Genetic Differentiation of *Puccinia triticina* Populations in Central Asia and the Caucasus

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ABSTRACT

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Isolates of *Puccinia triticina* collected from common wheat in the Central Asia countries of Kazakhstan, Uzbekistan, Tajikistan, and Kyrgyzstan and the Caucasus countries of Azerbaijan, Georgia, and Armenia were tested for virulence to 20 isolines of Thatcher wheat with different leaf rust resistance genes and molecular genotype at 23 simple sequence repeat (SSR) loci. After clone correction within each country, 99 isolates were analyzed for measures of population diversity, variation at single SSR loci, and for genetic differentiation of virulence phenotypes and SSR genotypes. Isolates from Central Asia and the Caucasus were also compared with 16 *P. triticina* isolates collected from common wheat in North America that were representative of the virulence and molecular variation

in this region and two isolates collected from durum wheat in France and the United States. Populations from the Caucasus, Uzbekistan, Tajikistan, and Kyrgyzstan were not significantly ($P > 0.05$) differentiated for SSR variation with F_{st} and R_{st} statistics. Populations from the Caucasus, Uzbekistan, Tajikistan, and Kyrgyzstan were significantly ($P < 0.05$) differentiated from the populations in South and North Kazakhstan for SSR variation. All populations from Central Asia and the Caucasus were significantly differentiated from the North American isolates and isolates from durum wheat for SSR variation and virulence phenotypes. There was a correlation between virulence phenotype and SSR genotype among individual isolates and at the population level. Mountain barriers may account for the differentiation of *P. triticina* geographic populations in Central Asia and the Caucasus.

Additional keywords: brown rust, *Triticum aestivum,* wheat leaf rust.

Genetic variation in plant pathogen populations is shaped by sexual recombination (16), mutation resulting in new genetic variants (33), migration of genetically distinct individuals between and within crop production regions (35), genetic drift and extinction events (15), and agricultural practices such as the cultivation of crops that have selective effects on their associated pathogens (19). The rusts of wheat (*Triticum aestivum* L) are notorious as highly diverse and mobile pathogens that can afflict large areas of wheat production on a continental scale (21). Leaf rust of wheat, caused by *Puccinia triticina* Eriks., is widespread throughout the wheat-producing regions of the world (39). *P. triticina* is highly diverse for virulence to leaf rust resistance genes in wheat. On an annual basis, more than 50 different virulence phenotypes of *P. triticina* are described in the United States (24). Mutation is a recurrent event and is likely the primary source of variation in *P. triticina* populations. *P. triticina* phenotypes with virulence to newly deployed resistance genes are often detected within a few years of release of resistant wheat cultivars. The primary alternate host of *P. triticina*, *Thalictrum speciosissimum* Loefl. (2,14), is native to Spain and Portugal, where pycnial infections with resultant aeciospores have been observed. Pycnial infections of *P. triticina* on *Thalictrum* spp. have also been observed in north-

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east Kazakhstan (J. A. Kolmer, *unpublished data*) and in Siberia on *Isopyrum fumarioides* (6). However, throughout the rest of the world, *P. triticina* is thought to reproduce by the clonal production of urediniospores, cycling on only wheat, the uredinial-telial host. Parasexual recombination in *P. triticina* has been documented in Australia (34).

Urediniospores of *P. triticina* can be carried by the prevailing winds hundreds or potentially thousands of kilometers from the site of initial infection. This highly effective long distance dispersal can result in the introduction of new *P. triticina* genotypes into wheat-production areas. New genotypes of *P. triticina* with virulence to common wheat have recently been introduced to Australia (35) and North America (20). Isolates of *P. triticina* with virulence to CIMMYT durum wheats (40) that are nearly identical for virulence phenotype (31) and molecular genotype (32), have been found in Spain, France, Mexico, Argentina, and the United States. The recent development of microsatellite locus specific markers for *P. triticina* (7,43) has made it possible to determine multilocus dikaryotic genotypes for population studies of this pathogen. Previously, random amplified polymorphic DNA (RAPDs) and amplified fragment length polymorphism (AFLPs), which are dominant markers, had been used to characterize populations of *P. triticina* in North America (20,23) and Europe (36).

Wheat is an important food staple and export crop in the Central Asia Region of Kazakhstan, Uzbekistan, Tajikistan, and Kyrgyzstan and in the Caucasus Region of Armenia, Azerbaijan, and Georgia (Fig. 1). From 2004 to 2005, Kazakhstan produced 9.95 million metric tons of wheat (USDA-Foreign Agricultural Service Statistics). In the Caucasus Region (1) and southern Kazakhstan, fall-seeded winter wheat is most commonly grown, while in northern Kazakhstan (28), spring wheat is most common. Leaf rust is a common disease on wheat throughout this region, particularly on the spring wheat in northern Kazakhstan. The objectives of this study were to characterize populations of *P. triticina*

from Central Asia, which were collected from common wheat, for virulence and molecular diversity. We wished to test the hypothesis that *P. triticina* migrates on a regular basis from the winter wheat region in the Caucasus, Tajikistan, Uzbekistan, and Kyrgyzstan, into southern and northern Kazakhstan, thus constituting a single large population throughout the region. Furthermore, we wanted to determine if there was a relationship between virulence to the resistance genes *Lr2a* and *Lr2c* and molecular genotype in the *P. triticina* populations in Central Asia and Caucasus, as had been previously found in North America (20,23). We also compared *P. triticina* isolates from Central Asia with representative isolates collected from common wheat in North America and durum wheat, to determine the degree of genetic relatedness between the different sets of isolates.

