Differentiation Among *Blumeria graminis* f. sp. *tritici* Isolates Originating from Wild Versus Domesticated *Triticum* Species in Israel

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Accepted for publication 14 March 2016.

ABSTRACT

Ben-David, R., Parks, R., Dinoor, A., Kosman, E., Wicker, T., Keller, B., and Cowger, C. 2016. Differentiation among *Blumeria graminis* f. sp. *tritici* isolates originating from wild versus domesticated *Triticum* species in Israel. *Phytopathology* 106:861–870.

Israel and its vicinity constitute a center of diversity of domesticated wheat species (*Triticum aestivum* and *T. durum*) and their sympatrically growing wild relatives, including wild emmer wheat (*T. dicoccoides*). We investigated differentiation within the forma specialis of their obligate powdery mildew pathogen, *Blumeria graminis* f. sp. *tritici*. A total of 61 *B. graminis* f. sp. *tritici* isolates were collected from the three host species in four geographic regions of Israel. Genetic relatedness of the isolates was characterized using both virulence patterns on 38 wheat lines (including 21 resistance gene differentials) and presumptively neutral molecular markers

(simple-sequence repeats and single-nucleotide polymorphisms). All isolates were virulent on at least some genotypes of all three wheat species tested. All assays divided the *B. graminis* f. sp. *tritici* collection into two distinct groups, those from domesticated hosts and those from wild emmer wheat. One-way migration was detected from the domestic wheat *B. graminis* f. sp. *tritici* population to the wild emmer *B. graminis* f. sp. *tritici* population at a rate of five to six migrants per generation. This gene flow may help explain the overlap between the distinct domestic and wild *B. graminis* f. sp. *tritici* groups. Overall, *B. graminis* f. sp. *tritici* is significantly differentiated into wild-emmer and domesticated-wheat populations, although the results do not support the existence of a separate f. sp. *dicocci*.

Additional keywords: *Triticum turgidum* subsp. *dicoccoides*, virulence analysis tool.

The high degree of host specialization found in powdery mildews reflects the advanced evolution of these obligate biotrophic pathogens (Eshed and Wahl 1970; Wyand and Brown 2003). The cereal powdery mildews (formerly *Erysiphe graminis*, now *Blumeria graminis*) are thought to have spread and evolved in tandem with their hosts (Oberhaensli et al. 2011), radiating phylogenetically in response to the development and geographic spread of cereal crops since Neolithic times (Zohary and Hopf 2000). One of the earliest cereal mildew taxonomic schemes, developed by Marchal (1902), assumed that infection by one physiological form was limited to one single host genus. Based on this, seven formae speciales were delimited: *B. graminis* f. sp. *hordei* on *Hordeum* species, f. sp. *tritici* on *Triticum* spp., f. sp. *secalis* on *Secale* spp., f. sp. *avenae* on *Avena* spp., and three formae speciales on wild grasses (this was extended to eight formae speciales by Oku et al. [1985]).

With the aid of molecular markers, nine distinct groups within *B. graminis* have been identified, and isolates from *Secale*, *Triticum*, and *Agropyron* species are closely related (Inuma et al. 2007; Troch et al. 2014; Wyand and Brown 2003), indicating that these taxa have only recently diverged from one another. In fact, *B. graminis* host range is continually evolving, as evidenced by

separate clusters (based on virulence spectra) detected for isolates from wheat versus triticale ($\times$ *Triticosecale*) (Walker et al. 2011). Based on β -tubulin phylogeny, isolates from *T. aestivum*, *T. durum*, and *T. dicoccoides* were closely related to each other and to those from genera *Secale*, *Triticale*, and *Agropyron* (Troch et al. 2014). Troch et al. (2014) proposed that the crop-infecting *B. graminis* clades should remain formae speciales but those infecting wild relatives should not.

Using seedling inoculations, Eshed and Wahl (1970) determined that Israeli *B. graminis* f. sp. *tritici* populations had a wide host range (16 of 60 host genera they tested). They proposed that the host range in Israel was broader than elsewhere and attributed this extra breadth to correlated coevolution, under which the genetic interrelatedness and extreme diversification of grasses in their center of origin called forth a correspondingly high level of genetic differentiation and variability in the matching *B. graminis* population. Further divergence of *B. graminis* into two subformae (from wild emmer and cultivated wheat) was suggested by Eshed et al. (1994), based on a detailed study of *B. graminis* f. sp. *tritici* isolates.

Using neutral markers, Parks et al. (2009) assayed a set of 28 *B. graminis* f. sp. *tritici* isolates from Israel in a comparison with United States and United Kingdom powdery mildew populations. In contrast to their expectation of high diversity among the Israeli *B. graminis* f. sp. *tritici* isolates, originating in or near the center of origin of wheat, the authors found that haplotype richness was greater in the United Kingdom sample. This finding was attributed to the larger effective populations in the cooler climate of the United Kingdom, which is more conducive to powdery mildew than the arid Middle East.

Israel and vicinity is a center of diversity of wild emmer wheat (*T. dicoccoides* = *Triticum turgidum* subsp. *dicoccoides* Desf.)

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*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary tables are published online.

<http://dx.doi.org/10.1094/PHYTO-07-15-0177-R>

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(Harlan and Zohary 1966; Salamini et al. 2002), which is distributed sympatrically with commercial fields of domesticated wheat (both *T. aestivum* and *T. durum* = *T. turgidum* subsp. *durum*). *T. dicoccoides* was first cultivated around 10,000 years ago in this region, giving rise to *T. durum* and, through a cross with *Aegilops tauschii*, to *T. aestivum* about 7,000 to 9,000 years ago (Peng et al. 2011; Salamini et al. 2002). Both *Triticum* species serve as hosts of *B. graminis* f. sp. *tritici*. Israel grows about 100,000 hectares of *T. aestivum* and 4,000 hectares of *T. durum* annually, with wild *T. dicoccoides* populations often occurring adjacent to or near commercial fields (Fig. 1), often in intimate mixture with other wild cereals, such as wild barley (*Hordeum spontaneum*) and wild oat (*Avena* spp.) (Fig. 2). Recent phenotypic screening of a broad, diverse collection of *Triticum* germplasm with several isolates of *B. graminis* f. sp. *tritici* resulted in a clear pattern of host adaptation; isolates sampled from wild wheat had higher frequencies of virulence to wild wheat accessions than to domesticated wheat lines, while the reverse was true for isolates from domesticated wheat (Ben-David 2011; Ben-David et al. 2008). This pattern is probably the result of long-term plant-pathogen coevolution, and it is consistent with previously observed host specialization by *B. graminis*. Recently, Menardo et al. (2016) determined the genomic sequences of 19 *B. graminis* f. sp. *tritici* isolates. Based on the strong, host-based molecular differentiation within the *B. graminis* f. sp. *tritici* clade, they designated a new forma specialis, *B. graminis* f. sp. *dicocci*.

