Changes of Races and Virulence Genes in *Puccinia striiformis* f. sp. *tritici*, the Wheat Stripe Rust Pathogen, in the United States from 1968 to 2009

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Abstract

Stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a serious disease of wheat in the world. The obligate biotrophic fungal pathogen changes its virulence rapidly, which can circumvent resistance in wheat cultivars and cause severe epidemics. Because *P. striiformis* f. sp. *tritici* races have been identified in the United States using different wheat genotypes in different time periods, it is difficult to make direct comparisons of the current population with historical populations. The objective of this study was to characterize historical populations with 18 *Yr* single-gene lines that are currently used to differentiate *P. striiformis* f. sp. *tritici* races in order to understand virulence and race changes of the pathogen over 40 years in the United States. From 908 *P. striiformis* f. sp. *tritici* isolates collected from 1968 to 2009 in the United States, 171 races were identified and their frequencies were

determined. More races, more new races, and races with more virulence genes were detected since the year 2000 than prior to 2000. None of the races were virulent to *Yr5* and *Yr15*, indicating that these genes have been effective since the late 1960s. Virulence genes to the remaining 16 *Yr* genes were detected in different periods, and most of them increased in frequency over time. Some virulence genes such as those to *Yr17*, *Yr27*, *Yr32*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2* appeared 14 to 37 years earlier than previously reported, indicating the greater value of using *Yr* single-gene lines as differentials. Positive and negative associations were detected between virulence genes. The continual information on virulence and races in the *P. striiformis* f. sp. *tritici* populations is useful for understanding the evolution of the pathogen and for breeding wheat cultivars with effective resistance to stripe rust.

Puccinia striiformis Westend f. sp. *tritici* Erikss. is an obligate biotrophic basidiomycete fungus. It causes wheat stripe (yellow) rust, one of the most serious diseases in the United States and worldwide (Chen 2005; Line 2002; Wellings 2011). The pathogen was first recognized in the United States in 1915 (Carleton 1915) and has caused severe epidemics in the western United States, particularly in the Pacific Northwest and California (Chen 2005; Line 2002; Shaner and Powelson 1971; Tollenaar and Houston 1967). In the eastern United States, stripe rust was significant in the Great Plains in the late 1950s (Daly et al. 1957; Futrell 1957; Futrell et al. 1959; Pady et al. 1957), increased in frequency in 1980s (Line and Qayoum 1992), and has become more devastating in the regions east of Rocky Mountains since 2000 (Chen 2007; Chen et al. 2002; Wan and Chen 2014).

Control of stripe rust has been largely through developing resistant cultivars (Chen 2005, 2013; Line 2002). However, race-specific resistance can be overcome by new virulence genes or new combinations of previously existing virulence genes that are present in new races of the pathogen. Virulence is race-specific pathogenicity at the level of host cultivars. It is the ability of a *P. striiformis* f. sp. *tritici* isolate to produce a compatible interaction on a wheat line or cultivar with a single or more resistance genes. The opposite is avirulence, the ability of a *P. striiformis* f. sp. *tritici* isolate to produce

an incompatible interaction on a wheat genotype with a resistance gene corresponding to the avirulence gene. The wheat–*P. striiformis* f. sp. *tritici* pathosystem follows the gene-for-gene relationship, in which, for each gene conferring race-specific resistance in the host, the pathogen has a corresponding gene conferring avirulence (Flor 1971). A race or pathotype can be defined by its virulence and avirulence pattern on a set of differential genotypes with different resistance genes.

On wheat, a single urediniospore infection can asexually produce millions of identical urediniospores, which can be disseminated by wind to a long distance. Because a large number of progeny urediniospores can be produced from one infection cycle and even more from multiple cycles of infection in a crop growth season, *P. striiformis* f. sp. *tritici* is highly capable of producing new races through mutation (Hovmøller and Justesen 2007; Steele et al. 2001; Wellings and McIntosh 1990). Basidiospores of *P. striiformis* f. sp. *tritici* can infect barberry (*Berberis* spp.) and Oregon grape (*Mahonia* spp.) under controlled conditions (Jin et al. 2010; Wang and Chen 2013) but natural infection of barberry was reported only in China at very low frequencies (Wang et al. 2015; Zhao et al. 2016). Sexual reproduction of *P. striiformis* f. sp. *tritici* under natural conditions in the U.S. Pacific Northwest has been ruled out due to degradation of teliospores and phenology of alternate hosts (Wang and Chen 2015). Somatic recombination may contribute to the production of new *P. striiformis* f. sp. *tritici* races with new combinations of virulence genes but nothing is known about to what extent this happens in nature in the United States (Lei et al. 2017; Little and Manners 1969; Wright and Lennard 1980). New races can be introduced from region to region, country to country, and continent to continent by wind and human activities (Ali et al. 2014; Brown and Hovmøller 2002; Hovmøller et al. 2002, 2016; Wellings and McIntosh 1990). New races often cause severe epidemics on previously resistant cultivars, and cultivars without race-specific resistance also can be continually damaged by existing races (Chen 2005, 2007, 2013, 2014; Line 2002; Wellings 2011). Moreover, clear identification of nonrace-specific resistance, such as high-temperature adult-plant (HTAP) resistance, relies on clear characterization of *P. striiformis* f. sp. *tritici* races (Chen 2005, 2013). Therefore, identifying races and monitoring virulence changes in the *P. striiformis* f. sp. *tritici* population is essential for effective control of stripe rust.

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Accepted for publication 16 April 2017.

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In the United States, specialization of *P. striiformis* f. sp. *tritici* on wheat cultivars has been recognized since the early 1920s (Bever 1934; Hungerford and Owens 1923) but a relatively stable system for identifying races was not available until 1969, when Line et al. (1970) established the first set of differentials. The set initially consisted of seven wheat cultivars in 1969, and the number was gradually increased to 20 with mixed wheat cultivars and yellow rust resistance (*Yr*) single resistance gene lines by the year 2000 (Chen et al. 2002; Line and Qayoum 1992). By 2009, 140 races (named from PST-1 to PST-140) were identified using the differential system in the United States (Wan and Chen 2012). The set of cultivar differentials has several drawbacks. First, most of the differential cultivars have more than one *Yr* gene, and virulence to one resistance gene can be masked by avirulence to another resistance gene. Second, many of the differentials are wheat cultivars grown in the Pacific Northwest, and races identified by these cultivars might not be useful for other regions. Third, most of the differentials are old wheat cultivars, and the virulence information based on these cultivars may not be very useful for current and future breeding programs and stripe rust management. To solve these problems, Wan and Chen (2014) established a new system using *Yr* single-gene lines as differentials in 2010. This new set of differentials consists of 18 *Yr* single-gene lines (Wan and Chen 2014; Wan et al. 2016). For distinguishing the races identified with the different sets of differentials, the races identified with the old differentials are named using the PST series (Chen et al. 2002) and those identified with the new set are named using a PSTv series (Wan and Chen 2014; Wan et al. 2016). Meanwhile, an octal system was used to describe the virulence and avirulence patterns of the PSTv races, and the system is easy for managing a large number of isolates, identifying different races, and analyzing virulence data. Through testing more than 2,000 isolates from the United States and more than 500 isolates from several other countries since 2010, it has been shown that the virulence and race information obtained using the new system is more useful for understanding virulence variation and race changes (Sharma-Poudyal et al. 2013; Wan and Chen 2014; Wan et al. 2016, 2017) (A. M. Wan and X. M. Chen, unpublished data).

