Structure and Migration in U.S. *Blumeria graminis* f. sp. *tritici* Populations

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Accepted for publication 19 November 2015.

ABSTRACT

Cowger, C., Parks, R., and Kosman, E. 2016. Structure and migration in U.S. *Blumeria graminis* f. sp. *tritici* populations. *Phytopathology* 106:295-304.

While wheat powdery mildew occurs throughout the south-central and eastern United States, epidemics are especially damaging in the Mid-Atlantic states. The structure of the U.S. *Blumeria graminis* f. sp. *tritici* population was assessed based on a sample of 238 single-spored isolates. The isolates were collected from 16 locations in 12 states (18 site-years) as chasmothecial samples in 2003 or 2005, or as conidial samples in 2007 or 2010. DNA was evaluated using nine single nucleotide polymorphism (SNP) markers in four housekeeping genes, and 10 simple sequence repeat (SSR) markers. The SSR markers were variably polymorphic, with allele numbers ranging from 3 to 39 per locus. Genotypic diversity was high (210 haplotypes) and in eight of the site-years, every isolate had a different SSR genotype. SNP haplotypic diversity was lower; although 15 haplotypes were identified, the majority of isolates possessed one of two haplotypes. The chasmothecial samples showed no evidence of linkage disequilibrium ($P = 0.36$),

while the conidial samples did ($P = 0.001$), but the two groups had nearly identical mean levels of genetic diversity, which was moderate. There was a weakly positive relationship between genetic distance and geographic distance ($R^2 = 0.25$, $P = 0.001$), indicating modest isolation by distance. Most locations in the Mid-Atlantic and Great Lakes regions clustered together genetically, while Southeast locations formed a distinct but adjacent cluster; all of these were genetically separated from Southern Plains locations and an intermediate location in Kentucky. One-way migration was detected at a rate of approximately five individuals per generation from populations west of the Appalachian Mountains to those to the east, despite the fact that the Atlantic states experience more frequent and damaging wheat mildew epidemics. Overall, the evidence argues for a large-scale mosaic of overlapping populations that re-establish themselves from local sources, rather than continental-scale extinction and re-establishment, and a low rate of long-distance dispersal roughly from west to east, consistent with prevailing wind directions.

Blumeria graminis (DC.) E. O. Speer f. sp. *tritici* (Em. Marchal) causes powdery mildew of wheat (*Triticum aestivum* L.) and is a major pathogen in the eastern winter wheat region of the United States and in temperate, moist wheat-growing areas worldwide. The U.S. Mid-Atlantic states (Delaware, Maryland, Virginia, North Carolina, and South Carolina) are frequently subject to damaging wheat powdery mildew epidemics, while economically important epidemics occur less frequently in the Southeast, Northeast, and Great Plains states, and recently in the Pacific Northwest. On susceptible varieties, yield losses up to 34% have been reported (Niewoehner and Leath 1998).

B. graminis f. sp. *tritici* is an obligate, biotrophic ascomycete that reproduces both sexually and asexually. On winter cereals, sexual reproduction occurs in late spring prior to host senescence (Götz et al. 1996), when compatible mating types combine to form chasmothecia. Within chasmothecia, the fungus may remain dormant and survive the summer in the absence of a living host. Ascospores may be released from chasmothecia to serve as primary inoculum for epidemic initiation in fall-sown cereals (Götz et al. 1996; Koltin and Kenneth 1970), although chasmothecia often fail to mature and/or release ascospores (authors' personal observation; Turner 1956), possibly due to poor nutrient availability for fungal development (Götz and Boyle 1998), shortness of the epidemic, or weather conditions (Turner 1956). After initial infections in late fall or winter, mycelial growth and sporulation is slow but continuous during the

winter except during frosts (Cherewick 1944; Turner 1956), and the infections become more active during milder, humid weather. Under peak conditions in early spring, multiple cycles of asexual reproduction yield copious quantities of locally adapted, clonal conidia (Wolfe and Schwarzbach 1978).

Recent years have seen significant discoveries in *Blumeria* genomics. *B. graminis* f. sp. *tritici* was recently revealed to have an unusually large genome for a plant-parasitic fungus (~174 Mb), composed over 90% of transposable DNA element (TE) sequences (Parlange et al. 2011; Wicker et al. 2013). Similarly, the closely related barley powdery mildew form *specialis* (*B. graminis* f. sp. *hordei*) has a very large genome (~120 Mb) consisting to an estimated 64% of TEs (Spanu et al. 2010). Massive genome expansion in the mildews to sizes over four times the median of other ascomycetous genomes is proposed to be a consequence of obligate biotrophy (Spanu et al. 2010). The high rate of similarity among repeat copies in *B. graminis* f. sp. *tritici* (Parlange et al. 2011) and comparisons with *B. graminis* f. sp. *hordei* sequences (Oberhaensli et al. 2011) suggest that *B. graminis* f. sp. *tritici* TE proliferation and genome expansion occurred after the two formae *speciales* diverged (Parlange et al. 2011), although the date of that divergence is controversial (Troch et al. 2014).

Historically, surveys of wheat powdery mildew in the United States and Europe have focused on distribution of virulence frequencies (Jevtic et al. 1991; Leath and Murphy 1985; Limpert et al. 1987; Limpert et al. 1999; Namuco et al. 1987; Niewoehner and Leath 1998; Parks et al. 2008; Persaud and Lipps 1995; Svec and Miklovicova 1998). These results are useful for wheat growers and resistance breeding but have limited utility for inferences about population structure, as regional differences in virulence frequencies likely reflect the selective effects of locally grown cultivars and provide little insight into population-structuring forces such as genetic drift or gene flow (Parks et al. 2009). Recently, molecular

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<http://dx.doi.org/10.1094/PHYTO-03-15-0066-R>

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markers amplifying specific *B. graminis* f. sp. *tritici* regions with single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) have been developed to assess population structure of powdery mildew (Parks et al. 2009, 2011). Selectively neutral SSRs and SNPs allow for model-based analysis of population history and structuring forces (Beerli and Palczewski 2010).

