Genetic Diversity of Barley Foliar Fungal Pathogens

Arzu Çelik Oğuz * and Aziz Karakaya

Department of Plant Protection, Faculty of Agriculture, Ankara University, Dişkapı, Ankara 06110, Turkey; karakaya@agri.ankara.edu.tr
* Correspondence: acelik@agri.ankara.edu.tr

Abstract: Powdery mildew, net blotch, scald, spot blotch, barley stripe, and leaf rust are important foliar fungal pathogens of barley. Fungal leaf pathogens negatively affect the yield and quality in barley plant. Virulence changes, which can occur in various ways, may render resistant plants to susceptible ones. Factors such as mutation, population size and random genetic drift, gene and genotype flow, reproduction and mating systems, selection imposed by major gene resistance, and quantitative resistance can affect the genetic diversity of the pathogenic fungi. The use of fungicide or disease-resistant barley genotypes is an effective method of disease control. However, the evolutionary potential of pathogens poses a risk to overcome resistance genes in the plant and to neutralize fungicide applications. Factors affecting the genetic diversity of the pathogen fungus may lead to the emergence of more virulent new pathotypes in the population. Understanding the factors affecting pathogen evolution, monitoring pathogen biology, and genetic diversity will help to develop effective control strategies.

Keywords: barley; Hordeum vulgare; Blumeria graminis; Pyrenophora teres; Rhynchosporium commune; Cochliobolus sativus; Pyrenophora graminea; Puccinia hordei; genetic diversity

1. Introduction

Barley (Hordeum vulgare L.) is one of the most important cereal crops that has been grown for thousands of years since prehistoric times, and is used in animal feed, malt products, and the food industry. Globally, it ranks fourth in grain production with approximately 150 million tons of production after wheat, rice, and maize [1].

Barley leaf diseases cause significant decreases in yield in all areas where barley is cultivated, and at the same time, reduces the quality. The use of fungicides or disease-resistant barley genotypes is effective in disease control, but the evolutionary potential of pathogens poses a risk of overcoming resistance genes in the plant and neutralizing fungicide applications [2–4]. Information about the evolutionary potential of pathogens is useful in developing control strategies [5–7].

The genetic makeup of a pathogen population is determined by the evolutionary history of that population. It is assumed that genetic makeup information also gives an idea of the evolutionary potential of pathogen populations in the future. Genetic structure refers to the distribution and amount of genetic diversity among and within populations. Genotype diversity and gene diversity are components of genetic makeup. Gene diversity refers to the number and frequencies of alleles in a population at individual loci, while genotype diversity refers to the genetically distinct individuals or the number and frequencies of multilocus genotypes in a population [7].

Changes in pathogen populations have been witnessed many times over the years [8]. The most devastating changes in history have been reported in pathogenic populations capable of breaking major resistance genes [9–11]. Most of these cases have resulted from the spread of a host plant carrying a single major resistance gene over a wide geographic area and occurred as a result of the pathogen population developing a means to overcome this resistance gene [7].
To understand the process by which the effectiveness of the resistance gene is broken down, the processes governing pathogen evolution must be understood. The genetic makeup and evolution of populations is a result of the interaction between the five forces: mutation, reproduction and mating system, gene and genotype flow, population size and random drift, major gene resistance, and selection imposed by major gene resistance and quantitative resistance. Mutation is one of the sources of genetic variation. As a result of mutations, changes in the DNA sequence of individual genes occur and these generate new alleles in populations. New virulent strains of plant pathogens can be formed through the mutations and these could break the major gene resistances. Population size affects the likelihood that mutants will be present. More mutants are observed in large populations compared to small populations and these can affect the diversity of genes in a population through random genetic drift. Disease management programs or climatic extremes that keep pathogen population size small, limit gene diversity and help to control the disease [4,6,7].

Gene flow is a process in which certain genotypes or genes are exchanged among populations. Greater genetic diversity is possible with pathogens that display a high degree of genotype/gene flow. Anthropogenic activities can affect the size of the genetic neighborhood. Humans have transported many different pathogens well beyond distribution borders through agriculture and intercontinental travel and trade [7]. Distribution of gene diversity among and within populations is affected by reproduction and mating systems. Reproduction can be mixed, asexual, or sexual. Pathogens that undergo regular recombination may pose greater risks [7,12].

Female fertility is much more important than the relative numbers of strains of different mating types in determining the given effective population size obtained from Gibberella species. Even when mating-type ratios differ significantly from 1:1, sexual reproduction can still occur regularly. In field populations, polymorphism occurs when the female is sterile or hermaphrodite, and only those female sterile mutants that function as males during sexual reproduction can make up the majority of the population. When a high frequency of female sterile strains is observed in field populations, this indicates that vegetative propagation is an important component of the natural history of the fungus. This theory suggests that there may be significant differences in female fertility between populations of the same species, and within the same species, the frequency of hermaphrodites in local populations may vary significantly. Additionally, if there are significant environmental differences that support sexual reproduction or vegetative reproduction in different places, these differences may become apparent, and the number of loci where female sterile mutations can occur might be large and mutations can be found in more than one locus rather than a single locus. These hypotheses will provide an insight into the evolution of asexual fungus species and how fungi maintain mixed modes of vegetative and sexual reproduction [13].

Selection cause changes in the mutant allele frequencies. When a major resistance gene is widely distributed over a wide geographic area, directional selection occurs [4,14]. Quantitative resistance is another option for obtaining resistant varieties. This is also called partial resistance or minor-gene resistance. Quantitative resistance does not exhibit the boom-and-bust cycle, which is characteristic of major resistance genes. Both minor and major gene resistances can be sensitive to environmental conditions [6,7].

Classification of genetic variation in fungi based on morphological characteristics affected by environmental conditions is very difficult. For characterization of genetic variation and phylogenetic relationships in fungal plant pathogenic populations, techniques such as isozyme and ribosomal DNA analyses, restriction fragment length polymorphism (RFLP), restriction of PCR-amplified internal transcribed spacers of the rDNA (ITS-RFLP), random amplified polymorphic DNA (RAPD) markers, amplified fragment length polymorphism (AFLP), universal rice primer-polymerase chain reaction (URP-PCR), sequence-specific amplified polymorphism (S-SAP), inter-retrotransposon amplified polymorphism
(IRAP), inter simple sequence repeat (ISSR) markers, single nucleotide polymorphism (SNP) markers in housekeeping genes and simple sequence repeat (SSR) markers are used. The result of understanding genetic variation in fungal pathogens is to understand the risk of pathogen evolution and planning resistance breeding strategies. The genetic variation of common barley fungal leaf pathogens is discussed in this review.

1.1. *Blumeria graminis* (DC.) E. O. Speer f. sp. *hordei* emend. É. J. Marchal (anamorph: *Oidium monilioides* Link)

Powdery mildew disease caused by the ascomycetous fungus *Blumeria graminis* (DC) Speer (Syn. *Erysiphe graminis* DC) is one of the most destructive pathogens of barley crops in many barley production areas of the world [15,16]. In connection with the spread of agriculture, it spread from Near East Neolithic agriculture to Europe and Central Asia and to the whole world [17]. The disease reduces the yield and the quality of the harvested grain [18,19].

Many plant pathogenic fungi species are classified into specific forms below the species level (formae speciales, ff. spp). In this taxonomic grouping, each form (f. sp) is adapted to a specific host species and does not differ morphologically from its closest relatives at the species level [20,21]. The concept of form specialis has been useful to pathologists. A form specialis has a direct impact on the possibilities for disease control through genetic resistance. In the process of testing genotypes, ensuring that the true potential of the pathogen is taken into account is crucial to establishing a stable germplasm [22]. In addition, some ff.spps that can show hybridization offer new virulence spectra and a high potential for the emergence of new pathotypes [23].

*Blumeria graminis* has been named and classified according to the host species (formae specialis, ff. spp). The formae specialis infecting barley, wheat, oat, rye, and tetraploid wheat were named as *B. graminis* f. sp. *hordei*, *B. graminis* f. sp. *tritici*, *B. graminis* f. sp. *avenae*, *B. graminis* f. sp. *secalis*, and *B. graminis* f. sp. *dicocci*, respectively. In addition, a few formae specialis affecting wild grasses exist [23].

The validity of the forma specialis category in evolutionary analysis is debatable [24]. Due to the relatively simple morphology of *B. graminis* and lack of polymorphic characteristics between different lineages, evolutionary analyses of ff. spp have been possible with phylogenetic studies carried out using molecular data. However, in cluster analyses, results contradicting the co-evolution of the plant-pathogen hypothesis of formae specialis infecting cereals were reported. For example, the line belonging to the *tritici* clade is estimated to consist of several lineages with different host ranges, and the exchange of genetic material between these lineages through hybridization and introgression can be possible. These results are not consistent with the patterns expected if the host and pathogen have co-evolved. In addition, it is thought that different forms (ff.spp) will not mate due to their specialization to the host, but sometimes they can mate on alternative hosts [20]. However, hybridization with experimental (in-lab) crosses has been successful for some ff.spp (e.g., *B. g. tritici*, *B. g. secalis*-*B. g. tritici* and *B. g. triticale*) [21,25]. These conditions offer a high potential for new virulence spectra and the emergence of new pathotypes [23]. As *B. graminis* is an essential biotroph, the evolution of the pathogen is affected by the spread of various host species [21].

*B. graminis* f. sp. *hordei* causes powdery mildew on barley plants. It has been reported that the genetic makeup of *B. graminis* f. sp. *hordei* (*Bgh*) populations are constantly changing. The level of variability depends on many factors such as long-distance spore distribution, presence of resistance genes in the host, asexual spores production, sexual recombination, and mutation frequency [26–28]. Fungus frequently reproduces asexually, conidia disperse with wind easily, and cause epiphytotics. The barley powdery mildew pathogen has a high potential for gene flow and is considered a high-risk pathogen [29–31]. This pathogen survives on barley plants as asexual colonies or sexual ascospores within the chasmothecia. An annual sexual cycle and subsequent asexual reproduction cycles can increase the frequencies of new allele combinations. Pathogens that undergo regular
sexual recombination pose an evolutionary greater risk. In Australia, high levels of genetic variation have been reported in Blumeria graminis populations, potentially resulting from random mating and propagation. In addition, another factor that seems to have an important effect on genetic diversity is the mixed mating system detected in the region [32]. High allele and haplotype diversity in Blumeria graminis populations are generated by processes such as migration and genetic drift [33,34].

Wind-driven dispersion is the most likely cause of genetic differences between geographic regions, but the movement of conidia through infected plant parts and spread by agricultural machinery and tools is also possible. Although asexual conidia can be transported across continents, it has generally been emphasized that most conidia were deposited close to where they were produced [34]. Blumeria graminis populations may show high genetic diversity if they originate from resistant and evolving local isolates, and lower genetic diversity if they result from isolates migrating from other regions [35], however, this may not be true as a general rule. In a study conducted in China, no correlation was found between isolates from seven different areas in terms of genetic distance and geographical distances [36]. Similarly, Wyand and Brown [37] emphasized that there was a general lack of correlation between genetic and geographical distances in isolates from Asia, Europe, and North America. These results can be related to the long distance distribution exhibited by Blumeria graminis and the selection of different host species [27,28]. One possible reason for the greater genetic distance between genetically close populations may be that the structure of these populations is also partially affected by random genetic drift [36]. Pathogenic variability in Blumeria graminis populations may be related to mutation frequency, sexual recombination, long-distance spore dispersal, and the presence of resistance genes in the host [27,28,38].

Isolates from Finland to Hungary were found to be significantly different when compared to southern European populations [39]. Similarly, Tucker et al. [40] observed significant haplotype differentiation in Western Australian Blumeria graminis isolates. In another study conducted in the British Isles, the majority of the British Blumeria graminis population was genotypically quite diverse, although several isolates sharing the same haplotype in more than one region were collected at 450 km intervals [41]. Despite a long geographic distance between some regions, values of higher gene flow were found. It has been emphasized that in China, Blumeria graminis populations have high genetic diversity and among populations, frequent long-distance gene flow was observed [36]. Among each Blumeria graminis population pair in China, origins of Blumeria graminis isolates and the genetic groups were closely related and the populations differed significantly. These results are evidence that despite the host local adaptation of the Blumeria graminis populations, there is frequent long-range gene flow among these populations [35].

