Ancient variation of the AvrPm17 gene in powdery mildew limits the effectiveness of the introgressed rye Pm17 resistance gene in wheat

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Introductions of chromosomal segments from related species into wheat are important sources of resistance against fungal diseases. The durability and effectiveness of introgressed resistance genes upon agricultural deployment is highly variable—a phenomenon that remains poorly understood, as the corresponding fungal avirulence genes are largely unknown. Until its breakdown, the Pm17 resistance gene introgressed from rye to wheat provided broad resistance against powdery mildew (Blumeria graminis). Here, we used quantitative trait locus (QTL) mapping to identify the corresponding wheat mildew avirulence effector AvrPm17. It is encoded by two paralogous genes that exhibit signatures of reoccurring gene conversion events and are members of a mildew sublineage-specific effector cluster. Extensive haplotype mining in wheat mildew and related sublineages identified several ancient virulent AvrPm17 variants that were present as standing genetic variation in wheat powdery mildew prior to the Pm17 introgression, thereby paving the way for the rapid breakdown of the Pm17 resistance. QTL mapping in mildew identified a second genetic component likely corresponding to an additional resistance gene present on the 1AL.1RS translocation carrying Pm17. This gene remained previously undetected due to suppressed recombination within the introgressed rye chromosomal segment. We conclude that the initial effectiveness of 1AL.1RS was based on simultaneous introgression of two genetically linked resistance genes. Our results demonstrate the relevance of pathogen-based genetic approaches to disentangling complex resistance loci in wheat. We propose that identification and monitoring of avirulence gene diversity in pathogen populations become an integral part of introgression breeding to ensure effective and durable resistance in wheat.

Wheat is the most widely cultivated food crop and is susceptible to a number of fungal diseases. For more than a century, breeding for genetically resistant cultivars has been a major focus for sustainable wheat production globally. Introgressions of chromosomal segments from closely related wild grasses such as Aegilops or Agropyron species (1, 2) and other crop species such as rye (Secale cereale) have been highly valuable sources of new resistance gene specificities (3). Specifically, the 1BL.1RS or 1AL.1RS translocations of the rye chromosome 1R introgressed into hexaploid (AABBDD) wheat (reviewed in ref. 4) were of great relevance for wheat resistance breeding. Genes present on these translocations are widely used in wheat breeding and confer resistance to leaf rust (Lr28), stripe rust (Yr9), stem rust (Sr31, Sr50/SrR, Sr1RAvr), and powdery mildew (the allelic Pm8/Pm17 pair) (5, 6).

It has been proposed that introgressed resistance genes provide more effective and potentially more durable resistance, since pathogens specialized on wheat have previously not been exposed and therefore have not adapted to the resistance specificities that evolved in other species (7). This is exemplified by the rye Sr31 gene, which was deployed worldwide. It provided effective and broad resistance against Puccinia graminis f. sp. tritici, the causal agent of wheat stem rust for over 30 y before being overcome by the virulent African strain Ug99 (8), demonstrating both the huge benefit of an introgressed rye gene as well as the constant need for new broadly active resistance genes (9). In contrast to Sr31 and the general hypothesis, many introgressed resistance genes were overcome quickly by wheat pathogens (10). For example, the rye introgressions with Pm8 and Pm17 became ineffective against wheat powdery mildew Blumeria graminis f. sp. tritici (B.g. tritici) within a few years after their deployment in large-scale agricultural settings (11–14).

Thus, it remains one of the most pressing questions in the field of plant breeding research why a few introgressed genes such as Sr31 remained effective over a long timeframe and despite worldwide deployment, whereas others are overcome quickly (7).

Significance

Domesticated and wild wheat relatives provide an important source of new immune receptors for wheat resistance breeding against fungal pathogens. The durability of these resistance genes is variable and difficult to predict, yet it is crucial for effective resistance breeding. We identified a fungal effector protein recognized by an immune receptor introgressed from rye to wheat. We found that variants of the effector allowing the fungus to overcome the resistance are ancient. They were already present in the wheat powdery mildew gene pool before the introgression of the immune receptor and are therefore responsible for the rapid resistance breakdown. Our study demonstrates that the effort to identify durable resistance genes cannot be dissociated from studies of pathogen avirulence genes.
The allelic *Pm8* and *Pm17* genes encode for nucleotide-binding leucine-rich repeat (NLR) proteins that were introgressed into wheat from *Petkus* and *Insave* rye cultivars, respectively (15, 16). It was demonstrated that both genes represent rye homologs of the wheat *Pm3* resistance gene (15, 16), which encodes for a high number of different NLR alleles that confer race-specific resistance against wheat powdery mildew through recognition of mildew encoded avirulence proteins (17–19).

In wheat powdery mildew, recent studies using map-based cloning, genome-wide association studies, and effector benchmarking approaches have identified several avirulence genes, among them *AvrPm3a*292, *AvrPm3a*292, and *AvrPm3*185 recognized by *Pm3a/Pm3f, Pm3b/Pm3c, and Pm3d*, respectively (17, 18). Sequence analysis of wheat mildew *Avr* genes revealed that they all encode small secreted candidate effector proteins (17, 18, 20, 21) and exhibit high levels of sequence variation on a population level, including the independent evolution of numerous gain-of-virulence alleles by diverse molecular mechanisms (17, 18, 20, 22). The identification and functional characterization of mildew avirulence genes has therefore significantly broadened our understanding of race-specific resistance and resistance gene breakdown in the wheat—mildew pathosystem.

Grass powdery mildews exist in many sublineages also called formae specialis (f. sp.) that are highly host specific, such as mildew on wheat (*B. graminis triticum*, rye (*B. graminis secalis*), or the wheat/rye hybrid triticale (*B. graminis secalis* triticum) which emerged recently and was attributed to a hybridization event between wheat and rye mildew sublineages (23, 24). Due to the strict host barrier, it is assumed that nonadapted mildew sublineages have not been exposed to NLR resistance specificities of an incompatible host and therefore have not evolved to evade recognition. Indeed, several *Pm3* alleles have been found to contribute to nonhost resistance through recognition of conserved avirulence effectors in nonadapted mildew sublineages such as *B. graminis secalis* (18). Given these observations, the rapid breakdown of *Pm8* and *Pm17* resistance after introgression into wheat remains puzzling and provides an opportunity to study evolutionary dynamics of wheat mildew in the context of introgression breeding. The *Pm7* introgression is especially suited for this purpose since the associated 1AL.1RS translocation, first described in 1976 in Oklahoma (25), was not used before the end of the 20th century, and has been deployed in large-scale agricultural setting only in the beginning of the 21st century in the United States, where it provided resistance against wheat mildew in bread wheat (26, 27). In contrast, the deployment in other wheat growing areas globally started only after the year 2000 (11, 27–29), and breakdown of *Pm17* resistance was generally observed within few years and has been well documented in several wheat growing regions such as the United States, China, and Switzerland (11, 13, 27, 30).