Researchers in Europe and the UK have inferred that populations of *B. graminis* are strongly influenced by long-distance dispersal (LDD). Although they are ephemeral (Cherewick 1944), both conidia and ascospores of *B. graminis* f. sp. *tritici* are wind-dispersed and reportedly have potential for LDD, e.g., the ~650 km from the UK to Denmark (Hermansen et al. 1978; Wolfe and Schwarzbach 1978). Similarities in the early-1980s evolution of *B. graminis* f. sp. *hordei* virulence profiles in widely separated European countries led observers to implicate migration, although it was uncertain whether the transport occurred in a single event or by multiple steps (Limbert et al. 1999; Wolfe et al. 1992). A “European mildew path” was proposed, with prevailing west-to-east winds accounting for virulence complexity gradients (Limbert et al. 1999). On the basis of small *B. graminis* f. sp. *tritici* samples and larger *B. graminis* f. sp. *hordei* samples, evaluated with amplified fragment length polymorphisms (AFLPs), Wyand and Brown (2003) concluded that LDD accounted for the lesser differentiation between populations on different continents than between those in more proximal locations within Europe.

In the New World, analysis of eastern U.S. *B. graminis* f. sp. *tritici* isolates with SNP markers provided evidence for recent introduction of the pathogen into the United States, perhaps concurrent with the introduction of wheat by European colonists (Parks et al. 2009). In addition, population subdivision between the southeastern and Mid-Atlantic states was found. A high level of homoplasy between unlinked SNP loci was attributed to the significant role played by sexual recombination (Parks et al. 2009).

In the United States, wheat powdery mildew is primarily managed through fungicide applications and resistance (*Pm*) genes deployed in partially resistant genetic backgrounds (Cowger et al. 2012; Leath

and Bowen 1989). The powdery mildew population in the U.S. Mid-Atlantic region has been highly adaptable, and qualitative resistance has not remained effective for long in wheat cultivars widely grown in this region. For example, cultivars bearing *Pm17* (Griffey et al. 2005a,b) had nearly complete resistance to powdery mildew when released in 2002 (Bowman 2004). However, by the spring of 2005, isolates virulent to *Pm17* were sampled at low frequency in Alabama and Georgia (Parks et al. 2009). By 2009, *Pm17* virulence was present at unusually high levels in Virginia (C. Griffey, *personal communication*) and also in North Carolina (Cowger et al. 2009).

An introduced pathogen with limited genetic diversity due to founder effects should not be highly adaptable, although multiple sequential introductions can mitigate the effects of genetic drift (Mack et al. 2002). One explanation for this pathogen’s adaptability may be the high levels of TE activity, which are thought to provide functional, “tuning knob” variation (King 2011). TEs have been associated with SSR formation (Buschiazzi and Gemmell 2006), which could facilitate evolution, as SSRs are found both within and outside genes, including in introns and regulatory regions.

The current study had two main objectives. First, we sought to evaluate the distribution of presumptively neutral genetic variability across the main areas of the central and eastern United States where *B. graminis* f. sp. *tritici* occurs. Previously, only samples from the Mid-Atlantic and Southeastern United States had been assayed and only with SNP markers (Parks et al. 2009). The current study applied what we expected to be a more informative marker system to those regions, but also for the first time to regions north and west of the Appalachian Mountains, using polymorphic SSR markers and four polymorphic SNP loci. Based on the higher mutation rate of SSRs compared with SNPs, we expected to find a high level of SSR polymorphism, as well as greater haplotypic diversity. We also expected to find population differentiation, particularly between populations east and west of the Appalachian Mountains.

Second, we wanted to assess migration within the U.S. area where wheat powdery mildew epidemics occur. Our hypothesis was that the Appalachian Mountains served as a significant barrier to migration, both due to their physical elevation and also because wheat is not grown in or near them. Historically, wheat powdery mildew epidemics have been most frequent on the east side of the Appalachians, where mildew resistance is an important breeding objective, and less frequent on the west side, where mildew resistance is a lesser priority. We expected to find diminished or nonexistent migration across the mountains.

MATERIALS AND METHODS

Mildew isolate collection. Samples were collected from 16 locations in 12 states in 2003, 2005, 2007, or 2010, with two locations (Kinston, NC and Warsaw, VA) yielding samples in each of 2 years (Fig. 1; Table 1). For the most part, the sampled cultivars were susceptible or moderately susceptible to powdery mildew, although in a few cases cultivars possessed moderate levels of partial resistance (Table 1). Sample collection methods for isolates from Alabama, Delaware, Georgia, North Carolina, and Virginia were previously described (Parks et al. 2008, 2009). Briefly, in 2003 and 2005, samples were collected from senescing wheat plants bearing chasmothecia. Chasmothecial maturation was induced in the lab; ascospores were allowed to infect detached leaves of the wheat cultivar Chancellor, which has no genes effective against U.S. *B. graminis* f. sp. *tritici* isolates; and the resulting infections were subcultured using needles to ensure that only one genotype was present. Spores were washed from infected leaves with a solution of 90% ethanol, pelleted by centrifugation, air-dried, and frozen at –20°C until DNA extraction.

Samples from Kentucky, Nebraska, Oklahoma, and Texas were collected in 2007, and those from New York, Michigan, and Wisconsin were collected in 2010. These samples were obtained as viable conidia on living wheat tissue. Samples of chasmothecia from Kentucky, Oklahoma, and Nebraska had been obtained in

Fig. 1. U.S. locations from which samples of the wheat pathogen *Blumeria graminis* f. sp. *tritici* were derived for population structure study. The circles/ovals indicate geographic regions; collections from 2003 and/or 2005 were Southeast region (1, Headland, AL; 2, Plains, GA; and 3, Griffin, GA) and Mid-Atlantic region (4, Salisbury, NC; 5, Kinston, NC; 6, Chocowinity, NC; 7, Warsaw, VA; 8, Painter, VA; and 9, Felton, DE). Collections in 2007 or 2010: 10, Lexington, KY, Great Lakes region (11, Ithaca, NY; 12, Sandusky, MI; and 13, Arlington, WI) and Southern Plains region (14, Red Cloud, NE; 15, Stillwater, OK; and 16, Chillicothe, TX).

2003 and 2005, but numerous attempts to derive isolates failed to produce viable cultures. Thus, because of the difficulty and unreliability of maturing and hatching chasmothecia in the lab, and the greater ease of acquiring conidial samples from collaborators, all further isolates were created from conidia. These conidia were transferred by shaking infected leaves over detached leaves of mildew-susceptible cultivar Chancellor (CI 12333). Isolates were subcultured and spores were collected as described above.