In the United States, *P. striiformis* f. sp. *tritici* races have been studied using the old differential system from 1960 to 2009 (Chen et al. 2002, 2010; Line and Qayoum 1992; Wan and Chen 2012) and using the new system since 2010 (Wan and Chen 2014; Wan et al. 2016). Although PSTv races identified with the new system have been also determined as corresponding PST races, only one or a few isolates of each PSTv race have been tested on the old differentials. Isolates of a single PSTv race can be determined as different PST races, and similarly, isolates of the same PST race can be different PSTv races. Moreover, as the number of differentials were gradually increased over 32 years from 7 in 1968 to 20 in 2000 (Chen et al. 2002; Line et al. 1970), not all isolates were tested with a same set of differentials. The lack of consistent tests is a serious obstacle for understanding the evolution of the *P. striiformis* f. sp. *tritici* population in the United States. Therefore, the objectives of this study were to identify races of the *P. striiformis* f. sp. *tritici* collections from 1968 to 2009 using the *Yr* single-gene differentials and determine race and virulence changes of the pathogen. Together with the races identified in recent years (Wan and Chen 2014; Wan et al. 2016), the results of the present study should provide continual information on virulence genes and races and lead to a better understanding of the virulence evolution of the pathogen. The virulence and race information also should be useful for more effective use of resistance to achieve sustainable control of stripe rust.

Materials and Methods

Recovery and increase of urediniospores of historical *P. striiformis* f. sp. *tritici* isolates. Glass vials and foil bags containing urediniospores of *P. striiformis* f. sp. *tritici* isolates removed from liquid nitrogen were heat shocked in a water bath at 50°C for 2 min. The heat-shocked urediniospores were then used to inoculate two-leaf seedlings of ‘Nugaines’ wheat, which is susceptible to all *P. striiformis* f. sp. *tritici* races identified thus far in the United States, for

recovering the isolates and increasing urediniospores. Inoculated plants were kept in a dew chamber for 24 h at 10°C in the dark, then transferred to a temperature-controlled growth chamber with diurnal temperatures gradually changing from 4°C at 2:00 A.M. to 20°C at 2:00 P.M. with a 16-h photoperiod (Chen et al. 2010). Plants inoculated with different isolates were isolated with plastic booths to prevent cross-contamination (Wan and Chen 2014). Urediniospores produced on Nugaines plants were collected 16 days after inoculation and were kept in a desiccator at 4°C. Spore collections were repeated several times until a sufficient quantity was obtained for differential testing and storage for each isolate. Many isolates were increased twice on Nugaines due to the low viability of urediniospores stored for so long in liquid nitrogen. Fresh urediniospores or those kept dry at 4°C were used within a month for testing on differentials. For long-term storage, urediniospores were dried in a desiccator at 4°C for at least 3 days but not longer than 10 days, then sealed in foil bags and stored in liquid nitrogen. The isolates used in this study were collected from 1968 to 2009 in the United States. If the number of isolates in a year was ≤50, all of the isolates were subject to recovery; if the number was >50, 50 isolates were randomly picked, with consideration for covering different states, races, and originated cultivars if the information was available. If the recovered isolate number of a year was <30 in the first recovery experiment, additional isolates were taken out for recovery. For the 42 years from 1968 to 2009, there were no isolates in the storage for 9 years (1969, 1976,

Table 1. Numbers of isolates, races, and new races since 1968 and mean numbers of virulence genes per race and standard deviations based on all isolates and new races from 1968 to 2009 in the United States^a

Year	All isolates				New races		
	Isolates	Races	Virulence genes		Races	Virulence genes	
			Mean	SD		Mean	SD
1968	26	9	0.8	0.6	9	0.8	0.6
1970	19	8	1.0	1.2	4	2.5	0.7
1971	26	11	1.9	1.6	5	4.3	1.3
1972	14	7	1.7	1.8	2	4.5	1.5
1973	10	8	1.9	1.4	2	3.5	1.0
1974	20	12	2.1	1.6	6	4.3	1.2
1975	37	22	2.6	1.9	12	4.7	0.7
1978	25	15	2.1	1.6	4	3.5	0.7
1981	12	11	2.8	2.1	3	4.7	0.6
1982	11	8	2.7	1.9	2	4.5	0.7
1983	37	20	2.6	1.8	1	4.0	0.0
1984	26	15	2.3	1.8	1	5.0	0.0
1985	48	18	2.6	1.9	2	5.0	0.0
1986	7	5	1.1	1.1	0	NA	NA
1987	39	24	2.8	1.7	2	2.5	1.0
1988	25	16	2.5	1.6	0	NA	NA
1989	15	12	2.7	1.5	0	NA	NA
1991	11	8	3.0	2.0	2	6.0	2.1
1992	22	13	2.4	1.4	1	2.0	0.0
1993	26	14	2.0	1.4	3	2.0	1.0
1997	50	27	3.8	2.0	5	7.3	1.0
1998	41	28	3.9	2.2	11	5.3	2.5
1999	32	18	3.2	1.7	5	4.4	1.3
2000	42	34	6.5	3.0	21	8.1	2.5
2001	19	17	6.3	2.5	8	7.8	2.6
2002	35	25	5.7	3.9	11	7.4	3.1
2003	33	28	7.0	3.1	16	8.4	2.3
2004	31	28	6.7	3.5	8	8.4	2.1
2005	31	28	7.4	3.4	8	7.6	1.9
2006	28	22	7.5	3.0	4	8.3	1.5
2007	30	19	9.0	4.0	5	11.8	3.3
2008	36	19	9.4	2.3	5	10.0	1.8
2009	44	20	10.3	2.1	3	9.5	2.1
1968 to 2009	908	171	4.4	3.5	171	6.8	2.9

^a SD = standard deviation and NA = not applicable.

1977, 1979, 1980, 1990, 1994, 1995, and 1996), and 7 to 50 isolates were recovered for each of the remaining 33 years, with an average of 28 isolates per year. Initially, 1,152 isolates were used to inoculate Nugaines, of which 908 produced urediniospores and were used in this study (Table 1).

Virulence testing on differentials. All 908 recovered isolates were tested using the 18 *Yr* single-gene lines that differentiate *P. striiformis* f. sp. *tritici* races, following the method previously described (Wan and Chen 2014). These 18 *Yr* single-gene lines are near-isogenic or recombinant inbred lines in the background of ‘Avocet S’, except ‘Tyee’, which is a winter wheat possessing a single gene, *YrTyee* (Chen and Line 1992b; Wan and Chen 2014). In the differential test for each isolate, Avocet S and Nugaines were included as susceptible references for ensuring adequate infection. In brief, two-leaf seedlings of the differentials were inoculated with urediniospores mixed with talcum at a 1:20 ratio and incubated in a dew chamber at 10°C in darkness for 20 to 24 h, then grown in a

growth chamber with the 4 to 20°C diurnal temperature and 16-h photoperiod described above. Each set of inoculated differential plants was covered with a plastic booth to prevent contamination. Infection types (IT) were recorded at 18 to 20 days after inoculation based on a 0-to-9 scale (Line and Qayoum 1992) and converted to avirulence or virulence. An isolate was considered avirulent on a specific differential line when there were no symptoms (IT 0) or there were necrotic or chlorotic flecks (IT 1), necrotic or chlorotic blotches without sporulation (IT 2), or necrotic or chlorotic blotches with only a trace to moderate sporulation (IT 3 to 6). An isolate was considered to be virulent if it caused abundant sporulation, with or without necrosis or chlorosis (IT 7, 8, or 9) (Wan and Chen 2014).

