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Geographic substructure of *Fusarium asiaticum* isolates collected from barley in China

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Abstract Fusarium head blight (FHB) can affect wheat and barley and is a devastating disease caused by a complex of *Fusarium* species. Here we report on a large-scale survey on the genetic diversity of isolates collected from barley in China. Ten VNTR markers were tested on a representative set of 40 isolates covering 14 sampling areas along the Yangtze River. VNTR4 and VNTR7, with 13 and 6 alleles, each were applied to a total of 1106 single-spore isolates to reveal the population structure of *F. asiaticum*. The *F. asiaticum* population showed high genetic diversity and a clear genotypic substructure within China. Pair-wise comparisons of allele frequencies between the mountainous provinces of Sichuan and Chongqing in Western China, Hubei Province in the centre or the eastern provinces of Zhejiang, Jiangsu and Shanghai showed significant differences. Even between counties of the same province, significant differences between allele frequencies were found ($P<0.001$). Our results indicate serious constraints for migration of this pathogen in the major cereal-growing areas of China.

Keywords FHB · *Fusarium graminearum* · Genotypic diversity · VNTR

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Introduction

Fusarium head blight (FHB) or scab is a significant fungal disease of wheat, barley and other small cereal grains all over the world (Jennings et al. 2004; Parry et al. 1995; McMullen et al. 1997; D'Mello et al. 1999). Although a number of fusaria can cause FHB, its primary etiological agents belong to the *Fusarium graminearum* species complex (Fg complex), which consists of phylogenetically distinct species that can not be discriminated based on their morphology (O'Donnell et al. 2004, 2008; Starkey et al. 2007). In addition to quantitative losses, *F. graminearum* causes a reduction in grain quality due to production of the type B trichothecene mycotoxins nivalenol (NIV), deoxynivalenol (DON) and several acetylated

derivatives of NIV and DON (Mirocha et al. 1989). These mycotoxins are detected frequently in cereal grain samples globally and represent a significant health threat to human and animal consumers (D' Mello et al. 1999; Gutleb et al. 2002).

FHB is a serious problem in China and several epidemics have been described since 1936, when the first severe outbreak was recorded (Xu and Chen 1993). Since then, FHB has been observed frequently in winter wheat in regions along the middle and lower reaches of the Yangtze River in central China, including the provinces of Jiangsu, Zhejiang, Anhui, Hubei, and the municipality of Shanghai. In previous years, an increasing number of wheat-growing regions throughout the country, including the provinces of Henan, Shaanxi, Ningxia, Gansu, Qinghai, Hebei, Shandong, Sichuan and the municipality of Chongqing have been affected by the disease (Chen et al. 2000). Between 1987 and 1997, five serious FHB epidemics occurred in Sichuan Province, a frequency much higher than those in other areas (Liu 1997). All available data indicate that FHB epidemics are becoming more frequent, more severe, and more widespread in China.

In China, FHB epidemics can cause great, direct yield losses. During severe epidemics, the percentage of scabbed spikes is usually 30% to 50%, but can exceed 60% to 70% in the most susceptible cultivars during the most severe epidemics (Zhang 1998; Shi and Wang 1999). The outbreak of FHB during 1996 resulted in a yield loss of about 78,000 metric tonnes (Li 1996) in Sichuan Province. In China, cereals are mostly grown by small-scale farming and FHB can have a dramatic impact on local communities. Although barley suffers less frequently from FHB than wheat, under conducive conditions, yield and quality losses may occur. As the consumption of beer in China is rising dramatically, there is an increasing demand for barley. In addition, barley also is used for animal feed, which exacerbates the need for this commodity.

In an extensive survey covering 21 Chinese provinces, 95% of *Fusarium* cultures that were isolated from 2,450 diseased wheat heads were morphologically characterized as *F. graminearum* without the assessment of chemotypes or use of molecular markers (CWSCG 1984). However, recently it was shown that what previously was called *F. graminearum* is a species complex consisting of at least 12 species collectively called the *Fusarium graminearum* clade (Fg-clade) and many of these species cannot be distinguished morphologically (O'Donnell et al. 2000, 2008; Starkey et al. 2007).