DNA extraction and marker amplification. For isolates sampled in 2003 and 2005, DNA was extracted from conidia using a modified Qiagen DNeasy blood and tissue kit (Qiagen, Valencia, CA) (Parks et al. 2009). For samples collected after 2005, DNA was obtained using a modified CTAB protocol (Stein et al. 2001) as follows. Five-centimeter leaf sections of 10-day-old plants were placed in 2-ml screw-cap microcentrifuge tubes and frozen at -20°C until DNA extraction. Cells were disrupted by adding eight nickel-plated lead beads to the tube with leaf material, flash-freezing in liquid nitrogen, and vigorously shaking tissue on a vortex lysis adapter (Mo Bio, Carlsbad, CA) for 15 s. If tissue was not fully ground, tubes were refrozen in liquid nitrogen and ground an additional 15 s. When lysis was sufficient, 600 μl of CTAB extraction buffer (1% [wt/vol] cetyltrimethylammonium bromide [CTAB], 0.7 M NaCl, 0.1 M Tris/HCl, pH 7.5, 50 mM EDTA, pH 8, and 1% [vol/vol] β -mercaptoethanol) was added and samples were incubated at 65°C . After 4 h incubation, 600 μl of chloroform/isoamyl alcohol (24:1) was added to the lysis solution and gently mixed for 15 min on an inversion table at the lowest setting. Samples were centrifuged at

10,000 rpm for 8 min, supernatant was transferred to a new microcentrifuge tube, and DNA was precipitated by adding 350 μl of 2-propanol and inverting 10 times. Precipitated DNA was centrifuged for 8 min at 15,000 rpm and the resulting DNA pellet was washed with a solution of 76% ethanol and 200 mM sodium acetate, followed by another wash with 76% ethanol. The clean pellet was air-dried and rehydrated using 100 μl of TE buffer.

DNA sequence data for samples collected in 2003 and 2005 had been previously reported (Parks et al. 2009); unused DNA was maintained at -80°C , and then used for SSR marker amplification following published protocols (Parks et al. 2011). Polymerase chain reaction amplification of 10 SSR and four SNP markers for samples collected post-2005 was performed as previously described (Parks et al. 2009, 2011). SNPs were found at a total of nine variable sites in four genes (two to five sites per gene; Table 2). The SSR markers are species-specific, and reproducibly generate one band per isolate. A handful of isolates with more than one band were assumed to consist of more than one *B. graminis* f. sp. *tritici* genotype, and were not included. DNA sequencing and SSR fragment analysis were carried out by Eton Biosciences, Inc. (Research Triangle Park, NC). Sequences were manually aligned and edited using Sequencher 4.7 (Genecodes Corporation, Ann Arbor, MI).

SSR fragment sizing was performed using PeakScanner version 1.0 (Applied Biosystems, Carlsbad, CA). SSR fragment sizes were binned into discrete alleles following expected periodicity using TANDEM (Matschiner and Salzburger 2009). In the entire data set, three extreme allele values were deleted because the repeat numbers

TABLE 1. Locations, years, plots and cultivars from which samples of *Blumeria graminis* f. sp. *tritici* were collected

City, state	Year sampled ^a	Location number (Fig. 1)	Plots sampled	Cultivars sampled	
				Cultivar name (number of isolates)	Susceptibility to powdery mildew ^b
Griffin, GA	2003	3	2	Coker 9663 (9)	S
Salisbury, NC	2003	4	3	Coker 9663 (27)	S
Kinston, NC	2003	5	3	Coker 9663 (24)	S
	2005		3	Roane (22)	S
Warsaw, VA	2003	7	2	Coker 9663 (11)	S
	2005		2	Unknown susceptible line (7)	S
Headland, AL	2005	1	2	GA951216-2E26 (9)	MR
Plains, GA	2005	2	2	P26R61 (16)	MR
Chocowinity, NC	2005	6	1	Roane (9)	S
Painter, VA	2005	8	1	Roane (5)	S
	2005		1	Unknown susceptible bread wheat (8)	S
Felton, DE	2005	9	1	Roane (10)	S
Lexington, KY	2007	10	1	Becker (7)	S ^c
	2007		1	Jagalene (10)	S
Red Cloud, NE	2007	14	3	Jagalene (6)	S
Stillwater, OK	2007	15	1	JEI 110 (4)	?
	2007		1	2145 (3)	S
	2007		1	Danby (5)	S
	2007		1	OK Bullet (4)	S
	2007		1	OK02522W (2)	?
	2007		1	Overley (3)	S
Chillicothe, TX	2007	16	1	TAMW-101 (5)	S ^d
	2007		1	Jagalene (4)	S
Ithaca, NY	2010	11	1	Brundage 96 (3)	?
	2010		1	Harus (3)	MS ^e
	2010		1	Madsen (1)	MS
	2010		1	Saranac (8)	MR
Sandusky, MI	2010	12	3	Hopewell (7)	MR
Arlington, WI	2010	13	1	Hopewell (2)	MR
	2010		1	Red Ruby (2)	MS
	2010		1	Kaskaskia (1)	S
	2010		1	KW 62 (1)	MS

^a Isolates derived in 2003 and 2005 were from chasmothecia; those from 2007 and 2010 were from conidia.

^b S = susceptible; MS = moderately susceptible; and MR = moderately resistant. Sources: Cornell University, Kansas State University, Michigan State University, North Carolina State University, Ohio State University, Oklahoma State University, University of Georgia, University of Wisconsin, Washington Crop Improvement Association, authors' observations, and as further footnoted. ? = no information available.

^c According to Liu et al. (2001).

^d According to Worrall et al. (1995).

^e According to Teich (1986).

were so distant from others at that locus that they were suspected of resulting from chromosome rearrangement or experimental error.

Genetic diversity analysis. As *B. graminis* f. sp. *tritici* populations are strongly structured by sexual reproduction, haplotypes are often short-lived and recombined by sex, and there are no true races. Analysis of genetic diversity in this study was thus based on comparisons of allelic profiles of isolates, rather than genotype frequencies.

Nei's unbiased diversity was assessed by locus within populations, across loci for each population, and for the overall sample, using GenAlEx version 6.5. Allelic structure of populations based on SSR data was used for migration analysis and assessment of the probability for any specific population that it was in fact effectively random-mating. To evaluate allelic diversity using the SNP data, overall and per-location estimates of nucleotide diversity and Watterson's population mutation rate (Θ_w) were calculated using DnaSP 5.10 (Librado and Rozas 2009).

In order to increase the statistical power of tests, locations were grouped into regions based on geographic proximity, as well as the prior finding of subdivision in the *B. graminis* f. sp. *tritici* population between the Mid-Atlantic and Southeastern United States (Parks et al. 2009). The four initial regions chosen, and the locations comprising them as numbered in Figure 1, were as follows: the Southeast (SOE, 1 to 3), Mid-Atlantic (MID, 4 to 9), Great Lakes (GLK, 11 to 13), and the Southern Plains (SPL, 14 to 16). Ithaca, NY (location 11) was approximately equidistant from the GLK and MID regions; isolates from Ithaca differed significantly in a Fisher's exact test from those in the MID ($P = 0.0002$) but not the GLK ($P = 0.44$), and thus Ithaca was assigned to the GLK region. Isolates from Lexington, KY (Location 10) did not fit into any of the four regions above ($P \leq 0.0001$) and were considered to represent (albeit very partially) a fifth region.