Thus, in the Israeli *B. graminis* f. sp. *tritici* population, evidence has been found for broad host range, limited genotypic diversity (at nonselected loci), and host specialization. Little is yet known about the evolutionary processes affecting the *B. graminis* f. sp. *tritici* population in its presumed center of origin. The close physical mixing of the three *Triticum* host species provides a unique opportunity to examine pathogenic host range at the host species level.

In this study, we have evaluated a diverse collection of *B. graminis* f. sp. *tritici* isolates from Israel, using both phenotypic and molecular tools in order to (i) characterize the virulence and molecular diversity of the collection, (ii) test whether this diversity was associated with host and geographic origin, and (iii) determine the degree of host species specificity in *B. graminis* f. sp. *tritici* isolates originating from wild and domesticated hosts.

MATERIALS AND METHODS

Plant material and powdery mildew isolates. A set of 61 *B. graminis* f. sp. *tritici* isolates was collected from wild and domesticated *Triticum* species growing in or near Israel: 26 from *T. aestivum*, 16 from *T. durum*, and 19 from *T. dicoccoides* (Table 1;

Fig. 3). They originated from across Israel's Mediterranean zone (31°25' to 33°07') and are being maintained at the Hebrew University as part of the Eshed-Dinor living mildew collection. All isolates were collected in Israel with one exception, isolate 52, which was collected from Dir-El-Balakh in the Gaza Strip (Table 1). Of the 61 isolates, 41 were collected in 1990 to 1993 and 20 in 2010. Isolates collected prior to 2010 were previously used in an analysis of population diversity comparing single-nucleotide polymorphisms (SNP) from the United States, United Kingdom, and Israel, and the remaining DNA was used for the present marker analysis (Parks et al. 2009). In 2010, additional isolates were collected in order to reduce potential bias from sample size differences among geographic regions and hosts of origin.

In addition, two *T. aestivum*-derived isolates that originated in the United Kingdom and Switzerland, respectively, were used in the study (JIW2 and 96224). The isolate A6 of *B. graminis* f. sp. *hordei*, the barley forma of the pathogen, came from the UZH collection (originally obtained from P. Schulze-Lefert, MPI Cologne) and was used as a reference in the SNP assay described below.

The Israeli isolates were collected from 38 locations (Table 1). In several locations, more than one isolate was collected: Amiad ($n = 4$, *T. dicoccoides*), Ein Hanaziv ($n = 4$, of which two were from

Fig. 2. Wild emmer wheat, *Triticum turgidum* subsp. *dicoccoides*, growing in its natural habitat (Mt. Gilboa, Israel) in a mixture with nonhost species of *Blumeria graminis* f. sp. *tritici*. A dotted black line frames wild wheat (*T. dicoccoides*) and a dashed black line frames wild barley (*Hordeum spontaneum*). Wild oats are also prominent.

Fig. 1. Sympatric distribution of wild emmer wheat (*Triticum dicoccoides*) populations and cultivated wheat in Mount Gilboa, Israel.

T. aestivum and two from *T. durum*), Hula ($n = 7$, of which six were from *T. aestivum* and one from *T. durum*), Gilboa ($n = 2$, *T. dicoccoides*), Nahal Oz ($n = 7$, of which five were from *T. aestivum* and two from *T. durum*), Negba ($n = 2$, one from *T. aestivum* and one from *T. durum*), and Tel-Aviv ($n = 2$, from a *T. dicoccoides* nursery, an artificial environment different from a natural wild emmer wheat habitat). In those locations where more than one isolate was

collected from *T. aestivum* or *T. durum*, they were from different varieties at each location.

The four geographic regions and numbers of isolates collected in each were as follows: Northern Coastal Plain ($n = 11$), Eastern Galilee ($n = 12$), Emek Bet Shean ($n = 10$), and Mt. Judea and Plain ($n = 28$). The first three regions are in northern Israel. The Northern Coastal Plain is mainly lowlands (<100 m elevation) adjacent to the

TABLE 1. *Blumeria graminis* f. sp. *tritici* isolates from wild or domesticated *Triticum* species included in the current study