Identification of races and determination of race and virulence frequencies. Based on the IT of each isolate on the 18 *Yr* single-gene differentials, the virulence/avirulence pattern of each isolate was determined. An isolate was assigned to a PSTv

Table 2. Octal codes, names, virulence groups and formulae, number of isolates, first and last years detected, and frequencies of 82 races of *Puccinia striiformis* f. sp. *tritici*, each identified from two or more isolates collected in the United States from 1968 to 2009

Race	Code	Virulence formula ^b	Number of		Year detected		Frequency (%) ^a		
			Genes ^c	Isolates	First	Last	High	M1	M2
PSTv-18	000000	–	0	83	1968	2007	42.1	13.5	9.1
PSTv-1	400000	1	1	52	1968	2005	21.4	11.9	5.7
PSTv-19	100000	6	1	77	1968	2007	27.3	13.2	8.5
PSTv-20	001000	17	1	15	1968	2003	11.5	5.9	1.7
PSTv-147	440000	1,7	2	2	1968	1987	3.8	4.2	0.2
PSTv-64	110000	6,9	2	5	1998	2001	5.3	3.9	0.6
PSTv-65	104000	6,10	2	9	1970	2002	9.1	4.7	1.0
PSTv-21	101000	6,17	2	28	1970	2007	11.5	5.0	3.1
PSTv-173	100001	6,Tye	2	6	1987	1999	2.1	2.1	0.7
PSTv-63	040002	7,Exp2	2	37	1968	2005	20.0	7.6	4.1
PSTv-22	021000	8,17	2	6	1999	2004	6.5	4.6	0.7
PSTv-130	000022	44,Exp2	2	8	1974	1998	5.0	3.9	0.9
PSTv-199	420002	1,8,Exp2	3	2	2002	2006	3.6	4.2	0.2
PSTv-70	101001	6,17,Tye	3	14	1973	1999	10.0	5.9	1.5
PSTv-150	440002	1,7,Exp2	3	7	1971	1989	10.0	8.4	0.8
PSTv-62	041002	7,17,Exp2	3	11	1975	2002	9.1	4.9	1.2
PSTv-24	040022	7,44,Exp2	3	28	1972	2005	14.3	6.2	3.1
PSTv-58	040006	7,Tr1,Exp2	3	9	1987	2003	13.3	7.2	1.0
PSTv-23	004500	10,24,32	3	30	1970	2006	9.1	5.4	3.3
PSTv-152	540002	1,6,7,Exp2	4	3	1971	1984	7.1	4.9	0.3
PSTv-193	510020	1,6,9,44	4	3	2000	2001	10.5	7.5	0.3
PSTv-48	510001	1,6,9,Tye	4	4	2003	2003	6.1	3.8	0.4
PSTv-79	450020	1,7,9,44	4	2	2000	2001	5.3	3.8	0.2
PSTv-57	141200	6,7,17,27	4	14	1974	1997	9.1	5.2	1.5
PSTv-153	441020	6,7,17,44	4	2	1971	1975	3.8	3.3	0.2
PSTv-183	140022	6,7,44,Exp2	4	4	1998	1999	2.4	2.4	0.4
PSTv-66	104500	6,10,24,32	4	15	1983	2004	9.1	5.2	1.7
PSTv-54	101204	6,17,27,Tr1	4	10	1975	1998	8.1	4.7	1.1
PSTv-59	101005	6,17,Tr1,Tye	4	4	1982	1993	9.1	7.8	0.4
PSTv-165	100023	6,44,Exp2,Tye	4	2	1975	1978	4.0	4.4	0.2
PSTv-25	041022	7,17,44,Exp2	4	16	1973	2006	10.0	4.7	1.8
PSTv-55	541002	1,6,7,17,Exp2	5	16	1981	2003	9.1	4.7	1.8
PSTv-2	511001	1,6,9,17,Tye	5	3	2002	2009	3.2	2.8	0.3
PSTv-94	160022	6,7,8,44,Exp2	5	3	1999	2000	6.3	3.6	0.3
PSTv-56	141204	6,7,17,27,Tr1	5	13	1978	1999	9.1	5.8	1.4
PSTv-90	141022	6,7,17,44,Exp2	5	6	1985	2000	4.0	3.3	0.7
PSTv-67	105500	6,10,17,24,32	5	12	1998	2003	9.4	4.1	1.3
PSTv-26	104700	6,10,24,27,32	5	12	1981	2008	8.3	5.2	1.3
PSTv-60	041206	7,17,27,Tr1,Exp2	5	5	1984	1998	18.2	6.6	0.6

(continued on next page)

^a High = a single-year highest frequency, M1 = the mean frequency of the years when the race was detected, and M2 = the mean frequency based on 908 isolates from 1968 to 2009.

^b Virulence formulae were based on the 18 *Yr* single-gene differentials: *Yr1*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr43*, *Yr44*, *YrSP*, *YrTr1*, *YrExp2*, and *YrTyee* (Wan and Chen 2014; Wan et al. 2016); – denotes no virulence to the 18 *Yr* genes.

^c Number of virulence genes.

race if it produced the same virulence/avirulence pattern as a previously designated race (Wan and Chen 2014; Wan et al. 2016). If an isolate produced a virulence/avirulence pattern different from previously described races, two or more single-uredinium isolates were obtained and tested on the differentials to clarify or confirm the unique virulence pattern. Once the unique pattern was confirmed, a new race name was designated following the PSTv series and accompanied with an octal code (Wan and Chen 2014; Wan et al. 2016). The frequency of each of the races or each of the virulence genes was calculated for each year, a 5-year period, and 1968 to 2009 to determine the frequency changes.

Determination of virulence diversity and changes of races from 1968 to 2009. Kosman index (Kosman 1996, 2014), Gleason index of richness (Gleason 1922), evenness (Sheldon 1969), genetic distances (Nei 1972) between different periods, and associations among virulence genes were analyzed using the Virulence Analysis Tools program (Kosman et al. 2008; Schachtel et al. 2012). Coefficient of association ϕ between pairs of differentials D_1 and D_2 was

calculated using the formula of Sokal and Rohlf (1995): $\phi(D_1, D_2) = (\alpha\delta - \beta\gamma) / [(\alpha + \beta)(\gamma + \delta)(\alpha + \gamma)(\beta + \delta)]^{1/2}$, where α = the number of isolates with shared virulence to both D_1 and D_2 , β = the number of isolates with virulence to D_1 but avirulence to D_2 , γ = the number of isolates with avirulence to D_1 but virulence to D_2 , and δ = the number of isolates with shared avirulence to both D_1 and D_2 .

For some of these analyses, periods were also used to facilitate comparisons. Each period consisted of five continuous years, even with some years without isolates, except the period 1998 to 1999. The general treatment of 5 years as a period was based on previous reports and observations of race changes and wheat cultivar changes (Chen 2005, 2007; Chen et al. 2010; Line 2002; Line and Qayoum 1992). The two years of 1998 and 1999 was treated as a period to separate these years from 2000 and later years because the year 2000 was a turning point for *P. striiformis* f. sp. *tritici* races and stripe rust epidemics (Chen 2005, 2007; Chen et al. 2002, 2010). The treatment of 5 years as a period did not dilute much of the data because the virulence and race data of individual years were also provided

Table 2. (continued from preceding page)