In this study, we report the molecular basis underlying the resistance breakdown of the introgressed *Pm17* gene in wheat. Using quantitative trait locus (QTL) mapping in a biparental mildew population, we demonstrate that the corresponding avirulence effector *AvrPm17* is encoded by a paralogous effector gene pair, residing in a dynamic effector cluster, specific to the wheat and rye mildew sublineages. Moreover, we describe the identification of numerous ancient virulence alleles of the *AvrPm17* gene that have been present as standing genetic variation in *B. graminis triticic* even before the introgression of *Pm17* into the wheat breeding pool. Lastly, we provide genetic evidence for the existence of a so far unidentified resistance gene against wheat mildew that was cointrogressed with *Pm17* from rye, which could be revealed through careful dissection of resistance specificities based on genetic studies in the pathogen.

### Results

**QTL Mapping Identifies a Single Avirulence Locus for *Pm17* in Wheat Powdery Mildew.** To understand the breakdown of the rye NLR *Pm17* in wheat, we aimed at identifying its corresponding avirulence gene by taking advantage of the recent cloning of *Pm17* and its validation in transgenic wheat lines (16). We used a preexisting, sequenced F1 mapping population derived from a cross of the avirulent *B. graminis triticale* isolate THUN-12 and *B. graminis tritici* isolate Bgt_96224, which exhibits a virulent phenotype on the independent transgenic lines *Pm17*#34 and *Pm17*#181 (*SI Appendix, Fig. S1 A and B*) (31). A single interval QTL mapping approach using 55 randomly selected progeny of the Bgt_96224 × THUN-12 cross identified a single locus on chromosome 1 at an identical map position (164.8 centimorgan [cM]) with highly significant logarithm of the odds (LOD) scores of 9.2 for wheat genotype *Pm17*#34 and 7.0 for *Pm17*#181, respectively (Fig. 1 A–C and *SI Appendix, Fig. S1 C and D and Table S1*). The pericentromeric location of the mapped *AvrPm17* locus contrasts with the location of previously identified wheat mildew avirulence genes that tend to reside near the telomeric region or on the chromosome arms (Fig. 1B and *SI Appendix, Fig. S2*; 17, 18, 20).

To identify *AvrPm17* candidate genes, we analyzed the physical region underlying the QTL (with a confidence interval [CI] of 1.5 LOD) on chromosome 1 in the chromosome-scale assemblies of the parental isolates Bgt_96224 and THUN-12 (31, 32). The genetic CI corresponded to a 61.8-kb region in the assembly of THUN-12 and a much larger region of 114.3 kb in the assembly of Bgt_96224. This striking difference in size is explained by a large 50-kb deletion in the THUN-12 genome (Fig. 1 D–F). The interval in the *Pm17* avirulent isolate THUN-12 only encodes the paralogous effector gene pair *BgTH12-04537* and *BgTH12-04538* (Fig. 1F). The two effector genes encode for identical proteins and differ by two synonymous single nucleotide polymorphisms (SNPs). The two gene copies are encoded by two inverted duplicated segments of 2,300 bp separated by a 4,709-bp intergenic region (Fig. 1B). The gene duplication is also present in the corresponding region of the virulent parent Bgt_96224 (Fig. 1F and *SI Appendix, Table S2*). There, the duplicated effector genes *Bgt*51729 and *Bgt*51731 are identical and encode for proteins that each carry two amino acid changes (A53V, R80S) compared with *BgTH12-04537* and *BgTH12-04538*, respectively (Figs. 1 D and E and 2 A and *SI Appendix, Fig. S3*). The interval in the Bgt_96224 genome encodes an additional effector gene, *Bgt*As-SP31098, that lies within the 50-kb deleted region in THUN-12. In the absence of additional genes in the locus of the avirulent parent THUN-12, we predicted that *BgTH12-04537* and *BgTH12-04538* encode for *AvrPm17*. Using RNA-sequencing data from the parental isolates, we found that *BgTH12-04537/BgTH12-04538* and *Bgt*51729/*Bgt*51731 are highly expressed at early stages of infection, corresponding to the establishment of the haustorial feeding structure at 2 d post infection (dpi), reminiscent of other wheat mildew *Avr* genes (*SI Appendix, Figs. S4 and S5*). The *AvrPm17* candidates are not differentially expressed between the two isolates (logFC < 1.5; *SI Appendix, Fig. S4*), therefore indicating that the amino acid polymorphisms observed between Bgt_96224 and THUN-12 must account for the difference in phenotype.

A previous study found that the hybrid genome of *B. graminis trititicale* isolates consists of distinct genomic segments inherited from either wheat or rye mildew (23). Due to the rye origin of the *Pm17* resistance gene, the origin of the avirulence locus in triticale mildew THUN-12 is of special interest. Following the approach of Menardo et al. (23) based on the analysis of fixed
polymorphism between wheat and rye mildew, we found that the physical region underlying the AvrPm17 QTL in THUN-12 is a segment inherited from wheat mildew (SI Appendix, Fig. S6 A–C). This indicates that the rye Pm17 gene recognizes an avirulence component originating from the nonadapted wheat powdery mildew donor, and not from the adapted rye mildew, in the triticale mildew hybrid.

Functional Validation of AvrPm17. To functionally validate AvrPm17, we transiently coexpressed the BgTH12-04537/BgTH12-04538 Avr candidate with Pm17-HA in Nicotiana benthamiana by Agrobacterium tumefaciens–mediated transient overexpression (18, 20). All effector constructs were expressed without the signal peptide and codon optimized for expression in N. benthamiana to ensure optimal translation in planta. BgTH12-04537/BgTH12-04538