MATERIALS AND METHODS

Isolate collections and determination of virulence phenotypes. During 2002, collections of *P. triticina* at 11 locations were made from commonly grown winter wheat cultivars in research plots and fields in southern Kazakhstan from Almaty to Shymkent (Fig. 1). During 2004, collections from seven locations in the same area were obtained from southern Kazakhstan. Collections of *P. triticina* were also obtained during 2004 from winter wheat plots and fields in Armenia, Azerbaijan, Georgia, Uzbekistan, Tajikistan, and Kyrgyzstan (Table 1). During 2005, collections from seven locations were obtained from commonly grown spring and winter wheat cultivars in northern Kazakhstan in the region northwest of Pavlodar.

The *P. triticina* collections from Central Asia were increased on seedlings of the susceptible wheats Thatcher and Little Club, and three single-uredinial isolates were increased from each viable collection. The collections and single-uredinial isolates were increased as previously described (24). The single-uredinial isolates (137 from Central Asia) were tested for virulence/avirulence on seedling plants of Thatcher isogenic lines with leaf rust resistance genes, *Lr1* (isogenic line no. RL6003), *Lr2a* (RL6000), *Lr2c* (RL6047), *Lr3* (RL6002), *Lr3ka* (RL6007), *Lr3bg* (RL6042), *Lr9* (RL6010), *Lr10* (RL6004), *Lr11* (RL6053), *Lr14a* (RL6013),

Lr14b (RL6006), *Lr16* (RL6005), *Lr17a* (RL6008), *Lr18* (RL6009), *Lr20* (RL6092), *Lr24* (RL6064), *Lr26* (RL6078), *Lr28* (RL6079), *Lr30* (RL6049), and *LrB* (RL6047). Inoculation, incubation, and greenhouse conditions were as previously described for determining virulence phenotypes of *P. triticina* (24). Seedlings were scored for infection type 10 to 12 days after inoculation. Infection types 0 to 2⁺ were considered as avirulent and infections 3 to 4 were considered as virulent, also as previously described (24). Virulence phenotypes of *P. triticina* isolates from Central Asia were described with the four-letter code used in the nomenclature for leaf rust race identification (25) and with a fifth letter based on virulent/avirulent infection types to lines with genes *Lr3bg*, *Lr14b*, *Lr20*, and *Lr28*.

Sixteen *P. triticina* isolates from the United States and Canada that were collected from spring and winter wheats were also included in the study for comparison with the Central Asia isolates. These isolates all differed for virulence phenotype and were previously shown to be representative of different groups of *P. triticina* isolates in North America on the basis of virulence, RAPD, and AFLP phenotypes (20,23). In addition, two isolates collected from durum wheat in France and the United States were also included. These isolates were representative of *P. triticina*

TABLE 1. Collections and number of *Puccina triticina* isolates from Central Asia used for analysis of microsatellite allele and virulence variation

Year/Country	No. of locations	No. of isolates
2002		
Southern Kazakhstan	11	14
2004		
Armenia	1	2
Azerbaijan	6	10
Georgia	1	1
Uzbekistan	5	7
Tajikistan	3	12
Kyrgyzstan	1	3
Southern Kazakhstan	7	25
2005		
Northern Kazakhstan	7	25

Fig. 1. Political map of Central Asia and the Caucasus.

populations in Mexico, the United States, France, and Spain that were virulent to durum wheat and nearly identical for simple sequence repeat (SSR) genotypes (32). All virulence phenotypes were recorded as binary numbers based on virulence/avirulence to the 20 Thatcher isolines.

Determination of molecular (SSR) genotypes. Twenty to thirty milligrams of urediniospores of each single-uredinial isolate were ground by shaking with 25 mg of glass beads for 20 s in a Savant FastPrep shaker (FP120; Holbrook, New York). DNA was extracted with the OmniPrep extraction kit (GenoTech, St. Louis, MO) according to instructions. DNA was quantified with a NanoDrop (Wilmington, DE) ND-100 spectrophotometer.

Microsatellite primer pairs PtSSR 3, PtSSR 13, PtSSR 50, PtSSR 55, PtSSR 61, PtSSR 76, PtSSR 91, PtSSR 92, PtSSR 151A, PtSSR 152, PtSSR 154, PtSSR 158, PtSSR 161, PtSSR 164, PtSSR 173, PtSSR 186, PtSSR 68-1, and PtSSR 184, (43) were used for characterization of SSR genotypes in the *P. triticina* isolates. In addition, microsatellite primer pairs RB 1, RB 8, RB 11, RB 26, and RB 35 (7) were also used for SSR genotyping for a total of 23 microsatellite loci. Polymerase chain reaction (PCR) conditions for amplification of SSR alleles were as previously described (43). Forward primers were fluorescently labeled (IRD700). Amplification products were separated using a LI-COR (Lincoln, NE) 4200 or 4300 DNA sequencer with a 7% polyacrylamide gel and visualized with SAGA software (LI-COR). Consistent alleles sizes for each primer pair were obtained from DNA extractions of different isolates from common wheat and durum wheat that had been collected from Central Asia and North America. Allele sizes were determined using IRDye 700 size standards (50 to 350 bp and 50 to 700bp; LI-COR). Individual isolates were scored for dikaryotic genotypes for each SSR locus by recording allele sizes in base pairs for both alleles at each locus.