In barley, little was known about the importance of FHB in China and the causal organisms until a recent survey. In this survey 1,894 single-spore isolates of *Fusarium* obtained from infected barley ears collected in 23 counties of seven provinces and two municipalities along the Yangtze River showed that in all regions *F. asiaticum* was the predominant species causing FHB on barley, while in the upper valleys of the Yangtze River also *F. graminearum sensu stricto* and *F. meridionale* were found (Yang et al. 2008). Dramatic differences in the distribution of chemotypes were found in the *F. asiaticum* populations, from a very high percentage of NIV producers in the mountainous upper valleys of the Yangtze River to predominantly DON producers in the middle and lower valleys. From these results it was suggested that a shift from NIV to DON producers had occurred recently in the lower valleys. However, these isolates have not been subjected to molecular genetic diversity analysis required to understand the population dynamics.

Molecular markers are valuable tools in population studies because they allow tracking and tracing of genetic variation. Various genetic marker systems such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), simple-sequence repeat (SSR) and amplified fragment length polymorphism (AFLP, Vos et al. 1995) have been employed over the past few years to analyze the population genetic structure, reproductive behavior and biogeographic structure of *F. graminearum* (Carter et al. 2000, 2002; Gale et al. 2002; Zeller et al. 2003; Mishra et al. 2004). In this paper, we have used VNTR markers that were developed by Suga et al. (2004). In the past, the development of SSR/VNTR markers was costly and cumbersome, but using the genome sequence of *F. graminearum* strain PH-1 (NRRL 31084) Suga et al. (2004) identified ten pairs of VNTR primers that are highly polymorphic in a small reference set representing different origins. These VNTRs are therefore likely to be effective for population genetic studies.

In this paper we describe the genetic divergence within and between populations of *F. asiaticum* collected from the upper (Sichuan and Chongqing),

middle (Hubei) and lower reaches (Jiangsu, Zhejiang and Shanghai) of the Yangtze River. The dramatic gradient previously observed for chemotype (Yang et al. 2008) prompted us to test the hypothesis that this gradient was also reflected in the population structure as determined by presumably neutral VNTR markers.

Materials and methods

Biological materials

In total, 1106 single-spore *F. asiaticum* isolates were genotyped in this study. The sampling sites (n=14) are listed in Table 1. A core set of 40 isolates was assembled for VNTR marker selection by taking two or three isolates from each location. Methods for culturing strains, DNA isolation and species characterization were described by Yang et al. (2008).

VNTR fingerprinting and gel electrophoresis

Genotyping of the isolates was performed by VNTR-PCR with the ten pairs of VNTR primers (Table 2) designed by Suga et al. (2004). The reaction mixture was 20 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTP, 1 μm of each primer, 0.5 unit of Taq DNA polymerase (Takara, Japan) and 10 ng of genomic DNA. Cycling conditions were: 94°C for 1 min; then 30 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 30 s; and a final extension at 72°C for 5 min. The PCR products were separated in 6% polyacrylamide denaturing gels. Before samples were loaded, the PCR products were mixed with loading buffer (Takara, Japan) and denatured at 95°C for 5 min. After electrophoresis, fragments were visualized using silver staining.

PCR products generated by the primers 4FS (GAAGCGTTGTCGGAG) and 4RS (CTATCTACTACC CACC) or primers 7FS (CAGTGGCACTGGTCTC) and 7RS (CACATTCGTTGAGAGCTCC) were separated by 3% MetaPhor agarose (Cambrex) including 0.5 μg/ml ethidium bromide and visualized on a UV transilluminator. Sequencing reactions were done on the different allelic amplicons for VNTR4 and VNTR7. Amplicons were purified via ethanol precipitation, sequenced using standard procedures, and then run on an ABI 3730 genetic analyzer. DNA sequences were edited using Sequencher ver. 4.1.2 (Gene Codes, Ann Arbor, MI) and alignments were performed manually.