Isolate no.	Geographic region	Location	<i>Triticum</i> sp. ^y	Cultivar name	Analysis ^z	Collection year
1	Eastern Galilee	Hula	aes	Dari'el	SN, SS	1990
4	Eastern Galilee	Hula	aes	Deganit	SS	1990
6	Eastern Galilee	Hula	aes	Atir	SN, SS	1990
7	Eastern Galilee	Hula	aes	Shafir	SN, SS	1990
8	Eastern Galilee	Hula	dur	Bareket	SN, SS	1990
9	Eastern Galilee	Hula	aes	652	SN, SS	1990
13	Eastern Galilee	Hula	aes	509	SN, SS	1990
15	Northern Coastal Plain	Yavor	dur	Inbar	SN, SS	1990
16	Mt. Judea, Plain	Nahal Oz	dur	Inbar	SS	1990
20	Emek Bet Shean	Ein Hanaziv	dur	Inbar	SS	1991
25	Mt. Judea, Plain	Bet Dagan	dur	Inbar	SN, SS	1991
29	Emek Bet Shean	Ein Hanaziv	aes	Shafir	SS	1990
30	Mt. Judea, Plain	Talm Yafe	aes	Shafir	SS	1991
33	Mt. Judea, Plain	Erez	aes	Deganit		1990
36	Mt. Judea, Plain	Lahav	aes	Deganit	SS	1990
37	Mt. Judea, Plain	Nahal Oz	aes	Deganit	SS	1990
43	Mt. Judea, Plain	Yesodot	aes	BetHashita	SS	1991
44	Mt. Judea, Plain	Negev	aes	Miriam	SS	1991
47	Mt. Judea, Plain	Saad	aes	BetLehem	SS	1990
50	Mt. Judea, Plain	Nahal Oz	aes	Barkay		1990
52	Mt. Judea, Plain	DirElBalakh	dur			1990
58	Eastern Galilee	Amiad	dic		SN, SS	1990
61	Eastern Galilee	Tabha	dur	Zenati Bouteille	SN, SS	1990
63	Emek Bet Shean	Gilboa	dic		SN, SS	1990
66	Eastern Galilee	Amiad	dic		SN, SS	1990
67	Mt. Judea, Plain	Lahav	dur	Inbar	SS	1990
68	Mt. Judea, Plain	Bet Dagan	aes	BetHash	SS	1990
70	Mt. Judea, Plain	Beeri	aes	Deganit	SN, SS	1990
91	Emek Bet Shean	Sde Eliahu	dur	Inbar	SN, SS	1990
92	Mt. Judea, Plain	Tel Aviv	dic		SS	1992
94	Emek Bet Shean	Ein Hanaziv	aes	Deganit	SS	1990
95	Emek Bet Shean	Ein Hanaziv	dur	Bareket	SN, SS	1990
96	Mt. Judea, Plain	Negba	aes	sw50	SN, SS	1990
97	Mt. Judea, Plain	Negba	dur	50	SN, SS	1990
101	Mt. Judea, Plain	Nahal Oz	aes	Dari'el	SS	1990
103	Eastern Galilee	Amiad	dic		SN, SS	1990
106	Mt. Judea, Plain	Nahal Oz	aes	Atir	SS	1990
107	Mt. Judea, Plain	Nahal Oz	aes	Shafir		1990
108	Mt. Judea, Plain	Nahal Oz	dur	Bareket	SS	1990
109	Mt. Judea, Plain	Tel Aviv	dic			1993
113	Eastern Galilee	Amiad	dic	W-B-1	SN, SS	1990
201	Northern Coastal Plain	Tzurit	dic		SN, SS	2010
202	Northern Coastal Plain	Haifa University	dic		SN, SS	2010
203	Northern Coastal Plain	Misgav	dic		SN, SS	2010
204	Northern Coastal Plain	Kfar-Masaryk	dur	Eylon	SN, SS	2010
205	Mt. Judea, Plain	Kfar-Menahem	dur	Eylon	SN, SS	2010
206	Mt. Judea, Plain	Neve Michael	dic		SN, SS	2010
207	Mt. Judea, Plain	Bet Meir	dic		SN, SS	2010
208	Emek Bet Shean	Gilboa	dic		SS	2010
209	Emek Bet Shean	K. Revhaya	dic		SN, SS	2010
210	Emek Bet Shean	G. Hamore	dic		SN, SS	2010
211	Emek Bet Shean	Merav	dic		SN, SS	2010
212	Mt. Judea, Plain	Shilat	dic		SN, SS	2010
213	Mt. Judea, Plain	Modihin West	dic		SN, SS	2010
214	Northern Coastal Plain	Akko	dur	Eylon	SS	2010
215	Northern Coastal Plain	Barkai	dur	Eylon	SN, SS	2010
216	Northern Coastal Plain	Ein Shemer	aes	Yuval	SS	2010
217	Northern Coastal Plain	Kfar-Hasidim	aes	Galil	SN, SS	2010
218	Northern Coastal Plain	Tel Far	aes	Ariel	SN, SS	2010
219	Mt. Judea, Plain	Bizaron	aes		SS	2010
220	Northern Coastal Plain	Bet Oren	dic		SN, SS	2010
JIW2	–	United Kingdom	aes		SN, SS	1990
96224	–	Switzerland	aes		SN, SS	1996

^y Wheat host: aes = *T. aestivum*, dur = *T. durum*, dic = *T. dicoccoides*.

^z Type of assay: SN = single-nucleotide polymorphism analysis, SS = simple-sequence repeat analysis.

Mediterranean Sea, the exception is Mount Carmel (546 m elevation). Eastern Galilee includes the lower eastern parts of the upper Galilee Mountains (200 m elevation) and their eastern border, the Hula Valley (<70 m). Emek Bet Shean includes the eastern valley farming region (~150 m elevation) with Mt. Gilboa (400 m elevation) on its southwest. Mt. Judea and Plain is a relatively vast region in central and southern Israel including the western foothills of Judean Mountains (400 to 600 m elevation), the Judea Plain, and the northern and western parts of the Negev Desert (50 to 150 m elevation).

Virulence characterization. The isolates from the Eshed-Dinoor mildew collection were characterized for virulence by inoculating a set of wheat powdery mildew (*Pm*) differential lines ($n = 21$), bread wheat cultivars ($n = 5$), durum wheat cultivars ($n = 8$), and wild emmer wheat accessions ($n = 4$) (Supplementary Table S1). The *Pm* differential lines mostly contained one *Pm* gene each, but in two cases, possessed two or three genes each. Bread wheat and durum wheat cultivars were chosen to represent cultivated Israeli germplasm (including *T. durum* landrace Zenati Bouteille). The

tested genotypes also included several cultivars from outside of Israel (e.g., bread wheat cultivars Chinese Spring, Arina, and Forno; and durum wheat cultivars Cerco and Langdon). Wild emmer wheat accessions from Israel were selected arbitrarily.

All 61 isolates were tested on all cultivars. Inoculation and disease assessment were performed according to Hsam and Zeller (1997) and Ben-David et al. (2014) with two to four replicates. The infection types of powdery mildew were recorded on a scale of 0 to 4: 0 indicated no visible symptoms, while a score of '0;' was recorded for necrotic flecks (hypersensitive response), and values of 1, 2, 3, and 4 signified highly resistant, resistant, susceptible, and highly susceptible reactions, respectively (Mains and Dietz 1930). Scores of 0 to 2 were grouped as resistant and scores of 3 to 4 as susceptible.