Fig. 1. Avirulence on wheat genotypes with Pm17 is controlled by a single locus in a biparental mapping population between B.g. tritici Bgt_96224 × B.g. triticale THUN-12. (A) Single interval QTL mapping of 55 progeny of the cross Bgt_96224 × THUN-12 on two transgenic lines expressing Pm17-HA under control of the maize ubiquitin promoter (Ubi) promoter. The genetic map of Bgt_96224 × THUN-12 based on 119,023 markers was reprinted from ref. 31, which is licensed under CC BY 4.0, and contains 11 linkage groups that correspond to the 11 chromosomes of B.g. tritici Bgt_96224 and B.g. triticale THUN-12 (31, 32). The significance level of the LOD (logarithm of the odds) value was determined using 1,000 permutations and is indicated by a red line. (B) Location of the QTLs identified in the pericentromeric region of chromosome 1 of B.g. tritici Bgt_96224. The centromeric region is indicated. Vertical bars indicate effector gene density in 50-kb windows following a gradient indicated in the color key. The line above the chromosome indicates the recombinant rate in cM/50 kb as described in ref. 31. The remaining 10 chromosomes are depicted in SI Appendix, Fig. S2. (C) Informative markers in the 7.44-cM genetic CI (1.5 LOD) and their cM position relative to the left flanking marker. Flanking markers and the best associated marker of the QTL are depicted in yellow. (D and E) Physical interval underlying the genetic interval in B.g. tritici Bgt_96224 (D) and B.g. triticale THUN-12 (E). Gene and gene orientation are indicated with blue arrows (gene length not drawn to scale). Nonsynonymous SNPs in THUN-12 versus isolate Bgt_96224 are indicated by a red bar within the gene. Green bars indicate the presence of transposable elements in the interval. (F) Alignment showing the region flanking 100 kb up and downstream of the AvrPm17 locus in the reference assemblies of B.g. tritici Bgt_96224 and B.g. triticale THUN-12. The location of the paralogous effectors BgTH12-04537/BgTH12-04538 and Bgt-51729/Bgt-51731 is indicated by a blue box. The 50-kb deletion in THUN-12 compared with Bgt_96224 is highlighted in yellow.
elicited a strong hypersensitive response (HR) upon coexpression with Pm17-HA but not when expressed alone, confirming that these polymorphic effector genes are AvrPm17 (Fig. 2 B and C). Coexpression of AvrPm17_THUN12 with either the Pm8 gene from rye or the Pm17 orthologs from wheat (Pm3a-f, Pm3CS) did not result in a HR in N. benthamiana (SI Appendix, Fig. S7), demonstrating the specificity of AvrPm17_THUN12 recognition by Pm17.

Interestingly, coexpression of AvrPm17_96224 with Pm17 also resulted in a HR (Fig. 2 B and C). The extent of cell death was, however, significantly reduced compared with the AvrPm17_THUN12 variant (paired Wilcoxon rank test \( P = 4.657\times 10^{-10} \) (Fig. 2 D)). We therefore concluded that Pm17 can weakly recognize AvrPm17_96224, at least in a heterologous overexpression system. To address the question of whether the weak recognition of AvrPm17_96224 translates into phenotypes on Pm17 wheat, we made use of the above-mentioned transgenic lines Pm17#181 and Pm17#34 which were previously shown to exhibit differences in Pm17 protein abundance, with Pm17#181 representing the stronger line (16). Consistent with a prediction of a quantitative difference in AVR recognition, we observed reduced mildew leaf coverage upon infection of both transgenic Pm17 wheat lines with isolate Bgt_96224 and with progeny of the Bgt_96224 variant (paired Wilcoxon rank test \( P = 1.145\times 10^{-4} \)), consistent with the model predictions. However, at least in a heterologous overexpression system, the differences in AVR protein abundance translate into reduced disease severity quantitatively. In contrast, the recognition of the AvrPm17_THUN12 haplotype conferred complete disease resistance in both transgenic lines (SI Appendix, Fig. S1 A–D).

We therefore also analyzed differences in AVR protein abundance using C-terminal FLAG epitope-tagged AVRPM17 variants as well as PM17-HA were detectable on a Western blot (SI Appendix, Fig. S8 A and B). Interestingly, expression of AVRPM17_THUN12 in N. benthamiana resulted in higher protein abundance than for the weakly recognized AVRPM17_96224 variant (SI Appendix, Fig. S8F). This suggests that the amino acid polymorphisms between Bgt_96224 and THUN-12 affect protein levels of AVRPM17, at least in the heterologous Nicotiana system. Similar observations were recently described for AvrPm3\( ^{23,24} \), where polymorphism in the AVR was found to affect the protein amount and thereby directly influence recognition by Pm3a (33).

AVRPM17 is part of effector family E003, the second largest effector family found in B. graminis (31). The family is composed of small proteins of approximately 110 amino acids that contain a predicted signal peptide, an N-terminal Y/FxC motif and the C-terminal cysteine residue highlighted in red and yellow, respectively. Functional validation of AvrPm17 in N. benthamiana. (A) Protein alignment of the AVRPM17 candidate in THUN-12 and Bgt_96224. The sequence corresponding to the predicted signal peptide, the Y/FxC motif and the C-terminal cysteine residue are highlighted in red and yellow, respectively. Polymorphic amino acid residues between Bgt_96224 and THUN-12 are highlighted in blue. (B) and (C) Coexpression of Pm17-HA and AVRPM17_THUN12 (BgtTH12-04537/BgtTH12-04538) and AvrPm17_96224 (Bgt-S1729/Bgt-S1731) by transient Agrobacterium-mediated expression in N. benthamiana, imaged by the Fusion FX imager system (B) or a conventional camera (C) at 5 dpi. Coinfiltrations were done at a ratio of 1:4 R:Avr. Coexpression of Pm17 with AvrPm17_THUN12 and AvrPm17_96224 induced HR in \( n = 18 \) leaves in three independent experiments. No HR was observed when AvrPm17_THUN12, AvrPm17_96224, or Pm17 was expressed alone \( (n = 18) \). (D) Difference in HR induction between the two AVRPM17 variants AVRPM17_THUN12 and AVRPM17_96224 in a ratio of R:Avr of 1:1. The y-axis represents quantitative measurement of HR based on the Fusion FX imager system. Individual datapoints are color coded based on three independent experiments with at least \( n = 8 \) leaves per experiment. The \( P \) value of the paired Wilcoxon-ranked sum test is indicated above the panel.

Fig. 2. Functional validation of AvrPm17 in N. benthamiana. (A) Protein alignment of the AVRPM17 candidate in THUN-12 and Bgt_96224. The sequence corresponding to the predicted signal peptide, the Y/FxC motif and the C-terminal cysteine residue are highlighted in red and yellow, respectively. Polymorphic amino acid residues between Bgt_96224 and THUN-12 are highlighted in blue. (B) and (C) Coexpression of Pm17-HA and AVRPM17_THUN12 (BgtTH12-04537/BgtTH12-04538) and AvrPm17_96224 (Bgt-S1729/Bgt-S1731) by transient Agrobacterium-mediated expression in N. benthamiana, imaged by the Fusion FX imager system (B) or a conventional camera (C) at 5 dpi. Coinfiltrations were done at a ratio of 1:4 R:Avr. Coexpression of Pm17 with AvrPm17_THUN12 and AvrPm17_96224 induced HR in \( n = 18 \) leaves in three independent experiments. No HR was observed when AvrPm17_THUN12, AvrPm17_96224, or Pm17 was expressed alone \( (n = 18) \). (D) Difference in HR induction between the two AVRPM17 variants AVRPM17_THUN12 and AVRPM17_96224 in a ratio of R:Avr of 1:1. The y-axis represents quantitative measurement of HR based on the Fusion FX imager system. Individual datapoints are color coded based on three independent experiments with at least \( n = 8 \) leaves per experiment. The \( P \) value of the paired Wilcoxon-ranked sum test is indicated above the panel.
encode for structurally similar proteins, despite little similarity on the primary amino acid sequences (SI Appendix, Fig. S12), and that the wheat Pm3 allelic series and its rye ortholog, Pm17 recognize structurally related effectors.