Data analysis

Population genetic analyses were performed using TFPGA 1.3 (http://www.marks geneticsoftware.net/). Weir and Cockerham's (1984) F-statistics were applied to estimate partitioning of genetic diversity

Table 1 Origin of 1106 *Fusarium asiaticum* isolates from 14 counties in four provinces and two municipalities in China

Province or Municipality	Site	County	Number of isolates
Sichuan	1	Mianzhu	82
	2	Mianyang	52
	3	Xinsheng	83
Chongqing	4	Tongnan	27
	5	Yongchuan	72
Hubei	6	Jingzhou	92
	7	Shayang	77
	8	Maliang	94
	9	Wuchang	150
	10	Huanggang	9
Jiangsu	11	Yancheng	16
Zhejiang	12	Hangzhou	200
	13	Jiaxing	80
Shanghai	14	Yongfeng	72
Total			1,106

Table 2 Results of the ten VNTR markers on 40 isolates

VNTR No	Locus[a]	Chromosome	Size (bp)[b]	No. of alleles				
				Sichuan Chongqing	Hubei	Zhejiang Jiangsu Shanghai		Total
1	HK1043	I	286	2	2	3		3
2	HK913	I	234	2	2	2		3
3	HK917	I	234	1	1	1		1
4	HK957	I	298					
			215	7	6	8		13
5	HK965	II	252	3	3	1		4
6	HK967	II	215	2	2	2		2
7	HK1059	III	248					
			137	5	4	2		6
8	HK977	III	210	2	1	1		2
9	HK630	IV	239	1	1	1		1
10	HK1073	IV	221	7	4	4		14
			total	32	26	25		49

[a] Locus name according to Suga et al. (2004)

[b] Based on the genome sequence of isolate PH-1 (NRRL 31084). The genome sequence of *F. graminearum* was obtained from http://www.broad.mit.edu

among provinces as well as among counties within provinces. Pairwise F_{ST} distance estimates were calculated based on the number of different alleles, and the statistical significance of pairwise Fst estimates was assessed using a permutation test with 1,000 permutations. Exact tests of population differentiation were conducted using the log-likelihood statistic G (Goudet et al. 1996), and were consistent with permutation test results.

Results

VNTR markers selection with a representative set of isolates

All ten VNTR primer pairs were used to detect genetic diversity among a core set of 40 *F. asiaticum* isolates, collected from the 14 counties in four provinces and two municipalities shown in Fig. 1. In total, the ten primer pairs identified 49 alleles demonstrating the high level of allele diversity for these VNTR markers in our Chinese collection. However, in this core set of 40 isolates belonging to the *F. asiaticum* no diversity was found for VNTR3 and VNTR9. The other eight VNTR markers generated robust, scoreable profiles with multiple alleles (Table 2). VNTR10 displayed the highest number of alleles (14). VNTR4 and VNTR7 also showed high polymorphisms, with 13 and 6 alleles detected, respectively (Fig. 2, Table 2). The other VNTR markers displayed 2 to 4 alleles each. Isolates from Sichuan/Chongqing showed the highest level of polymorphism, accounting for 32 alleles (Table 2). Although VNTR10 showed the highest number of alleles, its repeat motif is short (2 bp) and therefore alleles are more difficult to distinguish on gels. Consequently, we chose VNTR4 and VNTR7, with repeat sizes of 10 and 15 bp, respectively, for further analysis of the complete set of 1106 *F. asiaticum* isolates.

Optimizing the scoring for VNTR4 and VNTR7 and nomenclature

With VNTR4, 13 sizes of fragments were generated and sequenced. The longest fragment was 378 bp, and contained 25 units of the 10-bp repeat motif (GGGAGTCAAT). The shortest fragment was 158 bp, containing three units of the VNTR4 motif. The difference between the largest and the smallest allele was 22 repeat motifs (exactly 220 bp) and