Population differentiation and clustering. A principal coordinates analysis (PCoA) of pairwise genetic distances between populations was conducted using GenAlEx version 6.5 to assess patterns of clustering and differentiation among populations.

Isolation by distance was assessed for the SSR data using the Mantel test (Mantel 1967; Smouse and Long 1992) in GenAlEx version 6.5 (Peakall and Smouse 2006). Matrices were plotted with geographic distance on the X axis and Nei's genetic distance among locations on the Y axis. Potential correlation between the two data matrices was tested using 9,999 permutations of the data set under a

null hypothesis of no correlation between genetic and geographic distance.

Linkage disequilibrium and random-mating. To evaluate potential deviations from random-mating, tests of linkage equilibrium were conducted on site-years (i.e., with Kinston and Warsaw samples included separately by year). SSR data were clone-corrected before multilocus associations were tested. For clone-correction, two isolates were considered to be clones if their SSR and SNP genotypes (and virulence profiles where available) were identical and they were sampled from the same location in the same year. This resulted in the omission for this analysis of a total of nine isolates: two from Lexington, one from Red Cloud, and six from Stillwater. There were three cases in which two isolates shared the same SSR-SNP genotype yet came from different locations, and they were considered not to be clones (Brewer et al. 2012).

Multilocus linkage disequilibrium (LD) was determined using MultiLocus v. 1.3b (Agapow and Burt 2001). The index of association (I_A) and $\bar{r}_d$ were calculated, and their P values were estimated by 1,000 random permutations of the data. The complete, 229-isolate clone-corrected sample was analyzed for LD. Separate LD analyses were also performed on the chasmothecial and conidial fractions of the total sample. Finally, LD was also analyzed separately for (i) the Lexington, Red Cloud, Stillwater, and Chillicothe locations (locations 10, 14, 15, and 16) that clustered separately in the PCoA analysis, and (ii) the remaining populations.

Migration. Directional gene flow (migration) was estimated using the SSR data. In order to assure adequate sample sizes, two hypotheses were tested that each involved separating the sample into two groups. For hypothesis 1, that the Appalachian Mountains form a significant barrier to migration, locations in Figure 1 were divided into groups east (locations 1 to 9) and west (locations 10 to 16) of the Appalachian Mountains. Hypothesis 2 relied upon the results of the PCoA clustering analysis for a somewhat different east-west partition of locations into these two groups: Lexington, Red Cloud, Stillwater, and Chillicothe (locations 10, 14, 15, and 16) versus all other locations. For each hypothesis, four models were tested: unidirectional (asymmetric) migration in each direction; two-way migration; and panmixia.

In both hypotheses, the western sample was two to three times smaller than the eastern sample. To address this problem, a random selection was made from each eastern location of 10 and 5 isolates for the first and second hypotheses, respectively. Isolates with more than one missing value were passed over in randomized order. This

TABLE 2. Distribution of *Blumeria graminis* f. sp. *tritici* single nucleotide polymorphism haplotypes by geographic region of the United States^a

Haplotype	AOX				PKA			PPA			TUB			Number of isolates ^b					
	C	A	G	T	C	T	C	C	T	C	T	C		MID	GLK	SPL	SOE	KY	Total
	—	i	—	i	i	i	—	—	i	i	i	i							
H10	.	.	.	.	.	.	.	.	.	.	.	.		55	12	25	3	11	106
H1	.	.	.	A	.	.	.	.	.	.	.	.		18	12	2	20	3	55
H6	.	.	.	.	.	.	.	.	.	.	C	.		8	1	2			11
H11	.	.	.	.	.	.	.	.	.	.	.	G		7					7
H14	.	.	.	A	.	.	.	.	.	.	C	.		5			4		9
H7	.	.	.	.	T	C	.	.	.	.	.	.		3	3	3		3	12
H15	.	.	.	A	.	.	.	.	.	.	.	G		5					5
H12	.	.	.	A	T	C	.	.	.	.	.	.		1		1			2
H4	.	T	.	.	.	.	.	.	.	.	.	.		1					1
H8	.	.	.	.	T	.	.	.	.	.	C	.		1					1
H9	.	.	.	.	T	.	.	.	.	.	.	G		1					1
H5	.	.	T	.	.	.	.	.	.	.	.	.			1				1
H13	.	.	.	A	T	C	A	C	C	.	.	.					1		1
H3	.	T	.	.	.	.	.	.	.	.	C	.				1			1
H2	T	.	.	.	.	.	.	.	.	.	.	.				1	2		3
Total																			216

^a AOX = alpha oxidase, PKA = protein kinase A, PPA = protein phosphatase type 2A, and TUB = β -tubulin; i = phylogenetically informative and — = uninformative. Consensus nucleotides are given just below gene names.

^b Collections in 2003 and/or 2005: Mid-Atlantic (MID) region (Salisbury, NC; Kinston, NC; Chocowinity, NC; Warsaw, VA; Painter, VA; and Felton, DE) and Southeast (SOE) region (Headland, AL; Plains, GA; and Griffin, GA). Collections in 2007 or 2010: Lexington, KY; Great Lakes (GLK) region (Ithaca, NY; Sandusky, MI; and Arlington, WI) and Southern Plains (SPL) region (Red Cloud, NE; Stillwater, OK; and Chillicothe, TX).

selection brought the east/west ratios of isolates to 81/87 and 53/60 for hypothesis 1 and hypothesis 2, respectively. To test the repeatability of the analysis, migration in both directions was evaluated three times, each with its own sample selected randomly as just described, for each hypothesis.

Migrate-N uses a coalescent approach to estimate theta (Θ), the mutation-scaled effective population size, and M , the mutation-scaled effective immigration rate. For haploids, $\Theta = 2N_e\mu$, where $2N_e$ is the effective population size and μ is the mutation rate per marker per generation. 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 (and division by 2 for a haploid organism) provides a per-generation estimate of the number of immigrants between two populations. Comparisons of models were performed via ratios of marginal likelihoods (Bayes factors), using the Bezier approximation score to calculate model probabilities (Beerli and Palczewski 2010).

Fig. 2. Mean number of simple sequence repeats, averaged across 238 *Blumeria graminis* f. sp. *tritici* isolates, plotted against the total number of simple sequence repeat alleles at each locus.