Virulence and changes in genetic evolution affect many countries due to the movement of Blumeria graminis through airborne spores [42]. For example, when comparing the Moroccan, Tunisian, European, Middle Eastern, and Chinese populations, Moroccan isolates shared more prevalent trends in avirulence/virulence with East China isolates compared to isolates from Europe. Similar populations are produced in geographically distant regions with similar selection environments [42]. High allele and haplotype diversity with significant gene flow has been reported in the Blumeria graminis population obtained from Australia, however, there was a low level of genetic geographic differentiation in the population, suggesting that gene flow may not be restricted between geographic regions [32].

The virulence allele frequencies in Blumeria graminis populations are driven by selection exerted by host resistance genes. Blumeria graminis has a long history of defeating single, major resistance genes and pyramids of resistance genes. Barley varieties with major gene resistance may disappear through the corresponding evolution of virulence in the pathogen. Many barley varieties with this type of resistance gene may quickly become susceptible [31]. As Blumeria graminis isolates adapt to barley resistance genes, by direct selection, virulence changes in the pathogen population are induced. The Blumeria graminis genetic variation has been reported to be very high in natural populations, especially in areas where the disease is common [43–45]. For example, in Morocco, although some R genes are present in traditional varieties, virulence pathotypes are expected to evolve into different R genes and it is expected to result in
higher diversity, as resistant varieties are not systematically bred [42]. Factors affecting the distribution and/or identity of R genes in the cultivated barley plant could cause a rapid change in the evolutionary trajectory of Bgh in the region. Using varieties with previously unused R genes in the region enables virulence alleles at low frequencies in the population to reach high frequencies [42]. Due to different selection pressures, different evolutionary results may arise between traditional varieties and modern varieties, and even between varieties planted in mountainous and coastal regions [46–48].

The composition of the host barley population as well as climate, ecological, agricultural factors, and methods used to control plant pathogens affect the genetic diversity and virulence of the pathogen across regions and even continents. Unlike Europe, dry and hot summers in the Mediterranean climate support a compulsory sexual phase [42,49]. This is expected to generate a high variety of pathotypes and genotypes (combinations of virulence genes), thus providing materials for natural selection [29]. In addition, the use of systemic fungicides is expected to lead to the selection of fungicide resistance [50]. New virulence produced by mutations in nearby regions can rapidly combine with existing genotypes through genetic recombination. As a result, the pathogen has the potential to rapidly adapt to host resistance. The wide range of host resistance in grown barley varieties affects the directional selection of migrated or newly mutated virulent strains [31]. In addition, the spores of powdery mildew can spread to any direction with the wind, therefore, directional selection in the region can protect and expand virulence resulting from local mutations. However, in order to successfully migrate over longer distances, virulent pathotypes likely need a suitable host plant with appropriate susceptibility growing along the migration path [51].

Powdery mildew isolates exposed to continuous selection pressures through the varieties with effective major resistance genes or fungicide applications over successive generations will have high adaptability [31]. Furthermore, as a result of gene flow in Bgh populations, haplotype diversity within the population increases significantly. This, combined with Bgh’s other evolutionary capabilities, presents a challenge in the pathogen control method. As virulent individuals increase in the pathogen population, the effectiveness of the respective resistance will decrease. Combining two or more fully effective resistances in one cultivar can delay the emergence of virulent individuals. Mutation-induced recessive alleles of the Mlo locus in barley (mlo) are thought to provide a broad spectrum of resistance to E. graminis f. sp. hordei [52]. The role of Mlo susceptibility genes in plant-pathogen interactions is still unclear, but they are known to be strongly upregulated following Bgh infection [53]. Research should focus on achieving persistent resistance through the Mlo gene commonly seen in breeding new varieties [54]. In most susceptible barley varieties, genes with small effects (quantitative resistance) should be pyramided [55]. With a better understanding of pathogen evolution, the development of complex pathotypes and the adaptation process of pathogen populations will be better understood.

1.2. Pyrenophora teres Drechsler (anamorph: Drechslera teres (Sacc.) Shoem.)

Net blotch of barley caused by Pyrenophora teres Drechsler (anamorph: Drechslera teres (Sacc.) Shoem.) is an economically important barley disease. The pathogen has two forms: P. teres f. maculata (Ptm) causes the spot form and P. teres f. teres (Ptt) causes the net form of the disease [56–58]. Pyrenophora teres survives on plant debris in winter. The disease reduces the green leaf area and grain size, reduces the malt quality, and causes significant yield losses. The two forms of the fungus are morphologically the same, but genetically and symptomatically different [59]. Different researchers have examined the genetic variation of P. teres populations together or according to forms separately. Genetic variation in net blotch fungus has been studied in North America [60], Italy [61], Sweden [62], the Northern Hemisphere and Austria [63], Lithuania [64], Australia and South Africa [65–67], western Canada [2,58], Turkey [68] and Israel [69] by using different molecular markers.
Most of the life cycle of *P. teres* is haploid. Two mating types are required for *P. teres*, a heterotelic fungus, to reproduce sexually [70]. The pathogen has great evolutionary potential as it can be spread both sexually and asexually [7,71,72]. Sexual reproduction between two forms is possible [61,65,67,73].

Net form and spot form of net blotch isolates obtained from five different geographical regions of Italy were genotyped using amplified fragment length polymorphism (AFLP). As a result of cluster analysis, isolates were divided into two different groups according to the form. The absence of intermediate genotypes and the small number of bands shared between the two forms indicated that hybridization between the two forms was rare or absent in Sardinian field conditions. In addition, multilocus linkage disequilibrium and digenic analyses showed that sexual reproduction occurred at significant levels in *Ptt* and *Ptm* populations [61]. In another study, population structure and genetic diversity of *Ptt* and *Ptm* isolates were examined with AFLP analysis in Australia and the southwestern Cape of South Africa. The samples of *P. tritici-repentis*, *Bipolaris sorokiniana*, and *Exserohilum rostratum* were also included in the experiment. *Ptt* and *Ptm* samples formed two different genetic groups [65]. The results showed that sexual reproduction between the two forms is unlikely and reproduction occurs primarily asexually in the Australian and South African *Ptt* and *Ptm* populations. However, as a result of the AFLP analysis of *P. teres*, *P. graminea*, *P. tritici-repentis*, and *Helminthosporium sativum* isolates from the Czech Republic, Leisova et al. [74] showed that although *Ptt* and *Ptm* isolates were in different clusters, intermediate haplotypes with a relatively high number of co-markers were found between the two groups. These results showed that hybridization may occur between *P. teres* forms, but possibly coincided with selection pressure or genetic drift. Their results showed that the variability in *Ptm* and *Ptt* isolates may possibly have been influenced more by the year of sampling rather than the isolate geographic origin. McLean et al. [67] obtained *P. teres* f. *maculata* isolates from different regions of Australia and inoculated them onto a recently developed differential set. Barley lines Barque, Arimont, Chebec, CI5791, CI5286, CI16150, CI9214, Esperance Orge 289, Dairokkaku, Galleon, Skiff, Keel, TR250, and Torrens exhibited differential response. Thirty-three pathotypes were found and some of them were related to geographic regions. In their study, abundant diversity was observed. One isolate contained both AFLP bands unique to *P. teres* f. *teres* and *P. teres* f. *maculata*, suggesting that sexual recombination between these two forms may have occurred in the field. Although they could be identified based on disease symptoms, form specific single nucleotide polymorphism (SNP) primers can also be used to distinguish two forms [75]. In addition, using susceptible cultivars the two forms can be separated easily [76,77]. Using different barley net blotch differential sets, the researchers identified different numbers of pathotypes of *Ptm* and *Ptt* [58,65,67,76,78,79].

In studies examining two forms of *P. teres* together, the genetic variation and differentiation of the *P. teres* f. *teres* population have been reported to be higher than the *P. teres* f. *maculata* population. This may be due to the lower migration rate and the presence of sexual or asexual reproduction in populations [63,65,80].

Akhavan et al. [58] analyzed *Ptm* and *Ptt* populations obtained from three different provinces of western Canada using SSR markers. Among the 110 distinct alleles identified, 16 were specific to *Ptm*, 75 were specific to *Ptt*, and 19 alleles shared between *Ptt* and *Ptm*. A relatively high genotypic diversity was observed. Among all populations, significant genetic differentiation was found. Significant but lower genetic differentiation was found in *Ptt*. Among the *Ptm* populations, no significant genetic differentiation was observed. Isolates clustered in two distinct groups, *Ptm* or *Ptt*. No intermediate cluster was observed. A high number of haplotypes and an equal mating type ratio for both *Ptt* and *Ptm* was observed. According to the authors, both forms of *P. teres* may go through regular cycles of sexual recombination in western Canada. Similarly, Serenius et al. [63] detected high genetic differentiation as a result of AFLP analysis in *Ptt* samples obtained from North America, Northern Europe, Australia, and Russia. Cluster analysis showed that the *P. teres* population obtained from Austria was divided into two subgroups as *Ptt* and *Ptm*.
while *Ptt* samples were reported to have higher variation. In their research, with the exception of Krasnodar, Russia, where only MAT-2 was found, both mating types were equally common (1:1) in several locations in Finland and in Australia. Their results showed that the occurrence of forms of *P. teres*, prevalence of sexual reproduction, and genetic differentiation between geographical regions were highly variable.

However, many researchers found no correlation between the geographical origin and genetic variation of *Ptt* and *Ptm* isolates obtained from different locations [64,66,81,82]. When the pathogen is spread by asexual spores over long distances, this increases the risk of epidemics through migration and gene flow [7,83]. *P. teres* is thought to have limited conidial spread potential [84]. However, both seed-borne infections and seed mobility could potentially increase gene flow [85]. Serenius et al. [63] could not find a clear distinction between *P. teres f. teres* populations obtained from Northern Europe, North America, Russia, and Australia. However, significant differences were observed between the South African and Australian *Ptt* and *Ptm* populations and between the sampling locations for the *Ptt* samples [65]. Similarly, Çelik Oğuz et al. [68] reported that *Ptt* populations clustered more than the *Ptm* populations by location in Turkey.

Leisova et al. [73] and Liu et al. [86] emphasized that the variation in the populations of *P. teres* in the Czech Republic and North Dakota was affected more by the sampling year rather than the geographic origin of the isolates. On the other hand, no differences between *P. teres* populations obtained from Canada [58], Lithuania [64], and Turkey [68] were observed by collection year.

Barley net blotch disease can spread to distant areas with infected seeds, and *P. teres* populations can contain many pathotypes and create new pathotypes [57,64,79]. McLean et al. [66] investigated pathogenic and genetic diversity of *P. teres f. maculata* isolates obtained from Victoria (Australia). In their study, the sexual reproduction hypothesis with a mating ratio of 1:1 in the Victorian pathogen population was supported. Presumably, as a result of random mating and sexual recombination, each isolate displayed a unique genotype pattern. However, the high genetic variation found was not associated with pathogenic variation. The high level of genetic variation points to an important potential for the *Ptm* population to evolve rapidly to adapt to changes in selective pressures such as the deployment of host resistance.

Mutations that occur in pathogen populations can also break the gene resistance of the host and reveal new virulent pathotypes [2,58], Ellwood et al. [2] emphasized that the genotyping of fungicide resistant Cyp51A isolates using SSR markers revealed that a single mutation event occurred, followed by recombination and long-distance regional distribution over hundreds of kilometers.

It is thought that *P. teres* isolates obtained from different hosts also have an effect on the genetic diversity of the pathogen population. In Israel, Ronen et al. [69] genetically and phenotypically characterized the barley net blotch (*Ptt* and *Ptm*) populations obtained from cultivated barley (*H. vulgare*) and wild barleys (*H. vulgare* ssp. *spontaneum* and *H. murinum* ssp. *glaucum*). In their study, isolates did not differ significantly according to host, geographical origin, or form (*Ptt* vs. *Ptm*). However, significant virulence differences were found between isolates. Isolates from wild hosts were found to be more virulent compared to isolates from cultivated barley and it was emphasized that host diversity plays an important role in epidemiology in the barley center of origin. Similarly, from different districts of Turkey and in different years, Çelik Oğuz et al. [68] obtained *P. teres* isolates from different *Hordeum* species (*H. vulgare*, *H. spontaneum*, and *H. bulbosum*) and identified the genetic variations of the *P. teres* isolates by the polymorphic inter simple sequence repeat (ISSR) markers. They found that the similarity rate of *Ptm* isolates was lower than that of the *Ptt* isolates. No definite distinction was found between isolates by year, mating type, or geographic location. Although some isolates from the same wild host and location showed low similarity, some isolates from different wild hosts showed higher similarity to each other. This diversity in isolates from wild barley is thought to contribute to the genetic diversity of the *P. teres* population.
Infection of different *Hordeum* spp. may accelerate virulence evolution. In Australia, Linde and Smith [87] found that genetic diversity of *P. teres* f. *teres* in *H. vulgare* populations was higher than in populations from the *H. murinum* species complex. They found that host-associated *Ptt* isolates could not cross-infect hosts. In their study, barley *Ptt* populations were more diverse than barley grass *Ptt* populations. More frequent sexual reproduction of *Ptt* on the *H. murinum* species complex suggested that the evolutionary potential of pathogens on diverse hosts was high. Extensive gene flow of *Ptt* between regions in Australia was observed. This might show a panmictic population structure, and apparently human-mediated dispersal aided in the virulence evolution of *Ptt* on barley.