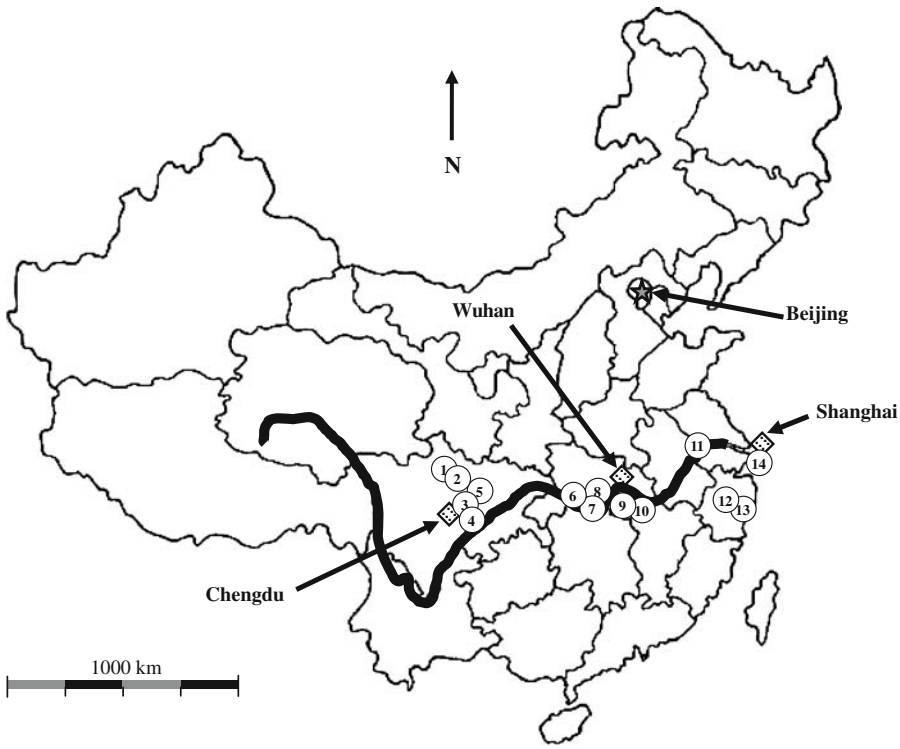

Fig. 1 Map of China indicating the sampling sites and course of the Yangtze River (thick black line). Numbers indicate the counties and correspond with the numbering in Table 1. Major cities are also indicated

repeat numbers accounted for all size differences found.

With VNTR7, six amplification fragments were obtained. Sequencing results showed that the longest allele was 263 bp and contained six units of the 15-bp repeat motif (AGCAGTGGTGGTCTC), while the shortest fragment was 188 bp with only one unit of the VNTR7 motif. There were only five repeat motifs (exactly 75 bp) difference between these alleles and again no additional insertions were found. For VNTR4 and VNTR7, new primers were designed, named 4S and 7S, which generate smaller fragments, and therefore allow convenient discrimination of alleles on agarose gels (Fig. 2). Because the number of repeat units is known, alleles can be scored as the number of the repeat units rather than the size of the amplicon, which simplifies coherent data exchange between research groups. Data are named by the locus (VNTR) and the allele (number of repeat units), e.g., VNTR4/12 indicates that an isolate has 12 repeat units for VNTR4.

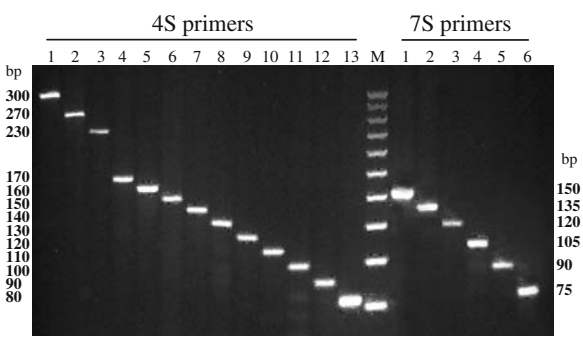

Fig. 2 Amplification products generated in isolates of Fusarium asiaticumby using shortened VNTR primers, showing 13 alleles generated by the 4S primers (left) for locus HK957 and six alleles generated by the 7S primers (right) for locus HK1059. M; 25-bp DNA ladder

Large-scale genotyping with the most informative VNTR markers

A large-scale study was performed on 1106 isolates that were genotyped with VNTR4 and VNTR7. Again, 13 alleles were displayed with VNTR4, and six with VNTR7, so compared to the core set of 40 representative isolates, no additional alleles for these loci were found among the 1106 isolates (Table 3). Allele frequencies are not distributed randomly along the Yangtze River. Of the 19 VNTR4 and VNTR7 alleles found in total, three alleles for VNTR4 (VNTR4/18, VNTR4/22, VNTR4/25) and one for VNTR7 (VNTR7/2) were found uniquely in Sichuan

Table 3 Allele groups for VNTR4 (GGGAGTCAAT repeat) and VNTR7 (AGCAGTGGTGGTCTC repeat) among 1106 *Fusarium asiaticum* isolates from Chinese barley