Data analysis. On the basis of geographic proximity and similarity of virulence phenotypes, the isolates from Tajikistan and Kyrgyzstan were pooled for the analysis. Likewise, the isolates from Azerbaijan, Armenia, and Georgia were pooled together and are referred to as the Caucasus population. Isolates from Uzbekistan, north Kazakhstan, south Kazakhstan, North America, and the two isolates from durum wheat were considered as separate populations. The average single-locus statistics for the SSR data: number of alleles, number of effective alleles, number of private alleles, Shannon's I, Nei's observed and expected heterozygosity (30), and F_{is} were calculated with GENALEX 6 (37). Allelic richness was calculated using FSTAT (J. Goudet, Lausanne, Switzerland). The isolates from durum wheat were not included in the single-locus statistics, since there were only two of these isolates. Analysis of molecular variance (AMOVA) using the method of Excoffier et al. (10) was done with GENALEX 6 with 999 permutations of the data set. Measures of population differentiation with F statistics (44), which assume the infinite allele model, and R statistics (42), which assume the stepwise mutation model, were conducted for SSR genotypic variation of isolates within populations, variation between populations within regions, and variation

between different regions. Population pairwise values of F_{st} and R_{st} were calculated with the AMOVA option in GENALEX 6. Nei's genetic distance (D) (29) was also calculated between the populations. An analysis of variation was also conducted for virulence variation using Φ statistics (27), which are analogous to F statistics for binary data. Pairwise values of Φ_{st}, and Nei's D between populations were calculated on the basis of virulence phenotypes. The F_{st}, R_{st}, Φ_{st}, and Nei's D values from the pairwise comparisons of populations were tested for correlation using the Mantel coefficient (26) calculated in GENALEX 6, with 999 permutations of the dataset.

The pairwise values between the populations of F_{st} for microsatellite variation and Φ_{st} for virulence variation were plotted in unrooted trees using PHYLIP 3.6 (11) with the NEIGHBOR clustering option. A neighbor-joining dendrogram of the individual isolates on the basis of microsatellite variation was plotted in NTSYS-pc version 2.1 (Exeter Software, Seatauket NY) using a matrix of number of allele differences between pairs of isolates that was exported from GENALEX 6. Clusters of isolates based on SSR genotypes were also derived on the basis of the model presented in Pritchard et al. (38). The number of SSR genotypes in each population and the Shannon indexes of diversity of SSR genotypes were calculated with GENCLONE (3).

RESULTS

Of the 137 *P. triticina* isolates from Central Asia that were tested for virulence phenotype and SSR genotypes, 99 were included in the analyses after removing clones within each of the five populations. Each population consisted of individual isolates that had unique virulence phenotypes or SSR genotypes. Of the 117 isolates from Central Asia, North America, and from durum wheat that were used in the analysis, there were 88 virulence phenotypes and 91 SSR genotypes (Table 2). There were a total of 71 virulence phenotypes and 75 SSR genotypes among the Central Asian isolates. In the 16 North American isolates, there were 16 virulence phenotypes and 14 SSR genotypes. The two isolates from durum wheat had identical virulence phenotypes on the Thatcher lines and differed for alleles at two loci for SSR genotype. The number of isolates in the five Central Asian populations varied from seven in Uzbekistan to 39 in south Kazakhstan. As a result, the south Kazakhstan and Uzbekistan populations had the highest and lowest number of virulence phenotypes and SSR genotypes, respectively. The south Kazakhstan population had the highest Shannon index of 3.44 and the Uzbekistan population had the lowest diversity of 1.47. All populations had normalized Shannon index values of more than 0.90, except for isolates from Tajikistan + Kyrgyzstan. The North American isolates had a Shannon index of 2.48 and a normalized Shannon index of 0.98 for SSR genotypes. The normalized Shannon index is also reflective of the evenness of genotype frequency distribution in each population (12).

The single-locus statistics averaged over the 23 SSR loci for each population are presented in Table 3. The isolates from north-

TABLE 2. Genotypic diversity for 23 microsatellite loci and virulence variation to 20 Thatcher isolines in populations of *Puccinia triticina* from Central Asia and North America

	Caucasus	North Kazakhstan	South Kazakhstan	Tajikistan + Kyrgyzstan	Uzbekistan	North America	Durum	Total
No. of isolates	13	25	39	15	7	16	2	117
No. of virulence phenotypes	12	21	37	15	7	16	1	88
No. of simple sequence repeat (SSR) genotypes	11	21	34	9	5	14	2	91
Shannon diversity[y] (SSR)	2.35	2.98	3.44	1.89	1.47	2.60	…	4.32
Normalized Shannon diversity[z] (SSR)	0.98	0.97	0.98	0.86	0.91	0.98	…	0.96

[y] Shannon index = $-\Sigma p_i(ln p_i)$ in which p_i = frequency of ith SSR genotype.