Resistance genes in alternative hosts should be investigated and the status of the pathogen in these plants should be monitored. Understanding the evolution of the pathogen on other hosts is essential to breeding strategies. When the high variability of *P. teres* is combined with the potential of sexual reproduction and long-distance spread through infected seeds, it is thought that the emergence capacity of new virulent pathotypes is high. The relatively high rate of differentiation between populations may be a result of the limited migration of *P. teres* [60]. If this is the case, genetic resistance to the net blotch pathogen may be more permanent due to the slow spread of virulent isolates. However, given the level of diversity within populations, the population should be regularly monitored for the emergence of new virulence genes and their combinations, the migration of pathotypes, and the spread of fungicide resistance mutations. The integration and combination of a broad resistance source base resulting from different resistant barley genotypes will likely be beneficial, but will not guarantee full resistance because it is thought that with high adaptation potential of the pathogen, the resistance in genotypes will be constantly changing in response to pathogen adaptation.

1.3. *Rhynchosporium commune* Zaffarano, McDonald, and Linde

*Rhynchosporium commune* Zaffarano, McDonald, and Linde causes scald disease of barley. A teleomorphic stage has not been reported yet. This fungus was first named by Oudemans in 1897 [88] as *Marsonia secalis* and later named *Rhynchosporium secalis* (Oud.) by Davis [89]. Zaffarano et al. [90] renamed the *Rhynchosporium* species. Isolates that cause disease on rye and triticale were renamed as *R. secalis* and isolates causing disease on barley, other *Hordeum* spp., and *Bromus diandrus* were named as *R. commune*.

*R. commune* does not form resistant spores, but spends the winter in the form of stroma in the host plant. Mycelia in the seed coat and pericarp of infected seeds can be the first source of infection. Symptoms of the disease first appear as gray-green-bluish oval and irregular greasy spots, then whitish gray in the middle, dark brown on the edges, and in different sizes. Symptoms are most common in the leaf blade, but can be seen in the sheath, nodes, stem, and heads [59,91].

Barley scald disease has been reported in over 50 countries from Asia, Europe, Africa, Oceania, North, and Latin America [91,92]. The disease is seen in areas where barley production is made, and it is more common in cooler and semihumid regions. This fungus can cause a 35–40% yield loss in barley growing areas [59,91].

*R. commune* populations are genetically and phenotypically diverse [15,93–96]. Pathogenic variation in *R. secalis* populations was tested using different barley genotypes. High pathogenic variation reported in numerous studies and two to 75 pathotypes have been reported by different researchers [96–103]. However, pathogenicity tests depend on the barley variety used, and the response of the variety to the pathogen may change over time as a result of a change affecting the virulence of the pathogen [104].

*Rhynchosporium commune* is an imperfect fungus with a haploid structure. Even though its sexual stage is unknown, the presence of two mating types has been reported by many researchers. Although both mating types are seen in some countries, mating type ratios indicate asexual reproduction (Iran [105], Syria [106], and Turkey [107]), while in some countries and states (Switzerland, Australia, Ethiopia, South Africa, Scandinavia, and California) [108–111], they indicate sexual reproduction.
asexual reproduction is proposed, according to the null hypothesis, mating type ratios in some regions confirmed the sexual reproduction hypothesis (Turkey and Iran). Foster and Fitt [112] found both mating types in *R. secalis* populations obtained from different parts of England. *R. secalis* may be a heterothallic dyscomycete, although the teleomorphic phase has not been identified yet. If present, the teleomorph of *R. secalis* is, with high possibility, a small apothecium produced on infected tissues [103].

Brunner et al. [113] and Zaffarano et al. [109] hypothesized that the origin of the fungus *R. secalis* was not the Fertile Crescent or the secondary gene center of the barley plant, but that the origin of the fungus was northern Europe and spread from there to the south, North America, and Australia by various commercial or anthropogenic vectors. In addition, it has been reported by various researchers [106,107,109,113] that the genetic diversity of *R. commune* is low in regions where barley originates, and the genetic diversity is high in regions without barley gene centers such as Europe, Australia, and America.

East of the Iranian Plateau is the secondary gene center of barley. In a study comparing *R. commune* populations obtained from Iran and Syria, it was reported that the Iranian population had lower genetic diversity than the Syrian population and reproduced asexually. It is thought that the pathogen has entered some parts of Iran through infected seeds. The results of this study also show that *R. commune* and barley did not evolve together in the origin of cultivated barley [106]. Similarly, Turkey is one of the most important gene centers of barley. Southern and southeastern Turkey are located in the Fertile Crescent region. Low genetic variation was found in a study in which Turkish *R. commune* populations were analyzed with ISSR markers. This study also supports the hypotheses of Zaffarano et al. [109] and Brunner et al. [113]. However, while mating type ratios support asexual reproduction in the total population, the sexual reproduction hypothesis was supported despite the limited number of isolates from the southeastern Anatolia region. This information shows that genetic variation in the region is likely to change over time [107].

Bouajila et al. [94] analyzed *R. secalis* isolates collected from different hosts from Tunisia with AFLP and microsatellite markers and emphasized that the variation was not associated with pathotype, host, or agro-ecological regions. In another study, *R. secalis* isolates obtained from West Asia and North Africa were analyzed with RAPD and AFLP molecular markers and high genetic similarity was observed between isolates obtained from the same region. This similarity is thought to be the result of the spread of the pathogen’s conidia by rain splashes. Rain provides the distribution of spores in the field to certain distances [114]. On the other hand, high gene and genotype variation was found in Australia, California, Finland, and Norway *R. secalis* populations [93,115]. Zaffarano et al. [109] found a significant relationship between genetic variation and geographical distance in *R. secalis* isolates obtained from barley, wild barley (*Hordeum spontaneum*), and rye, emphasizing that gene flow is locally common but rare across continents. Mating type ratios supported the sexual reproduction hypothesis in most populations ($\chi^2$ values of the populations: Australia 0.082, Eritrea 0.360, Ethiopia 0.043, Finland 4.412 *, Jordan 0.040, Norway 0.800, South Africa 1.152, Switzerland 1.688, Syria 4.310, and United States 6.000 * (p < 0.05)). The highest genetic variation was found in populations of Scandinavia followed by Swiss populations. In addition, research on the genetic structure of *R. commune* revealed that the pathogen retains a high level of genetic diversity on a microscale [109–111].

Generally, the center of genetic variation is the origin of the pathogen’s host. A high genetic variation is expected in the barley pathogen *R. commune* in the Fertile Crescent region where barley originated. However, while high genetic variation has been reported from *R. secalis* populations of Australia, California, Finland, Norway, Scandinavia, and Switzerland, low genetic variation and high genetic similarity between populations of North Africa, West Asia, Tunisia, Syria, Iran, and Turkey were observed. Results from these studies supported the hypothesis outlined in Brunner et al. [113] and Zaffarano et al. [109].

Genetic variation in *R. commune* populations responds rapidly to selection pressure caused by the use of novel fungicides. *R. commune* develops resistance against benzimidazole fungicides faster and slower against triazole fungicides [116,117]. Populations exposed
to tebuconazole, epoxiconazole, and flusilazole were found to have lower susceptibility compared to populations not previously exposed and a decrease in the effectiveness of these fungicides against the pathogen was emphasized [118,119].

Variation and mutation in the CYP51 gene family play a very important role inazole fungicide resistance in various fungus species [120]. One mechanism known to contribute to azole resistance in \textit{R. commune} has led to the emergence of CYP51A, the parologue of CYP51 [3,4,120]. It is suggested that in \textit{R. commune} populations, CYP51A is the most important source of fungicide resistance. CYP51A was found in the most resistant \textit{R. commune} populations from Switzerland and New Zealand and it is thought that fungicide selection pressure may have an impact on the genetic variation in \textit{R. commune} populations [4].

The hypothesis that the origin of \textit{R. commune} is not the same as the origin of barley has been supported by studies showing that high levels of gene flow and recombination occur in regions where the pathogen originates. For this reason, regions with high genetic variation carry significant risks in terms of regional breakdown of resistance genes or rapid spread of fungicide resistance. In northern Europe, which is thought to be the origin of the pathogen, additional host resistance sources can be found. If there is specialization in the host, it will be necessary to determine whether genetic variation will occur among the specialized populations. Sequence information about the genomes of the \textit{Rhynchosporium} species can be used with comparative genomic techniques and it is thought that these techniques will provide information about the host-specific interaction and the sexual evolution of the pathogen. Since the pathogen is also commonly observed in wild barley species [121,122], the role of these plants in virulence evolution should be studied.

\textbf{1.4. Cochliobolus sativus (Ito and Kurib.) Drechs. ex. Dastur) (anamorph: Bipolaris sorokiniana (Sacc.) Shoem.)}

\textit{Cochliobolus sativus} (Ito and Kurib.) Drechs. ex Dastur) (anamorph: Bipolaris sorokiniana (Sacc.) Shoem.) is the causal agent of spot blotch disease. This fungal pathogen also causes root rot, black point, and seedling blight [123]. The host range of the fungus is wide. It is pathogenic on barley, bread and durum wheats, triticale, rye, corn, rice, and some grass species [124,125]. It is more prevalent in cereal growing regions with hot and humid conditions and its importance has increased in Europe and America in recent years [125]. Although the yield losses vary according to the country and host, the yield loss can be up to 70–100\% in short crop rotations or rotations consisting predominantly with cereals [126,127]. Spot blotch is one of the most important diseases of barley. Symptoms appear as dark brown necrotic spots on the leaves of above-ground plant parts [59,124].

\textit{Bipolaris sorokiniana} survives on host plants and on infected plant debris in the field as conidia and in seeds, soil, or infected plant tissues as mycelia. Infected seeds are considered the primary inoculum source. Fungus isolates show high variability, especially in interactions with \textit{H. vulgare} [125]. Pseudothecia, the sexual stage of the fungus, is rare in nature [128].

Generally, in \textit{B. sorokiniana}, genetic diversity has been considered together with pathogenic diversity. The presence of pathotypes in the fungus was first described by Valjavec Gratian and Steffenson [129] with three pathotypes named 0, 1, and 2. Arabi and Jawhar [130,131] identified three different pathotypes from Syria, and Meldrum et al. [132] identified six pathotypes from Australia. In Canada, Ghazvini and Tekauz [133] evaluated the virulence diversity of 127 \textit{B. sorokiniana} isolates from Canada and other countries and identified eight virulence groups. Next, Leng et al. [134] identified a new pathotype, which they called pathotype 7. In studies showing that \textit{B. sorokiniana} populations have a high level of genetic variability, it is thought that migration, mutation and gene flow, and recombination, which are three general sources of variation, are the cause of genetic variability [83,135].

Zhong and Steffenson [136] identified three pathotypes (0, 1, and 2) among \textit{C. sativus} isolates obtained from different regions. In their study, pathotype 2 showed high virulence, but considering the genetic variation of the population, the allelic diversity in the pathotype
2 group was lower than the pathotype 1 and pathotype 0 groups. Although two AFLP markers specific to pathotype 2 isolates have been identified, no close correlation between pathotypes and AFLP groups were observed. The authors further suggested that genetic change in the fungus population may have occurred via parasexual recombination.

The presence of two mating types [137,138] and somatic hybridization [139] suggest the possibility of both asexual and sexual recombination as a source of variation in the B. sorokiniana population. B. sorokiniana isolates collected from different parts of Canada and other countries have been found to have a high level of genetic variation [135]. In this study, while isolates with different virulence in barley genotypes can be clearly distinguished, the molecular analysis did not show a strong distinction between six different virulence groups defined by the classical pathotype identification method. A closer correlation was found between virulence and AFLP patterns than between the AFLP pattern and the geographic origin of the isolates. The authors hypothesized that the genetic profiles of three groups of isolates, which are among the high virulence and low virulence isolates, may be the result of genetic recombination.