Locus[a]	Allele[a]	Size (bp)	Frequency[b]			
			Sichuan Chongqing	Hubei	Zhejiang Jiangsu Shanghai	
VNTR4	3	80	37/316	0	4/368	
	4	90	9/316	0	13/368	
	5	100	19/316	0	97/368	
	6	110	0	18/422	54/368	
	7	120	0	94/422	48/368	
	8	130	123/316	92/422	87/368	
	9	140	31/316	11/422	31/368	
	10	150	11/316	0	17/368	
	11	160	23/316	74/422	6/368	
	12	170	42/316	133/422	11/368	
	18	230	14/316	0	0	
	22	270	5/316	0	0	
	25	300	2/316	0	0	
VNTR7	1	75	173/316	286/422	268/368	
	2	90	17/316	0	0	
	3	105	66/316	0	94/368	
	4	120	5/316	34/422	0	
	5	135	10/316	53/422	6/368	
	6	150	45/316	49/422	0	

[a] Alleles are named based on the number of repeat units found by sequencing

[b] Number of isolates carrying the allele among 1106 *F. asiaticum* isolates tested

or Chongqing, in the upper valleys of the Yangtze River. In addition, nine alleles were only found in two of the three geographic areas (Table 3). Based on a *t*-test, the number of alleles in the upper valley was significantly higher (P=0.045) compared to the lower reaches.

In this study, all 1106 isolates were identified as *F. asiaticum* and we tested whether the *F. asiaticum* population should be regarded as a single metapopulation or if we could observe a substructure. The result showed that the average gene diversity was highest in the mountainous province of Sichuan (H'=0.69) and the lowest at sea level in Jiangsu and Shanghai (H'=0.54). The average number of alleles per population was also highest in Sichuan (A=7.50) (Table 4). Significant population differentiation (P<0.001) was observed between each population pair, with F_{ST} values ranging from 0.035 (Zhejing/Shanghai and Jiangsu, <250 km apart) to 0.200 (Chongqing/Shanghai and Jiangsu, >1,500 km apart) (Table 5). For both VNTR4 and VNTR7 the differentiation between counties is larger than the differentiation between provinces (F_{SC}-values 0.24 and 0.17, respectively). When both loci are combined, the differentiation among counties within provinces also showed a high F_{ST} value (F_{ST}=0.22) and pairwise comparisons between the 14 counties showed significant differentiation among all counties (Table 6). These results indicated that the genetic diversity of *F. asiaticum* showed a clear substructure even within provinces.

Since we were faced with substantial differences in the population size between provinces (88–421), and particularly between counties (9–200), we selected 20–30 isolates per county (excluding counties with N<20) randomly and ran the F_{ST} analyses again to validate these results. We again observed significant population differentiation (P<0.001) between each pair of provinces. The F_{ST} values ranged from 0.061 (Zhejing/Shanghai and Jiangsu) to 0.322 (Chongqing/Shanghai and Jiangsu) (Supplemental Table 1), which was consistent with the result of the complete set of 1106 isolates. The differentiation between counties (F_{ST}-values = 0.201) was also larger than the differentiation between provinces (F_{SC}-values = 0.155). Pairwise comparisons between the 12 counties showed significant differentiation in all comparisons (Supplemental Table 2).

Table 4 Diversity within the 1106 *Fusarium asiaticum* isolates collected from the different regions of China

Province	N [a]	Mean gene diversity H' [b]	Allelic diversity [c]
Hubei	421	0.65	5.50
Chongqing	99	0.59	4.00
Sichuan	218	0.69	7.50
Jiangsu/Shanghai	88	0.54	4.50
Zhejiang	280	0.64	6.50

[a] Number of *F. asiaticum* isolates

[b] Mean gene diversity in each population calculated according to Nei (1978)

[c] Number of alleles in each population calculated according to Nei (1978)