Race	Code	Virulence formula ^b	Number of		Year detected		Frequency (%) ^a		
			Genes ^c	Isolates	First	Last	High	M1	M2
PSTv-27	005504	10,17,24,32,Tr1	5	8	1982	1999	9.1	3.7	0.9
PSTv-61	004504	10,24,32,Tr1	5	6	1978	2004	9.1	4.4	0.7
PSTv-46	511201	1,6,9,17,27,Tye	6	2	2003	2006	3.6	3.3	0.2
PSTv-121	450031	1,7,9,44,SP,Tye	6	2	2000	2001	5.3	3.8	0.2
PSTv-99	161022	6,7,8,17,44,Exp2	6	2	1998	1998	4.9	4.9	0.2
PSTv-190	160023	6,7,8,44,Exp2,Tye	6	3	2000	2004	4.8	5.0	0.3
PSTv-28	151022	6,7,9,17,44,Exp2	6	7	2000	2008	10.5	5.0	0.8
PSTv-29	141222	6,7,17,27,44,Exp2	6	22	1971	2009	8.3	5.1	2.4
PSTv-4	511211	1,6,9,17,27,SP,Tye	7	17	2000	2009	10.7	5.6	1.9
PSTv-43	470221	1,7,8,9,27,44,Tye	7	2	2001	2009	5.3	3.8	0.2
PSTv-30	170026	6,7,8,9,44,Tr1,Exp2	7	3	2003	2004	6.1	4.6	0.3
PSTv-78	160262	6,7,8,27,43,44,Exp2	7	2	2000	2004	3.2	2.8	0.2
PSTv-5	571220	1,6,7,8,9,17,27,44	8	3	2002	2009	3.6	2.9	0.3
PSTv-71	550263	1,6,7,9,43,44,Exp2,Tye	8	2	2000	2000	4.8	4.8	0.2
PSTv-77	511035	1,6,9,17,44,SP,Tr1,Tye	8	2	2000	2002	2.9	2.6	0.2
PSTv-44	470223	1,7,8,9,27,44,Exp2,Tye	8	2	2005	2005	6.5	6.5	0.2
PSTv-31	171026	6,7,8,9,17,44,Tr1,Exp2	8	5	2004	2009	3.6	3.1	0.6
PSTv-33	170226	6,7,8,9,27,44,Tr1,Exp2	8	5	2005	2008	5.6	3.9	0.6
PSTv-32	170066	6,7,8,9,43,44,Tr1,Exp2	8	9	1998	2009	3.6	3.2	1.0
PSTv-87	101722	6,7,17,24,27,32,44,Exp2	8	4	1997	1998	6.0	4.1	0.4
PSTv-7	531262	1,6,8,9,17,27,43,44,Exp2	9	4	2002	2007	3.3	3.1	0.4
PSTv-52	171262	6,7,8,9,17,27,43,44,Exp2	9	3	2007	2008	5.6	4.4	0.3
PSTv-34	171226	6,7,8,9,17,27,44,Tr1,Exp2	9	2	2002	2008	2.9	2.8	0.2
PSTv-35	171066	6,7,8,9,17,43,44,Tr1,Exp2	9	5	2003	2009	4.5	3.5	0.6
PSTv-36	170266	6,7,8,9,27,43,44,Tr1,Exp2	9	7	2000	2007	5.3	3.8	0.8
PSTv-9	575221	1,6,7,8,9,10,17,27,44,Tye	10	2	2003	2004	3.2	3.1	0.2
PSTv-10	531253	1,6,8,9,17,27,43,SP,Exp2,Tye	10	4	2004	2009	3.2	2.9	0.4
PSTv-37	171266	6,7,8,9,17,27,43,44,Tr1,Exp2	10	33	2002	2009	25.0	11.8	3.6
PSTv-13	571271	1,6,7,8,9,17,27,43,44,SP,Tye	11	3	2000	2009	3.2	2.9	0.3
PSTv-47	571266	1,6,7,8,9,17,27,43,44,Tr1,Exp2	11	2	2005	2006	3.6	3.4	0.2
PSTv-11	571263	1,6,7,8,9,17,27,43,44,Exp2,Tye	11	16	2007	2009	18.2	14.2	1.8
PSTv-12	571067	1,6,7,8,9,17,43,44,Tr1,Exp2,Tye	11	6	2001	2009	5.3	3.5	0.7
PSTv-85	570267	1,6,7,8,9,27,43,44,Tr1,Exp2,Tye	11	2	2003	2005	3.2	3.1	0.2
PSTv-15	551273	1,6,7,9,17,27,43,44,SP,Exp2,Tye	11	2	2004	2005	3.2	3.2	0.2
PSTv-192	550277	1,6,7,9,27,43,44,SP,Tr1,Exp2,Tye	11	2	2000	2008	2.4	2.4	0.2
PSTv-39	175266	6,7,8,9,10,17,27,43,44,Tr1,Exp2	11	2	2008	2009	2.8	2.5	0.2
PSTv-38	171666	6,7,8,9,17,24,27,43,44,Tr1,Exp2	11	2	2001	2007	5.3	4.3	0.2
PSTv-16	574566	1,6,7,8,9,10,24,32,43,44,Tr1,Exp2	12	4	2000	2005	4.8	3.7	0.4
PSTv-17	571273	1,6,7,8,9,17,27,43,44,SP,Exp2,Tye	12	2	2002	2002	5.7	5.7	0.2
PSTv-14	571267	1,6,7,8,9,17,27,43,44,Tr1,Exp2,Tye	12	12	2007	2009	13.6	10.8	1.3
PSTv-40	174766	6,7,8,9,10,24,27,32,43,44,Tr1,Exp2	12	9	2002	2009	7.1	5.5	1.0
PSTv-41	175766	6,7,8,9,10,17,24,27,32,43,44,Tr1,Exp2	13	6	2000	2009	6.7	4.1	0.7
PSTv-51	575777	1,6,7,8,9,10,17,24,27,32,43,44,SP,Tr1,Exp2,Tye	16	3	2007	2009	3.3	2.8	0.3
Total	...	...	...	819	...	...	...	...	90.2

in supplementary files, but it does make it easier to present the results.

Results

From the total of 908 isolates, 171 *P. striiformis* f. sp. *tritici* races were identified (Table 1), of which 89 were detected from one isolate (Supplementary Table S1) and 82 were detected from multiple (2 to 83) isolates (Table 2), with frequencies ranging from 0.1 to 9.7% of the 908 isolates. In all, 93 races were detected in only 1 year while the remaining 78 races were detected in two or more years. In a single year, the highest frequency was obtained for PSTv-18 (42.1% in 1970), followed by PSTv-19 (27.3% in 1982), PSTv-1 (21.4% in 1972), PSTv-37 (20.5% in 2009), and PSTv-63 (20.0% in 1973) (Table 2; Supplementary Table S2). From 1968 to 2009, there were 27 races having overall mean frequencies greater than 1.0% (Table 2). PSTv-18, avirulent to all 18 *Yr* genes, was the most frequent race (9.1%) over the 42 years, followed by PSTv-19 (8.5%), virulent only to *Yr6*, and PSTv-1 (5.7%), virulent only to *Yr1* in the differentials (Table 2). PSTv-18 and PSTv-19 were the top two predominant races from 1968 to 1987, with frequencies of 13.4 to 27.1 and 10.8 to 18.8%, respectively, in each 5-year period (Table 3). PSTv-18 remained among the top five most frequent races in the periods from 1968–72 to 2000–04, PSTv-19 in the periods from 1968–72 to 1998–99, and PSTv-1 in the periods from 1968–72 to 1993–97 (Table 3), indicating that they were long lasting. PSTv-1 was the third most frequent race in 1968–72 (15.3%), 1973–77 (9.0%), and 1983–87 (9.6%); the fourth in 1978–82 (4.2%); and the first in 1988–92 (13.7%). Since the period 2000–04, the top two races were replaced by PSTv-4 and PSTv-37, which were not detected before 2000.