Genetic variation among microorganisms may occur through parasexual recombination, and recombination may result in the appearance of new types of virulence in the microorganism populations [140,141]. The mutation can be a strong variation source in B. sorokiniana and its isolates are prone to spontaneous or induced mutation [135,140,142]. It has been suggested that chromosomal rearrangements are the main diversity source in B. sorokiniana populations [136,143].

High levels of genetic variability were found within groups of isolates collected from the same location, and even among isolates of B. sorokiniana from a single lesion [135,144]. In a study investigating the pathogenic and genetic variation of C. sativus from North China, 19 pathotypes from 71 isolates were found [145]. In the dendrogram formed in their study using the AFLP method, no relationship was found between the isolates and their origin. The same pathotype isolates or isolates obtained from the same location did not group into clusters. On the other hand, common genetic profiles between isolates collected from different regions of Canada [135], and North Dakota and Poland [136] were found.

Al-Sadi [146] investigated the infection response of B. sorokiniana isolates to wheat and barley cultivars. Necrotic lesions were observed on inoculated wheat and barley cultivars. No significant differences were seen in the necrotic lesion size with the majority of isolates. However, varying levels of chlorotic lesions and spores were observed. The author concluded that a lack of host specialization of the pathogen on wheat and barley was present.

In Australia, Knight et al. [147] determined the differences in Australian B. sorokiniana populations. Spot blotch isolates clustered apart from common root rot infections. B. sorokiniana isolates collected from barley spot blotch exhibited a high level of pathogenic variability in the differential set genotypes. Isolates formed three clusters showing low, intermediate, or high pathogenicity levels. The authors suggested that in Australia, population divergence within the pathogen isolates related to host specificity was present.

Baturo-Ciesniewska [148] investigated the genetic variation among the B. sorokiniana isolates obtained in different years from different areas of barley plants (roots, grains and leaves), and from different agricultural systems (conventional, organic, and integrated farming systems). Genetic diversity was found among the isolates, but a clear effect of any factor was not revealed. The source of variation is thought to be formed by the interaction of several factors, rather than a single factor. It is thought that the geographical origin, cultivation system, host variety, and in some cases, the plant organ from which the isolate is obtained, may affect the genetic diversity. A significant difference among the pathogenicity of the isolates was observed. Most isolates exhibited more lesions on the roots compared to leaves. There were no significant correlations between pathogenicity and origin of isolates from different plant parts or mycelium morphology.

Weikert-Oliveira et al. [149] compared B. sorokiniana isolated from wheat, B. oryzae from rice, B. maydis, and Exserohilum turcicum from maize using random amplified polymorphic
DNA (RAPD) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analyses. Genetic differences among the isolates were found. A significant level of polymorphism among different species was present, on the other hand, low level of polymorphism among isolates of the same species were observed. Isolates were grouped according to species and their host. No directly related polymorphism correlated with a geographic source of the isolates of *B. oryzae* and *B. sorokiniana* and climatic factors were observed.

Universal rice primers were found to be sensitive and they could be used to analyze the genetic variability of *B. sorokiniana*. Aggarwal et al. [150] grouped the *B. sorokiniana* isolates obtained from wheat plants by geographic origin using universal rice primer-polymerase chain reaction (URP-PCR). This analysis also provided important information about the relationship among the isolates and degree of genetic variability. However, the separation of the isolates by their geographic origin was not observed in the dendrogram. The authors suggested that gene transfer through parasexual combination may have occurred in the *B. sorokiniana* population. Patterns that characterize the profile of *B. sorokiniana* were not possible. Zhong and Steffenson [138] analyzed 54 field isolates of *C. sativus* collected worldwide using DNA hybridization and PCR amplification analysis and found that both mating types existed in populations around the world. Low frequency of successful backcrosses of progeny to parents was found in a cross of MAT-1 and MAT-2 isolates and many crosses between opposite mating type isolates were unsuccessful. The authors concluded that genetic factors other than MAT genes may affect the fertility of the fungus.

Studies have shown that genetic variation in the *B. sorokiniana* population is often handled with pathogenic diversity. Identification of the isolates of this pathogen, which is increasing especially in hot and humid regions in the world, by using molecular tools, and the use of specific isolates can help breeders or pathologists in choosing the appropriate cultivars. In order to avoid disease outbreaks in the future, efforts should be made to use and develop new genetic resources against spot blotch in barley germplasm by using spot blotch pathotypes identified in these studies. In other words, to face the threat posed by newly arisen highly virulent isolates, the isolates that create variation must be constantly observed.

1.5. *Pyrenophora graminea* Ito and Kuribayashi (anamorph: *Drechslera graminea* (Rabenh. ex. Schlech.) Shoemaker)

Barley stripe disease is caused by the fungal pathogen *Pyrenophora graminea* Ito and Kuribayashi (anamorph: *Drechslera graminea* (Rabenh. ex. Schlech.) Shoemaker). Barley stripe is a single cycle and seed-borne disease. It is present in many regions of the world including Europe, China, Russia, India, North Africa, Turkey, and North America [151–155]. Significant yield losses can occur in areas with the disease. The disease manifests itself as chlorotic and necrotic areas in the leaves and heads are also impaired [59]. Variation in virulence, morphological, and physiological characteristics of *P. graminea* has been reported [156–160]. The fungus has a wide variation in conidial and cultural characteristics, virulence, and fungicide response [156,161–163]. However, there are limited studies related to population structure and molecular genetic variation of the fungus [161,164].

*P. graminea* is a heterothallic fungus with sexual and asexual reproduction ability [59, 80,165–167]. This mixed reproduction system and long-distance dispersal capability of the fungus make the genetic variation of the populations of *P. graminea* important [158]. Rapid adaptation of new pathogen genotypes that possess rich genetic variation to new hosts or fungicides is important in pathogen control [162]. The observation of the sexual stage in the field suggests that genetic recombination may be responsible for variation [156,168,169].

Genetic recombination as a result of sexual reproduction has a dominant effect on the adaptation and dynamics of a species. The way to evaluate the existence of sexual reproduction is to determine the frequency and occurrence of mating type genes. While this is insufficient to prove an active sexual cycle, according to null hypothesis, it is possible for sexual recombination to occur if the two types of mating occur approximately or equally frequently within a given population. [12,170]. Sexual stage of *P. graminea* may occur on
stubble or field debris between growing seasons and plays a role in the formation of the future population and then only asexually spread [166]. This hypothesis can be tested by monitoring changes in the genetic makeup of field populations during the growing season. Dokhanchi et al. [166] reported an almost equal distribution of mating type alleles within and between different *P. graminea* populations in Iran. They frequently observed MAT-1 and MAT-2 types from different regions, different fields in the same region, from the same field, and even within the same lesion of each leaf from a single barley plant. The authors concluded that this pathogen undergoes regular cycles of sexual recombination in most of the regions examined.

High genetic variation, probably resulting from the active sexual cycle in the Syrian *P. graminea* population, has been reported [164,171]. On the other hand, Bayraktar and Akan [172] reported low genetic variation in Turkish *P. graminea* populations. In their study, 45 isolates obtained from different geographic origins and barley genotypes produced identical RFLP patterns. Among the isolates, ISSR analysis revealed little genetic variability. Isolates were separated into four clusters and Turkish isolates were clustered separately from the Italian isolates. The authors concluded that Turkish *P. graminea* populations were genetically homogeneous and may be derived from a single gene pool. *Pyrenophora graminea* isolates were collected from Syria and evaluated using ITS-RFLP formed two phylogenetic groups. These groups were not divided into clusters or classes according to the origin or color of the isolate [151]. Al-Daoude et al. [171] tested *P. graminea* isolates using RAPD and ISSR markers in Syria and found no correlation between the geographical distribution of isolates and their genetic relationships and virulence levels and genetic relationships. In another study conducted in Syria, *P. graminea* isolates were evaluated using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). In their study, isolates were divided into three groups. No significant correlation was found between the number of protein subunits and the virulence levels of the isolates [173]. Arabi and Jawhar [174] studied *P. graminea* isolates obtained from diverse geographical regions of Syria in relation to vegetative compatibility groups (VCGs), pathogenicity, and variation within the internal transcribed spacers (ITS) of ribosomal DNA. They observed variation in the pathogenicity and in the resistance level to isolates and among genotypes. Isolates were grouped into three VCGs. No self-incompatibility was seen in the tested isolates. The authors concluded that the virulence level was related to VCG. Isolates within a VCG subgroup were molecularly similar and clustering of isolates were correlated with VCG subgroups regardless of the geographic origin and pathogenicity. VCGs also differed in molecular variability.

Information on the sexual cycle, variation, and distribution of *P. graminea* will be useful for identifying suitable isolates to be used to screen for the resistant barley lines, and for future sampling and mapping studies. Furthermore, detection of the source of infection is necessary to estimate the spread of the disease to locations and to study the nascent colonization. There is no genetic mapping study for *P. graminea*. A sufficient number of lineages will need to be obtained from hybrids in order to create a genetic map [175]. Mapping studies can be helpful in pathogenicity differentiation, resistance breeding, and disease control.

1.6. *Puccinia hordei* G. Otth.

*Puccinia hordei* G. Otth., the causal agent of barley leaf rust, is macrocyclic and heteroecious rust. *P. hordei* belongs to the genus *Puccinia*, the largest genus of the Pucciniales order with 3000 to 4000 species [59,176].

Barley leaf rust pathogen *P. hordei* forms spherical light orange-brown pustules on leaves. In many regions around the world, it is an economically important disease of barley, lowering the yield up to 62% in susceptible cultivars during an epidemic [177]. Significant yield losses due to this pathogen have been reported from Australia [177–180], New Zealand [181], Europe, and the USA [182].
Alternative hosts of *P. hordei* are species in the genera of *Ornithogalum*, *Leopoldia*, and *Dipodi*. In addition to cultivated barley (*H. vulgare*), the pathogen can cause disease on *H. vulgare* ssp. *spontaneum*, *H. bulbosum*, and *H. murinum* [121, 122, 183, 184].

The importance of the alternative host in the life cycle of the fungus varies according to the region. For example, it is insignificant in places such as Europe where the growth of *Ornithogalum* does not coincide with the germination of the fungus teliospores [185]. Additionally, in regions such as North America, the role of the alternative host is considered insignificant to generate genetic variation, and the source of variation is considered to be mutations. However, in Israel, where *Ornithogalum* species and wild *Hordeum* species coexist, an alternative host is known to play a role in the pathogen survival and recombination that creates genetic diversity [183].

In Australia, *P. hordei* is the only cereal rust pathogen reported to be sexually recombined [186]. *Ornithogalum umbellatum*, an alternative host of *P. hordei* known to play a role in creating pathogenic variability, has been reported to be widespread in the Yorke Peninsula in South Australia [187, 188]. The conditions in this area are suitable for pycniospore, aeciospore and basidiospore production [187]. However, the telial stage is more important in the survival of the pathogen [189].

Alternative hosts can contribute to pathogenic variation through sexual recombination. Six *P. hordei* pathotypes were identified from aeciospores collected from infected *O. umbellatum* plants in South Australia and a high diversity of *P. hordei* pathotypes has been reported in pathogenicity surveys in South Australia [187, 189].

Limited research has been done using various marker systems to assess the genetic variation of the barley leaf rust pathogen at a global level [190–194].

Newton et al. [190] examined the variation in dsRNA and isozymes within and between *P. hordei*, *Puccinia striformis*, and *P. recondita*. Among the wheat-attacking form of *P. striiformis* (WYR), no difference was observed. Barley-attacking form (BYR) of *P. recondita* and of *P. hordei* exhibited similar intra-group uniformity in smaller numbers of isolates. The researchers found major differences in isozyme phenotypes between the three species. All isolates within each group showed the same or a similar phenotype for WYR and BYR. On the other hand, each isolate of *P. hordei* and *P. recondita* had a unique phenotype. Both between the three species and between BYR and WYR, differences in dsRNA phenotypes were observed.

Isolates of *P. hordei* and *P. striiformis* f. sp. *hordei* obtained from different countries and isolates of *P. striiformis* f. sp. *tritici* and *P. recondita* obtained from the UK were characterized using RAPDs, RFLPs, rDNA intergenic sequence, or virulence on differential cultivars. Rust species were readily distinguished by both RAPDs and RFLPs. Variability was observed using differential cultivars, RFLPs, and RAPDs with probes from the rDNA region within a species. However, using a wide range of random probes, the researchers were unable to detect RFLPs from a *P. hordei* genomic library [191].