Discussion

Our study demonstrated that the VNTR markers identified by Suga et al. (2004) in the genome sequence of *F. graminearum* isolate PH-1 isolated from maize (NRRL 31084) are also useful tools for population genetic analyses of *F. asiaticum* on Chinese barley. Some VNTR markers appear more informative than others. For example, for the markers VNTR4, VNTR7 and VNTR10, Suga et al. (2004) detected ten, four and ten alleles, respectively, among ten isolates of *F. graminearum* from two continents (USA, Italy) and five isolates of *F. asiaticum* sampled in China. Using the same three markers, among 40 *F. asiaticum* isolates we obtained 13, six and 14 alleles for VNTR4, VNTR7 and VNTR10, respectively. This demonstrates that the genetic diversity for these loci in *F. asiaticum* derived from barley-growing areas in China was high as Suga and co-workers observed in a combined set of *F. graminearum* and *F. asiaticum* isolates from wheat, maize and cyclamen collected in USA, Italy and China. In contrast, we detected no polymorphisms in the core set of 40 isolates for VNTR3 and VNTR9, whereas Suga et al. (2004) identified two and three alleles for these VNTR markers, respectively. Furthermore, among the Chinese *F. asiaticum* isolates analyzed by Suga et al. (2004) these markers showed two alleles among five isolates collected from two fields in a single county, whereas in this study we detected no polymorphisms among 40 isolates from 14 counties in five provinces and two municipalities. The fact that no additional alleles for VNTR4 and VNTR7 were found among 1106 isolates compared to the core set of 40 isolates indicates that the core set was indeed representative for the entire *Fusarium* population studied. The strategy of taking three isolates for each of the 14 sampled counties seemed to have been efficient. Previously, Yang et al. (2008) and others (e.g., Ward et al. 2008) reported that the different members of the *F. graminearum* clade occur sympatrically as can be expected from their phylogenies. Therefore, we performed additional analysis on the entire *F. asiaticum* population (N=1,106) studied. A clear genetic substructure also was found within the *F. asiaticum* population. To our knowledge this is the first time that such a clear substructure in *F. asiaticum* was identified from a comprehensive collection obtained in a single year and a single host.

Previously, Miedaner et al. (2001) found no geographical structuring among 207 isolates of *F. graminearum* collected from various parts of the world. These contrasting observations may be due to several factors. Firstly, the number of isolates ana-

Table 5 Pairwise F_{ST} (above diagonal) and permutation test probability (below diagonal) estimates with 1106 *Fusarium asiaticum* isolates collected from the different provinces of China

	Sichuan	Chongqing	Hubei	Jiangsu/Shanghai	Zhejiang
Sichuan	****	0.151	0.082	0.126	0.056
Chongqing	<0.001	****	0.159	0.200	0.145
Hubei	<0.001	<0.001	****	0.123	0.103
Jiangsu/Shanghai	<0.001	<0.001	< 0.001	****	0.035
Zhejiang	<0.001	<0.001	<0.001	<0.001	****

Table 6 Pairwise F_{ST} (above diagonal) and permutation test probability (below diagonal) estimates with 1106 *F. asiaticum* isolates collected from the different counties

	Mianzhu	Mianyang	Xinsheng	Tongnan	Yongchuan	Jingzhou	Shayang	Maliang	Wuchang	Huanggang	Yancheng	Hangzhou	Jiaxing	Yongfeng
Mianzhu	****	0.202	0.109	0.456	0.195	0.131	0.170	0.140	0.151	0.213	0.405	0.130	0.157	0.151
Mianyang	<0.001	****	0.102	0.403	0.202	0.372	0.400	0.328	0.146	0.428	0.561	0.145	0.128	0.197
Xinsheng	<0.001	<0.001	****	0.378	0.118	0.238	0.250	0.193	0.143	0.194	0.355	0.056	0.078	0.097
Tongnan	<0.001	<0.001	<0.001	****	0.292	0.495	0.633	0.494	0.306	0.775	0.875	0.399	0.420	0.458
Yongchuan	<0.001	<0.001	<0.001	<0.001	****	0.263	0.290	0.212	0.114	0.203	0.319	0.080	0.144	0.118
Jingzhou	<0.001	<0.001	<0.001	<0.001	<0.001	****	0.338	0.185	0.212	0.290	0.488	0.241	0.279	0.252
Shayang	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	****	0.204	0.248	0.376	0.534	0.215	0.294	0.208
Maliang	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	****	0.180	0.241	0.416	0.170	0.191	0.170
Wuchang	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	****	0.276	0.420	0.119	0.139	0.130
Huanggang	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	****	0.814	0.160	0.296	0.244
Yancheng	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	****	0.259	0.315	0.258
Hangzhou	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	****	0.048	0.019
Jiaxing	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	****	0.061
Yongfeng	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	****

Shaded cells indicate comparisons of counties within the same province or municipality

lyzed in this study is much larger, enhancing the statistical power of the analysis. Secondly, the isolates in this study came from a single host collected in the same year and all 1106 isolates were identified as *F. asiaticum*.