Molecular markers. Simple-sequence repeat (SSR) alleles were determined for a total of 58 isolates (Table 1). DNA extraction for these isolates followed a CTAB protocol as previously described (Cowger et al. 2016). Ten SSR markers (BGT1 to BGT10) were amplified with fluorescent labels as previously described (Parks et al. 2011). Resulting fragments were analyzed by Eton Biosciences, Inc. (Durham, NC) on an ABI 3730xl with size standard GS500LIZ. Fragments were manually sized using Peak Scanner version 1.0 (Applied Biosystems Inc., Carlsbad, CA). Raw fragment sizes with decimals were binned into alleles of expected periodicity, using TANDEM (Matschiner and Salzburger 2009). All analyses were performed on binned fragment sizes.

Four loci were investigated (two genic and two intergenic) based on two characterized loci in the *B. graminis* f. sp. *tritici* genome (Oberhaensli et al. 2011). SNPs were identified for 37 of the total sample of 63 isolates; the 37 isolates were those for which high-quality sequence information was available at all four loci. Genomic DNA samples were extracted using a Qiagen DNeasy plant mini kit. On average, 500 to 600 bp of good-quality sequence were obtained for each primer (about 1 kb per locus). Sequences were aligned using BioEdit software (www.mbio.ncsu.edu/BioEdit/page2.html). Analysis was performed using clustalW multiple alignment as implemented in BioEdit 7.1.3. The sequences were clustered and ordered by their similarity.

Statistical analyses. Virulence. The virulence scores were analyzed using the virulence analysis tool (VAT software) to characterize diversity within the collection and to identify different pathotypes (Schachtel et al. 2012) (<https://en-lifesci.tau.ac.il/profile/kosman/vat>). In addition to assessment of descriptive parameters such as virulence frequency, pathotype frequency, and virulence complexity, the assignment-based approach to diversity analysis (Kosman 1996, 2014; Kosman and Leonard 2007) was employed for comparison of isolates from different host species and from different geographic regions. Dissimilarity between virulence patterns of isolates was estimated with the simple mismatch coefficient, and the corresponding Kosman's estimates were calculated for diversity within (*KW*) and distance between (*KB*) populations. A permutation test was performed with 1,000 random partitions of the data for differentiation statistics based on the *KW* diversity according to Equations 20 to 22 in Kosman (2014) as explained by Kosman et al. (2014). Diversity (always nonnegative for *KW*) within a total pool of isolates and the weighted average diversity within *k* populations were estimated for the original subdivision and for 1,000 random partitions of the total population into *k* groups containing the same numbers of isolates as in the original populations. The null hypothesis that the populations were not genetically differentiated was rejected if, in at least 950 of the 1,000 cases, the differentiation statistic obtained for the random partitions was less than the differentiation statistic obtained for the original partition ($P < 0.05$).

Using the unweighted pair group method with arithmetic means, a dendrogram for 61 isolates was derived based on simple mismatch dissimilarity and using the SAHN module of the NTSYSpc package, v. 2.1 (Exeter Software). The cophenetic correlation was calculated (COPH and MXCOMP modules of NTSYSpc) to assess correspondence between the dendrogram structure and the original distance

Fig. 3. Locations across Israel and surrounding regions where *Blumeria graminis* f. sp. *tritici* samples were collected from wild emmer wheat (filled circles) or cultivated wheat fields (open circles). The four geographic regions are marked with dashed black line: A, Northern Coastal Plain; B, Eastern Galilee; C, Emek Bet Shean; and D, Mt. Judea and Plain.

matrix upon which the clustering was based (goodness of fit of clustering by the dendrogram).

Molecular data. Analyses with the SSR markers were conducted on the 58 isolates for which data were of high quality. Relationships among isolates were analyzed using the stepwise mutation model. Dissimilarity between SSR patterns was estimated on the basis of allele sizes (number of tandem repeats) under the assumption that the mutation rate varied from locus to locus (Appendix, Equation A1). To evaluate potential separation of isolates derived from different hosts, the obtained matrix of dissimilarities was subjected to a principal coordinate analysis (PCoA), and the corresponding plot was generated with GenAlex 4 software. Population subdivision based on host geographic origin was assessed by applying to SSR fragment sizes an exact G-test as implemented in Genepop version 4.0.11, with 10,000 de-memorization steps in 100 batches and 5,000 iterations per batch.

An additional test of population subdivision was performed, using the SSR data in Structure 2.3.3, under a model assuming independent allele frequencies among populations and genetically admixed individuals. Data were fitted to a model assuming K values of 1 to 5, where K is the optimum number of populations given the data. Ten independent analyses per K value were executed, and each independent run consisted of 1,000,000 steps with a 10% burn in. The optimal K was determined using the Evanno method (Evanno et al. 2005) via Structure Harvester (Earl and vonHoldt 2012).

Migration. Comparisons of effective population sizes and estimates of migration among host populations were made using Migrate-N version 3.3.1 (Beerli and Palczewski 2010). The SSR and SNP markers were analyzed separately, as the evolution of the markers was presumed to have occurred on different evolutionary time scales. Migrate-N utilizes a coalescent approach for estimation of the mutation-scaled effective immigration rate (Θ) and the mutation-scaled effective population size (M). For haploids, $\Theta = 2N\mu$, where $2N\mu$ is the effective population size and μ is the mutation rate per marker per generation (per site per generation for SNPs). Parameter M is a ratio of immigration to mutation (m/μ , where m is the immigration rate per generation from a source to a sink population). Multiplication of estimates Θ and M (divided by 2 for a haploid organism) provides a per-generation estimate of the number of migrants between two populations.

Due to the data requirements of Migrate-N, DNA sequences were edited and polymorphisms were only analyzed for that portion of each sequence having base calls with high reliability in all

individuals. The resulting sequences were as follows: 349 bp for primer I12G2-F, 349 bp from primer I12I9-F, 316 bp from I12I9-R, 500 bp from C19G5-F, 520 bp from C19G5-R, 483 bp from C19I4-F, and 406 bp from C19I4-R. Data were concatenated in chromosomal order into two contigs numbered 12 and 19. Gaps between concatenated sequences were ignored.