[z] Normalized Shannon index = $-\Sigma p_i(ln p_i)/ln N$ in which N = number of SSR genotypes.

ern Kazakhstan had the highest number of alleles per locus at 3.35 and the isolates from Uzbekistan had the lowest number of alleles at 2.48 per locus. There was little variation among the populations in number of effective alleles, ranging from 2.04 effective alleles per locus in the south Kazakhstan isolates to 1.87 alleles in the North American isolates. For allelic richness, the isolates from the Caucasus, Tajikistan + Kyrgyzstan, and Uzbekistan populations were pooled together since isolates in these regions had similar virulence phenotypes and SSR genotypes. The north Kazakhstan isolates had the highest allelic richness at 3.03 per locus and the North American isolates had the lowest at 2.57 per locus. Private SSR alleles were detected in all populations except in the Tajikistan + Kyrgyzstan and Uzbekistan isolates. The south Kazakhstan isolates were the most diverse on a locus basis with a Shannon I of 0.762 and the North American isolates had the lowest I statistic of 0.658. Isolates from all populations had higher observed levels of heterozygosity per locus (H_o) compared with the expected values (H_e). The Tajikistan + Kyrgyzstan isolates deviated the most from the expected heterozygosity with an F_{is} value of –0.563 and the isolates from south Kazakhstan deviated the least with an F_{is} of –0.070.

In the AMOVA, with isolates from the five Central Asia populations, the North American isolates, and the two isolates from durum wheat, the variation for SSR genotypes within populations for F_{st} and R_{st} statistics was 81 and 74% of the total variation, respectively (Table 4). Four regional groups of populations were defined for the AMOVA: the Caucasus region; the Kazakhstan, Tajikistan, Kyrgyzstan, and Uzbekistan region; the North American isolates; and the durum isolates. The SSR variation among populations within regions was 5% of the total variation with F_{sr} compared with 13% with R_{sr}. Among the four regions, F_{rt} accounted for 14% of the total variation compared with 13% for R_{rt}. Using Φ statistics, the variation in virulence to the 20 Thatcher isolines had a similar distribution, with 68% of the total variation within populations, 14% of the total variation among populations within regions, and 18% of the total variation among the regions (Table 4).

Genetic differentiation was also assessed for pairwise comparisons of populations with F_{st} and R_{st} statistics for SSR genotypes and the Φ_{st} statistic for virulence phenotypes. For SSR genotypes, the north Kazakhstan–south Kazakhstan, Caucasus-Tajikistan + Kyrgyzstan, Caucasus-Uzbekistan, and Tajikistan + Kyrgyzstan-Uzbekistan populations were not significantly genetically differentiated ($P > 0.05$) according to F_{st} and R_{st}. All other pairwise comparisons of populations with both measures of genetic differentiation were significant ($P < 0.05$). The Φ_{st} statistics for genetic differentiation based on virulence were very similar to the SSR data. The north Kazakhstan-south Kazakhstan, Caucasus-Uzbekistan, and Tajikistan + Kyrgyzstan-Uzbekistan populations were not significantly genetically differentiated for virulence ($P < 0.05$), while the Caucasus-Tajikistan-Kyrgyzstan populations were borderline ($P = 0.049$) for significance. All other population

TABLE 3. Average of single-locus statistics of *Puccinia triticina* populations from Central Asia and North America at 23 microsatellite loci

	Caucasus	North Kazakhstan	South Kazakhstan	Tajikistan + Kyrgyzstan	Uzbekistan	North America
No. of alleles	2.74 (0.26)[t]	3.35 (0.32)	3.30 (0.34)	2.74 (0.23)	2.48 (0.22)	2.565 (0.22)
No. of effective alleles[u]	1.96 (0.16)	1.93 (0.19)	2.04 (0.18)	1.97 (0.13)	1.95 (0.14)	1.87 (0.12)
Allelic richness[v]	2.813 (0.25)	3.03 (0.28)	3.02 (0.28)	2.813 (0.25)	2.813 (0.25)	2.57 (0.20)
No. of private alleles	0.087 (0.60)	0.174 (0.81)	0.261 (0.13)	0.00 (0.00)	0.00 (0.00)	0.261 (0.14)
I[w]	0.703 (0.084)	0.687 (0.093)	0.762 (0.093)	0.701 (0.078)	0.683 (0.078)	0.658 (0.067)
H_e[x]	0.417 (0.046)	0.381 (0.051)	0.423 (0.048)	0.428 (0.046)	0.424 (0.045)	0.409 (0.041)
H_o[y]	0.582 (0.067)	0.441 (0.068)	0.453 (0.053)	0.669 (0.076)	0.639 (0.073)	0.620 (0.071)
F_{is}[z]	–0.371	–0.157	–0.070	–0.563	–0.507	–0.516

[t] Standard error.

[u] Number of effective alleles = $1/1 – H_e$.

[v] Allelic richness = $\Sigma[1–((N – N_i)/n/(N/n))]$ in which N_i = number of ith SSR genotype among the N sampled individuals within a population, and n = smallest population size.

[w] Shannon $I = –\Sigma p_i(lnp_i)$ in which p_i = frequency of ith allele.

[x] Expected heterozygosity, $H_e = 1 –\Sigma p_i^2$ in which p_i = frequency of ith allele.

[y] Observed heterozygosity, H_o = number of heterozygotes/N.

[z] Fixation index, $F_{is} = H_e – H_o/H_e$.

