 6:573.
Reineke LC, Komar AA, Caprara MG, Merrick WC. 2008. A small stem–loop element directs internal initiation of the URE2 internal ribosome entry site in Saccharomyces cerevisiae. Journal of Biological Chemistry 283:19011–19025 DOI 10.1074/jbc.M803109200.

Richards TA, Soanes DM, Jones MD, Vasieva O, Leonard G, Paszkiewicz K, Foster PG, Hall N, Talbot NJ. 2011. Horizontal gene transfer facilitated the evolution of plant parasitic mechanisms in the oomycetes. Proceedings of the National Academy of Sciences of the United States of America 108:15258–15263 DOI 10.1073/pnas.1105100108.

Rokas A, Wisecaver JH, Lind AL. 2018. The birth, evolution and death of metabolic gene clusters in fungi. Nature Reviews Microbiology 16:731–744 DOI 10.1038/s41579-018-0075-3.

Salcedo A, Rutter W, Wang S, Akhunova A, Bolus S, Chao S, Anderson N, De Soto MF, Rouse M, Szabo L. 2017. Variation in the AvrSr35 gene determines Sr35 resistance against wheat stem rust race Ug99. Science 358:1604–1606 DOI 10.1126/science.aao7294.

Savory F, Leonard G, Richards TA. 2015. The role of horizontal gene transfer in the evolution of the oomycetes. PLOS Pathogens 11(5):e1004805 DOI 10.1371/journal.ppat.1004805.

Seidl MF, Faino L, Shi-Kunne X, van den Berg GCM, Bolton MD, Thomma BPHJ. 2015. The genome of the saprophytic fungus Verticillium tricorpus reveals a complex effector repertoire resembling that of its pathogenic relatives. Molecular Plant-Microbe Interactions 28:362–373 DOI 10.1094/MPMI-06-14-0173-R.

Sharma R, Mishra B, Runge F, Thines M. 2014. Gene loss rather than gene gain is associated with a host jump from monocots to dicots in the smut fungus Melanopsichium pennsylvanicum. Genome Biology and Evolution 6:2034–2049 DOI 10.1093/gbe/evu148.

Singh US, Doughty KJ, Nashaat NI, Bennett RN, Kolte SJ. 1999. Induction of systemic resistance to Albugo candida in Brassica juncea by pre- or coinoculation with an incompatible isolate. Phytopathology 89:1226–1232 DOI 10.1094/PHYTO.1999.89.12.1226.

Singh US, Nashaat NI, Doughty KJ, Awasthi RP. 2002. Altered phenotypic response to Peronospora parasitica in Brassica juncea seedlings following prior inoculation with an avirulent or virulent isolate of Albugo candida. European Journal of Plant Pathology 108:555–564 DOI 10.1023/A:1019937115378.

Slot JC, Hibbett DS. 2007. Horizontal transfer of a nitrate assimilation gene cluster and ecological transitions in fungi: a phylogenetic study. PLOS ONE 2:e1097 DOI 10.1371/journal.pone.0001097.

Soanes D, Richards TA. 2014. Horizontal gene transfer in eukaryotic plant pathogens. Annual Review of Phytopathology 52:583–614 DOI 10.1146/annurev-phyto-102313-050127.
Sohn J, Voegele RT, Mendgen T, Hahn M. 2000. High level activation of vitamin b1 biosynthesis genes in haustoria of the rust fungus Uromyces fabae. Molecular Plant–Microbe Interactions 13:629–636 DOI 10.1094/MPMI.2000.13.6.629.

Spanu PD. 2012. The genomics of obligate (and nonobligate) biotrophs. Annual Review of Phytopathology 50:91–109 DOI 10.1146/annurev-phyto-081211-173024.

Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stüber K, van Themaat EverLoren, Brown JK, Butcher SA, Gurr SJ, Lebrun MH, Ridout CJ, Schulze-Lefert P, Talbot NJ, Ahmadinejad N, Ametz C, Barton GR, Benjdia M, Bidzinski P, Bindschedler LV, Both M, Brewer MT, Cadle-Davidson L, Cadle-Davidson MM, Collemare J, Cramer R, Frenkel O, Godfrey D, Harriman J, Hoede C, King BC, Klages S, Kleemann J, Knoll D, Koti PS, Kreplak J, López-Ruiz FJ, Lu X, Maekawa T, Mahanil S, Micali C, Milgroom MG, Montana G, Noir S, O’Connell RJ, Oberhaensli S, Parlane F, Pedersen C, Quesneville H, Reinhardt R, Rott M, Sacristán S, Schmidt SM, Schön M, Skamnioti P, Sommer H, Stephens A, Takahara H, Thordal-Christensen H, Vigouroux M, Wessling R, Wicker T, Panstruga R. 2010. Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. Science 330(6010):1543–1546 DOI 10.1126/science.1194573.

Sperschneider J, Dodds PN, Gardiner DM, Singh KB, Taylor JM. 2018. Improved prediction of fungal effector proteins from secretomes with effector P 2.0. Molecular Plant Pathology 19:2094–2110 DOI 10.1111/mpp.12682.

Staples RC. 2000. Research on the rust fungi during the twentieth century. Annual Review of Phytopathology 38:49–69 DOI 10.1146/annurev.phyto.38.1.49.

Stern DL. 2013. The genetic causes of convergent evolution. Nature Reviews Genetics 14:751–764.

Szabo LJ, Bushnell WR. 2001. Hidden robbers: the role of fungal haustoria in parasitism of plants. Proceedings of the National Academy of Sciences of the United States of America 98(14):7654–7655 DOI 10.1073/pnas.151262398.

Talbot NJ, Kershaw MJ, Wakley GE, de Vries OMH, Wessels JGH, Hamer JE. 1996. Mpg1 encodes a fungal hydrophobin involved in surface interactions during infection-related development of Magnaporthe grisea. The Plant Cell 8:985–999 DOI 10.2307/3870210.

Tao SQ, Cao B, Tian CM, Liang YM. 2017. Comparative transcriptome analysis and identification of candidate effectors in two related rust species (Gymnosporangium yamadae and Gymnosporangium asiaticum). BMC Genomics 18:651 DOI 10.1186/s12864-017-4059-x.

Thines M, Kamoun S. 2010. Oomycete-plant coevolution: recent advances and future prospects. Current Opinion in Plant Biology 13:427–433 DOI 10.1016/j.pbi.2010.04.001.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Fredit Frey N, Gianinazzi-Pearson V, Gilbert LB, Handa Y, Herr JR, Hijri M, Koul R, Kawaguchi M, Krajinski F, Lammers PJ, Masclaux FG, Murat C, Morin E, Ndkumana S, Pagni M, Petitpierre D, Requena N, Rosikiewicz P,
Riley R, Saito K, San Clemente H, Shapiro H, van Tuinen D, Bécard G, Bonfante P, Paszkowski U, Shachar-Hill Y, Tuskan GA, Young JPW, Sanders IR, Henrissat B, Rensing SA, Grigoriev IV, Corradi N, Roux C, Martin F. 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* 110:20117–20122 DOI 10.1073/pnas.1313452110.

Trepanier M, Becard G, Moutoglis P, Willemot C, Gagne S, Avis TJ, Rioux J-A. 2005. Dependence of arbuscular-mycorrhizal fungi on their plant host for palmitic acid synthesis. *Applied Environmental Microbiology* 71:5341–5347 DOI 10.1128/AEM.71.9.5341-5347.2005.

Tucker SL, Talbot NJ. 2001. Surface attachment and pre-penetration stage development by plant pathogenic fungi. *Annual Review of Phytopathology* 39:385–417 DOI 10.1146/annurev.phyto.39.1.385.