Changes in the number of races and new races. The number of races varied from year to year, ranging from 5 in 1986 to 34 in 2000, with an average of 17 races a year (Table 1). The number of races per year was significantly correlated to the number of isolates ($r = 0.64$, $P < 0.01$). On average, more races were detected in a year from 2000 to 2009 (24 races) than from 1968 to 1999 (14 races). New races were identified in each of the 33 years from 1968 to 2009, except 1986, 1988, and 1989, at an average of five new races per year. More than 10 new races were identified in 1975 ($n = 12$), 1998 ($n = 11$), 2000 ($n = 21$), 2002 ($n = 11$), and 2003 ($n = 16$). The highest number of races ($n = 86$) and the highest number of new races ($n = 64$) were detected in the period 2000–04, followed by the period 2005–09 (59 races and 25 new races), and the third highest number of new races ($n = 20$) was identified in the period 1973–77 (Table 4). More new races were identified during the 10 years of 2000–09 (89 new races) than the 23 years from 1968 to 1999 (82 new races) with viable isolates, indicating that the *P. striiformis* f. sp. *tritici* pathogen has changed more rapidly since 2000.

Race changes in the number of virulence genes. Based on virulence to the 18 *Yr* genes, the number of virulence genes in the 171 races ranged from 0 (PSTv-18) to 16 (PSTv-51) (Table 2). The nine races identified in 1968 each had virulence to only 0, 1, or 2 of the 18 *Yr* genes in the differentials. New races identified after 1968 gradually increased the number of virulence genes. In 2007, race PSTv-51, with virulence to 16 of the 18 *Yr* genes, the highest number of virulence genes, was detected. In general, new races detected in 2000 and after had a higher number of virulence genes (an average of 8.3) than those detected prior to 2000 (an average of 4.5), indicating that the *P. striiformis* f. sp. *tritici* pathogen is able to accumulate a large number of virulence genes in a single isolate.

Virulence genes and their frequencies. Virulence genes of the 171 races are presented in Table 2, the frequencies of the virulence genes in each year are provided in Supplementary Table S3, and the frequencies of the virulence genes in each period are presented in Figure 1. Virulence genes to *Yr1*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr43*, *Yr44*, *YrSP*, *YrTr1*, *YrExp2*, and *YrTye* were identified, whereas no virulence genes were identified to overcome resistance genes *Yr5* and *Yr15*. Virulence genes to *Yr1*, *Yr6*, *Yr7*, *Yr17*, *Yr32*, *Yr44*, and *YrExp2* were first detected in 1968 and those to *Yr10* and *Yr24* were first detected in 1970. The *Yr27*

virulence was first detected in 1971 and virulence genes to *YrSP* and *YrTye* were first detected in 1972. The virulence gene to *Yr43* was first detected in 1974 and the virulence gene to *YrTr1* in 1975. No virulence to *Yr9* was detected until 1997 and to *Yr8* until 1998.

The frequencies of virulence genes varied greatly from year to year. For example, the virulence gene to *Yr1* was detected in all of the years with available isolates except in 1978, with the frequencies ranging from 5.0% in 1998 to 54.8% in 2005, at an average of 24.7%. The frequencies of virulence to *Yr6* ranged from 14.3% in 1972 to 97.7% in 2009, at a yearly mean of 60.8%. Similar to the virulence to *Yr6*, the virulence genes to *Yr7*, *Yr17*, and *YrExp2* had relatively high frequencies whereas those to *Yr10*, *Yr24*, *Yr32*, *YrSP*, and *YrTye* had relatively low frequencies throughout all periods (Fig. 1). Although first detected in 1997 or 1998, the frequencies of virulence genes to *Yr9* and *Yr8* increased rapidly after the year 2000.

Associations between virulence genes. Strong positive associations (≥ 0.50) were detected for the virulence to *Yr1* with that to *YrTye*; *Yr7* with *Yr8*, *Yr44*, and *YrExp2*; *Yr8* with *Yr9*, *Yr43*, and *Yr44*; *Yr9* with *Yr27*, *Yr43*, and *Yr44*; *Yr10* with *Yr24* and *Yr32*; *Yr24* with *Yr32*; *Yr27* with *Yr43*; *Yr43* with *Yr44* and *YrTr1*; and *Yr44* with *YrExp2* (Table 5). Although relatively low in values, negative associations were detected for the virulence gene to *Yr1* with those to *Yr10*, *Yr24*, *Yr32*, and *YrTr1*; *Yr7* with *Yr10* and *Yr32*; *Yr9* with *Yr24* and *Yr32*; *Yr10* with *Yr17*, *Yr44*, *YrSP*, *YrExp2*, and *YrTye*; *Yr17* with *Yr24* and *Yr32*; *Yr24* with *Yr44*, *YrSP*, *YrExp2*, and *YrTye*; *Yr32* with *Yr44*, *YrSP*, *YrTr1*, and *YrTye*; *YrSP* with *YrTr1* and *YrExp2*; and *YrTr1* with *YrTye*. All of the remaining possible associations were positive but below 0.50, excluding those of *Yr5* and *Yr15* because their virulence genes were not detected.

Population diversity and differentiation. When the isolates of each 5-year period were treated as a population, the diversity of each population was determined based on the virulence data. The 1968–72 population had the lowest Kosman index (0.15), Gleason index of richness (4.28), and evenness, whereas the 2000–04 population had the highest Kosman index (0.56), Gleason index of richness (17.76), and evenness (0.95) (Table 4). The second highest Kosman diversity index (0.47) and Gleason index of richness (11.70) were detected in the 2005–09 population but the second highest evenness (0.93) was detected for the 1993–97 and 1998–99 populations. Low diversity and richness were detected in the populations before 2000 and high diversities and richness were found in the populations after 2000.

Populations that were more temporally close were more closely related. For example, the populations of 1983–87 and 1988–92 had the lowest genetic distance (0.001), whereas the populations of 1968–72 and 2005–09 had the highest genetic distance (0.352) (Table 6). Low genetic distances (<0.010) were detected for all pairwise comparisons of population next to each other, except those of 1998–99 versus 2000–04 (0.072) and 2000–04 versus 2005–09 (0.040). Populations of 1973–77, 1978–82, and 1983–87 had very low genetic distances (0.001 to 0.004), indicating little change during these periods. High distances (>0.07) were detected between 2000–04 or 2005–09 populations and all other populations, except between the 2000–04 and 2005–09 populations, again indicating that significant changes occurred after the year 2000.

From 1968 to 2009, the *P. striiformis* f. sp. *tritici* populations changed from simple races with low numbers of virulence genes to complex races with high numbers of virulence genes. For example, the mean number of virulence genes of the population of 1968 was just 0.8, whereas that of the 2009 population was more than 10.0 (Table 1). The complexity of the *P. striiformis* f. sp. *tritici* population increased over the years but more rapid increases occurred during the 10 years from 2000 to 2009 (Fig. 2). Over the nine periods, the mean numbers of virulence genes in new races were significantly higher than those of the populations ($P = 0.05$). The differences were larger from 1973–77 to 1993–97 but became smaller after 1998–99; the mean number of new races and the mean number of virulence genes of new races were almost identical in the period 2005–09. These results indicated that new races were produced with more virulence

genes, and new races quickly became predominant races after the late 1990s.

Discussion

The stripe rust pathogen is capable of changing rapidly to produce new races and adapting to wheat cultivars with different race-specific resistance genes, and races can be selected by cultivars with race-specific resistance for further changes in frequency and distribution. In the present study, we focused on characterizing *P. striiformis* f. sp. *tritici* isolates collected from 1968 to 2009 using the new set of *Yr* single-gene differentials, which was recently established by Wan and Chen (2014). The data of the present study allow us to compare *P. striiformis* f. sp. *tritici* populations in the United States and find changes in races over the past 40 years, as discussed below. Our results from the present study provide continual information on virulence genes and races in the United States. The information on frequencies of races and virulence genes should be useful for understanding the evolution of the *P. striiformis* f. sp. *tritici* population over the last half-century in the United States and for developing resistant cultivars for control of stripe rust.