TABLE 4. Analysis of genetic variance for populations of *Puccinia triticina* from Central Asia and North America for genetic differentiation on the basis of allele frequencies at 23 microsatellite loci and virulence to 20 Thatcher isogenic lines of wheat

Source	df	Statistic	Variance (%)	Value	Probability
Single sequence repeat variation					
Among regions	3	F_{rt}[r]	14	0.136	0.001
Among populations/regions	3	F_{sr}[s]	5	0.059	0.001
Within populations	227	F_{st}[t]	81	0.188	0.001
Among regions	3	R_{rt}[u]	13	0.131	0.001
Among populations/regions	3	R_{sr}[v]	13	0.147	0.001
Within populations	227	R_{st}[w]	74	0.259	0.001
Virulence variation					
Among regions	3	Φ_{pt}[x]	18	0.183	0.001
Among populations/regions	3	Φ_{pr}[y]	14	0.168	0.001
Within populations	110	Φ_{pt}[z]	68	0.320	0.001

[r] $F_{rt} = V_{AR}/(V_{WP} + V_{AP} + V_{AR})$, where V_{AR} = variance among regions, V_{WP} = variance within populations, and V_{AP} = variance among populations within regions.

[s] $F_{sr} = V_{AP}/(V_{WP} + V_{AP})$.

[t] $F_{st} = V_{AP} + V_{AR} / (V_{WP} + V_{AP} + V_{AR})$.

[u] $R_{rt} = V_{AR}/(V_{WP} + V_{AP} + V_{AR})$.

[v] $R_{sr} = V_{AP}/(V_{WP} + V_{AP})$.

[w] $R_{st} = V_{AP} + V_{AR} / (V_{WP} + V_{AP} + V_{AR})$.

[x] $\Phi_{pt} = V_{AR}/(V_{WP} + V_{AP} + V_{AR})$.

[y] $\Phi_{pr} = V_{AP}/(V_{WP} + V_{AP})$.

[z] $\Phi_{pt} = V_{AP} + V_{AR} / (V_{WP} + V_{AP} + V_{AR})$.

pairs were significantly genetically differentiated for virulence ($P < 0.05$).

There were significant correlations ($P < 0.05$) between the F_{st} and R_{st} population pairwise values for SSR variation and Φ_{st} population pairwise values for virulence variation (Table 5). The F_{st} values for SSR variation were also very strongly correlated (>0.90) with Nei's D for pairs of populations for both SSR variation and virulence variation.

The F_{st} population pairwise values for SSR variation (Fig. 2A) and Φ_{st} population pairwise values for virulence variation (Fig. 2B) were plotted as unrooted trees by the neighbor-joining method. The two plots were highly similar, as the Tajikistan + Kyrgyzstan, Uzbekistan, and Caucasus populations were grouped very close for both SSR and virulence variation and were moderately separated from the south and north Kazakhstan populations. The two distinct groups of Central Asia and Caucasus populations were highly distant from the North American population and the two durum isolates for both SSR and virulence variation.

Among the individual isolates there was a clear relationship between virulence phenotype for genes *Lr2a* and *Lr2c* and microsatellite genotypes. In a neighbor-joining dendrogram (Fig. 3; Table 6) of the 117 individual isolates based on microsatellite allele differences, there were seven major groups of isolates. Isolates in groups 1, 2, and 4 are all from Central Asia or the Caucasus and are all avirulent to *Lr2a* and virulent to *Lr2c*. Isolates in groups 3A, 3B, and 3C with one exception are all from Central Asia or the Caucasus and are all virulent to both *Lr2a* and *Lr2c*. Isolates from group 5 are from North America and are avirulent to *Lr2a* and virulent to *Lr2c*. Isolates from group 6 are from durum wheat and are avirulent to *Lr2a* and *Lr2c*, and isolates from group 7 are from North America and virulent to *Lr2a* and *Lr2c*. Isolates A and B were from south Kazakhstan and were virulent to *Lr2a* and *Lr2c*. Isolate C was from North Kazakhstan and was avirulent to *Lr2a* and virulent to *Lr2c*. Isolate D was from North America and was avirulent to *Lr2a* and *Lr2c*.

To determine if there was a significant relationship between virulence phenotype and SSR genotype, the number of SSR allele differences and number of virulence differences for all pairs of isolates were converted to the Dice and simple matching similarity coefficients, respectively in NTSYS 2.1. Two matrices of virulence differences were derived, the first was based on virulence to all 20 resistance genes used in this study and the second was based on virulence only to genes *Lr2a* and *Lr2c*. The virulence matrices were compared for correlation with the SSR genotype matrix with the Mantel coefficient (26) in NTSYS 2.1. There was a significant correlation ($r = 0.54$, $P < 0.001$) between virulence phenotype on the basis of all 20 resistance genes and SSR genotypes. The correlation between SSR genotypes and phenotype based on virulence to genes *Lr2a* and *Lr2c* was also significant ($r = 0.46$, $P < 0.001$).