RESULTS

Virulence analysis. Virulence frequencies to most *Pm* alleles were very similar between isolate populations from *T. aestivum* and *T. durum*, while frequencies of virulence of *T. dicoccoides* isolates to the same alleles were significantly lower (Fig. 4). The exceptions were *Pm1b*, *Pm5b*, and *Pm22*; virulence to those *Pm* genes was equal or higher among wild emmer-derived isolates. Among isolates originating from *T. aestivum* or *T. durum*, almost every isolate had a unique pathotype, while the proportion was lower for isolates from *T. dicoccoides* (Table 2). Complete virulence/avirulence data for the isolates are given in Supplementary Table S1.

No geographic pattern was observed in the virulence data. By the differentiation test based on KW, the null hypothesis that the

TABLE 2. Virulence complexities (VC) for *Blumeria graminis* f. sp. *tritici* isolates from two domestic and one wild *Triticum* species in Israel

Host	No. of isolates	No. of haplotypes	VC ^z
<i>T. aestivum</i>	26	25	13.34 a
<i>T. durum</i>	16	16	11.37 a
<i>T. dicoccoides</i>	19	12	5.47 b
TOTAL	61		

^z VC values followed by different letters are significantly different from each other ($P < 0.0001$).

TABLE 3. Kosman genetic distances between (KB) and diversity within (KW, along the diagonal) groups of *Blumeria graminis* f. sp. *tritici* originating from *Triticum aestivum*, *T. durum* and *T. dicoccoides* in Israel, based on virulence data

	<i>B. graminis</i> f. sp. <i>tritici</i> isolates from		
	<i>T. aestivum</i>	<i>T. durum</i>	<i>T. dicoccoides</i>
<i>T. aestivum</i>	0.321	0.189	0.384
<i>T. durum</i>	...	0.413	0.347
<i>T. dicoccoides</i>	...	...	0.377

Fig. 4. Frequencies of virulence to individual *Pm* genes of 61 *Blumeria graminis* f. sp. *tritici* isolates collected in Israel and vicinity from wild (*T. dicoccoides*) and domesticated *Triticum* species collected in 1990 to 1993 or 2010.

populations from the four regions were not genetically differentiated was not rejected (data not shown; $P = 0.235$).

However, isolates were clearly differentiated by host of origin. Larger genetic distances were observed between isolates from *T. dicoccoides* and those from both *T. aestivum* (0.38) and *T. durum* (0.35) than between the latter two populations (0.19) (Table 3). In addition, significant differentiation among the populations was demonstrated using the permutation test with KW diversity ($P = 0.001$), including less significant differentiation among isolates from *T. aestivum* and *T. durum* ($P = 0.05$).

SSR data. Of the 10 SSR markers tested, only seven amplified in a sufficient number of genotypes to be useful. BGT-2, BGT-8, and BGT-9 had more than 40% missing data and were not analyzed. This contrasted with the performance of the same SSR markers on isolates from the United States, where all 10 primer pairs amplified fragments from all isolates (Parks et al. 2011).

Pairwise exact G tests of population subdivision based on geographic origin found no significant differences between any sampling locations (Table 4). However, exact tests based on hosts indicated that, while isolates collected from *T. aestivum* and *T. durum* could not be distinguished from one another, both were significantly different from *T. dicoccoides*.

Structure Harvester analysis of Structure 2.3.3 runs provided additional support for an optimal K value of 2, based on Delta K. Data from 10 runs were summarized using CLUMPP and were visualized using distruct 1.1 (Fig. 5). A clear clustering of isolates into two groups, wild and domesticated, was observed, although some isolates from the wild-origin hosts were assigned to the domesticated group with a high probability and, in one case, vice versa. PCoA of the dissimilarity matrix largely mirrored the Structure barplot results (Fig. 6). They both indicated that *T. aestivum* and *T. durum* isolates tended to cluster with each other, as did *T. dicoccoides* isolates with each other, although the two groupings overlapped. For example, three *T. durum*-originated *B. graminis* f. sp. *tritici* isolates (25, 61, and 97) were positioned with the wild-origin *B. graminis* f. sp. *tritici* group. Similarly, three wild-origin *B. graminis* f. sp. *tritici* isolates (92, 103, and 208) were distributed proximal to the domestic-origin *B. graminis* f. sp. *tritici* group (Fig. 6).

SNP analysis. The *B. graminis* f. sp. *hordei* isolate A6 produced a polymerase chain reaction product only at one locus (I12_G2). At that locus, a strong SNP polymorphism was detected between this isolate and the *B. graminis* f. sp. *tritici* isolates (data not shown).

In general, the molecular diversity in the *B. graminis* f. sp. *tritici* assay was low. Thirty-two SNPs were detected: 11 in the genic sequences, 21 in the intergenic regions. Four SNPs divided the collection into two major haplotypes (data not shown). This discrimination was only partly based on wheat host species. The rest of the SNPs divided a small group of several isolates from the rest

of the collection. In seven cases, a single isolate was separated from the rest of the collection based on a SNP.

There was a strong congruence in the clustering of isolates by virulence and SNP data (Fig. 7). Among the 34 isolates for which both virulence profiles and SNP haplotypes were available, there were only two isolates that did not appear either above or below the dividing line for both types of data (Fig. 7).

Migration. Using Migrate-N, a test for subdivision of the *T. aestivum* and *T. durum* populations determined that they form a single population, i.e., panmixia best fit the data from those two populations alone ($P > 0.99$ for both marker types). In subsequent analyses, the *T. aestivum* and *T. durum* data were pooled, and migration was assessed between the two populations originating from domestic wheat and *T. dicoccoides*, respectively. Four models each were tested for SNPs and SSRs: panmixia, two-way migration, one-way migration from domestic wheat to *T. dicoccoides*, and one-way migration in the other direction. For both marker types, the best-fitting model was one-way migration of mildew from domestic wheat to *T. dicoccoides* ($P \geq 0.99$).