Sun et al. [192] evaluated the genetic diversity of *P. hordei* isolates using amplified fragment length polymorphism markers in Australia. *P. graminis* f. sp. *secalis* and *P. graminis* f. sp. *tritici* pathotypes were included for comparison. *P. hordei* was distinctly different from *P. graminis* f. sp. *secalis* and *P. graminis* f. sp. *tritici*. Five groups of *P. hordei* isolates were recognized. The authors concluded that *P. hordei* molecular diversity could be associated with virulence, but not well with the geographic origin of the isolates.

Microsatellite markers available for similar rust types were not found to be polymorphic in *P. hordei* [195, 196]. However, in Australia, Karaoglu and Park [193] tested *P. hordei* isolates with seventy-six polymorphic microsatellite loci. Although the number of isolates was low, the markers displayed relatively high polymorphic information content (PIC) values, indicating a high degree of genetic variation among Australian *P. hordei* population isolates. The authors reported that these microsatellite markers, which were used for the first time in population genetics studies for *P. hordei*, are promising.

Another useful tool for the evaluation of genetic variation in *P. hordei* is the PCR fingerprinting technique. Sandhu et al. [194] used PCR-fingerprinting primers (GACA)₄
and M13 to assess the genetic variability in *P. hordei*. Polymorphisms were observed with two primers, however, more polymorphism was reported with the use of primer (GACA)$_4$. Clonality among the pathotypes of *P. hordei* was observed. This supported the hypothesis that some were the result of mutational changes of a founding *P. hordei* genotype. The authors obtained evidence of sexual recombination within *P. hordei* on the *O. umbellatum* in Australia.

Results from long-term studies of pathogenic variability in wheat rust pathogens in New Zealand and Australia have provided important evidence of rust migration between these two geographic regions [197]. However, the differences in the Australian pathotypes found in barley rust *P. hordei* show that the Australian and New Zealand populations are different [194].

*P. hordei* can survive in different hosts, which provides the opportunity to create a high level of variability. A large number of isolates must be studied in order to better characterize the genetic relationships between and within the pathotypes. It will be useful to monitor the movement of *P. hordei* on a global level, particularly exotic isolates. Genome sequence data and strong discriminatory markers such as locus-specific SSRs can better address evolutionary and diagnostic questions, contributing to the development of sustainable rust control, surveillance, and management strategies for this important barley pathogen.

### 1.7. Effect of Genomic Structure of Barley Leaf Pathogens on Genetic Variation

Möller and Stukenbrock [198] reviewed the genome architecture and evolution in fungal plant pathogens. Interaction between pathogens and plants activates a co-evolutionary dynamic in which pathogens evade from defense systems of plants and plants recognize pathogens. In both pathogens and plants, genetic variation at the population and genomic levels is shaped by these evolutionary processes. Fungal species can rapidly evolve and adapt to new environmental conditions. In fungi, genome plasticity and transposable elements have been identified as important forces of rapid evolution. In plant pathogenic fungi, virulence-related genes are typically found on specific chromosomes or in genomic compartments that are rich in transposable elements. Genes found in genomic environments with high recombination and/or mutation rates may evolve faster and effector genes found in regions rich in transposable elements may accumulate more mutations. The genomic data of the pathogenic fungus population allows for an understanding of the mechanisms of traits involved in host–pathogen interactions, genome size changes, their history, and adaptive evolution. Loci or genes that are subject to strong selection pressures can be identified by the distribution of substitutions and polymorphisms and rates of mutations in coding sequences. Coevolution of effectors and resistance genes may have effects on genetic diversity. Recombination and mutations contribute to the genomic variation. Natural selection and genetic drift affect the fate of any allele or mutation in the genome, and the effective population size of the species contributes to these processes.

Although progress has been made, the underlying population genetics processes important in shaping genome structure in pathogens are still not fully understood. Advanced models can help to better understand them. New studies to be conducted in the light of existing studies will provide a better understanding of the evolution of pathogens. Some important studies on the genomes of major barley pathogens discussed in our current review are shown in Table 1.
Table 1. Some important studies on the genomes of major barley pathogens.

| Pathogen                                      | Study Details                                                                 |
|-----------------------------------------------|------------------------------------------------------------------------------|
| \textit{Blumeria graminis} (DC.) E. O. Speer f. sp. \textit{hordei} emend. É. J. Marchal (anamorph: \textit{Oidium monilioides} Link) | A genetic map based on functional genes, avirulence genes, and molecular markers Pedersen et al., 2002 [199] |
|                                               | Gene expression profiles                                                     | Both et al., 2005 [200] |
|                                               | Sequencing of the \textit{Bgh} genome                                       | Spanu et al., 2010 [201] |
|                                               | Genome structure and transcriptional programs in divergent hosts             | Hacquard et al., 2013 [202] |
| Transposable elements (TEs)                   | Different formae speciales of \textit{B. graminis}                          | Oberhaensli et al., 2011 [203] |
| Unique and reliable presence/absence variation (PAV) markers | Komínková et al., 2016 [204] |
| Lifestyles of pathogens                       | Amselem et al., 2015 [205] |
| Update of genome sequence and transposable element (TE) families | Frantzeskakis et al., 2018 [206] |
| Proteome analysis                             |                                                                                | Kusch et al., 2014 [207] |
| Evolutionary history                          |                                                                                | Menardo et al., 2017 [23] |
| Effector genes                                | Schmidt et al., 2014 [208] |
|                                               | Aguilar et al., 2016 [209] |
|                                               | Menardo et al., 2017 [210] |
|                                               | Pham et al., 2019 [211] |
| \textit{Pyrenophora teres} Drechsler (anamorph: \textit{Drechslera teres} (Sacc.) Shoem.) |                                                                                | Halterman et al., 2001 [212] |
|                                               | Pedersen et al., 2002 [199] |
|                                               | Skamnioti et al., 2008 [213] |
|                                               | Lu et al., 2016 [214] |
|                                               | Dreiseitl, 2017 [215] |
| \textit{Pyrenophora teres} f. teres           | Genome assembly using Illumina sequencing                                     | Ellwood et al., 2010 [216] |
|                                               | Effector genes                                                               | Friesen et al., 2008 [217] |
|                                               |                                                                                | Ismail et al., 2014 [218,219] |
|                                               |                                                                                | Liu et al., 2015 [220] |
|                                               |                                                                                | Wyatt et al., 2018 [221] |
|                                               | Avr genes                                                                    | Weiland et al. 1999 [222] |
|                                               |                                                                                | Lai et al., 2007 [223] |
|                                               |                                                                                | Beattie et al., 2007 [224] |
|                                               |                                                                                | Wyatt et al., 2018 [221] |
|                                               |                                                                                | Wyatt et al., 2020 [225] |
|                                               | Genomic analysis comparison and identification sub-telomeric regions          |                                                                                |
|                                               | Updated isolate 0-1 reference genome assembly and annotation                 | Wyatt et al., 2018 [221] |
|                                               | Transposable elements (TEs)                                                  | Syme et al., 2018 [226] |
|                                               | In vitro secretome profile                                                   | Muria-Gonzalez et al., 2020 [227] |
| \textit{Pyrenophora teres} f. maculata        | The first \textit{P. teres} f. \textit{maculata} genome sequence and transposable elements (TEs) | Syme et al., 2018 [226] |
|                                               | Four reference quality genome assemblies                                     | Wyatt and Friesen 2020 [228] |
| \textit{Rhynchosporium commune} Zaffarano, McDonald, and Linde | Necrosis inducing peptides (NIP1, NIP2, and NIP3) were identified | Wevelsiep et al. 1993 [229] |
|                                               | A phylogeographical analysis using nuclear DNA sequences                     | Zaffarano et al., 2009 [230] |
|                                               | Necrosis-inducing protein (NIP) effectors                                    | Schürch et al., 2004 [231] |
|                                               |                                                                                | Kirsten et al., 2012 [232] |
|                                               |                                                                                | Stefansson et al., 2014 [95] |
|                                               |                                                                                | Gamble et al., 2016 [233] |
|                                               |                                                                                | Mohd-Assaad et al., 2019 [234] |
Table 1. Cont.

| Rhynchosporium commune | Zaffarano, McDonald, and Linde |
|------------------------|--------------------------------|
| Identification and characterization of the PFP1 gene | Siersleben et al., 2014 [235] |
| Sequencing and annotation of the complete mitochondrial (mt) genomes of four closely related Rhynchosporium species | Torriani et al., 2014 [236] |

| Comparative population genomics studies |
|----------------------------------------|
| Torriani et al., 2014 [236] |
| Penselin et al., 2016 [237] |
| Mobd-Assaad et al., 2019 [234] |

| Genome plasticity |
|-------------------|
| von Felten et al., 2011 [238] |
| Zhu et al., 2018 [239] |

| Avr genes |
|-----------|
| Rohe et al., 1995 [240] |

| Cochliobolus sativus (Ito and Kurib.) Drechs. ex. Dasturi (anamorph: Bipolaris sorokiniana (Sacc.) Shoem.) |
|--------------------------------------------------------|
| Genome sequence (34.4 mbp, isolate ND90Pr) | Ohm et al., 2012 [241] |

| Extracellular enzymes |
|-----------------------|
| Chand et al., 2014 [243] |

| Identities and role of the cell wall-degrading enzymes (CWDE) |
|-------------------------------------------------------------|
| Aich et al., 2017 [244] |

| Detection of the virulence gene ToxA in the wheat and barley |
|-------------------------------------------------------------|
| McDonald et al., 2018 [245] |

| Isolation and structural elucidation of chlorinated metabolites |
|---------------------------------------------------------------|
| Han et al., 2019 [246] |

| The mitochondrial genome and phylogeny |
|----------------------------------------|
| Song et al., 2020 [247] |

| Secretome analysis |
|--------------------|
| Pathak et al., 2020 [248] |

| Pyrenophora graminea | Ito and Kuribayashi (anamorph: Drechslera graminea (Rabenh. ex. Schlech.) Shoemaker) |
|---------------------|-----------------------------------------------|
| Candidate gene screening | Ghannam et al., 2016 [153] |
| Transposable elements | Taylor et al., 2004 [161] |
| Development and characterization of microsatellite markers based on whole-genome sequences and pathogenicity differentiation | Si et al., 2019 [175] |
| Characterization of the pgpbs gene, a mitogen-activated protein kinase kinase | Liang et al., 2019 [249] |
| The first genome resource | Si et al., 2020 [250] |

| Puccinia hordei | G. Otth. |
|----------------|---------|
| Puccinia hordei genome predicted by cytometry study | Kullman and Teterin 2006 [251] |
| Effector genes, genomic and transcriptomic comparisons | Park 2015 [252] |
| Diagnostic simple sequence repeat (SSR) markers | Karaoglu and Park 2014 [193] |
| Avr genes and the first genome assembly | Chen et al., 2019 [253] |

2. Conclusions

Finding effective and sustainable control measures poses a major challenge in crop protection research. It is important to have knowledge about the genetic variation of local fungal populations and the mechanisms that lead to the generation of new pathotypes/strains, in order to ensure the longevity of newly bred resistant barley varieties. Knowledge of the amount of genetic variation and its distribution among populations will assist in understanding the biology of the pathogen and developing effective control measures. A better understanding of evolutionary forces helps to predict the growth potential of pathogen populations in agricultural ecosystems. Pathogens that pose the greatest risk to overcome resistance genes in the host are the ones that have a mixed breeding system (sexual + asexual), high genotype flow potential, high mutation rates, and large effective population sizes. Pathogen populations possessing high evolutionary potential are more likely to overcome genetic resistance in the host [7]. Selection is the evolutionary force that can be most easily controlled through human intervention and, therefore, offers the chance to inhibit the evolutionary process in the pathogen. Agricultural systems that place the
major resistance genes in rotations as well as other non-major forms of gene resistance, and properly used fungicides will reduce the effectiveness of selection. Disruption of selection will slow the rate of increase in the frequency of more virulent or fungicide resistant mutants [32]. The populations should be monitored regularly for the emergence of new virulence genes and their combinations, the spread of fungicide resistance mutations, and the migration of pathotypes. It should not be forgotten that as Fahrenholz said “parasite phylogeny mirrors host phylogeny” [254].