The isolates were also clustered for SSR genotype using the program *Structure* version 2 (38). A burn-in period of 10,000 and 10,000 reps were used with the admixture model. Under these parameters and model, the optimal k value (number of
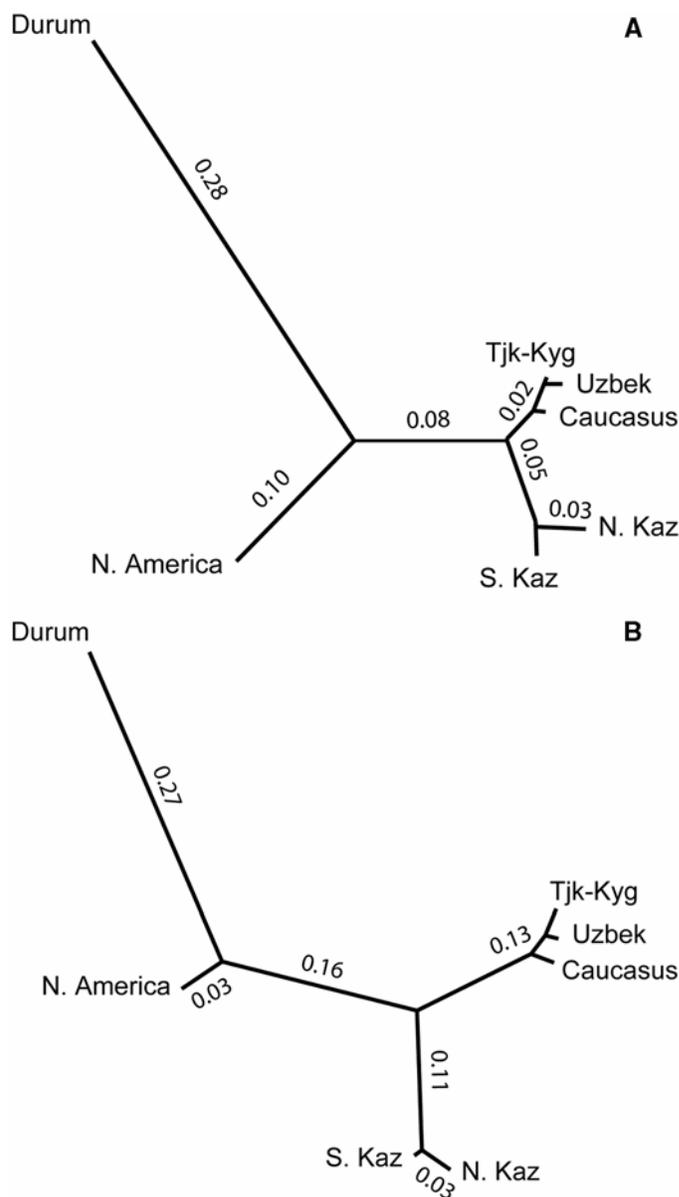

Fig. 2. Unrooted neighbor-joining dendrogram of **A,** F_{st} values between pairs of populations based on variation at SSR loci of *Puccinia triticina* isolates in Central Asia, the Caucasus, North America, and durum wheat: Tjk-Kyg = Tajikistan and Kyrgyzstan; Uzbek = Uzbekistan; S. Kaz = South Kazakhstan;, N. Kaz = North Kazakhstan; and N. America = North America; and **B,** Φ_{st} values between pairs of populations based on virulence variation of *P. triticina* isolates in Central Asia, the Caucasus, North America, and from durum wheat: Tjk-Kyg = Tajikistan and Kyrgyzstan; Uzbek = Uzbekistan; S. Kaz = South Kazakhstan; N. Kaz = North Kazakhstan; and N. America = North America. Values of F_{st} and $\Phi_{st} > 0.01$ are given for the major branches.

TABLE 5. Correlation coefficients[y] between F_{st}, R_{st}, and Φ_{pt} measures of genetic differentiation and Nei's (D) genetic distance based on allelic variation at 23 simple sequence repeat (SSR) microsatellite loci and for virulence to 20 lines of Thatcher wheat isogenic for leaf rust resistance genes for pairs of *Puccinia triticina* populations from Central Asia and North America

SSR variation	F_{st} (SSR)	R_{st} (SSR)	Nei D (SSR)	Φ_{pt} (Virulence)
F_{st}				
R_{st}	0.45 (0.009)[z]			
Nei D	0.94 (0.001)	0.49 (0. 018)		
Virulence variation				
Φ_{pt}	0.82 (0.001)	0.60 (0.004)	0.76 (0.001)	
Nei D	0.93 (0.001)	0.58 (0.003)	0.92 (0.001)	0.89 (0.001)

[y] Mantel correlation coefficient (26).

[z] Probability, correlation is significant if probability is < 0.05.

clusters) was seven with a probability of 0.999. In Table 6, the relationship between the clusters defined by the *Structure* program and the grouping obtained from the neighbor-joining procedure in Figure 3 is shown. Isolates in groups 1 and 2 from Figure 3 were placed in cluster 1 by *Structure*. Isolates in groups 3A, 3B, and 3C in Figure 3 corresponded to clusters 2, 3, and 4, respectively; isolates in groups 4, 5, and 6 in Figure 3 corresponded to clusters 5, 6, and 7, respectively; isolates in

Fig. 3. Unrooted neighbor-joining dendrogram of 117 isolates of *Puccinia triticina* from Central Asia, the Caucasus, North America, and durum wheat. Isolate origins are designated as: Arm = Armenia; Azb = Azerbaijan; Geo = Georgia; Tjk = Tajikistan; Kyg = Kyrgyzstan; Uzb = Uzbekistan; S Kaz = south Kazakhstan; N Kaz = north Kazakhstan; and NA = North America. Virulence of the isolates to genes *Lr2a* and *Lr2c* are designated by the following letters: F, P = avirulent to *Lr2a* and virulent to *Lr2c*; T, S = virulent to *Lr2a* and *Lr2c*; and M, B = avirulent to *Lr2a* and *Lr2c*. The scale is number of allele differences.

group 7 and individual isolates A, B, C, and D in Figure 3, corresponded to cluster 6.