**AvrPm17 Is Encoded in a Mildew Sublineage-Specific Effector Cluster and Exhibits Signs of Reoccurring Gene Conversion Events.** Candidate effector genes in wheat and barley powdery mildew have been grouped into 235 families based on sequence similarity (31). AvrPm17 belongs to family E003, which is represented with 69 members in B. g. tritici (isolate Bgt_96224), 70 members in B. g. tritici (isolate THUN-12), and 59 members in B. g. borderi (isolate DH14) (31, 35). E003 is physically organized in gene clusters distributed over 7 of the 11 chromosomes of wheat and triticale mildew (SI Appendix, Fig. S11). Family members encoded in the same chromosomal location form phylogenetically related clades, consistent with the previously proposed expansion mechanism of effector genes through local duplication (SI Appendix, Fig. S11; ref. 22). Interestingly, the AvrPm17 clade is encoded in a gene cluster that spans more than 1.3 Mb on chromosome 1 and contains seven and eight members in triticale and wheat mildew, respectively, as well as a solitary family member on chromosome 8 (Fig. 3 A and B and SI Appendix, Fig. S11). The locus also harbors an additional effector cluster from family E011, consisting of 11 members, that has expanded within the E003 cluster (Fig. 3B). To study the evolutionary history of the AvrPm17 clade, we identified the region corresponding to the AvrPm17 clusters on scaffold_27 in barley mildew isolate DH14 based on conserved syntenic flanking genes. Strikingly, the region in DH14 is only 200 kb in size and harbors only two E003 family members of the AvrPm17 clade as well as a single effector from family E011 (Fig. 3B). This indicates that both effector clades have been significantly expanded in wheat mildew after the divergence from the barley mildew lineage (Fig. 3B). Using sequencing data from five rye mildew strains, we could also show that rye mildew has six out of the eight E003 family members in the clade, whereas it lacks most of the E011 genes (Fig. 3B). These findings indicate that the E003 expansion has happened in a progenitor of rye/wheat mildew, whereas the E011 family expansion in this region is wheat mildew specific. Strikingly, rye mildew does not encode for an AvrPm17 gene, consistent with the virulent phenotype of the five rye mildew isolates on the Pm17-donor line ‘Insave’ (SI Appendix, Fig. S13). This might indicate that AvrPm17 was lost in rye mildew possibly due to the selection pressure imposed by the Pm17 gene in the rye gene pool (SI Appendix, Table S3).

Given the highly dynamic genomic context of the AvrPm17 locus, it is striking that the avirulent AVRPM17_THUN12 variant and the partial gain-of-virulence variant AVRPM17_96224 are encoded by near identical (BgtTH12-04537/BgtTH12-04538, two synonymous SNPs) or identical (Bgt-51729/Bgt-51731) paralogous gene copies within the isolates THUN-12 and Bgt_96224, respectively (SI Appendix, Fig. S14). Most importantly, the three nonsynonymous SNPs (two affecting the same codon) that differentiate AvrPm17_96224 from the avirulent AvrPm17_THUN12 are identical in both genes Bgt-51729 and Bgt-51731 (Fig. 3C). Congruently there is an identical SNP in the intron of Bgt-51729 and Bgt-51731 when compared with both genes in THUN-12 (BgtTH12-04537/BgtTH12-04538). Strikingly, the AvrPm17 locus in the newly sequenced genome assembly of another B. g. tritici isolate IRS7 also contains two AvrPm17 gene copies that are encoded in the same inverted direction (SI Appendix, Fig. S15A). Both AvrPm17_ISR7 gene copies also encode again for identical proteins that share the A53V substitution with AVRPM17_96224. There are three potential explanations for how paralogous genes copies in three isolates are more similar to each other than to their respective ortholog: 1) independent accumulation of identical mutations in both genes, 2) recurrent gene duplication, and 3) transfer of mutation from one gene to the other by gene conversion. It is highly unlikely that the exact same four mutations have occurred independently in both genes. Furthermore, the duplicated region in Bgt_96224, THUN-12, and IRS7 is identical in size and position and therefore must have occurred in the ancestor of the three isolates, predating the accumulation of the mutations differentiating the AvrPm17 genes (Fig. 3C and SI Appendix, Fig. S15A). Consistent with the hypothesis of a more ancient duplication event, we found that flanking regions of the duplicated segment are significantly more divergent (Fig. 3D and SI Appendix, Fig. S16). Importantly, the duplicated segments have acquired insertions in the noncoding region (Fig. 3E and SI Appendix, Fig. S15A). These insertions are specific for one copy of the duplicated segments and are present and identical in all three haplotypes (Fig. 3E and SI Appendix, Figs. S15 and S17 and Text 1). The presence of these insertions corroborates that AvrPm17_96224, AvrPm17_THUN-12, and AvrPm17_ISR7 gene copies originate from the same gene duplication event and not from independent duplications. Therefore, the nucleotide polymorphisms defining the differences between AvrPm17_THUN12, AvrPm17_96224, and AvrPm17_ISR7 have most probably occurred in one gene copy and were then transferred to its duplicate by gene conversion (for further details, see SI Appendix, Text 1). We propose that gene conversion event(s) contribute to the evolutionary potential of AvrPm17 as an efficient way to transfer beneficial mutations to both gene copies.

**Virulent AvrPm17 Haplovariants Are Ancient and Predate Pm17 Introgression into Wheat.** A haplotype mining approach in a diversity panel of 151 resequenced isolates of wheat mildew (129 isolates) and triticale mildew (22 isolates) for AvrPm17 revealed there are three dominant AVRPM17 variants in the gene pool (Fig. 4 A and B). Two of these are the above-described avirulent AVRPM17_THUN12 (varA) and the weakly recognized AVRPM17_96224 (varB). The most frequent haplotype is varC (AVRPM17_ISR7) that contains a single amino acid polymorphism (A53V) and induces weaker HR in the Nicotiana coexpression assays as compared with the functional varA found in THUN-12 (Fig. 4C). In addition, 11 isolates originating from China encode for the only complete loss-of-recognition haplotype found (varD) with three amino acid changes (A53V, E55R, G61A) compared with varA (Fig. 4 A–C and SI Appendix, Fig. S18). Importantly, all AvrPm17 variants were found to be expressed during early infection of wheat (i.e., the haustorial stage) in isolates containing each of the four haplovariants varA to varD (SI Appendix, Fig. S19).