Estimates of population size (Θ) and migration rate (M) diverged in opposite directions for the two types of markers, with SNPs leading to a larger estimated effective population for *B. graminis* f. sp. *tritici* derived from domestic hosts, and SSRs producing a larger estimated effective population for strains from wild emmer (Table 5). Overall, SNPs yielded estimates of small effective populations but a high migration rate, and the opposite was true for SSRs. Consequently, the estimated numbers of migrants per generation for the two marker types were very similar (five to six migrants per generation from domesticated wheats to *T. dicoccoides*).

DISCUSSION

To our knowledge, this is one of the first in-depth analyses of host-range differentiation within a *Blumeria graminis* forma specialis. *B. graminis* f. sp. *tritici* isolates separated into two distinct groups based on host of origin: domesticated wheat (*T. durum* and *T. aestivum*) and wild wheat (*T. dicoccoides*). This pattern was consistent across every analysis performed on virulence, microsatellite, or sequence data. Given that the two types of hosts have coexisted sympatrically in Israel for around 8,000 years, this genetic separation by host species could be evidence of separate coevolution of *B. graminis* f. sp. *tritici* on its ancestral and cultivated hosts following domestication of the latter. However, given the significant one-way migration that was detected, the genetic separation is more likely due, at least in part, to strong genetic drift in populations of wild *B. graminis* f. sp. *tritici* founded by those relatively few “domesticated” immigrants able to survive and reproduce on *T. dicoccoides*. Today, the distribution of

TABLE 4. Exact tests of population subdivision by geographic location and host of origin of 58 *Blumeria graminis* f. sp. *tritici* isolates collected in Israel, analyzed with simple-sequence repeat markers

Populations	χ^2 value	df	P value
Geographic regions ^y			
EBS and EGAL	18.9	14	0.17
EBS and J&P	17.5	14	0.23
EGAL and J&P	16.0	14	0.31
EBS and NCP	8.0	12	0.79
EGAL and NCP	13.6	12	0.33
J&P and NCP	21.9	14	0.08
Host of origin ^z			
AES and DIC	75.2	14	0.00
AES and DUR	11.4	14	0.66
DIC and DUR	40.5	14	0.00

^y EBS = Emek Bet Shean, EGAL = Eastern Galilee, J&P = Mt. Judea and Plains, and NCP = Northern Coastal Plain.

^z AES = *Triticum aestivum*, DIC = *T. dicoccum*, and DUR = *T. durum*.

Fig. 5. Clustering of 61 *Blumeria graminis* f. sp. *tritici* isolates from Israel by STRUCTURE, showing two primary groups, wild origin (from *Triticum dicoccoides*) and domestic origin (from *T. aestivum* or *T. durum*).

wild emmer wheat habitats is limited, in most cases, to higher elevations and heavy soils. This, in addition to other factors, has contributed to the formation of eco-geographically distinct wheat niches in the wild and in domesticated fields. The specific attributes of each niche represent different adaptive challenges to wheat and its obligate biotroph that underlie the differentiation of wild and domesticated *B. graminis* f. sp. *tritici*.

Menardo et al. (2016) analyzed full genomic sequences of 17 of the isolates in our dataset ($n = 61$) and found that most isolates originating from *T. dicoccoides* (six of eight) were strongly differentiated from other *B. graminis* f. sp. *tritici* isolates. Based on these results, they defined a new forma specialis, *B. graminis* f. sp. *dicocci*, growing exclusively on tetraploid wheat. However, in the current study, which included the isolates studied by

Fig. 6. Genetic diversity analysis of 58 *Blumeria graminis* f. sp. *tritici* isolates based on data from the seven simple-sequence repeat (SSR) markers. Principal coordinates analysis was implemented using the step-wise mutation model to measure dissimilarities between SSR profiles. *B. graminis* f. sp. *tritici* isolates originated from wild wheat (*Triticum dicoccoides*, green circles), domesticated durum wheat (*T. durum*, red triangles), or bread wheat (*T. aestivum*, dark squares). Together, the two principal coordinates account for 55% of the genetic variance among isolates. Isolates mentioned in the text are labeled.

Fig. 7. Congruence of virulence and single-nucleotide polymorphism profiles of *Blumeria graminis* f. sp. *tritici* isolates from Israel and vicinity. **A**, Simple-mismatch dissimilarity dendrogram of virulence scores of 61 isolates created with the virulence analysis tool (VAT). Of the 61 isolates, 19 were sampled from wild emmer wheat and 42 from domesticated wheat. **B**, Multilocus clustalW analysis of blue genic (C19_G5 and I12_G2) and red intergenic (C19_I4 and I12_I9) sequences of 37 *B. graminis* f. sp. *tritici* isolates. Of the 37 isolates, 15 were sampled from wild emmer wheat and 22 from domesticated wheat. *B. graminis* f. sp. *tritici* isolates from domesticated and wild wheat are consistently either above or below the red line, respectively, with few exceptions. Solid black lines = isolate aligned similarly by both analyses; dotted black lines = isolates aligned differently.

Menardo et al. (2016), we did not find *B. graminis* f. sp. *tritici* isolates that grew exclusively on tetraploid wheat species, and thus our results do not support the conclusion of Menardo et al. (2016) that there is a distinct forma specialis pathogenic uniquely on tetraploid wheat. This discrepancy could be at least partly explained by the limited overlap between the two studies in terms of virulence data. Of the 36 wheat lines that were included in our virulence study, only three were also tested by Menardo et al. (2016).

In addition to the existence of two distinct groups of *B. graminis* f. sp. *tritici*, domestic and wild, some outlier genotypes were observed, i.e., isolates from *T. dicoccoides* that clustered with isolates from domesticated hosts and vice versa. These may be the result of the significant gene flow that was detected from populations in cultivated wheat fields to populations on wild emmer. Despite this level of one-way gene flow, the virulence profile of the wild emmer-derived *B. graminis* f. sp. *tritici* population still diverged significantly from that of the domestic wheat-derived population. The virulence profile in our study was determined on the basis of interactions with several *Pm* genes that probably do not exist in wild emmer and, thus, the virulence complexity (VC) of the wild wheat-derived isolates was naturally low. The isolates derived from domesticated wheat were more adapted to at least several of the *Pm* genes, and therefore their VC was higher.