DISCUSSION

The results of this study indicated that two large distinct regional populations of *P. triticina* are present in Central Asia and the Caucasus. Isolates from the Caucasus Region of Azerbaijan, Georgia, and Armenia and also Uzbekistan, Tajikistan, and Kyrgyzstan were highly similar for virulence to the Thatcher isolines and also for SSR genotypes. Isolates from southern and northern Kazakhstan were highly similar for virulence phenotype and SSR genotypes and were genetically differentiated from isolates in the other countries. The two regional populations in Central Asia and the Caucasus were very distinct from the *P. triticina* isolates in North America and were also distinct from the two isolates from durum wheat.

The SSR genotypes showed a significant relationship with virulence to resistance genes *Lr2a* and *Lr2c*. The isolates from Central Asia and the Caucasus with virulence to *Lr2a* and *Lr2c* were closely related for SSR genotype and were clearly separated from other groups of closely related isolates that were avirulent to *Lr2a* and virulent to *Lr2c*. Isolates that were avirulent to *Lr2a* and virulent to *Lr2c* were the predominant virulence phenotypes in the Caucasus, Uzbekistan, and Tajikistan + Kyrgyzstan populations. Isolates with virulence to *Lr2a* and *Lr2c* were the predominant virulence phenotypes in south and north Kazakhstan. Using RAPDs (22,23) and AFLPs (20) as molecular markers, there was also a very strong relationship between virulence to the *Lr2a* and *Lr2c* resistance genes and molecular phenotype in *P. triticina* populations in Canada and in international collections. Resistance genes *Lr2a* and *Lr2c* are alleles at the same locus in wheat (8,18). Dyck and Samborski (9) examined the genetics of virulence in *P. triticina* to the *Lr2* locus. In isolates that were derived from sexual recombination, they found that isolates that were virulent to *Lr2a* were always virulent to *Lr2c* and were homozygous for the recessive allele *p2* that conditioned virulence to both genes; isolates that were avirulent to both alleles were either homozygous or heterozygous for the dominant avirulence allele *P2*; and isolates that were avirulent to *Lr2a* and virulent to *Lr2c* were heterozygous for *P2* and for another dominant independent gene that inhibited the expression of avirulence to *Lr2a*. The groups of *P. triticina* isolates that differ for virulence for *Lr2a* and *Lr2c* may have originated from different introductions of the fungus that are also genetically distinct at a large number of loci throughout the genome. The association between *Lr2a* and *Lr2c* virulence phenotypes and SSR genotypes would be maintained by the asexual production of urediniospores.

One isolate from North America was grouped for SSR variation with the isolates from Central Asia that were virulent or avirulent to both *Lr2a* and *Lr2c*. Isolates with this virulence phenotype are currently found almost exclusively on *Triticum cylindricum* in Kansas and Oklahoma (24) and had very distinct RADP and AFLP phenotypes in previous studies (20,23). These isolates may have some degree of telial host specialization, as also seen with the two *P. triticina* isolates collected from durum wheat used in this study.

The two regional populations of *P. triticina* in Central Asia most likely can be attributed to geographical barriers that have restricted movement of urediniospores between the two regions. The Tien Shan Mountains run continuously along the border between southern Kazakhstan and Kyrgyzstan and partially along the border between Uzbekistan and southern Kazakhstan. The mountain barrier would effectively restrict movement of urediniospores from Tajikistan, Kyrgyzstan, and Uzbekistan into southern Kazakhstan. There are no geographical barriers between the steppe lands of southern and northern Kazakhstan, thus urediniospores can be easily transported in a south to north direction on the southerly winds in spring and summer. This would account for the lack of genetic differentiation between isolates from south and north Kazakhstan.

Some limited migration of *P. triticina* between the two regions must still be occurring since a few isolates with the same virulence phenotypes were found in the two regions. Two isolates with virulence to genes *Lr2c*, *Lr3*, *Lr17a*, *Lr30*, *LrB*, *Lr10*, *Lr14a*, *Lr3bg*, and *Lr14b* that also had identical SSR genotypes were found in Uzbekistan and northern Kazakhstan. Isolates of four virulence phenotypes that were found in the Caucasus Region, Uzbekistan, or Tajikistan were also found in south or north Kazakhstan.

In the Caucasus and Uzbekistan, Tajikistan, Kyrgyzstan, and southern Kazakhstan, fall-planted winter wheat cultivars are the predominant wheat types. Cultivars with resistance genes *Lr3*, *Lr3ka*, *Lr10*, *Lr23*, and *Lr26* are grown in these countries (41). Virulence was common to all of these genes in isolates from the Caucasus and Uzbekistan, Tajikistan, Kyrgyzstan, and south Kazakhstan. In northern Kazakhstan, spring wheats are the predominant type grown. It is very likely that the spring wheat and winter wheat cultivars grown in Central Asia differ for leaf rust resistance genes as is the case in North America, although the *P. triticina* populations in southern and northern Kazakhstan were not significantly differentiated.