The genetic structure of *F. asiaticum* observed in this study may reflect restrictions on migration. This can be due to geographic barriers such as the high mountain ridges that surround Sichuan and Chongqing. We also observed genetic structuring between populations from Hubei compared with those from Jiangsu, Shanghai and Zhejiang. This may reflect the small-scale, regional farming. In addition, members of the *F. graminearum* clade, such as *F. asiaticum*, are homothallic and can therefore complete the sexual cycle without a partner which would increase the probabilities of local fixation of alleles by allowing essentially clonal propagation of a successful genotype. Taken together this may explain the emergence of area-specific clusters within the *F. asiaticum* populations from the upper valleys of the Yangtze River. For example, the alleles VNTR4/25, VNTR4/22, VNTR4/18 and VNTR7/2 were observed exclusively in Sichuan and Chongqing. This is in agreement with a recent observation showing the occurrence of genetic differentiation along environmental gradients (Doebeli and Dieckmann 2003).

Our study using VNTR markers showed that in each county the *F. asiaticum* population was significantly different and this contrasts to the dramatic gradient of NIV versus DON chemotypes along the Yangtze River (Yang et al. 2008) that basically splits this geographic area into two regions. The presence of a cline in chemotypes in *Fusarium* populations was also found in other regions of the world. Analysis of the diversity of FHB pathogens revealed that 3ADON-producing *F. graminearum* isolates are prevalent in North America (Ward et al. 2008) and significant population structure associated with trichothecene chemotype differences was identified (F_{ST}>0.285; P<0.001). Based on the profile of the DON/NIV chemotype gradient along the Yangtze River (Yang et al. 2008), we previously suggested that this cline was caused by a recent shift in the *Fusarium* population. We found further evidence for such a recent shift in the *Fusarium* population. We demonstrate that the number of alleles for VNTR4 and VNTR7 is lower in the lower valleys and that some VNTR4 and VNTR7 alleles are highly over-represented. This could be explained by founder effects following recent migration. Nevertheless, the genetic diversity found in the middle and lower valleys is still high. This would indicate that the previously proposed displacement (Yang et al. 2008) was slow, allowing genetic exchange between the old and new populations by occasional outcrossing events which would mask invasion by new genotypes.

The first outbreak of FHB in China was reported in 1936 in regions along the middle and lower reaches of the Yangtze River in central China (Xu and Chen 1993). Since then, FHB has occurred frequently in winter wheat in central China, including the provinces of Jiangsu, Zhejiang, Hubei, and the municipality of Shanghai. This has forced farmers in these areas to shift to other crops including barley. Subsequently, an increasing number of wheat-growing regions throughout the country, including Sichuan province and the municipality of Chongqing, have been affected by the disease (Chen et al. 2000). From 1987 to 1997 in Sichuan Province and the municipality of Chongqing,

there were five serious FHB epidemics during the years 1989, 1990, 1992, 1996, and 1997, a frequency much higher than that observed in other areas (Li 1996; Liu 1997). Previously Yang et al. (2008) showed that although FHB symptoms are similar, clear differences in the causal species can be found. In this study we demonstrated also that clear differences in genotypes can be observed in different regions which may affect the severity of FHB epidemics. We believe that the incidence and severity of FHB may have gradually increased in China because of migration and selection for *F. asiaticum* isolates that produce DON and our current study on population diversity supports this view. In other regions in the world migration of FHB pathogens may be higher due to regional and global transportation of cereal commodities. Similarly, the migration of FHB pathogens within China is likely to increase in the future. Therefore, monitoring and identification of *Fusarium* species is important and we hope that the currently characterized gene bank will contribute to the understanding of pathogen migration and selection and can be used as a reference for future studies.

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