Migrate-N analyses employed the Bayesian search strategy with 10 independent replications. Starting parameters for analysis were derived from F_{ST} , and all loci were assumed to have a constant mutation rate. To evaluate more genealogies, a static heating scheme with four temperatures of 1,000,000, 3, 1.5, and 1 was used, as recommended in the Migrate-N manual. Runs included a burn-in of 50,000 steps to avoid biasing the results based on the starting parameter. After the burn-in, 10,000 steps were recorded at intervals of 50 steps. Uniform prior distributions of Θ and M were restricted to the ranges of 0 to 70 and 0 to 300, respectively. Short preliminary runs (data not shown) determined appropriate prior boundaries.

RESULTS

In total, 238 *B. graminis* f. sp. *tritici* isolates were evaluated: 71 isolates collected from four locations in 2003, 86 from seven locations in 2005, 53 from four locations in 2007, and 28 from three locations in 2010 (Table 1). The sample sizes evaluated with SSR markers were different from those analyzed with SNPs due to DNA availability and quality, with 238 individuals genotyped for SSRs and 216 for SNPs.

Allelic diversity. Among the 10 SSR loci, allelic diversity differed considerably (Fig. 2). The loci BGT-1 and BGT-8 were highly polymorphic, with 39 and 28 alleles detected, respectively (after deletion of three out-of-range values, as explained above). At the other extreme, only three alleles each were found at BGT-3 and BGT-6, with most locations having only one or two alleles. Across locations and loci, there was a mean of 12.4 alleles per locus (N_a). By location, mean N_a values were positively associated with sample sizes ($P < 0.05$ by least-squares regression); however, relative to their sample sizes, Plains (GA) and Chocowinity (NC) had low mean N_a values (3.0 and 2.2, respectively), and Ithaca (NY) had a high value (4.9). There was a significant correlation between mean number of repeats, when simply averaged across the 238 isolates, and total number of alleles detected in all isolates (Fig. 2).

A moderate degree of genetic diversity was found within populations (Table 3). The overall mean of population diversities, weighted by sample size, was 0.494 (out of a maximum of 1.0).

TABLE 3. Genetic diversity of 238 *Blumeria graminis* f. sp. *tritici* isolates derived from 16 U.S. locations (18 site-years) in 2003, 2005, 2007, and 2010 and genotyped at 10 simple sequence repeat loci

Location ^a	Number of isolates	Number of haplotypes	Number of alleles at locus (N_a) ^b	Diversity within populations ^c
Hea (1)	9	8	2.5 (0.40)	0.415 (0.096)
Pla (2)	16	15	3.0 (0.45)	0.431 (0.079)
Gri (3)	9	8	2.4 (0.31)	0.416 (0.086)
Sal (4)	27	26	5.4 (1.15)	0.528 (0.103)
Kin03 (5)	24	24	5.8 (1.32)	0.539 (0.106)
Kin05 (5)	22	22	5.0 (1.33)	0.524 (0.104)
Cho (6)	9	8	2.2 (0.33)	0.383 (0.099)
War03 (7)	11	10	3.3 (0.60)	0.515 (0.090)
War05 (7)	7	7	2.7 (0.45)	0.492 (0.097)
Pai (8)	13	12	3.5 (0.81)	0.499 (0.109)
Fel (9)	10	10	3.3 (0.60)	0.538 (0.095)
Lex (10)	17	12	3.9 (0.82)	0.536 (0.107)
Ith (11)	15	15	4.9 (0.92)	0.583 (0.103)
San (12)	7	7	2.9 (0.64)	0.512 (0.108)
Arl (13)	6	6	2.6 (0.52)	0.443 (0.122)
Red (14)	6	5	2.5 (0.48)	0.463 (0.134)
Sti (15)	21	12	3.7 (0.75)	0.472 (0.092)
Chi (16)	9	9	3.1 (0.43)	0.594 (0.088)
Mean			3.5 (0.47)	0.494 (0.023)
Total ^d	238	210		

^a Number in parentheses = location number in Figure 1. Ascosporic samples obtained from locations 1 to 9 in 2003 and/or 2005, and conidial samples from locations 10 to 16 in 2007 or 2010.

^b Mean number of alleles across loci; standard error in parentheses.

^c Nei's unbiased genetic diversity by population, followed by the mean across loci and populations; standard errors in parentheses.

^d Total number of isolates and number of different haplotypes in the total 238-isolate sample (not the sum of per-location values).

With regard to the diversity of individual populations, Chocowinity with 0.383 was significantly less diverse than Chillicothe with 0.594, but most populations did not differ significantly from each other in diversity ($P \leq 0.05$).

There was no regional clustering of either low- or high-diversity populations. Mean diversity values for the regions were $SOE = 0.42$, $GLK = 0.51$, $SPL = 0.51$, $MID = 0.50$, and $KEN = 0.54$. Similarly, chasmothecial populations did not have higher genetic diversity than conidial populations; mean values were 0.48 and 0.51, respectively.

With respect to the DNA sequence data, calculated across all four SNP loci, nucleotide per-site diversity varied from 0.00035 in Stillwater (OK) to 0.00111 in Chillicothe (TX), with a mean of 0.00084 (Table 4). As expected, Watterson's joint estimator of mutation rate and population size (Θ_w) corresponded well to SNP nucleotide diversity (Table 4); locations with lower nucleotide diversity had a lower Θ_w . The Θ_w values ranged from 0.408 in Arlington (WI) to 1.663 in Sandusky (MI). Private SNP alleles in the European and Israeli samples studied previously in comparison with the smaller U.S. *B. graminis* f. sp. *tritici* sample (Parks et al. 2009) were also not found in the present, larger U.S. population.

A few locations had low genetic diversity based on both SSRs and SNPs: Arlington, (WI), Headland (AL), and Chocowinity (NC). Warsaw 2003 and Kinston (both years) had high values of both measures. But locations with intermediate SSR-based diversity had either high or low SNP-based diversity, and sample size was not associated with either.

Population diversity and structure. Based on the SSR data, genotypic diversity was extremely high, with 210 haplotypes identified in the total sample (Table 3). No haplotype was shared by more than three isolates (data not shown). In eight of the site-years, every isolate had a unique SSR haplotype (Table 3). Among the 21 isolates (15 after clone-correction) in the Stillwater, OK, population, there were 12 haplotypes.