The total number of 171 races identified in the present study is more than the total number of 140 races identified using the previous set of 7 to 20 cultivar differentials from the 1960s to 2009 (Chen 2005; Chen et al. 2002, 2010; Line and Chen 1996; Line and Qayoum 1992; Wan and Chen 2012) (A. Wan and X. M. Chen, unpublished data). The larger number of races identified from sampled isolates with the new set of differentials indicate that the *Yr* single-gene lines are more powerful in differentiating *P. striiformis* f. sp. *tritici* races. The earliest studies reported two races in 1932 in Canada (Newton et al. 1933) and in 1933 in the United States (Bever 1934). In Bever's study, the race from Montana was virulent on 'Chinese 166' (*Yr1*) and 'Red Russian' wheat and avirulent on 'Heines Kolben' (*Yr6* and *YrHK*), whereas the race from Idaho was virulent on Heines Kolben and avirulent on Chinese 166 and Red Russian. Later, Newton and Johnson (1936) found that their two races were similar to the two races reported by Bever (1934). These studies showed that the *P. striiformis* f. sp. *tritici* races in the 1930s could be differentiated by *Yr1* and *Yr6*, and virulence genes to these two resistance genes lasted into the late 1960s, as was found in the present study.

In the United States, there was no *P. striiformis* f. sp. *tritici* race study from the middle 1930s until the 1960s, when a set of five wheat cultivars was selected in 1968 and an additional two cultivars added in 1969 (Line et al. 1970). In the present study, more races were identified in the early years using the current set of differentials than in previous reports. Five races were previously identified up to 1968 (Line and Qayoum 1992). In contrast, nine races were identified from

the 1968 isolates alone in our study. From 1968 to 1987, in total, 39 races were identified using 13 wheat cultivar differentials (Line and Qayoum 1992). In the present study, we identified 55 races during this period using the 18 *Yr* single-gene differentials. In the previous studies, 81 races were identified from 2000 to 2009 using a set of 20 wheat cultivars, including *Yr* single-gene lines *Yr5*, *Yr8*, *Yr9*, and *YrTye* (Chen et al. 2002, 2010; Wan and Chen 2012), which were also included in the present study. In the present study, we identified 89 races from the isolates collected during this period using the 18 *Yr* single-gene differentials. The different numbers of races were due to the different differentials.

The races identified in the present study provide information on virulence directly related to individual resistance genes and reported virulence genes that were previously unable to be detected using the cultivar differentials. In addition to virulence genes to *Yr1* and *Yr6*, as discussed above, virulence genes to *Yr7*, *Yr17*, *Yr32*, *Yr44*, and *YrExp2* were detected in 1968, although their frequencies were much lower than those of *Yr1* and *Yr6*. In the present study, virulence genes to *Yr10* and *Yr24* were first detected in 1970, a virulence gene to *Yr27* in 1971, virulence genes to *YrSP* and *YrTye* in 1972, a virulence gene to *Yr43* in 1974, and a virulence gene to *YrTr1* in 1975, whereas virulence genes to *Yr8* and *Yr9* were not detected until 1998 and 1997. There were no previous reports on the existence of the virulence

Table 4. Numbers of isolates, detected races, and new races of *Puccinia striiformis* f. sp. *tritici* and diversity parameters in each 5-year period from 1968 to 2009 in the United States

Period ^b	Number of			Diversity parameter ^a		
	Isolates	Races	New races	Kosman	Gleason	Evenness
1968–72	85	19	19	0.15	4.28	0.79
1973–77	67	31	20	0.26	7.14	0.88
1978–82	48	23	10	0.27	5.68	0.89
1983–87	157	31	6	0.29	5.93	0.88
1988–92	73	28	3	0.29	6.29	0.92
1993–97	76	32	8	0.33	7.39	0.93
1998–99	73	36	16	0.34	8.16	0.93
2000–04	160	86	64	0.56	17.76	0.95
2005–09	169	59	25	0.47	11.70	0.86
1968–2009	908	NA ^c	171	0.46	26.87	0.89

^a Kosman index, Gleason index of richness, and Evenness were calculated based on Kosman (1996), Gleason (1922), and Sheldon (1969), respectively.

^b Every 5 years was a period, except 1998 to 1999, because the year 2000 was a year when a large number of new races were identified (Chen et al. 2002).

^c Not applicable.

Table 3. Predominant races of *Puccinia striiformis* f. sp. *tritici* and their frequency (%) in each period from 1968 to 2009 in the United States

Period ^a	Predominant races and their frequency (%)				
	1	2	3	4	5
1968–72	PSTv-18 (27.1)	PSTv-19 (16.5)	PSTv-1 (15.3)	PSTv-20 (8.2)	PSTv-63 (5.9)
1973–77	PSTv-18 (16.4)	PSTv-19 (13.4)	PSTv-1 (9.0)	PSTv-63 (7.5)	PSTv-54, PSTv-57, PSTv-150 (4.5)
1978–82	PSTv-19 (18.8)	PSTv-18 (14.6)	PSTv-56, PSTv-63 (8.3)	PSTv-1, PSTv-20, PSTv-23, PSTv-24, PSTv-55 (4.2)	PSTv-21, PSTv-25, PSTv-26, PSTv-27, PSTv-29, PSTv-57, PSTv-61, PSTv-62, PSTv-165, PSTv-169, PSTv-170, PSTv-171, PSTv-172 (2.1)
1983–87	PSTv-18 (13.4)	PSTv-19 (10.8)	PSTv-1 (9.6)	PSTv-63 (8.9)	PSTv-23 (6.4)
1988–92	PSTv-1 (13.7)	PSTv-19 (12.3)	PSTv-18, PSTv-21, PSTv-23, PSTv-66 (5.5)	PSTv-130, PSTv-24, PSTv-58, PSTv-62, PSTv-70 (4.1)	PSTv-25, PSTv-56, PSTv-57, PSTv-60, PSTv-61, PSTv-63 (2.7)
1993–97	PSTv-18 (9.2)	PSTv-19, PSTv-21, PSTv-55, PSTv-70 (6.6)	PSTv-23, PSTv-24, PSTv-58, PSTv-66 (5.3)	PSTv-1, PSTv-63, PSTv-87 (3.9)	PSTv-26, PSTv-59, PSTv-65, PSTv-90 (2.6)
1998–99	PSTv-19 (13.7)	PSTv-67 (8.2)	PSTv-56 (5.5)	PSTv-24, PSTv-29, PSTv-63, PSTv-64, PSTv-65, PSTv-174 (4.1)	PSTv-18, PSTv-22, PSTv-23, PSTv-55, PSTv-70, PSTv-94, PSTv-99, PSTv-171 (2.7)
2000–04	PSTv-4 (5.0)	PSTv-37 (4.4)	PSTv-18 (3.8)	PSTv-29, PSTv-36 (3.1)	PSTv-22, PSTv-25, PSTv-28, PSTv-32, PSTv-48, PSTv-62 (2.5)
2005–09	PSTv-37 (15.4)	PSTv-11 (9.5)	PSTv-14 (7.1)	PSTv-4 (5.3)	PSTv-40 (4.1)

^a Every 5 years was a period, except 1998 to 1999, because the year 2000 was a year when a large number of new races were identified (Chen et al. 2002).