Analysis of the genetic variation of the pathogen and knowledge of pathogen evolution are important for developing strategies in resistance breeding. Monitoring pathogen populations in combination with resistant cultivar spread can extend the life span of individual specific resistances and lead to a reduction in pesticide applications. The status of the pathogen in barley and alternative hosts should be monitored and resistance genes in these hosts should be investigated for the control of barley pathogens. In addition, determining the different virulence levels of the pathogen and utilizing molecular maps can facilitate the selection of resistant genotypes in the field. In studies conducted in Turkey, scald, powdery mildew, both forms of net blotch, semi loose smut, loose smut, leaf rust (brown rust), and barley stripe diseases were found on H. spontaneum and H. bulbosum plants [121,122]. The incidence and severity values of these diseases varied. Some of the populations were disease free. It appears that a wide range of variation exists in naturally growing H. spontaneum and H. bulbosum populations. Detailed studies in wild barley species are necessary to elucidate the roles of these species on virulence evolution of the pathogen. In addition, genomes of barley foliar pathogens and their evolution should be studied in detail.

Future work should focus on increasing the yield of less susceptible cultivars through breeding programs. In other words, to face the threat posed by any new more virulent isolates, the pathotypes/races that create variation must be constantly observed. In addition, strict implementation of quarantine measures for seed-borne pathogens and incorporation of pathogen-resistant barley germplasm as defined by international programs into breeding lines is essential.

Author Contributions: Conceptualization, A.Ç.O. and A.K.; writing original draft preparation, A.Ç.O. and A.K.; writing, review and editing, A.Ç.O. and A.K. Both authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. IGC. 2019. Available online: https://www.igc.int/en/default.aspx (accessed on 28 September 2020).
2. Ellwood, S.; Piscetek, V.; Mair, W.; Lawrence, J.; Lopez-Ruiz, F.; Rawlinson, C. Genetic variation of Pyrenophora teres f. teres isolates in Western Australia and emergence of a Cyp51A fungicide resistance mutation. Plant Pathol. 2019, 68, 135–142. [CrossRef]
3. Hawkins, N.J.; Cools, H.J.; Sierotzki, H.; Shaw, M.W.; Knoogge, W.; Kelly, S.L.; Kelly, D.E.; Fraaije, B.A. Paralog re-emergence: A novel, historically contingent mechanism in the evolution of antimicrobial resistance. Mol. Biol. Evol. 2014, 31, 1793–1802. [CrossRef] [PubMed]
4. Mohd-Assaad, N.; McDonald, B.A.; Croll, D. Multilocus resistance evolution to azole fungicides in fungal plant pathogen populations. Mol. Ecol. 2016, 25, 6124–6142. [CrossRef]
5. Palumbi, S.R. Humans as the world’s greatest evolutionary force. Science 2001, 293, 1786–1790. [CrossRef] [PubMed]
6. McDonald, B.A.; Linde, C. The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica 2002, 124, 163–180. [CrossRef]
7. McDonald, B.A.; Linde, C. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 2002, 40, 349–379. [CrossRef] [PubMed]
8. Biffen, R.H. Mendel’s laws of inheritance and wheat breeding. J. Agric. Sci. 1905, 1, 4–48. [CrossRef]
9. Kolmer, J. Genetics of resistance to wheat leaf rust. Annu. Rev. Phytopathol. 1996, 34, 435–455. [CrossRef]
10. Brown, J.; Foster, E.; O'hara, R. Adaptation of powdery mildew populations to cereal varieties in relation to durable and non-durable resistance. In *The Gene-for-Gene Relationship in Plant-Parasite Interaction*; Crute, I., Holub, E., Burdon, J., Eds.; CABI: Wallingford, UK, 1997; pp. 119–138.

11. Mes, J.J.; Haring, M.A.; Cornelissen, B.J. Foxy: An active family of short interspersed nuclear elements from *Fusarium oxysporum*. *Mol. Gen. Genet.* **2000**, *263*, 271–280. [CrossRef]

12. Milgroom, M.G. Recombination and the multilocus structure of fungal populations. *Annu. Rev. Phytopathol.* **1996**, *34*, 457–477. [CrossRef]

13. Leslie, J.F.; Klein, K.K. Female fertility and mating type effects on effective population size and evolution in filamentous fungi. *Genetics* **1996**, *144*, 557–567. [CrossRef] [PubMed]

14. Staskawicz, B.J.; Mudgett, M.B.; Dangl, J.L.; Galan, J.E. Common and contrasting themes of plant and animal diseases. *Science* **2001**, *292*, 2285–2289. [CrossRef] [PubMed]

15. Jørgensen, J.H.; Wolfe, M. Genetics of powdery mildew resistance in barley. *Annu. Rev. Phytopathol.* **2000**, *38*, 61–89. [CrossRef]

16. Dreiseitl, A.; Kosman, E. Virulence phenotypes of *Blumeria graminis* f. sp. *hordei* in South Africa. *Eur. J. Plant Pathol.* **2013**, *136*, 113–121. [CrossRef]

17. Zohary, D.; Hopf, M. *Domestication of plants in the Old World*; UK Clarendon Press: Oxford, UK, 1988.

18. Conry, M.; Dunne, B. Influence of number and timing of fungicide applications on the yield and quality of early and later-sown spring melting barley grown in the south-east of Ireland. *J. Agric. Sci.* **2001**, *136*, 159–167. [CrossRef]

19. Zhang, Z.; Henderson, C.; Perfect, E.; Carver, T.; Thomas, B.; Skamnioti, P.; Gurr, S. Of genes and genomes, needles and haystacks: *Blumeria graminis* and functionality. *Mol. Plant Pathol.* **2005**, *6*, 561–575. [CrossRef]

20. Schulze-Lefert, P.; Panstruga, R. A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends Plant Sci.* **2011**, *16*, 117–125. [CrossRef]

21. Troch, V.; Audenaert, K.; Wyand, R.A.; Haesaert, G.; Höfte, M.; Brown, J.K. Formae speciales of cereal powdery mildew: Close or distant relatives? *Mol. Plant Pathol.* **2014**, *15*, 304–314. [CrossRef]

22. Gordon, T.; Martyn, R. The evolutionary biology of *Fusarium oxysporum*. *Annu. Rev. Phytopathol.* **1997**, *35*, 111–128. [CrossRef] [PubMed]

23. Menardo, F.; Wicker, T.; Keller, B. Reconstructing the evolutionary history of powdery mildew lineages (*Blumeria graminis*) at different evolutionary time scales with NGS data. *Genome Biol. Evol.* **2017**, *9*, 446–456. [CrossRef]

24. Panstruga, R.; Spanu, P.D. Powdery mildew genomes reloaded. *New Phytol.* **2014**, *202*, 13–14. [CrossRef]

25. Menardo, F.; Praz, C.R.; Wyder, S.; Ben-David, R.; Bourras, S.; Matsumae, H.; McNally, K.E.; Par Lange, F.; Riba, A.; Roffler, S. Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species. *Nat. Genet.* **2016**, *48*, 201–205. [CrossRef]

26. Newton, A.C.; Hackett, C.A.; Guy, D.C. Diversity and complexity of *Erysiphe graminis* f. sp. *hordei* collected from barley cultivar mixtures or barley plots treated with a resistance elicitor. *Eur. J. Plant Pathol.* **1998**, *104*, 925–931. [CrossRef]

27. Dreiseitl, A.; Dinoor, A.; Kosman, E. Virulence and diversity of *Blumeria graminis* f. sp. *hordei* in Israel and in the Czech Republic. *Plant Dis.* **2006**, *90*, 1031–1038. [CrossRef] [PubMed]

28. Dreiseitl, A.; Wang, J. Virulence and diversity of *Blumeria graminis* f. sp. *hordei* in East China. *Eur. J. Plant Pathol.* **2007**, *117*, 357–368. [CrossRef]

29. Boussut, L.; de Vallavieille-Pope, C. Effect of sexual recombination on pathotype frequencies in barley powdery mildew populations of artificially inoculated field plots. *Eur. J. Plant Pathol.* **2003**, *109*, 13–24. [CrossRef]

30. Müller, K.; McDermott, J.M.; Wolfe, M.S.; Limpert, E. Analysis of diversity in populations of plant pathogens: The barley powdery mildew pathogen across Europe. *Eur. J. Plant Pathol.* **1996**, *102*, 385–395. [CrossRef]

31. Wolfe, M.; McDermott, J. Population genetics of plant pathogen interactions: The example of the *Erysiphe graminis-Hordeum vulgare* pathosystem. *Annu. Rev. Phytopathol.* **1994**, *32*, 89–113. [CrossRef]

32. Tucker, M.; Moffat, C.; Ellwood, S.; Tan, K.-C.; Jayasena, K.; Oliver, R. Development of genetic SSR markers in *Blumeria graminis* f. sp. *hordei* and application to isolates from Australia. *Plant Pathol.* **2015**, *64*, 337–343. [CrossRef]

33. Shaw, M. Modeling stochastic processes in plant pathology. *Annu. Rev. Phytopathol.* **1994**, *32*, 523–544. [CrossRef] [PubMed]

34. Brown, J.K.; Hovmøller, M.S. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **2002**, *297*, 537–541. [CrossRef]

35. Zhu, J.; Zhou, Y.; Shang, Y.; Hua, W.; Wang, J.; Jia, Q.; Liu, M.; Yang, J. Genetic evidence of local adaption and long distance migration in *Blumeria graminis* f. sp. *hordei* populations from China. *J. Gen. Plant Pathol.* **2016**, *82*, 69–81. [CrossRef]

36. Zhu, J.-H.; Wang, J.-M.; Jia, Q.-J.; Yang, J.-M.; Zhou, Y.-J.; Feng, L.; Wei, H.; Shang, Y. Pathotypes and genetic diversity of *Blumeria graminis* f. sp. *hordei* in the winter barley regions in China. *Agric. Sci. China* **2010**, *9*, 1787–1798. [CrossRef]

37. Wyand, R.A.; Brown, J.K. Genetic and forma specialis diversity in *Blumeria graminis* of cereals and its implications for host-pathogen co-evolution. *Mol. Plant Pathol.* **2003**, *4*, 187–198. [CrossRef] [PubMed]

38. Brown, J.K. Chance and selection in the evolution of barley mildew. *Trends Microbiol.* **1994**, *2*, 470–475. [CrossRef]

39. Hovmøller, M.; Caffier, V.; Jalli, M. The European barley powdery mildew virulence survey and disease nursery 1993–1999. *Agronomie* **2000**, *20*, 729–743. [CrossRef]

40. Tucker, M.; Jayasena, K.; Ellwood, S.; Oliver, R. Pathotype variation of barley powdery mildew in Western Australia. *Australas. Plant Pathol.* **2013**, *42*, 617–623. [CrossRef]
41. Brown, J.; Jørgensen, J.H. A catalogue of mildew resistance genes in European barley varieties. In Proceedings of the European Workshop on Integrated Control of Cereal Mildews: Virulence Patterns and Their Change, Riseø (Denmark), Roskilde, Denmark, 23–25 January 1990.

42. Jensen, H.R.; Dreiseitl, A.; Sadiki, M.; Schoen, D.J. High diversity, low spatial structure and rapid pathotype evolution in Moroccan populations of Blumeria graminis f. sp. hordei. Eur. J. Plant Pathol. 2013, 136, 323–336. [CrossRef]

43. Andrivon, D.; de Vallavieille-Pope, C. Racial diversity and complexity in regional populations of Erysiphe graminis f. sp. hordei in France over a 5-year period. Plant Pathol. 1993, 42, 443–464. [CrossRef]

44. Caffier, V.; de Vallavieille-Pope, C.; Brown, J. Segregation of avirulences and genetic basis of infection types in Erysiphe graminis f. sp. hordei. Phytopathology 1996, 86, 1122–1131. [CrossRef]

45. Dreiseitl, A. Adaptation of Blumeria graminis f. sp. hordei to barley resistance genes in the Czech Republic in 1971–2000. Plant Soil Environ. 2003, 49, 241–248. [CrossRef]

46. Moore, H.; Fox, H.; Harrouni, M.; Alami, A.E. Environmental challenges in the Rif mountains, northern Morocco. Environ. Conserv. 1998, 25, 354–365. [CrossRef]

47. Ceccarelli, S.; Grando, S.; Bailey, E.; Amri, A.; El-Felah, M.; Nassif, F.; Rezgui, S.; Yahyaoui, A. Farmer participation in barley breeding in Syria, Morocco and Tunisia. Euphytica 2001, 122, 521–536. [CrossRef]

48. Mboup, M.; Bahri, B.; Leconte, M.; De Vallavieille-Pope, C.; Kaltz, O.; Enjalbert, J. Genetic structure and local adaptation of European wheat yellow rust populations: The role of temperature-specific adaptation. Ecol. Appl. 2012, 5, 341–352. [CrossRef][PubMed]

49. Wolfe, M.; Brändle, U.; Koller, B.; Limpert, E.; McDermott, J.; Müller, K.; Schaffner, D. Barley mildew in Europe: Population biology and host resistance. Euphytica 1992, 63, 125–139. [CrossRef]

50. Brown, J.K.; Le Boulaire, S.; Evans, N. Genetics of responses to morpholine-type fungicides and of avirulences in Erysiphe graminis f. sp. hordei. Eur. J. Plant Pathol. 1996, 102, 479–490. [CrossRef]

51. Brown, J.K.; Jessop, A.C.; Rezanoor, N.H. Genetic uniformity in barley and its powdery mildew pathogen. Proc. R. Soc. London Ser. B Biol. Sci. 1991, 246, 83–90.