Based on sequencing coverage, we estimated that the majority of isolates encoding varA, varB, or varC contain two AvrPm17 copies, whereas isolates that encode varD encode for a single AvrPm17 gene (SI Appendix, Fig. S20 and Dataset S3). In addition, three isolates likely encode more than three AvrPm17 copies (SI Appendix, Fig. S20). Strikingly, 97% of the isolates with two AvrPm17 copies encode for two identical mature AVRPM17 proteins in one of the following combinations: varA/varA, varB/varB, and varC/varC (Fig. 4B). This supports the hypothesis that the AvrPm17 gene copies are kept identical by recurring gene conversion events (for details, see SI Appendix, Text 2; Figs. S15–S17 and S21; and Table S4). A PCR-based AvrPm17 locus
Fig. 3. AvrPm17 is a member of a highly expanded effector gene cluster. (A) Phylogenetic relationship of the AVRPM17 effector family. A shows a subsection of the phylogenetic tree based on protein sequences of E003 effector family members of B. gramineae (69 members), B. triticicola (70 members), and B. hordei (59 members). The full tree can be found in SI Appendix, Fig. S11. Effector family members are highlighted as follows: members in B. gramineae in yellow, members in B. triticicola in green, and members in B. hordei in blue. For each branch, the local support values calculated with the Shimodaira–Hasegawa test are indicated. (B) Schematic representation of the AvrPm17 effector cluster in the high-quality genomes of B. gramineae THUN-12, B. triticicola Bgt_96224, and B. hordei DH14. In the absence of a high-quality genome assembly for B. secalis, the presence/absence of genes was estimated by coverage analysis based on mappings of five resequenced isolates. Genes that are present in at least one B. secalis isolate were considered as present. Genes and their orientation are indicated by triangles. The white rectangle in the B. gramineae THUN-12 assembly indicates the position of the 50-kb deletion presented in Fig. 1E. The gene marked with an asterisk represents a collapsed gene duplication in the B. triticicola Bgt_96224 assembly that was resolved in the B. gramineae THUN-12 genome assembly. The syntetic relationship is indicated by dashed lines. The figure is not drawn to scale. (C–E) The AvrPm17 gene copies have evolved through gene conversion. (C) Analysis of SNPs in the AvrPm17 gene copies in the two parental isolates. SNPs in Bgt_96224 are shown in comparison with THUN-12. The AvrPm17 gene copies are represented schematically with gray boxes representing the two exons. The transcriptional orientation is indicated by the direction of the arrowhead in the second exon. SNPs are indicated in the coding sequences and the intron. Red bars represent SNPs that are shared between the two gene copies in Bgt_96224 and yellow bars indicate SNPs that are present in only one copy. (D) Visual representation of the duplication of AvrPm17 in isolate Bgt_96224. To allow alignment of the two sequences, the insertions in the downstream region of the two genes were spliced out. The x-axis shows the alignment position, while the y-axis shows the sequence identity calculated in 50-bp sliding windows. The position of the AvrPm17 gene is highlighted by a yellow box. (E) Schematic representation of the duplicated segments (as yellow boxes) containing AvrPm17 genes (indicated by gray arrows) in the three high-quality genomes of THUN-12, IS17, and Bgt_96224. SNPs are indicated compared with BgTH12-04537 as follows: blue indicates nonsynonymous SNPs, and black indicates synonymous SNPs or SNPs in the intron. White triangles indicate insertions.

dissection in 16 isolates representing the four most commonly found combinations varA/varA, varB/varB, varC/varC, or varD confirmed the presence and conserved position of the specific insertions that distinguish the two duplicated segments in all isolates containing two AvrPm17 copies (SI Appendix, Fig. S15 A–F). This further corroborates that identical AvrPm17 gene copies in these isolates did not evolve from independent duplications but are derived from the same ancient gene duplication.

In a next experiment, we analyzed the virulence phenotype of the above-mentioned 16 representative isolates covering the diversity of AvrPm17 in wheat mildew (varA to varD), on Pm17 transgenic wheat lines (Fig. 4D and SI Appendix, Table S5). Consistent with the strong HR induction of varA upon coexpression with Pm17 in N. benthamiana, isolates encoding the varA/varA variant were completely avirulent on both transgenic lines (Fig. 4D and SI Appendix, Fig. S1 A and B and Table S5). In accordance with the absence of HR in N. benthamiana, isolates encoding varD displayed full virulence on the transgenic Pm17 lines (Fig. 4D and SI Appendix, Fig. S18 and Table S5), demonstrating that the race specificity of the transgenic lines is retained despite Pm17 overexpression. Infections of the Pm17 transgenic lines with isolates containing the varB/varB or varC/varC variants resulted in a quantitative reduction of leaf coverage when compared with the corresponding sister lines (Fig. 4D and SI Appendix, Table S5). This reduction in infection success is consistent with the data from N. benthamiana, in which both the varB and varC variant induced an HR response that was however significantly weaker than by the avirulence variant varA (Figs. 2 B–D and 4C and SI Appendix, Fig. S18). To independently validate the observed quantitative differences in Pm17-mediated cell...
death, we measured wheat protoplast viability (i.e., cell death) upon cotransfection of \textit{Pm17} with different \textit{AvrPm17} variants. Cotransfection of \textit{Pm17} with \textit{AvrPm17\_varA} strongly reduced protoplast viability compared with the nonrecognized \textit{varD} \textit{(SI Appendix, Fig. S22A)}. In addition, we observed intermediate protoplast viability upon cotransfection of \textit{varB} or \textit{varC} with \textit{Pm17} compared with the nonrecognized \textit{varD} variant and recognized \textit{varA} \textit{(SI Appendix, Fig. S22A)}, which is consistent with the
intermediate reactions toward varB and varC observed in *N. benthamiana* (Fig. 4C) and on *Pm17* transgenic wheat lines (Fig. 4D). Taken together, the three assays ( *N. benthamiana* , wheat protoplast, wheat phenotyping) consistently show the strongest resistance reaction for varA followed by varC and varB (varC > varB) and no effect for the nonrecognized varD (Fig. 4 C and D and SI Appendix, Fig. S22 A and B). These data indicate quantitative activation of *Pm17*-mediated resistance by different *AvrPm17* variants. Furthermore, these findings indicate that *AvrPm17* varD and *AvrPm17* varB/varC indeed represent virulence or partial virulence alleles, respectively, that are likely responsible for the resistance breakdown of the *Pm17* gene in wheat.