genes to *Yr24*, *Yr32*, *Yr43*, *Yr44*, and *YrSP* because wheat genotypes with these genes were not used as differentials prior to 2010. The first year of virulence to *Yr10*, 1968, detected in the present study, was the same as reported by Line and Qayoum (1992). However, the detection of virulence to *YrTy*e in 1972 was 10 years earlier than the previous report of 1983, possibly because *YrTy*e (*YrTy*e) was not added to the set of differentials until 1983 (Line and Qayoum 1992). Our result also showed that virulence to *YrTy*e had existed for several years before *YrTy*e was released in 1979 (Allan et al. 1980). Only one gene, *YrTy*e, has been identified in differential *YrTy*e, and the gene has been recently mapped to the short arm of chromosome 3A and permanently named as *Yr76* (Xiang et al. 2016). The result indicates the necessity of monitoring the pathogen population and screening breeding lines with as many races as possible, even with low-frequency races. Similarly, the virulence to *YrTr1* was detected in 1975 in the present study, 14 years earlier than the previous report of virulence to the differential 'Tres' in 1989 (Line 2002). The previous studies did not detect the early occurrences of virulence genes to *Yr7*, *YrTr1*, *Yr17*, and *YrExp2*. This is due to the fact that the previous differentials 'Lee' (*Yr7*, *Yr22*, and *Yr23*), Tres (*YrTr1* and *YrTr2*), 'Hyak' (*Yr17* and *YrTy*e), and 'Express' (*YrExp1* and *YrExp2*) have additional genes that can mask the virulence to these *Yr* genes (Chen and Line 1992a,b; Lin and Chen 2008).

The present study confirmed the previous reports of no virulence to *Yr5* or *Yr15*, and these genes have been effective against all

P. striiformis f. sp. *tritici* races identified thus far in the United States (Chen et al. 2002, 2010; Wan and Chen 2012, 2014; Wan et al. 2017). These results are consistent with the reports of effectiveness of these genes against *P. striiformis* f. sp. *tritici* populations in many other countries (Bahri et al. 2011; Brar and Kutcher 2016; Sharma-Poudyal et al. 2013; Wan et al. 2017; Zhan et al. 2012) (<http://wheatrust.org/>). The virulence to *Yr5* has been identified in India (Nagarajan et al. 1986) and Australia (Wellings 2007; Wellings and McIntosh 1990) and the virulence to *Yr15* was reported in Afghanistan and other places (McIntosh et al. 1995). Although *Yr5* and *Yr15* are currently effective in the United States and worldwide, these genes should be used in breeding programs together with other genes for durable resistance.

All of the 16 virulence genes detected in the present study increased their frequencies from 1968 or their first year detected but several virulence genes were not detected every year. Since being detected in 1970, virulence to *Yr10* never had more than 30% frequency in a year, and races with this virulence appeared for a few years and then disappeared. Virulence to *Yr10* has been almost restricted in the western United States, especially the Pacific Northwest, because *Yr10* has been mostly used in breeding programs in this region (Chen et al. 2010; Wan and Chen 2012, 2014; Wan et al. 2016). *Yr10* has been largely effective in many other countries (Sharma-Poudyal et al. 2013; Thach et al. 2015; Wan et al. 2017) (<http://wheatrust.org/>). In the United States, the decrease of virulence to *Yr10* (0.9 to 5.5%)

Fig. 1. Frequency changes of virulence genes of *Puccinia striiformis* f. sp. *tritici* to 18 *Yr* genes in different periods from 1968 to 2009 in the United States. Each period contains 5 years, except the period 1998–99.

since 2009 has made wheat cultivars with *Yr10* become highly resistant again in the region (Wan and Chen 2014; Wan et al. 2016).

Virulence to *Yr8* and *Yr9* increased rapidly since the first detection in 1998 and 1997, respectively. The rapid increase of races with virulence to *Yr8* and *Yr9* may be associated with the following facts. First, many cultivars grown in the United States and other countries have virulence to *Yr9* (Graybosch 2001). Second, the *Yr8* virulence has been mostly coupled with the *Yr9* virulence in *P. striiformis* f. sp. *tritici*

in the United States since 2000 and in other countries in the recent years (Bahri et al. 2009; Chen et al. 2002, 2010; Sharma-Poudyal et al. 2013; Thach et al. 2015; Wan and Chen 2012, 2014; Wan et al. 2016; Zhan et al. 2012). Third, some races with virulence to these resistance genes are aggressive and adapted to a wide range of temperatures (Chen 2005; Hovmøller et al. 2008; Milus et al. 2006, 2009).

Our data suggested that the virulence genes to *Yr17* and *Yr27* might have existed long before their recent recognition. The *Yr17*

Table 5. Associations between virulence genes corresponding to *Yr* genes for 908 isolates of *Puccinia striiformis* f. sp. *tritici* collected from 1968 to 2009 in the United States

Virulence to	Correlation coefficient														
	<i>Yr6</i>	<i>Yr7</i>	<i>Yr8</i>	<i>Yr9</i>	<i>Yr10</i>	<i>Yr17</i>	<i>Yr24</i>	<i>Yr27</i>	<i>Yr32</i>	<i>Yr43</i>	<i>Yr44</i>	<i>YrSP</i>	<i>YrTr1</i>	<i>YrExp2</i>	<i>YrTy</i>
<i>Yr1</i>	0.10	0.10	0.20	0.40	-0.15	0.09	-0.16	0.21	-0.16	0.18	0.09	0.36	-0.08	0.03	0.51
<i>Yr6</i>	...	0.20	0.30	0.40	0.03	0.39	0.02	0.45	-0.00	0.35	0.25	0.15	0.24	0.10	0.28
<i>Yr7</i>	...	...	0.50	0.40	-0.16	0.29	-0.15	0.41	-0.16	0.42	0.62	0.01	0.33	0.79	0.05
<i>Yr8</i>	...	...	...	0.70	0.04	0.24	0.01	0.48	0.01	0.73	0.60	0.09	0.49	0.48	0.24
<i>Yr9</i>	...	...	...	...	0.00	0.29	-0.03	0.54	-0.03	0.65	0.55	0.34	0.44	0.37	0.45
<i>Yr10</i>	...	...	...	...	...	-0.11	0.87	-0.00	0.89	0.07	-0.07	-0.01	0.15	-0.16	-0.12
<i>Yr17</i>	...	...	...	...	...	...	-0.09	0.45	-0.09	0.24	0.23	0.15	0.22	0.22	0.26
<i>Yr24</i>	...	...	...	...	...	...	...	0.01	0.94	0.06	-0.05	-0.03	0.12	-0.12	-0.14
<i>Yr27</i>	...	...	...	...	...	...	...	...	0.01	0.52	0.39	0.26	0.41	0.28	0.31
<i>Yr32</i>	...	...	...	...	...	...	...	...	...	0.05	-0.06	-0.02	0.13	-0.12	-0.13
<i>Yr43</i>	...	...	...	...	...	...	...	...	...	...	0.55	0.15	0.50	0.47	0.22
<i>Yr44</i>	...	...	...	...	...	...	...	...	...	...	...	0.07	0.33	0.67	0.19
<i>YrSP</i>	...	...	...	...	...	...	...	...	...	...	...	...	-0.01	-0.02	0.45
<i>YrTr1</i>	...	...	...	...	...	...	...	...	...	...	...	...	...	0.31	-0.01
<i>YrExp2</i>	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.03

Table 6. Nei's genetic distances based on virulence among the *Puccinia striiformis* f. sp. *tritici* populations of every 5-year period from 1968 to 2009 in the United States

Period	Genetic distance ^a							
	1968–72	1973–77	1978–82	1983–87	1988–92	1993–97	1998–99	2000–04
1973–77	0.007	...	...	...	...	...	...	...
1978–82	0.010	0.004	...	...	...	...	...	...
1983–87	0.011	0.004	0.002	...	...	...	...	...
1988–92	0.010	0.004	0.002	0.001	...	...	...	...
1993–97	0.022	0.011	0.005	0.003	0.004	...	...	...
1998–99	0.036	0.017	0.012	0.012	0.015	0.007	...	...
2000–04	0.144	0.105	0.109	0.111	0.112	0.100	0.072	...
2005–09	0.352	0.281	0.282	0.288	0.288	0.262	0.211	0.040

^a Virulence distances were calculated using the Nei distance index (Nei 1972).