It could be hypothesized that the virulences to *Pm* genes found in cultivated wheats are not advantageous and entail a fitness cost to *B. graminis* f. sp. *tritici* isolates that are established on wild emmer, because the latter host possesses a partially differing suite of resistance genes. In that scenario, the domestic *B. graminis* f. sp. *tritici* strains lose the unnecessary virulences through sexual recombination once they become established on wild emmer. Alternatively, it is possible that *Pm*-virulent domestic *B. graminis* f. sp. *tritici* strains are selected against on wild emmer and it is mainly low-VC domestic *B. graminis* f. sp. *tritici* isolates that establish on wild emmer and contribute to the “wild” *B. graminis* f. sp. *tritici* gene pool. Either or both explanations are compatible with the lower virulence frequencies and lower VC on wild emmer-derived *B. graminis* f. sp. *tritici* isolates. In an evolutionary context, it is also possible that the wild emmer-derived *B. graminis* f. sp. *tritici* strains with higher VC represent the ancestral portion of the *B. graminis* f. sp. *tritici* population on wild wheat from which mildew of domesticated wheat eventually evolved. However, as indicated earlier, the VC data should be interpreted with caution, as the differential set contained only a few wild emmer wheat lines.

A notable finding of this study was the absence of support for *B. graminis* f. sp. *tritici* migration from *T. dicoccoides* into the domesticated wheat populations of *B. graminis* f. sp. *tritici*, despite the spatial proximity of these host populations. There were a few anomalous cases: one isolate (61) had the virulence, SSR, and SNP alleles of a wild strain and clustered with emmer-derived isolates, but was sampled from a *T. durum* landrace cultivar (Figs. 6 and 7). Interestingly, this isolate (61) was also an exception in a previous study (Eshed et al. 1994): originating from domesticated wheat but with virulence attributes and genetic profile similar to isolates from

wild emmer wheat. Isolate 61 was collected from a durum wheat landrace (sampled in 1990 from Zenati Bouteille) in the eastern Galilee, a region with a long history of durum-based traditional dryland farming. This region is also dominated by large populations of wild wheat. Thus, the region provides an intimate geographic proximity between wild and domesticated wheats, in which wild wheat plants invade wheat fields and grow as weeds on their margins. This pattern enables not only spontaneous introgressions of wild emmer into durum, but also the movement and adaptation of the mildew pathogen.

In order for a migrant to be successful, it must contribute alleles to the next generation, so isolate 61 does not, by itself, provide evidence for migration, but it still might have been the result of an appreciable amount of inoculum spreading from wild-emmer into wheat fields. Isolates 204 and 205 were sampled in 2010 from the *T. durum* cultivar Eylon in different geographical zones not so much in proximity to wild emmer. Their virulence haplotypes were similar to those of wild *B. graminis* f. sp. *tritici* isolates; they were avirulent to a number of *Pm* genes (e.g., *Pm1*, *Pm3*, and *Pm4*). However, isolates 204 and 205 were clearly classified by SSR and SNP data with isolates from domesticated hosts (Figs. 6 and 7), and so, these isolates could represent the successful contribution of avirulence alleles by a wild migrant or migrants to the domestic population, pending on the absence of the relevant *Pm* genes. Two other *T. dicoccoides*-derived isolates (103 and 113) clustered with the *T. aestivum*-derived isolates based on virulence data and could have been derived from migration from cultivated to wild populations. For isolate 103, this pattern was also evident in both molecular assays, while for isolate 113 it was present for SNP data (Fig. 7) but not for SSRs (Fig. 6). Isolate 92, which showed a pattern similar to that of 103, was collected from a wild emmer wheat plant in a nursery at Tel-Aviv University; its habitat was thus not natural and it could be considered an exception.

Two factors likely account for the dominance of one-way migration here: (i) the much larger production area of domesticated wheat in Israel and (ii) the contrast between the genotypically uniform expanses of cultivated bread and durum wheats, in which a virulent clone can build up very large spore populations within a season, and the genotypically diverse populations of *T. dicoccoides*, in which rates of epidemic expansion are more limited, due to the intimate mixtures of resistant (in most cases nonhost) and susceptible plants within natural habitats (Fig. 2). While our results are ambiguous with respect to whether domestic or wild hosts support a larger *B. graminis* f. sp. *tritici* effective population (Table 5), *B. graminis* f. sp. *tritici* on domesticated wheat clearly produces larger spore populations, ensuring a greater likelihood that any given new infection will be caused by a strain originating from domestic hosts.

Given the considerable gene flow detected, the genetic differentiation of cultivated and wild *B. graminis* f. sp. *tritici* populations requires an explanation. Evolutionary forces resulting in this differentiation may include (i) infrequent wild-to-domestic infection, as the population on wild emmer lacks the necessary virulence mutations, (ii) meager wild-to-domestic spread of mildew inoculum, due to the much smaller populations of both host and

TABLE 5. Migration of *Blumeria graminis* f. sp. *tritici* isolates collected in Israel from domestic *Triticum* species (source) to wild emmer wheat (sink) as estimated by two types of molecular markers^y

Host population	Single-nucleotide polymorphisms			Simple-sequence repeats		
	Population size ^z (Θ)	Migration rate (M)	Migrants per generation (m)	Population size ^z (Θ)	Migration rate (M)	Migrants per generation (m)
Domesticated (<i>T. aestivum</i> , <i>T. durum</i>)	0.0020	5,882	...	13.7	0.7	...
Wild (<i>T. dicoccoides</i>)	0.0013	...	5.8	49.1	...	4.8

^y Best-fitting migration model: *T. aestivum* and *T. durum* are a single population and all migration is from the domesticated hosts to *T. dicoccoides* ($P \geq 0.99$). Values are modes of parameter estimates.