Both partially virulent variants varB and varC were present in all major subpopulations (i.e., China, Europe, Israel, United States) (Fig. 4B). Given their global distribution and considering that some of the isolates were collected already in the 1990s (Dataset S3), this suggests that the partially virulent *AvrPm17* variants varB and varC were present as standing genetic variation in the wheat mildew population before large-scale agricultural deployment of wheat varieties carrying the *Pm17* introgression at the beginning of the 21st century in the United States, and only subsequently in other regions of the world (27, 29).

To further test this hypothesis, we extended our haplotype analysis to closely related formae speciales of *B. grisea*. Due to their distinct host range, *Blumeria graminis* f. sp. *dicocci*, a forma specialis sampled on wild tetraploid wheat, and *Blumeria graminis* f. sp. *dactylidis* infecting the wild grass *Dactylis glomerata* (23, 36) are unlikely to have previously been exposed to the *Pm17* resistance gene. Strikingly, we found that three isolates of *B. grisea* encode up to two copies of *AvrPm17* varB (Fig. 4B). Furthermore, we found three additional haplvariants (varE to varG) specific to *B. grisea* (Fig. 4 A and B). Coexpression of varE-G with *Pm17* in *N. benthamiana* resulted in significantly weaker HR responses compared with *AvrPm17* varA, suggesting that these variants might also represent partially virulent alleles (Fig. 4C and SI Appendix, Fig. S18). This is consistent with the observation that these haplvariants share the A53V, R80S mutation (varE,G) or the A53V mutation (varF) with *AVRPm17* varB (Fig. 4A). Since *B. grisea* does not grow on most hexaploid wheat cultivars, including ‘Bobwhite’ (23), we could not test the contribution of the varE-G recognition to *Pm17* virulence. In *B. grisea* , *dactylidis*, represented by two isolates, we found an additional haplvariant *AvrPm17* varBdg, which carries two substitutions (A53V and G61A) compared with *AVRPm17*_ varA and is only very weakly recognized by *Pm17* in *N. benthamiana* (Fig. 4 A and C). Most importantly, these mutations are shared with the nonrecognized Chinese haplotype *AvrPm17* varD, demonstrating that 1) the E55R substitution in the Chinese haplotype is the causative mutation leading to complete loss of recognition by *Pm17* (Fig. 4C and SI Appendix, Fig. S18) and 2) that part of the *AvrPm17* diversity found in wheat mildew is ancient and predates the split of *B. grisea* and *B. dactylidis*. Taken together, we found that a significant proportion of the *AvrPm17* sequence diversity found in *B. grisea*, including several gain-of-virulence mutations, is shared with its closely related formae speciales *B. grisea* or *B. dactylidis*. Combined with the observation of a global distribution of partially virulent *AvrPm17* variants varB and varC and their presence in isolates collected before the deployment of *Pm17* wheat in agriculture, our findings strongly indicate that these *AvrPm17* gain-of-virulence mutations represent standing genetic variation in wheat mildew which predates the introgression of *Pm17* into wheat.

While the existence of numerous virulent or partially virulent *AvrPm17* haplotypes in the global mildew population prior to *Pm17* introgression might explain rapid *Pm17* resistance breakdown, this observation is hardly compatible with the initially described broad resistance phenotype exerted by the 1AL.1RS translocation. Based on these considerations, we therefore hypothesized the 1AL.1RS translocation to harbor a second mildew resistance gene in addition to *Pm17*.

The 1RS.1AL Translocation Encodes for Two Powdery Mildew Resistance Specificities. To test for the predicted second resistance gene of the 1AL.1RS translocation, we characterized the genetic association of avirulence of the *Bgt* _96224 × THUN-12_ mapping population on the original 1AL.1RS translocation line ‘Amigo’ (16). The *Pm17* avirulent isolate THUN-12 showed an intermediate phenotype on ‘Amigo’, demonstrating that recognition of *AvrPm17* varA results in quantitative resistance in the presence of the endogenous *Pm17* gene (Fig. 5 A and B). In contrast, the *Pm17*-virulent isolate *Bgt* _96224_ was avirulent on ‘Amigo’, indicating that 1) this isolate carries an additional avirulence component recognized by ‘Amigo’ and 2) the *Bgt* _96224 × THUN-12_ biparental population is suited to validate the second resistance specificity in ‘Amigo’ (Fig. 5 C). Consistent with our hypothesis, a QTL mapping analysis based on 117 progeny of *Bgt* _96224 × THUN12_ identified two significant QTLs associated with the avirulence phenotype on cultivar ‘Amigo’ (for details, see Fig. 5D and SI Appendix, Text 3 and Table S6). One QTL on chromosome 1 corresponds to the *AvrPm17* locus, thereby verifying the activity of the *Pm17* gene in the original translocation line ‘Amigo’. In addition, we identified a highly significant QTL on chromosome 9 that was not detected in the QTL analysis on the transgenic *Pm17* lines (Figs. 1A and 5D), likely encoding the avirulence component recognized by the predicted second resistance gene of the 1AL.1RS translocation. The CI of the QTL on chromosome 9 encompasses 371 kb in the avirulent isolate *Bgt* _96224_ and harbors a total of 16 effector genes, of which four are polymorphic compared with THUN-12 (SI Appendix, Table S7 and Fig. S23A). The corresponding genomic region in the hybrid *B. grisea* _triticale_ THUN-12 is again inherited from *B. grisea* (SI Appendix, Table S6). Upon coexpression with *Pm17* in *N. benthamiana*, none of the effector candidates encoded by isolate *Bgt* _96224_ within the CI elicited a HR (SI Appendix, Fig. S23B). This finding demonstrates that the second QTL on chromosome 9 is independent of the *Pm17* resistance specificity. Most importantly, only progeny of the cross that carry the *AvrPm17* _96224_ haplvariant (*AvrPm17* varB and the THUN-12 genotype in the QTL on chromosome 9 are fully virulent (Fig. 5 D and E), further demonstrating that the simultaneous presence of both virulence alleles is necessary to overcome the resistance on ‘Amigo’.

We therefore conclude that the broad effectiveness of the 1AL.1RS translocation in providing resistance against wheat powdery mildew was based on two resistance gene specificities in the 1AL.1RS translocation. Since the *Pm17* resistance specificity has been attributed to a single locus based on segregation analysis, we hypothesize that the second locus is genetically linked to the 1AL.1RS region and has previously been genetically masked due to repressed recombination frequently associated with introgressed segments in wheat (37).