The *P. triticina* isolates were highly heterozygous for SSR loci as the observed average heterozygosity values exceeded the expected values in all populations. However, the north and south Kazakhstan populations had much lower F_{is} values compared with the Caucasus, Uzbekistan, and Tajikistan + Kyrgyzstan populations, which is likely directly related to the different *P. triticina* populations in these two regions. Balloux et al. (4) modeled

TABLE 6. Clusters of *Puccinia triticina* microsatellite genotypes of isolates from Central Asia, the Caucasus, and North America as determined by the program *structure version 2* (38) compared with grouping of microsatellite genotypes derived from neighbor joining procedure

Neighbor-joining group[z]	*Structure* cluster	Origin	Virulence
1	1	Central Asia, Caucasus	*Lr2a* – avirulent, *Lr2c* – virulent
2	1	Central Asia, Caucasus	*Lr2a* – avirulent, *Lr2c* – virulent
3A	2	Central Asia, Caucasus	*Lr2a* – virulent, *Lr2c* – virulent
3B	3	Central Asia, Caucasus	*Lr2a* – virulent, *Lr2c* – virulent
3C	4	Central Asia, Caucasus	*Lr2a* – virulent, *Lr2c* – virulent
4	5	Central Asia, Caucasus	*Lr2a* – avirulent, *Lr2c* – virulent
5	6	North America	*Lr2a* – avirulent, *Lr2c* – virulent
6	7	Durum wheat	*Lr2a* – avirulent, *Lr2c* – avirulent
7	6	North America	*Lr2a* – avirulent, *Lr2c* – avirulent
			Lr2a – virulent, *Lr2c* – virulent
Isolate A	6	Central Asia, Caucasus	*Lr2a* – virulent, *Lr2c* – virulent
Isolate B	6	Central Asia, Caucasus	*Lr2a* – virulent, *Lr2c* – virulent
Isolate C	6	Central Asia, Caucasus	*Lr2a* – virulent, *Lr2c* – avirulent
Isolate D	6	North America	*Lr2a* – avirulent, *Lr2c* – avirulent

[z] Neighbor-joining groups in Figure 3.

variable rates of clonal reproduction on population genetic attributes of diploid organisms as measured with neutral markers. Their model predicted that high rates of clonal reproduction would result in high levels of heterozygosity, since in the absence of sexual recombination there will be greater divergence between alleles within loci due to the cumulative effect of recurrent mutation, known as the "Meselson effect." Halkett et al. (13) considered high values of F_{is} to be the best indicator of clonal reproduction since it represents the deviation from random mating within populations. However, the model of Balloux et al. (4) also predicted that expected values of heterozygosity would be found with even very low rates of sexual recombination. Pycnial and aecial infections of *P. triticina* on *Isopyrum fumaroides* in Siberia have been noted (6), as well on *Thalictrum speciossisimum* in northeast Kazakhstan (J. Kolmer, *unpublished data*). There is a possibility that some degree of limited sexual recombination is occurring in the *P. triticina* populations in Kazakhstan since both populations had lower values of F_{is} compared with the other populations. As Balloux et al. (4) and Halkett et al. (13) indicate, separating the effects of limited sexual recombination in organisms that are largely clonal can be difficult. Isolates of *P. triticina* that were collected from durum wheat in different world regions also had higher levels of observed heterozygosity at SSR loci compared with expected values (32). Heterozygosity in *P. tritcina* populations for virulence alleles had been previously noted (17). Populations of *P. graminis* f. sp. *tritici* (wheat stem rust) (5) also had high levels of isozyme and virulence heterozygosity.

Four isolates did not group with any other isolates based on SSR variation. Two of these isolates were from south Kazakhstan and neither of these isolates were unusual for virulence phenotype compared with other isolates from south Kazakhstan. If sexual recombination on *Isopyrum* and *Thalictrum* is occurring to a limited degree in Kazakhstan, it is possible that these isolates resulted from a recombination event that reduced the disequilibria between virulence and SSR variation. A third isolate from north Kazakhstan did not group with any isolates from Central Asia and appeared to be most closely related for SSR variation to isolates from North America. This isolate was the only Central Asia isolate with virulence to *Lr28*, like most isolates found in North America. However, isolates with the same complete combination of virulence as this isolate have not been found in North America. This isolate may have also resulted from a recombination event. The fourth isolate was collected in North America in 1954 and had virulence only to *Lr14a*, *Lr14b*, and *Lr20*. These highly avirulent isolates are only rarely found in the United States.

In this study, two distinct regional populations of *P. triticina* were described in Central Asia and the Caucasus using virulence to isogenic lines and SSR markers. Distinct groups of isolates based on SSR genotypes that were associated with virulence to resistance genes *Lr2a* and *Lr2c* were also described. By using a standardized set of SSR markers and virulence to a standardized set of Thatcher isolines, it should be possible to characterize worldwide populations of *P. triticina* from the major wheat-producing regions to determine genetic relationships between the different populations and determine if newly important virulence phenotypes have arisen as a result of mutation from previous genotypes or by introduction of genetically dissimilar isolates from another region. Gene deployment strategies designed to prolong the effective life of leaf rust resistance genes will be dependent on knowledge of genetic variability and migration patterns of *P. triticina* within and between continental regions.

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