Turian G, Hamilton RH. 1960. Chemical detection of 3-indolylacetic acid in Ustilago zeae tumors. *Biochimica et Biophysica Acta* 41:148–150 DOI 10.1016/0006-3002(60)90381-4.

Upadhyaya NM, Garnica DP, Karaoglu H, Sperschneider J, Nemri A, Xu B, Mago R, Cuomo CA, Rathjen JP, Park RF, Ellis JG, Dodds PN. 2014. Comparative genomics of Australian isolates of the wheat stem rust pathogen *Puccinia graminis* f. sp. tritici reveals extensive polymorphism in candidate effector genes. *Frontiers in Plant Science* 5:759.

Voegele RT, Struck C, Hahn M, Mendgen K. 2001. The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. *Proceedings of the National Academy of Sciences of the United States of America* 98:8133–8138 DOI 10.1073/pnas.131186798.

Wang B, Sun Y, Song N, Zhao M, Liu R, Feng H, Wang X, Kang Z. 2017a. *Puccinia striiformis* f. sp. tritici microRNA-like RNA 1 (Pst-miR1), an important pathogenicity factor of *Pst*, impairs wheat resistance to *Pst* by suppressing the wheat pathogenesis-related 2 gene. *New Phytologist* 215:338–350 DOI 10.1111/nph.14577.

Wang M, Weiberg A, Dellota Jr E, Yamane D, Jin H. 2017b. Botrytis small RNA BcsiR37 suppresses plant defense genes by cross-kingdom RNAi. *RNA Biology* 14:421–428 DOI 10.1080/15476286.2017.1291112.

Weiberg A, Jin H. 2015. Small RNAs—the secret agents in the plant–pathogen interactions. *Current Opinion in Plant Biology* 26:87–94 DOI 10.1016/j.pbi.2015.05.033.

Wicker T, Oberhaensli S, Parlanege F, Buchmann JP, Shatalina MR, Roffler Stefan, Bendavid R, Doležel J. 2013. The wheat powdery mildew genome shows the unique evolution of an obligate biotroph. *Nature Genetics* 45:1092–1096 DOI 10.1038/ng.2704.

Xu Q, Tang C, Wang L, Zhao C, Kang Z, Wang X. 2020. Haustoria — arsenals during the interaction between wheat and *Puccinia striiformis* f. sp. tritici. *Molecular Plant Pathology* 21:83–94 DOI 10.1111/mpp.12882.

Yin C, Jurgenson JE, Hulbert SH. 2011. Development of a host-induced RNAi system in the wheat stripe rust fungus *Puccinia striiformis* f. sp. Triticum. *Molecular Plant-Microbe Interactions* 24:554–561 DOI 10.1094/MPMI-10-10-0229.
Zhang N, Cai G, Price DC, Crouch JA, Gladieux P, Hillman B, Khang CH, LeBrun MH, Lee YH, Luo J, Qiu H, Veltri D, Wisecaver JH, Zhu J, Bhattacharya D. 2018. Genome wide analysis of the transition to pathogenic lifestyles in *Magnaporthales* fungi. *Scientific Reports* 8:5862 DOI 10.1038/s41598-018-24301-6.

Zheng WM, Huang LL, Huang JQ, Wang XJ, Chen XM, Zhao J, Guo J, Zhuang H, Qiu CZ, Liu J, Liu HQ, Huang XL, Pei GL, Zhan GM, Tang CL, Cheng YL, Liu MJ, Zhang JS, Zhao ZT, Zhang SJ, Han QM, Han DJ, Zhang HC, Zhao J, Gao XN, Wang JF, Ni PX, Dong W, Yang LF, Yang HM, Xu JR, Zhang GY, Kang ZS. 2013. High genome heterozygosity and endemic genetic recombination in the wheat stripe rust fungus. *Nature Communications* 4:2673 DOI 10.1038/ncomms3673.