In summary, we here demonstrate that the *Pm17* introgression is genetically complex and that such complexity could only be revealed through accurate genetic dissection of the avirulence determinants in the pathogen distinguishing the two resistance specificities.
The recent identification of numerous Ave genes both in B. tritici and B. hordei has significantly advanced our understanding of NLR-mediated resistance in the cereal powdery mildew pathosystem (17, 18, 20–22, 35, 38). The functional cloning of Ave genes not only allowed molecular studies on recognition mechanisms (33, 35) but has also set the ground for genetic studies based on the natural diversity of avirulence components in local and global mildew collections. This has led to the discovery of numerous gain-of-virulence mechanisms exerted by Blumeria pathogens, including single amino acid polymorphisms, truncations, and deletions of Ave genes as well as a fungal encoded suppressor SvrPm3 acting on Pm3-mediated resistance through masking of AVRPM3 recognition (17, 18, 35). These findings highlight the importance of genetic and genomic studies in fungal plant pathogens in order to understand the mechanisms of resistance breakdown and allow us to adapt current breeding approaches toward more durable deployment of resistance genes in cereal crops.

After cloning and functional characterization of so far 10 Blumeria Ave genes, several patterns emerged. Blumeria AVR effectors were found to be small proteins with a length of 102 to 130 amino acids and to contain an N-terminal signal peptide, a largely conserved Y/FxC motif, and a conserved cysteine residue toward the C terminus (17, 18, 20–22, 35, 38), while otherwise exhibiting highly divergent amino acid sequences. Furthermore, wheat mildew Ave were consistently among the highest expressed genes within their effector gene family, indicating high abundance in host cells upon secretion presumably influencing the efficacy of their virulence function alongside NLR-mediated recognition in resistant cultivars (18). The newly identified AvePm17 exhibits all of the above-mentioned characteristics of Blumeria AVRs and therefore further corroborates the emerging patterns.

Despite showing little homology to proteins with a known function, more than a hundred Blumeria effectors are predicted to exhibit a ribonuclease-like fold (20, 34, 35, 39, 40). Notably, such a ribonuclease-like structure has recently been confirmed by protein crystallization of the barley powdery mildew effector BEC1054 (34). Similarly, despite highly divergent amino acid sequences, in silico protein modeling approaches predicted ribonuclease-like folds for most of the functionally verified avirulence proteins in barley

**Discussion**

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**Fig. 5.** QTL mapping in the Bgt_96224 × THUN-12 F1 population on the wheat cultivar ‘Amigo’ carrying a 1RS.1AL translocation from ‘Insave’ rye including the Pm17 resistance gene. (A) Representative photographs of phenotypes of the parental isolates Bgt_96224 and THUN-12 on ‘Amigo’ at 10 dpi. The susceptible wheat cultivar ‘Kanzler’ was used as an infection control. (B) Boxplot summarizing the phenotypes of Bgt_96224 and THUN-12 on ‘Amigo’. Leaf coverage of individual leaf segments was scored according to the following scale: avirulent = 0, avirulent/intermediate = 0.25, intermediate = 0.5, intermediate/virulent = 0.75, and virulent = 1 (n = 15, three independent experiments). (C) Distribution of phenotypes of the 117 progeny of the cross Bgt_96224 × THUN-12 on ‘Amigo’. Progeny phenotypes were scored as described in B and the average of at least six leaf segments for each progeny was plotted. (D) Single interval QTL mapping of Bgt_96224 × THUN-12 on cultivar ‘Amigo’. The black line indicates the LOD score of the association throughout the 11 chromosomes of wheat powdery mildew. The red line indicates the significance threshold determined by 1,000 permutations. (E) QTL effect plots summarizing phenotypes of the 117 progeny on ‘Amigo’. The phenotypes were plotted based on the genotypes of the best associated marker at the QTL location on chromosome 9 (QTLchr09) and chromosome 1 (AvrPm17 locus). (F) Photographs of representative progeny of the cross Bgt_96224 × THUN-12 with different genotype combinations (see A) for the QTLs identified in D. Phenotypes on wheat cultivar ‘Amigo’ and the transgenic lines expressing Pm17 at 10 dpi are shown.
and wheat mildew (20, 21, 35), including all AVRPM3 effectors (18) and AVRPM17 (this study). Based on the homology between rye Pm17 and wheat Pm3 NLRs combined with the predicted structural similarities of their corresponding AVR proteins, we propose a conserved recognition mechanism, likely leading to similar selection pressures acting on AVR genes for evasion of recognition.

Extensive haplotype mining in a global wheat mildew collection for A AvrPm3b, A AvrPm3c, and A AvrPm3d revealed that virulent alleles were exclusively based on single amino acid polymorphisms (18, 22). Even though copy number variation was common, disruption or deletion of the avirulence gene, as observed for many other AVRs, has never been detected. In line with these findings, our haplotype mining approach for the paralogous A AvrPm17 copies in a comparable wheat mildew diversity panel also failed to identify gene deletions or nonsense mutations and in return found four variants varA to varD, of which three represent partial or complete virulence alleles that are based on amino acid polymorphisms. In contrast, we found the A AvrPm17 genes to be absent in rye mildew, suggesting different gain-of-virulence mechanisms in these closely related mildew sublineages, likely due to differences in the exposure to the Pm17 gene. Alternatively, the rye lineage might have never possessed an A AvrPm17 gene. Therefore, expanding the diversity panel of rye mildew isolates will be crucial to disentangle the evolutionary history of the A AvrPm17 in this mildew lineage. Such analyses will help to determine if Pm17 in rye still provides race-specific resistance against certain rye mildew isolates.

Gene duplications in effector genes are common and considered advantageous for pathogens, as they allow the independent diversification of virulence factors (31). However, the presence of identical avirulence gene copies can represent a major liability, as gain-of-virulence mutations need to occur in both gene copies to effectively change the phenotypic outcome. This was described for wheat mildew A AvrPm3d in which gain-of-virulence mutations in one of the tandem duplicated gene copies was not sufficient to render the isolates virulent (18). Gene conversion, efficiently transferring beneficial mutations between gene copies, could provide pathogens with a molecular mechanism to mitigate the disadvantage of duplicated avirulence genes. Indeed, a case of gene conversion leading to gain of virulence was described for A Avr3c in the oomycete Phytophthora sojae (41). Similarly, we found evidence for gene conversion to have occurred between the paralogous copies of A AvrPm17 (SI Appendix, Text 1 and 2). The high frequency of wheat mildew isolates encoding for varA/varA, varB/varB, or varC/varC genotypes furthermore indicates repeated gene conversion events between the two paralogs. Whether this phenomenon is dependent on inherent predisposition of the locus to nonallelic gene conversion to occur or whether it reflects the existence of an additional selection pressure linked to the virulence function of A AvrPm17 to maintain the sequences identical will be subject to further studies.