Overall differentiation among populations and among regions was extremely high ($P < 0.001$ in both cases). Locations clustered by geographic region (Fig. 3). The Mid-Atlantic and Great Lakes regions formed a single, loose cluster; the three Southeast locations also formed a cluster; and Kentucky clustered loosely with the Southern Plains locations. The 2003 and 2005 Kinston populations were tightly clustered with each other, as were the 2003 and 2005 Warsaw populations.

A significant positive association between Nei's genetic distance and geographic distance was found (Mantel test, $R^2 = 0.25$, $P = 0.001$, Figure 4). This provided evidence of relatively weak isolation by distance; i.e., about 50% of the variation in genetic difference among populations could be explained by their physical separation.

Sexual recombination and LD. There was no significant LD in the chasmothecial populations sampled in 2003 and 2005 ($\bar{r}_d = 0.001$, $P = 0.357$), but LD was significant in the 72 isolates sampled as conidia in 2007 and 2010 ($\bar{r}_d = 0.041$, $P = 0.001$, Table 5). In the same vein, the populations from Chillicothe, Lexington, Stillwater, and Red Cloud that clustered separately from other locations (Fig. 3) exhibited significant LD ($\bar{r}_d = 0.058$, $P < 0.001$), while the remaining locations did not ($\bar{r}_d = 0.006$, $P = 0.19$). LD was not significant in the total clone-corrected sample of 229 isolates ($\bar{r}_d = 0.01$, $P = 0.10$).

Migration. The migration analysis discarded models of two-way migration and panmixia for both hypotheses (Table 6). When populations were divided into groups east and west of the Appalachians, the western group had a larger effective population size (Θ). The

Fig. 3. Principal coordinates analysis of genetic clustering based on simple sequence repeat data from 10 loci in 238 isolates of the wheat pathogen *Blumeria graminis* f. sp. *tritici*. Collections in 2003 and/or 2005: Sal = Salisbury, NC; K03 and K05 = Kinston, NC in 2003 and 2005; Cho = Chocowinity, NC; W03 and W05 = Warsaw, VA in 2003 and 2005; Pai = Painter, VA; Fel = Felton, DE; Hea = Headland, AL; Pla = Plains, GA; and Gri = Griffin, GA. Collections in 2007 or 2010: Lex = Lexington, KY; Ith = Ithaca, NY; San = Sandusky, MI; Arl = Arlington, WI; Red = Red Cloud, NE; Sti = Stillwater, OK; and Chi = Chillicothe, TX.

TABLE 4. Allelic diversity and number of haplotypes of 216 *Blumeria graminis* f. sp. *tritici* isolates from 16 U.S. locations, using single nucleotide polymorphism markers

Location ^a	Number of isolates	Nucleotide diversity (per site) ^b	Θ_w ^c	Number of haplotypes
Hea (1)	5	0.00031	0.480	2
Pla (2)	13	0.00075	1.289	3
Gri (3)	12	0.00061	0.662	3
Sal (4)	28	0.00077	0.771	6
Kin (5)	41	0.00089	1.402	9
Cho (6)	8	0.00038	0.771	2
War (7)	13	0.00095	1.289	7
Pai (8)	8	0.00074	0.771	3
Fel (9)	7	0.00066	1.224	3
Lex (10)	17	0.00071	0.887	3
Ith (11)	15	0.00085	1.230	4
San (12)	7	0.00110	1.633	4
Arl (13)	7	0.00044	0.408	2
Red (14)	5	0.00092	1.440	2
Sti (15)	21	0.00035	0.834	4
Chi (16)	9	0.00111	1.472	3
Total	216	0.00084	1.513	15

^a Number in parentheses = location number in Figure 1. Ascospore samples obtained from locations 1 to 9 in 2003 and/or 2005, and conidial samples from locations 10 to 16 in 2007 or 2010.

^b Calculated with Jukes and Cantor correction (estimated using DNAsP).

^c Watterson's population mutation rate per sequence (estimated using DNAsP).

direction of migration was west to east at a rate of approximately five migrants per generation. In the three replicate tests of west-to-east versus east-to-west, where each replicate involved random isolate picks to make the samples closer in size, the west-to-east model was always selected.

When populations were divided for migration analysis on the basis of the clustering results (Fig. 3) into a west-central group (KY, NE, OK, and TX) versus all other locations, the west-central group also had a larger effective population size (Table 6). Again, the direction of migration was from the west-central group toward the other locations, this time at a rate of approximately two migrants per generation. In this case, the west-to-east migration was selected over the east-to-west alternative in two of the three replicate comparisons. For both hypotheses, a *P* value of nearly 1.0 was associated with the best-fitting model.

DISCUSSION

This is the first reported population genetic analysis using neutral molecular markers on a sample of the *B. graminis* f. sp. *tritici* population in wheat powdery mildew-prone areas across the central and eastern United States. In broad strokes, the analysis revealed a population with high genetic and genotypic diversity, discernibly isolated by geographic distance. The most surprising finding was that the significant signal of migration within the overall population was from the region in which wheat powdery mildew is an episodic problem (the Southern Great Plains) to the region in which it occurs more regularly (the eastern United States), instead of the other way around.

Mean estimates of genic diversity in the U.S. *B. graminis* f. sp. *tritici* population were substantially higher using SSR markers than SNPs. This is unsurprising, since the mutation rate of SNPs is generally lower than those of SSRs.

SSR allelic diversity on a per-locus basis was in line with that reported for other plant-pathogenic fungi. For comparison, N_a was two to four, with an average of 2.3, across nine SSR loci in 12 *Zymoseptoria graminicola* isolates (Owen et al. 1998), two to nine per locus (average = 3.8) across 18 loci in 21 *Puccinia triticina* isolates (Szabo 2007), and 1.3 to 4.5 across eight SSR loci in 111 isolates of *B. graminis* f. sp. *hordei* (Tucker et al. 2014).

Given the high level of genomic rearrangement and TE activity previously demonstrated for this pathogen, we were surprised at the wide variation in per-locus SSR polymorphism, and the low levels of polymorphism detected at several loci. It is of course unknown whether any of the polymorphisms appeared recently, i.e., since the presumed introduction of the pathogen to the United States by European colonists, or more anciently.

The low diversity found at certain SSR loci could be accounted for by association with loci that are under selection in *B. graminis* f. sp. *tritici*, such as elicitors. Surveys of virulence frequencies have found that some virulence alleles are essentially fixed in the U.S. *B. graminis* f. sp. *tritici* population (e.g., virulence to *Pm3a*, *Pm3c*, *Pm3f*, *Pm5a*, *Pm6*, and *Pm7* (Niewoehner and Leath 1998; Parks et al. 2008). On the other hand, frequencies of *B. graminis* f. sp. *tritici* virulence to other *Pm* genes (e.g., *Pm2*, *Pm4b*) are intermediate (Niewoehner and Leath 1998; Parks et al. 2008), suggesting a role for

balancing selection in structuring the population. If mutations to virulence at a locus have a deleterious effect on the pathogen and cultivars possessing that resistance are grown only sporadically, selection and drift may have reached an equilibrium, maintaining a low or moderate frequency of these alleles in the population. It is also possible that the locus is evolutionarily young, and greater polymorphism has not yet had time to appear.