Fig. 2. Changes in the number of virulence genes per isolate and per new race in *Puccinia striiformis* f. sp. *tritici* in different periods from 1968 to 2009 in the United States.

virulence has been quite common, detected every year since 1968, except 1982. Its frequency was below 50% before 2001, increased to 86.4% by 2009, and maintained in the range of 73.9 to 90.0% from 2010 to 2015 (Wan and Chen 2014; Wan et al. 2016) (A. M. Wan and X. M. Chen, unpublished data). Similarly, the *Yr27* virulence increased to more than 50% after 2005 (Wan and Chen 2014; Wan et al. 2016). The increases of virulence genes to *Yr17* and *Yr27* are consistent with the situation in many other countries, and the increases are due to the host cultivar selection. A large number of cultivars with *Yr17* or *Yr27* have been developed and grown throughout the world and, as a result, virulence to these genes has developed in many countries (Brar and Kutcher 2016; Bayles et al. 2000; Boshoff et al. 2002; Fang et al. 2011; Milus et al. 2015; Sharma-Poudyal et al. 2013; Wan and Chen 2014; Wan et al. 2016, 2017; Wellings 2008). The previous unrecognition of virulence to these two genes was due to the fact that wheat cultivars with *Yr17* or *Yr27* also have other resistance genes. The additional resistance genes could mask the ineffectiveness of *Yr17* and *Yr27*. For example, Hyak, which was included in the previous set of wheat cultivar differentials (Line 2002), has *Yr17* and *YrTye*, and only the races with virulence to both genes in the cultivar can detect virulence to either *Yr17* or *YrTye*. The results of this study demonstrate the importance of using single-gene lines to differentiate races for virulence information directly related to individual resistance genes (Wan and Chen 2014).

The present study shows that *P. striiformis* f. sp. *tritici* is able to accumulate different virulence genes to produce complex races. Compared with the 1968 population, in which each race had a mean number of one virulence gene, the 2009 population had races each possessing more than 10 virulence genes on average. Many of the races with a large number of virulence genes have thrived in recent years, suggesting that races with more virulence genes have advantages in infecting and reproducing on wheat cultivars, and wheat cultivars with different resistance genes have selected those that are able to infect more cultivars (Chen 2005; Chen et al. 2010). Virulence is more important than any other traits for an obligate biotrophic fungus such as *P. striiformis* f. sp. *tritici*, because these fungi cannot survive on the primary crop host without virulence to resistance genes in cultivars. However, *P. striiformis* f. sp. *tritici* is able to infect many wild grass species serving as auxiliary hosts (Cheng et al. 2016), and grasses are able to keep races with fewer virulence genes. For example, PSTv-18 was one of the earliest races, does not have any virulence gene to the 18 *Yr* genes in the single-gene differentials, and was detected from 1968 to 2009. It remained among the top five most frequent races in all of the 5- or 2-year periods, except 2000–04. In 2015, PSTv-18 became the third most frequent race again (A. W. Wan and X. M. Chen, unpublished data). This race was often collected from grasses and wheat cultivars without race-specific all-stage resistance. Similarly, PSTv-19 remained among the top five most frequent races from 1968 to 1999 and was still detectable in the recent years (Wan and Chen 2014; Wan et al. 2016) (A. M. Wan and X. M. Chen, unpublished data). In addition to virulence to resistance genes other than the 18 *Yr* genes used in the differentials, these races with narrow spectra of virulence are able to infect many species of Gramineae grasses, susceptible wheat cultivars, and cultivars with only nonrace-specific HTAP resistance, which is often partial and, therefore, has less selection pressure for specific races (Chen 2005, 2013; Cheng et al. 2016).

In addition to mutation, other mechanisms may be involved in generating new races. Races might be produced through mutation from unidentified races, recombination of two previously existing races, or introduction. It is not possible to detect unidentified races in the past due to the lack of *Yr* single-gene lines and the limitation of the past collection and sampling in the present study. For recombination, both sexual recombination and somatic recombination have been demonstrated for *P. striiformis* f. sp. *tritici* under controlled conditions (Jin et al. 2010; Lei et al. 2017; Wang and Chen 2013, 2015). Because sexual reproduction has not been detected in the United States (Cheng and Chen 2014; Wang et al. 2015) and barberry does not function as an alternate host for *P. striiformis* f. sp. *tritici* due to teliospore degradation and barberry phenology (Wang and Chen 2015), it is unlikely that sexual recombination has played a role in

producing new races in the United States. Somatic recombination is a likely mechanism involved in the formation of new races, as clearly shown for *P. striiformis* f. sp. *tritici* under controlled conditions (Lei et al. 2017) and indicated by studies by natural populations (Cheng et al. 2016). However, to determine which races were produced through somatic recombination, molecular characterization of the isolates used in the present study is needed.

In the present study, significant positive and negative associations were identified between different virulence genes. Because the *P. striiformis* f. sp. *tritici* populations are largely clonal (Cheng and Chen 2014; Hovmøller et al. 2002), when two virulence or avirulence genes or a virulence gene and an avirulence gene are present in a race, the two genes will likely present together in races evolved from the early race. Although specific mechanisms for the associations require further studies, the results obtained in the present study can be used to choose *Yr* genes with negative associations for combinations such as *Yr1* with *Yr10*, *Yr24*, or *Yr32*; *Yr10* with *Yr7*, *Yr17*, *Yr44*, *YrSP*, *YrExp2*, or *YrTye*; *Yr24* with *Yr44*; *YrSP*, *YrExp2*, or *YrTye*; and *Yr32* with *YrExp2* or *YrTye*; and avoid using combinations of genes with positive associations such as *Yr1* with *YrSP*; *Yr10* with *Yr24* or *Yr32*; *Yr17* with *Yr27*; *Yr43* with *Yr44*, *YrTr1*, or *YrExp2*; and *YrSP* with *YrExp2*. However, genes such as *Yr5* and *Yr15* that are more effective against all races identified thus far and recently identified genes for effective all-stage resistance against all tested races such as *Yr45* (Li et al. 2011), *Yr53* (Xu et al. 2013), and *Yr64* and *Yr65* (Cheng et al. 2014) are preferred for use in combinations in breeding programs. It is more preferred to combine the effective all-stage resistance genes with genes for nonrace-specific HTAP resistance such as *Yr36* (Fu et al. 2009), *Yr39* (Lin and Chen 2007), *Yr52* (Ren et al. 2012), *Yr59* (Zhou et al. 2014), and *Yr62* (Lu et al. 2014) and many temporarily designed genes or quantitative trait loci (Chen 2013; Hou et al. 2015) for developing wheat cultivars with high-level and durable resistance to stripe rust.

Acknowledgments

This study was supported by the United States Department of Agriculture–Agricultural Research Service (project number 5348-22000-015-00D); Washington Grain Commission, and Idaho Wheat Commission PPNS number 0722; and Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences, Agricultural Research Center, project number WNP00663 (projects 13C-3061-5665 and 13C-3061-4232), Washington State University, Pullman 99164-6430.

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