It was hypothesized that introgressed R resistance genes provide effective and durable resistance by recognizing an effector gene which, in the absence of previously acting diversifying selection, is largely conserved (7). The opportunity to test this hypothesis for a fungal pathogen in wheat arose with the recent cloning of the introgressed stem rust resistance genes Sr35 [from Triticum monococcum (42)] and Sr50 [from S. cereale (43)] and their corresponding avirulence genes A AvrSr35 and A AvrSr50 in Puccinia graminis f. sp. tritici (Pgt) (44, 45). Virulent alleles for both genes were identified in Pgt races with diverse geographic origins. Whether these virulent alleles emerged before the introgression of Sr35 and Sr50 into wheat or as a consequence of their agricultural use was, however, not assessed. The identification of partially or fully virulent A AvrPm17 haplotypes (varA to varD) in a geographically diverse set of wheat mildew isolates collected over the last three decades and the identification of A AvrPm17 homologs in closely related mildew sublineages provided a unique opportunity to investigate a possible connection between the starting agricultural use of the Pm17 introgression in wheat at the beginning of the 21st century and the emergence of virulent A AvrPm17 alleles in wheat mildew. Using 1) a global mildew population with a unique temporal resolution including many isolates that were collected before Pm17 deployment or exhibit different host preferences, 2) careful phenotypic studies on transgenic Pm17 lines, and 3) functional studies of Avr recognition in transient protein expression assays, we could demonstrate that virulent A AvrPm17 variants were largely present in mildew populations prior to the deployment of Pm17. We propose that this genetic diversity has arisen from the evolutionary arms race between Blumeria and its host species potentially tracing back to a Pm17/Pm3-like gene in the progenitor of rye and wheat. This hypothesis is corroborated by the fact that the A AvrPm17 gene is encoded in a highly expanded gene cluster of effector family E003, which is exclusive to wheat and rye mildew, suggesting that the expansion of this cluster evolved prior to the split of the two mildew lineages 250,000 y ago (23). One of the mechanisms that is proposed to drive expansion of effector gene clusters is the continuous coevolution with the host immune system (46, 47). Thus, the presence of Pm17/Pm3-like genes in the progenitor of rye and wheat might have resulted in selection pressure leading to the expansion of the effector cluster on chromosome 1 in the progenitor of wheat and rye mildew, suggesting a long history of R gene-mediated effector evolution in natural ecosystems, long before the start of agricultural cultivation. Our findings highlight the importance for resistance durability to select introgressed resistance specificities based on the evolutionary history of donor and recipient species. In this work, we demonstrate the need to identify and monitor the genetic diversity of the corresponding avirulence factors in order to achieve effective and durable resistance. We propose that such studies are very timely, considering the current important efforts to introgress R genes into wheat from phylogenetically distant wild relatives or phylogenetically close diploid progenitor species.

An often-stated advantage of larger translocations from related species is the simultaneous introgression of several resistance specificities active against different plant pathogens, such as the most widely deployed rye translocation 1BL.1RS from ‘Petkus’ carrying Lr26, Yr9, Sr31, and Pm8 (4). For effective and durable resistance, the introgression of several resistance genes active against the same pathogen is highly desirable. By extending the mildew QTL mapping approach from Pm17 transgenic lines to the original 1RS.1AL translocation cultivar ‘Amigo’, we have found evidence for the presence of a second resistance gene potentially recognizing an avirulence gene of B. graminis isolate Bgt 96224. Historically, the Pm17 resistance associated with the 1RS.1AL translocation has been attributed to a single locus (48). The additional resistance specificity predicted by our QTL approach is therefore most likely genetically linked with the Pm17 gene and has been missed by genetic approaches solely applied on the plant side due to suppressed recombination within the translocated genomic region originating from ‘Insave’ rye (37). The simultaneous presence of two race-specific resistance genes in the 1RS.1AL translocation might explain the initially broad resistance exhibited by cultivars such as ‘Amigo’, despite the likely longstanding presence of several gain-of-virulence alleles for A AvrPm17.
in the *B. graminis* population. The identification of this second so far unknown AVR/R gene pair in the future will potentially provide further answers regarding the initial efficacy but also the quick breakdown of the powdery mildew resistance encoded on the 1RS.1AL translocation.

Identification and cloning of introgressed resistance genes has often been hampered by the absence of recombination throughout parts or the entirety of the alien chromatin regions (49). In recent years, several technological advances such as resistance gene enrichment sequencing or mutant chromosome sequencing (MutChromSeq) approaches that do not rely on fine mapping have helped to alleviate this phenomenon and led to the identification of numerous new resistance genes often residing in highly complex loci (50, 51). Here we show that genetic mapping populations of plant pathogens could provide an additional tool to dissect complex translocated genomic regions with low or absent recombination, thereby complementing recently developed, plant-focused approaches.

**Materials and Methods**

Complete details are provided in *SI Appendix, Materials and Methods* (individual sections are cited here). Constructs used in this study are listed in Dataset S1. Primer sequences are listed in Dataset S2. Details about powdery mildew isolates and their associated (Sequence Read Archive) accession numbers are listed in Dataset S3. Phenotyping and subsequent QTL analysis are described in *SI Appendix, section 1*. Candidate identification is described in *SI Appendix, section 2*. Construction of the expression plasmids is described in *SI Appendix, section 3*. Transient expression procedures using *A. tumefaciens* in *N. benthamiana* followed by HR measurement are described in *SI Appendix, section 4*.

Western blot detection of tagged avirulence and resistance genes can be found in *SI Appendix, section 5*. Expression analysis can be found in *SI Appendix, section 6*. PCR-based dissection of the AvrPm17 locus can be found in *SI Appendix, section 7*. Bioinformatic analyses are detailed in *SI Appendix, section 8*. The wheat protoplast assay is described in *SI Appendix, section 9*. The sequence of Pm17 is available in GenBank under accession number AYD60116.1. The AvrPm17 haplotypes are available in GenBank under accession numbers OMZ58717 to OMZ58731.

**Data Availability.** Genomic resequencing data for powdery mildew strains and PacBio sequencing data for *B. graminis* isolate ISR7 have been deposited in the Sequence Read Archive (accession nos. PRJNA625429 and PRJNA783175) (52, 53). The genome assembly of *B. graminis* isolate ISR7 was deposited in the European Nucleotide Archive (accession no. PRJEB41382) (54). The Pm17 sequence is available in GenBank (accession no. AYD60116.1) (55). The AvrPm17 haplotypes were deposited in GenBank (OMZ58717–OMZ58731) (56, 57). Scripts and data used to produce the figures have been deposited to GitHub ([https://github.com/MarionMcUeller/AvrPm17](https://github.com/MarionMcUeller/AvrPm17)) (58).

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