^z Mutation-scaled effective population size.

parasite, (iii) possible mating-type incompatibility, limiting sexual reproduction and transmittal of alleles when cross-host infections occur, and (iv) loss of migrant alleles due to population bottlenecks caused by low, sporadic mildew occurrence in the Israeli environment, especially in wild habitats (Fig. 2). It seems likely that, while relatively many domestic *B. graminis* f. sp. *tritici* spores land on *T. dicoccoides* plants, only a small percentage of them survive, due both to a hostile climate and to the suite of resistance genes in wild emmer. These genes may, indeed, be *Pm* genes of the nucleotide binding site-leucine-rich repeat type that constitute the bridge between host and nonhost resistance in closely related host species described by Schulze-Lefert and Panstruga (2011). The small set of domestic survivors may found wild populations subject to strong genetic drift, which could at least partially account for the differentiation of the two populations. Basically, even when successful domestic-to-wild migrants mate and supply genes to the next generation, the migrant alleles remain rare and can be lost in the event of a population bottleneck. Nevertheless, migration continues at a rate thus far sufficient to prevent greater divergence of the two populations, at least within the study area of sympatric wild and domestic *Triticum* spp. It would be interesting to expand the survey area to include the cultivated wheat and wild emmer *B. graminis* f. sp. *tritici* populations in the Turkish, Iraqi, and Iranian regions comprising the remainder of wild emmer's natural range (Özkan et al. 2011), in order to see if the differentiation we detected is also present there.

The two molecular assays produced similar results except with respect to the migration analyses. Based on SNPs, the domestic wheat *B. graminis* f. sp. *tritici* effective population was estimated to be about 50% larger than that derived from *T. dicoccoides*. With SSRs, however, the emmer-derived population was estimated to be nearly four times larger than the population originating on domestic wheat. The smaller effective population sizes and higher migration rate based on SNPs likely reflects the more ancient origins of these polymorphisms, as compared with the relatively recent SSR polymorphisms. Low diversity of SNPs was in line with what was previously found in mildew isolates from the U.S. (Parks et al. 2008). Due to their associations with transposable elements (Buschiazzi and Gemmell 2006), which constitute over 90% of the *B. graminis* f. sp. *tritici* genome (Parlange et al. 2011; Wicker et al. 2013), SSRs likely have a higher rate of evolution than SNPs, and the present isolate sample would thus give rise to a larger inferred effective population size using SSRs than SNPs.

In any case, the estimate of five to six migrants per generation is relatively high, indicating that there is substantial gene flow from *B. graminis* f. sp. *tritici* populations in domesticated wheat fields to those in the wild emmer populations of Israel. For comparison, estimates from other airborne ascomycetous plant pathosystems include 0.5 to 1.7 from SSRs and intergenic spacer sequences on *Sclerotinia sclerotiorum* populations in nine United Kingdom locations (Clarkson et al. 2013) and between 0.76 and 7 for *Dothistroma septosporum* in Estonia, Finland, and the Czech Republic (Drenkhan et al. 2013) (although values for the latter should apparently have been divided by two, the inheritance scalar for a haploid organism). Of course, the *B. graminis* f. sp. *tritici* populations studied here are intermixed on a much smaller and finer geographic scale than in the other examples.

The geographic factor of *B. graminis* f. sp. *tritici* isolates sampled from different regions within Israel was nonsignificant in explaining both molecular and pathotypic variation. This suggests that the *B. graminis* f. sp. *tritici* population of Israel is well-mixed and randomly mating across the study area, which makes sense given the small size of the region sampled.

In conclusion, the *B. graminis* f. sp. *tritici* population of Israel was found to be panmictic on durum and common wheat, but genetically differentiated between isolates originating from domestic and those from wild emmer wheat. Both virulence profiles and neutral genetic markers confirmed a partial separation into these two groups. Gene

flow occurs from the domestic wheat *B. graminis* f. sp. *tritici* population to the wild population and the rate may be sufficient to prevent the two groups from diverging into separate formae speciales.

APPENDIX

The analysis of genetic diversity with SSR markers requires an appropriate assessment of allelic dissimilarity. Given the step-wise mutation model, the first step is measuring differences between any two SSR alleles. Let as_{ij} be allele size of allele i at locus j , and ltr_j be the length of the tandem repeat unit at locus j ; then the difference between alleles i and k at that locus is calculated as $|as_{ij} - as_{kj}| / ltr_j$, which is the difference in the number of tandem repeats in the two alleles. This can be considered an approximation of the number of mutation events (tandem repeat insertions or deletions that are not reversed) that result in the transformation of allele i into allele k . If the maximum number of tandem repeats (mutation events) varies from locus to locus, one can assume that the mutation rate is also variable and locus-specific. Therefore, a particular value of the difference between two SSR alleles should have a greater impact on dissimilarity between isolates at loci where changes happen more slowly. In other words, a larger maximum number of tandem repeats $tr_max_j(as)$ at locus j as compared with the maximum at another locus, given the same interval of evolutionary time, is interpreted as a higher mutation rate at locus j . Thus, we normalize $|as_{ij} - as_{kj}| / ltr_j$ by $tr_max_j(as)$, so that the difference between two SSR alleles i and k at locus j equals

$$\rho_{ikj} = \frac{|as_{ij} - as_{kj}| / ltr_j}{tr_max_j(as)} = \frac{|as_{ij} - as_{kj}| / ltr_j}{[max_j(as) - min_j(as)] / ltr_j} \\ = \frac{|as_{ij} - as_{kj}|}{max_j(as) - min_j(as)}$$

where $max_j(as)$ and $min_j(as)$ are the maximum and minimum allele sizes, respectively, detected at locus j . Now, the dissimilarity between microsatellite patterns of two isolates i and k with profiles $(as_{i1}, as_{i2}, \dots, as_{in})$ and $(as_{k1}, as_{k2}, \dots, as_{kn})$ at n loci is determined as follows

$$\rho_{ik} = \frac{1}{n} \cdot \sum_{j=1}^n \rho_{ikj} = \frac{1}{n} \cdot \sum_{j=1}^n \frac{|as_{ij} - as_{kj}|}{max_j(as) - min_j(as)} \quad (A1)$$

ACKNOWLEDGMENTS

This work was supported by University Research Priority Program Systems Biology/Functional Genomics grants, University of Zurich, an Advanced Investigator Grant from the European Research Council (ERC-2009-AdG 249996, Durable resistance) and by the United States Department of Agriculture-Agricultural Research Service.

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