52. Büschges, R.; Holricher, K.; Panstruga, R.; Simons, G.; Wolter, M.; Frijters, A.; Van Daelen, R.; Diergaarde, P.; Groenendijk, J. The barley Mlo gene: A novel control element of plant pathogen resistance. Cell 1997, 88, 695–705. [CrossRef]

53. Andolfi, G.; Iovieno, P.; Ricciardi, L.; Lotti, C.; Filippone, E.; Pavan, S.; Ercolano, M.R. Evolutionary conservation of MLO gene promoter signatures. BMC Plant Biol. 2019, 19, 150. [CrossRef]

54. Dreiseitl, A. Genes for resistance to powdery mildew in European barley cultivars registered in the Czech Republic from 2011 to 2015. Plant Breed. 2017, 136, 351–356. [CrossRef]

55. Niks, R.E.; Qi, X.; Marcel, T.C. Quantitative resistance to biotrophic filamentous plant pathogens: Concepts, misconceptions, and mechanisms. Annu. Rev. Phytopathol. 2015, 53, 445–470. [CrossRef][PubMed]

56. McLean, M.; Howlett, B.; Hollaway, G. Epidemiology and control of spot form of net blotch (Pyrenophora teres f. maculata) of barley: A review. Crop Pasture Sci. 2009, 60, 303–315. [CrossRef][PubMed]

57. Liu, Z.; Ellwood, S.R.; Oliver, R.P.; Friesen, T.L. Pyrenophora teres: Profile of an increasingly damaging barley pathogen. Mol. Plant Pathol. 2011, 12, 1–19. [CrossRef]

58. Akhavan, A.; Turkington, T.K.; Askarian, H.; Tekauz, A.; Xi, K.; Tucker, J.R.; Kutcher, H.R.; Strelkov, S.E. Virulence of Pyrenophora teres populations in western Canada. Can. J. Plant Pathol. 2016, 38, 183–196. [CrossRef]

59. Matthre, D. Compendium of Barley Diseases; APS Press: Paul, MN, USA, 1997.

60. Peever, T.L.; Milgroom, M.G. Genetic structure of Pyrenophora teres populations determined with random amplified polymorphic DNA markers. Can. J. Bot. 1994, 72, 915–923. [CrossRef]

61. Rau, D.; Brown, A.H.; Brubaker, C.L.; Attene, G.; Balmas, V.; Saba, E.; Papa, R. Population genetic structure of Pyrenophora teres Drechs. the causal agent of net blotch in Sardinian landraces of barley (Hordeum vulgare L.). Theor. Appl. Genet. 2003, 106, 947–959. [CrossRef][PubMed]

62. Jonsson, R.; Sail, T.; Bryngelson, T. Genetic diversity for random amplified polymorphic DNA (RAPD) markers in two Swedish populations of Pyrenophora teres. Can. J. Plant Pathol. 2000, 22, 258–264. [CrossRef]

63. Serenius, M.; Manninen, O.; Wallwork, H.; Williams, K. Genetic differentiation in Pyrenophora teres populations measured with AFLP markers. Mol. Res. 2007, 111, 213–223. [CrossRef]

64. Statkevičius, G.; Brazauskas, G.; Semaškienė, R.; Leistrumaitė, A.; Dabkevičius, Z. Pyrenophora teres genetic diversity as detected by ISSR analysis. Agriculture 2010, 97, 91–98.

65. Lehmensiek, A.; Bester-Van Der Merwe, A.; Sutherland, M.; Platz, G.; Kriel, W.; Potgieter, G.; Prins, R. Population structure of South African and Australian Pyrenophora teres isolates. Plant Pathol. 2010, 59, 504–515. [CrossRef]

66. McLean, M.; Keiper, F.; Hollaway, G. Genetic and pathogenic diversity in Pyrenophora teres f. maculata in barley crops of Victoria, Australia. Australas. Plant Pathol. 2010, 39, 319–325. [CrossRef]

67. McLean, M.; Martin, A.; Gupta, S.; Sutherland, M.; Hollaway, G.; Platz, G. Validation of a new spot form of net blotch differential set and evidence for hybridisation between the spot and net forms of net blotch in Australia. Australas. Plant Pathol. 2014, 43, 223–233. [CrossRef]

68. Çelik Oğuz, A.; Ölmez, F.; Karakaya, A. Genetic diversity of net blotch pathogens of barley in Turkey. Int. J. Agric. Biol. 2019, 21, 1089–1096.
Agronomy 2021, 11, 434

100. Abbott, D.; Burdon, J.; Jarosz, A.; Brown, A.; Muller, W.; Read, B. The relationship between seedling infection types and field reactions to leaf scald in Clipper barley backcross lines. Aust. J. Agric. Res. 1991, 42, 801–809. [CrossRef]

101. Jørgensen, H.L. Pathogenic variation of *Rhynchosporium secalis* in Denmark and sources of resistance in barley. Plant Dis. 1995, 79, 297–301.

102. Tekauz, A. Pathogenic variation in *Rhynchosporium secalis* on barley in Canada. Can. J. Plant Pathol. 1991, 13, 298–304. [CrossRef]

103. Goodwin, S.B. The barley scald pathogen *Rhynchosporium secalis* is closely related to the discomycetes *Tapesia* and *Pyrenopeziza*. Mycol. Res. 2002, 106, 645–654. [CrossRef]

104. Williams, K.; Donnellan, S.; Smyl, C.; Scott, L.; Wallwork, H. Molecular variation in *Rhynchosporium secalis* isolates obtained from hotspots. Australas. Plant Pathol. 2003, 32, 257–262. [CrossRef]

105. Arzanlou, M.; Karimi, K.; Mirabi, F. Some evidence for skewed mating type distribution in Iranian populations of *Rhynchosporium commune*, the cause of barley scald disease. J. Plant Prot. Res. 2016, 56, 237–243. [CrossRef]

106. Seifollahi, E.; Sharifi-Nabii, B.; Javan-Nikkah, M.; Linde, C. Low genetic diversity of *Rhynchosporium commune* in Iran, a secondary centre of barley origin. Plant Pathol. 2018, 67, 1725–1734. [CrossRef]

107. Çelik Oğuz, A.; Ölmez, F.; Karakaya, A.; Azamparsa, M.R. Genetic variation and mating type distribution of *Rhynchosporium secalis* in Turkey. Physiol. Mol. Plant Pathol. 2021, 114, 101614. [CrossRef]

108. Linde, C.C.; Zala, M.; McDonald, B.A. Further evidence for sexual reproduction in *Rhynchosporium secalis* based on distribution and frequency of mating-type alleles. *Fungal Genet. Biol.* 2003, 40, 115–125. [CrossRef]

109. Zaffarano, P.L.; McDonald, B.A.; Zala, M.; Linde, C.C. Global hierarchical gene diversity analysis suggests the Fertile Crescent is not the center of origin of the barley scald pathogen *Rhynchosporium secalis*. *Phytopathology* 2006, 96, 941–950. [CrossRef]

110. Linde, C.C.; Zala, M.; McDonald, B.A. Molecular evidence for recent founder populations and human-mediated migration in the barley scald pathogen *Rhynchosporium secalis*. *Mol. Phylogenetics Evol.* 2009, 51, 454–464. [CrossRef] [PubMed]

111. McDonald, B.A. How can research on pathogen population biology suggest disease management strategies? The example of barley scald (*Rhynchosporium secalis*) culture. *Plant Pathol.* 2015, 64, 1005–1013. [CrossRef]

112. Foster, S.J.; Witt, S. Isolation and characterisation of the mating-type (MAT) locus from *Rhynchosporium secalis*. *Mol. Plant Pathol.* 2001, 2, 294–300. [CrossRef]

113. Brunner, P.C.; Schürch, S.; McDonald, B.A. The origin and colonization history of the barley scald pathogen *Rhynchosporium secalis*. *J. Evol. Biol.* 2007, 20, 1311–1321. [CrossRef]

114. Von Korff, M.; Udupa, S.; Yahyaoui, A.; Baum, M. Genetic variation among *Rhynchosporium secalis* populations of West Asia and North Africa as revealed by RAPD and AFLP analysis. *J. Phytopathol.* 2004, 152, 106–113. [CrossRef]

115. Salamati, S.; Zhan, J.; Burdon, J.J.; McDonald, B.A. The genetic structure of field populations of *Rhynchosporium secalis* from three continents suggests moderate gene flow and regular recombination. *Phytopathology* 2000, 90, 901–908. [CrossRef]

116. Locke, T.; Phillips, A. The occurrence of cabendazim resistance in *Rhynchosporium secalis* on winter barley in England and Wales in 1992 and 1993. *Plant Pathol.* 1995, 44, 294–300. [CrossRef]

117. Taggart, P.; Locke, T.; Phillips, A.; Pask, N.; Hollomon, D.; Kendall, S.; Cooke, L.; Mercer, P. Benzimidazole resistance in *Rhynchosporium secalis* and its effect on barley leaf blotch control in the UK. *Crop Prot.* 1999, 18, 239–243. [CrossRef]

118. Robbertse, B.; Van Der Rijst, M.; Van Aarde, I.; Lennox, C.; Crous, P. DMI sensitivity and cross-resistance patterns of *Rhynchosporium secalis* isolates from South Africa. *Crop Prot.* 2001, 20, 97–102. [CrossRef]

119. Cooke, L.; Locke, T.; Lockley, K.; Phillips, A.; Sadiq, M.; Coll, R.; Black, L.; Taggart, P.; Mercer, P. The effect of fungicide programmes based on epoxiconazole on the control and DMI sensitivity of *Rhynchosporium secalis* in winter barley. *Crop Prot.* 2004, 23, 393–406. [CrossRef]

120. Brunner, P.C.; Stefansson, T.S.; Fountaine, J.; Richina, V.; McDonald, B.A. A global analysis of CYP51 diversity and azole sensitivity in *Rhynchosporium commune*. *Phytopathology* 2016, 106, 355–361. [CrossRef]

121. Karakaya, A.; Mert, Z.; Oğuz, A.C.; Ertas, M.; Karagöz, A. Determination of the diseases occurring on naturally growing wild barley (*Hordeum spontaneum*) field populations. *Rad. Poljopr. Fak. Univ. U Sarajev.* (Work. Fac. Agric. Univ. Sarajevo) 2016, 61, 291–295.

122. Karakaya, A.; Çelik Oğuz, A.; Saraç Sivrikaya, I. Diseases occurring on Hordeum bulbosum field populations at Bingöl province of Turkey. *Rad. Poljopr. Fak. Univ. U Sarajev.* (Work. Fac. Agric. Univ. Sarajevo) 2020, 60, 57–71. [CrossRef]

123. Kumar, J.; Schäfer, P.; Hückelhoven, R.; Langen, G.; Baltruschat, H.; Stein, E.; Nagarajan, S.; Kogel, K.H. *Bipolaris sorokiniana*, a cereal pathogen of global concern: Cytological and molecular approaches towards better control. *Mol. Plant Pathol.* 2002, 3, 185–195. [CrossRef]

124. Acharya, K.; Dutta, A.K.; Pradhan, P. ‘Bipolaris sorokiniana’ (Sacc.) Shoem.: The most destructive wheat fungal pathogen in the warmer areas. *Aust. J. Crop Sci.* 2011, 5, 1064.

125. Gupta, P.; Chand, R.; Vasistha, N.; Pandey, S.; Kumar, U.; Mishra, V.; Joshi, A. Spot blotch disease of wheat: The current status of research on genetics and breeding. *Plant Pathol.* 2018, 67, 508–531. [CrossRef]

126. Murray, T.D.; Parry, D.W.; Cattlin, N.D. *A Color Handbook of Diseases of small grain Cereal Crops*; Iowa State University Press: Iowa, IA, USA, 1998.