Another factor potentially associated with the differences in diversity at SSR loci is repeat number. Mean fragment size per locus was correlated with mean N_a . Longer microsatellite alleles are more mutable than shorter alleles (Dettman and Taylor 2004). That is, long uninterrupted series of repeats have higher mutation rates than do short series, and are therefore more polymorphic (Buschiazzi and Gemmell 2006). Of course, other factors could also account for low allelic diversity, such as position of a locus on a chromosome.

It is interesting that genetic diversity estimates did not appear to vary according to whether samples were derived from conidia collected in the middle of a growing season, while plants are green, or from ascospores derived from senescent leaves collected at the end of the season. This suggests that selective forces during the growing season are not causing major bottlenecks that reduce diversity. Susceptible reservoirs may explain this outcome, as our samples mainly came from susceptible cultivars with no known effective resistance genes. Of course, bottlenecks are rare where resistant cultivars are only occasionally grown.

Even though genetic diversity did not differ between chasmothecial and conidial populations, the populations sampled from conidia exhibited significant linkage disequilibrium, while those originating from chasmothecia did not. LD is not expected when using neutral markers to assay populations that are undergoing regular sexual reproduction. The pooling of previously isolated, genetically

Fig. 4. Plot of Nei's genetic distance versus geographic distance, using simple sequence repeat markers from 238 isolates of the wheat pathogen *Blumeria graminis* f. sp. *tritici* originating from 16 locations in 12 U.S. states in 2003, 2005, 2007, and 2010. Geographic regions: Great Lakes, Kentucky, Mid-Atlantic, Southeast, and Southern Plains.

TABLE 5. Multilocus linkage disequilibrium for collection of *Blumeria graminis* f. sp. *tritici* isolates from 16 locations in the central and eastern United States in 2003 to 2010

Population ^a	Sample size ^b	I_A (<i>P</i> value)	$\bar{r}_d$ (<i>P</i> value)
Chasmothecial populations	157	0.008 (0.357)	0.001 (0.357)
Conidial populations	72	0.338 (0.001)	0.041 (0.001)
Chillicothe, Lexington, Red Cloud, Stillwater	44	0.454 (<0.001)	0.058 (<0.001)
All other locations	185	0.054 (0.192)	0.006 (0.192)
Total sample	229	0.083 (0.074)	0.010 (0.101)

^a Figure 1 and Table 3 provide chasmothecial and conidial populations.

^b Original population of 238 was clone-corrected and nine isolates were omitted.

different populations could have led to a spurious detection of LD in the conidial populations (Wolfe and Knott 1982). This would be consistent with our finding of isolation by distance, although it is noteworthy that LD was not found in the Mid-Atlantic and Southeast samples. The LD observed in the conidial populations may be the result of multiple cycles of asexual reproduction during the growing season, as was found with *Erysiphe necator*, the grape powdery mildew pathogen (Brewer et al. 2012). We also considered as a possibility that *B. graminis* f. sp. *tritici* populations were not maturing their ascospores in Great Plains conditions. However, in that case wheat powdery mildew epidemics would have to be significantly initiated by conidia and mycelium that overwintered in the hot, arid conditions of Oklahoma and Texas, which seems unlikely. Epistatic selection was also suggested as the explanation for LD in *B. graminis* f. sp. *hordei* (Brown and Wolfe 1990). While this cannot be ruled out here, it seems more likely the life stage sampled helps explain why we found LD in the populations derived from conidia.

Haplotypic diversity based on the 10 SSR markers was very high: 210 haplotypes among 238 isolates, with every isolate having a unique haplotype in eight of the site-years. Such high haplotypic diversity emanating from one or a few research plots per location reflects that this pathogen has an enormous effective population size and engages in frequent sexual recombination. This finding is in line with the work of O'Hara and Brown (1997), who found that in samples of 72 to 250 *B. graminis* f. sp. *hordei* isolates from 100-m² barley plots, virulence pathotypes were usually found only once per plot. It is also similar to the report of Tucker et al. (2014) on *B. graminis* f. sp. *hordei* in Australia; they found 97 unique haplotypes in 111 *B. graminis* f. sp. *hordei* isolates analyzed with eight SSR loci. A large amount of *B. graminis* genetic variability can be sampled within a small spatial area, likely due to the establishment of local epidemics by large, airborne and atmospherically mixed populations.

The relatively small number of SSR genotypes at Stillwater (OK) was noteworthy given that those isolates originated from six different plots, a relatively large number in this experiment. The *B. graminis* f. sp. *tritici* population may have undergone a bottleneck at this location, and this is consistent with a report that *Pm3a* was still effective there in 2009 (Chen et al. 2009), while it has not been effective east of the Appalachians since the 1990s (Niewoehner and Leath 1998). Oklahoma wheat powdery mildew populations may be isolated due to genetic drift from a founder effect that is too recent to be detected using SNP markers.

Despite the general correlation of geographic and genetic distance, the Great Lakes and Mid-Atlantic regions formed a

genetically similar cluster, if a loose one. This suggests a history of gene flow between those regions. The Southeastern locations were grouped nearby, slightly separated from the GLK and MID regions. In all these locations, wheat powdery mildew is commonly seen, although it is less often severe outside the Mid-Atlantic states of North Carolina, Virginia, Maryland, and Delaware. The genetic similarity of the GLK and MID locations, despite the samples having been collected as much as seven years apart, indicates that it is appropriate to evaluate such questions as migration using an overall population in which subpopulations were collected in different years.

While Kentucky and the Southern Plains clustered separately, the three Southern Plains locations did not form a tight cluster, with the population from Stillwater, OK, being more remote. In these locations west of the Appalachians Mountains, wheat powdery mildew appears more infrequently, which allows for bottlenecks and genetic isolation.

Within the individual MID locations Kinston (NC) and Warsaw (VA), in the heart of the U.S. region where *B. graminis* f. sp. *tritici* epidemics are most frequently severe, no differences were observed between 2003 and 2005 populations. Within these two locations, populations maintained their genetic makeup over this 2-year period. In the United States, there have not been detailed observations of where wheat powdery mildew survives year-round. Our finding is consistent with annual re-establishment from local sources. The lack of differentiation of Kinston or Warsaw populations over a 2-year period suggests that at least in the more conducive areas, populations survive year-to-year without detectable genetic drift.