127. Sharma, R.; Duveiller, E. Advancement toward new spot blotch resistant wheats in South Asia. *Crop Sci.* 2007, 47, 961–968. [CrossRef]
Agronomy 2021, 11, 434

214. Lu, X.; Kracher, B.; Saur, I.M.; Bauer, S.; Ellwood, S.R.; Wise, R.; Yaeno, T.; Maekawa, T.; Schulze-Lefert, P. Allelic barley MLA immune receptors recognize sequence-unrelated avirulence effectors of the powdery mildew pathogen. Proc. Natl. Acad. Sci. USA 2016, 113, E6486–E6495. [CrossRef]

215. Dreiseitl, A. Heterogeneity of powdery mildew resistance revealed in accessions of the ICARDA wild barley collection. Front. Plant Sci. 2017, 8, 202. [CrossRef] [PubMed]

216. Ellwood, S.R.; Liu, Z.; Syme, R.A.; Lai, Z.; Hane, J.K.; Keiper, F.; Moffat, C.S.; Oliver, R.P.; Friesen, T.L. A first genome assembly of the barley fungal pathogen Pyrenophora teres f. teres. Genome Biol. 2010, 11, 1–14. [CrossRef]

217. Friesen, T.L.; Faris, J.D.; Solomon, P.S.; Oliver, R.P. Host-specific toxins: Effectors of necrotrophic pathogenicity. Cell. Microbiol. 2008, 10, 1421–1428. [CrossRef]

218. Ismail, I.; Godfrey, D.; Able, A. Fungal growth, proteinaceous toxins and virulence of Pyrenophora teres f. teres on barley. Australas. Plant Pathol. 2014, 43, 535–546.

219. Ismail, I.; Godfrey, D.; Able, A. Proteomic analysis reveals the potential involvement of xylanase from Pyrenophora teres f. teres in net form net blotch disease of barley. Australas. Plant Pathol. 2014, 43, 715–726. [CrossRef]

220. Liu, Z.; Holmes, D.J.; Faris, J.D.; Chao, S.; Brueggeman, R.S.; Edwards, M.C.; Friesen, T.L. Necrotrophic effect-conferred susceptibility (NETS) underlies the barley—Pyrenophora teres f. teres interaction specific to chromosome 6H. Mol. Plant Pathol. 2015, 16, 188–200. [CrossRef]

221. Wyatt, N.A.; Richards, J.K.; Brueggeman, R.S.; Friesen, T.L. Reference assembly and annotation of the Pyrenophora teres f. teres isolate 0–1. G3 Genes Genomes Genet. 2018, 8, 1–8.

222. Weiland, J.J.; Steffenson, B.J.; Cartwright, R.D.; Webster, R.K. Identification of molecular genetic markers in Pyrenophora teres f. teres associated with low virulence on ‘Harbin’ barley. Phytopathology 1999, 89, 176–181. [CrossRef]

223. Lai, Z.; Faris, J.D.; Weiland, J.J.; Steffenson, B.J.; Friesen, T.L. Genetic mapping of Pyrenophora teres f. teres genes conferring avirulence on barley. Fungal Genet. Biol. 2007, 44, 323–329. [CrossRef]

224. Beattie, A.D.; Scoles, G.J.; Rossnagel, B.G. Identification of molecular markers linked to a Pyrenophora teres avirulence gene. Phytopathology 2007, 97, 842–849. [CrossRef] [PubMed]

225. Wyatt, N.A.; Richards, J.K.; Brueggeman, R.S.; Friesen, T.L. A comparative genomic analysis of the barley pathogen Pyrenophora teres f. teres identifies subtelomeric regions as drivers of virulence. Mol. Plant Microbe Interact. 2020, 33, 173–188. [CrossRef]

226. Syme, R.A.; Martin, A.; Wyatt, N.A.; Lawrence, J.A.; Muria-Gonzalez, M.J.; Friesen, T.L.; Ellwood, S.R. Transposable element genomic fissing in Pyrenophora teres is associated with genome expansion and dynamics of host-pathogen genetic interactions. Front. Genet. 2018, 9, 130. [CrossRef]

227. Muria-Gonzalez, M.J.; Zulak, K.G.; Allegaert, E.; Oliver, R.P.; Ellwood, S.R. Profile of the in vitro secretome of the barley net blotch fungus, Pyrenophora teres f. maculata: A resource for studying the barley spot form net blotch interaction. Mol. Plant Microbe Interact. 2020, 34, 135–139. [CrossRef] [PubMed]

228. Wevelsiep, L.; Rupping, E.; Knoege, W. Stimulation of barley plasmalemma H+-ATPase by phytotoxic peptides from the fungal pathogen Rhynchosporium secalis. Environ. Microbiol. 2019, 21, 2677–2695. [CrossRef] [PubMed]

229. Schürch, S.; Linde, C.C.; Knoegg, F.; Jackson, L.F.; McDonald, B.A. Molecular population genetic analysis differentiates two virulence mechanisms of the fungal avirulence gene NIP1. Mol. Plant Microbe Interact. 2004, 17, 1114–1125. [CrossRef]

230. Kirsten, S.; Navarro-Quezada, A.; Penselin, D.; Wenzel, C.; Matern, A.; Leitner, A.; Baum, T.; Seiffert, U.; Knoegg, W.; Nécrois-Inducing proteins of Rhynchosporium commune, effectors in quantitative disease resistance. Mol. Plant Microbe Interact. 2012, 25, 1314–1325. [CrossRef]

231. Gamble, M. Molecular characterisation of the Rhynchosporium commune interaction with barley. Ph.D. Thesis, University of Dundee, Dundee, Scotland, 2016.

232. Mohd-Assaad, N.; McDonald, B.A.; Croll, D. The emergence of the multi-species NIP1 effector in Rhynchosporium was accompanied by high rates of gene duplications and losses. Environ. Microbiol. 2019, 21, 2677–2695. [CrossRef] [PubMed]

233. Siersleben, S.; Penselin, D.; Wenzel, C.; Albert, S.; Knoegg, W. PFP1, a gene encoding an Epc-N domain-containing protein, is essential for pathogenicity of the barley pathogen Rhynchosporium commune. Eukaryot. Cell 2014, 13, 1026–1035. [CrossRef] [PubMed]

234. Torriani, S.F.; Penselin, D.; Knoegg, W.; Felder, M.; Taudien, S.; Platzer, M.; McDonald, B.A.; Brunner, P.C. Comparative analysis of mitochondrial genomes from closely related Rhynchosporium species reveals extensive intron invasion. Fungal Genet. Biol. 2014, 62, 34–42. [CrossRef]

235. Penselin, D.; Münsterkötter, M.; Kirsten, S.; Felder, M.; Taudien, S.; Platzer, M.; Ashelford, K.; Paskiewicz, K.H.; Harrison, R.J.; Hughes, D.J. Comparative genomics to explore phylogenetic relationship, cryptic sexual potential and host specificity of Rhynchosporium species on grasses. BMC Genom. 2016, 17, 953. [CrossRef]

236. Von Felten, A.; Zaffarrano, P.L.; McDonald, B.A. Electrophoretic karyotypes of Rhynchosporium commune, R. secalis and R. agropyri. Eur. J. Plant Pathol. 2011, 129, 529–537. [CrossRef]

237. Zhu, W.; Zhan, J.; McDonald, B.A. Evidence for local adaptation and pleiotropic effects associated with melanization in a plant pathogenic fungus. Fungal Genet. Biol. 2018, 115, 33–40. [CrossRef]
240. Rohe, M.; Gierlich, A.; Hermann, H.; Hahn, M.; Schmidt, B.; Rosahl, S.; Knogge, W. The race-specific elicitor, NIP1, from the barley pathogen, *Rhynchosporium secalis*, determines avirulence on host plants of the *Rrs1* resistance genotype. *EMBO J.* 1995, 14, 4168–4177. [CrossRef]

241. Ohm, R.A.; Feau, N.; Henriussat, B.; Schoch, C.L.; Horwitz, B.A.; Barry, K.W.; Condon, B.J.; Copeland, A.C.; Dhillon, B.; Glaser, F. Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. *PLoS Pathog.* 2012, 8, e1003037. [CrossRef] [PubMed]

242. Condon, B.J.; Leng, Y.; Wu, D.; Bushley, K.E.; Ohm, R.A.; Otillar, R.; Martin, J.; Schackwitz, W.; Grimwood, J.; MohdZainudin, N. Comparative genome structure, secondary metabolite, and effector coding capacity across *Cochliobolus* pathogens. *PLoS Genet.* 2013, 9, e1003233. [CrossRef]

243. Chand, R.; Kumar, M.; Kushwaha, C.; Shah, K.; Joshi, A.K. Role of melanin in release of extracellular enzymes and selection of aggressive isolates of *Bipolaris sorokiniana* in barley. *Curr. Microbiol.* 2014, 69, 202–211. [CrossRef] [PubMed]

244. Aich, S.; Singh, R.K.; Kundu, P.; Pandey, S.P.; Datta, S. Genome-wide characterization of cellulases from the hemi-biotrophic plant pathogen, *Bipolaris sorokiniana*, reveals the presence of a highly stable GH7 endoglucanase. *Biotechnol. Biofuels* 2017, 10, 1–14. [CrossRef] [PubMed]

245. McDonald, M.C.; Ahren, D.; Simpfendorfer, S.; Milgate, A.; Solomon, P.S. The discovery of the virulence gene ToxA in the wheat and barley pathogen *Bipolaris sorokiniana*. *Mol. Plant Pathol.* 2018, 19, 432–439. [CrossRef]

246. Han, J.; Zhang, J.; Song, Z.; Liu, M.; Hu, J.; Hou, C.; Zhu, G.; Jiang, L.; Xia, X.; Quinn, R.J. Genome-and MS-based mining of antibacterial chlorinated chromones and xanthones from the phytopathogenic fungus *Bipolaris sorokiniana* strain 11134. *Appl. Microbiol. Biotechnol.* 2019, 103, 5167–5181. [CrossRef]

247. Song, N.; Geng, Y.; Li, X. The mitochondrial genome of the phytopathogenic fungus *Bipolaris sorokiniana* and the utility of mitochondrial genome to infer phylogeny of Dothideomycetes. *Front. Microbiol.* 2020, 11, 863. [CrossRef]

248. Pathak, G.M.; Gurjar, G.S.; Kadoo, N.Y. Insights of *Bipolaris sorokiniana* secretome-an in silico approach. *Biologia* 2020, 75, 2367–2381. [CrossRef]

249. Liang, Q.; Li, B.; Wang, J.; Ren, P.; Yao, L.; Meng, Y.; Si, E.; Shang, X.; Wang, H. PGPBS, a mitogen-activated protein kinase kinase, is required for vegetative differentiation, cell wall integrity, and pathogenicity of the barley leaf stripe fungus *Pyrenophora graminea*. *Gene* 2019, 696, 95–104. [CrossRef] [PubMed]

250. Si, E.; Meng, Y.; Ma, X.; Li, B.; Wang, J.; Yao, L.; Yang, K.; Zhang, Y.; Shang, X.; Wang, H. Genome resource for barley leaf stripe pathogen *Pyrenophora graminea*. *Plant Dis.* 2020, 104, 320–322. [CrossRef] [PubMed]

251. Kullman, B.; Teterin, W. Estimation of fungal genome size: Comparison of image cytometry and photometric cytometry. *Folia Cryptog.* 2006, 42, 43–56.

252. Park, R.F.; Golegaonkar, P.G.; Derevnina, L.; Sandhu, K.S.; Karaoglu, H.; Elmansour, H.M.; Dracatos, P.M.; Singh, D. Leaf rust of cultivated barley: Pathology and control. *Annu. Rev. Phytopathol.* 2015, 53, 565–589. [CrossRef]

253. Chen, J.; Wu, J.; Zhang, P.; Dong, C.; Upadhyaya, N.M.; Zhou, Q.; Dodds, P.; Park, R.F. *De novo* genome assembly and comparative genomics of the barley leaf rust pathogen *Puccinia hordei* identifies candidates for three avirulence genes. *G3 Genes Genomes Genet.* 2019, 9, 3263–3271. [CrossRef] [PubMed]

254. Eichler, W. XLI.—Some rules in ectoparasitism. *J. Nat. Hist.* 1948, 1, 588–598. [CrossRef]