This first assessment of migration in the U.S. *B. graminis* f. sp. *tritici* population yielded a surprising result: migration was detected from Great Plains locations and the centrally located state of Kentucky toward the north, south, and east, rather than from the mildew-prone Atlantic states toward the west. Our initial hypothesis that the Appalachian Mountains form a barrier to migration was confirmed for east-to-west migration, but not for west-to-east migration. The migration out of the Great Plains that we detected does fit with the fact that *Pm3a*, a gene widely deployed in the 1980s in the eastern United States, was defeated there by the early 1990s but remains effective in Oklahoma (Chen et al. 2009; C. Cowger, unpublished data). The finding of a larger effective population in the west may reflect the larger wheat area there, and also the greater genetic drift associated with frequent extinction and recolonization in a less conducive physical environment.

Prevailing wind directions in the United States in the months November through May, when *B. graminis* f. sp. *tritici* infections occur, are a likely reason for our migration results. Winds in the

TABLE 6. Evidence of west-to-east migration from a set of 238 *Blumeria graminis* f. sp. *tritici* isolates collected from 16 U.S. locations in 2003, 2005, 2007, and 2010

Hypothesis	Groups (Fig. 1)	Population size (Θ) ^a	Models ^b	Likelihood score (Bezier) ^c	Effective migration rate <i>M</i>	Number of migrants per generation
1: Appalachian Mountains are a significant barrier to migration	West: locations 10 to 16 East: locations 1 to 9	9.5 2.3	West to east	-19,602	4.2	4.8
			East to west	-19,871		
			Two-way migration	-29,569		
2: Migration occurs between west-central locations and all others (based on Fig. 3)	Locations 10, 14, 15, 16 All other locations	6.6 3.0	Panmixia	-21,493	1.2	1.8
			10, 14, 15, 16, →other	-30,447		
			Other, →10, 14, 15, 16	-39,081		
			Two-way migration	-57,716		
			Panmixia	-47,125		

^a Mutation-scaled effective population size.

^b Each hypothesis was tested with three replicate runs, each with independent random subsampling of eastern locations to create more similar east/west sample ratios (see text for details). Likelihood scores for first two models are means of three replicates, while two-way migration and panmixia scores are from one replicate each. *M* and number of migrants per generation are also means of three replicates.

^c Within a hypothesis, larger (less negative) scores indicate better model fits. Calculated from the Bezier score, model probabilities (not shown) provided >99% support for the "west-to-east" and "west-central to all other locations" models compared with their respective alternatives.

Southern and Central Plains are largely from south to north (Climate Atlas of the United States 2015). In Kentucky and the Great Lakes, Mid-Atlantic, and Southeast regions, winds are primarily toward the north and east. Thus, wind direction likely helps account for the fact that migration only from the Plains and Kentucky toward the north and east was detected.

The U.S. *B. graminis* f. sp. *tritici* population is not panmictic, given the clustering and isolation by distance. It appears to consist of a set of discrete, overlapping subpopulations that oversummer locally. Thus, while there is interregional migration and there may be LDD events, annual population establishment in the United States likely occurs primarily from relatively local sources. This contrasts with the annual interregional extinction-recolonization cycles of other obligate biotrophic fungal pathogens. For example, the wheat rusts (*Puccinia triticina* and *Puccinia striiformis*) recolonize in the United States, India, and elsewhere annually via south-to-north wind dispersion, including long-distance spore transport (Brown and Hovmöller 2002; Kolmer 2005; Nagarajan and Singh 1990). Wheat leaf rust overwinters in the southern United States and Mexico, and the urediniospores arrive by wind in the wheat production areas of the Great Plains and the eastern United States (Kolmer 2005). Similarly, the downy mildews, another important group of obligate pathogens, regularly undergo extinction and “green-wave” recolonization. Cucurbit downy mildew (caused by *Pseudoperonospora cubensis*) is annually extinguished in the north-central and eastern United States and recolonized from below the 30th latitude (Holmes et al. 2004). Similarly, tobacco blue mold, the downy mildew caused by *Peronospora tabacina*, does not overwinter in northern growing regions such as Connecticut and Massachusetts, but rather is reintroduced each season from its southern range, following the green wave of tobacco north at a mean rate of 9 to 18 km/day (Aylor 1999, 2003). Our evidence suggests that *B. graminis* f. sp. *tritici* is different. Borrowing the metaphor of Brown and Hovmöller (2002), if the rusts and downy mildews are like migratory birds among biotrophic plant pathogens, U.S. cereal powdery mildew is a less nomadic, more year-round resident.

On the other hand, SSR alleles were not specific to geographic region, as would be expected if the population were strongly differentiated and shaped by drift. A possible explanation for this is that the allelic variation predates a single introduction or a small set of introductions of a genetically well-mixed founding *B. graminis* f. sp. *tritici* population by European colonists in the 17th and 18th centuries. In an alternative scenario, multiple founding populations were genetically distinct, but in the ensuing several hundred years, levels of gene flow among U.S. regions were sufficient to assure broad distribution of SSR allelic variation.

In conclusion, our study revealed a highly genetically diverse U.S. *B. graminis* f. sp. *tritici* population, in keeping with the huge population sizes, mixed reproductive mode, and enormous genomic plasticity of this parasite. Isolation by distance implies that populations are rekindled from relatively local sources, and is consistent with the model of a large, overlapping mosaic of local populations. This is not inconsistent with an important role for LDD. Indeed, while separation of populations east and west of the Appalachians was apparent, migration from the Southern Plains and Kentucky to the north, south and east was detected. These results suggest that novel virulences that arise anywhere along the eastern seaboard are likely to spread in that region and to the Great Lakes, but are not likely to appear in the Southern Plains nor elsewhere west of the Appalachians.

ACKNOWLEDGMENTS

We thank P. Beerli for consultation and especially P. Labadie for a large contribution to the Migrate analyses. We appreciate the technical assistance of M. Hargrove, M. Gromlich, and J. Essic. We also thank the contributors of mildew-infected wheat samples: J. Baker, G. Bergstrom, D. Bland,

B. Brown, K. Burch, C. Griffey, R. Hunger, J. Johnson, K. Lackermann, K. McCarty, B. Mulrooney, D. Van Sanford, M. Staton, and S. Wegulo. We greatly appreciate the input of reviewers, which substantially strengthened the analysis and its presentation.

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