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Selection Pressure and Founder Effects Constrain Genetic Variation in Differentiated Populations of Soilborne Bymovirus *Wheat yellow mosaic virus* (*Potyviridae*) in China

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ABSTRACT

Sun, B.-J., Sun, L.-Y. Tugume, A. K., Adams, M. J., Yang, J., Xie, L.-H., and Chen, J.-P. 2013. Selection pressure and founder effects constrain genetic variation in differentiated populations of soilborne bymovirus *Wheat yellow mosaic virus* (*Potyviridae*) in China. Phytopathology 103:949-959.

To study the population genetic structure and forces driving the evolution of *Wheat yellow mosaic virus* (WYMV), the nucleotide sequences encoding the coat protein (CP) (297 sequences) or the genome-linked virion protein (VPg) (87 sequences) were determined from wheat plants growing at 11 different locations distributed in five provinces in China. There were close phylogenetic relationships between all sequences but clustering on the phylogenetic trees was congruent with their provenance, suggesting an origin-dependent population genetic structure. There

were low levels of genetic diversity, ranging from 0.00035 ± 0.00019 to 0.01536 ± 0.00043 (CP), and 0.00086 ± 0.00039 to 0.00573 ± 0.00111 (VPg), indicating genetic stability or recent emergence of WYMV in China. The results may suggest that founder effects play a role in shaping the genetic structure of WYMV. Between-population diversity was consistently higher than within-population diversity, suggesting limited gene flow between subpopulations (average F_{ST} 0.6241 for the CP and 0.7981 for the VPg). Consistent amino acid substitutions correlated with the provenance of the sequences were observed at nine positions in the CP (but none in the VPg), indicating an advanced stage in population structuring. Strong negative (purifying) selection was implicated on both the CP and VPg but positive selection on a few codons in the CP, indicating an ongoing molecular adaptation.

Plant viruses threaten food security by inciting many damaging plant disease epidemics and the damage is predicted to increase, partly because climate change is expected to accelerate rates of evolution, emergence, and adaptability of viral and subviral parasites (21). This is especially critical because most plant viruses have RNA genomes, whose intrinsic properties of high replication speeds, lack of error correction during their RNA replication, short generation times, and vast within-plant population sizes dictate extremely high genetic variability of their populations (22). The inherently high evolutionary potential of plant viruses makes their population genetic structures more "plastic", which challenges the measures implemented for virus disease control (23). Therefore, it is very important, both fundamentally and practically, to understand the forces that drive the molecular evolution and determine the population genetic structures of plant viruses (20).

The family *Potyviridae* is a large group of plant viruses, many of which are of great agricultural significance (5,41). Viruses in this family have single-stranded positive-sense RNA (+ssRNA) genomes that are encapsidated in flexible, filamentous virions.

The genera in the family are differentiated according to genome composition and structure, sequence similarity, and the vectors transmitting the viruses (5). The genomes of most of the viruses are monopartite but those in the genus *Bymovirus* are bipartite. RNA 1 and RNA 2 are separately encapsidated into virions of different lengths and each encodes a single polyprotein (3). The bymovirus polyprotein encoded by RNA 1 shares homology with the central and C-terminal regions of the polyproteins encoded by monopartite members of the family and, by analogy with them, is assumed to be processed into eight proteins (P3, 7K, CI, 14K, genome-linked virion protein [VPg], NIa-Pro, NIb, and coat protein [CP]) (4).

Viruses in the genus *Bymovirus* only infect grasses and cereals and each has a very limited host range. They cause mosaic diseases of varying severity depending on the virus and host plant cultivar (30,34). Of the six current agriculturally important bymoviruses, *Wheat yellow mosaic virus* (WYMV) is the most economically important pathogen of winter wheat (*Triticum aestivum*) in Japan and China (26,34), accounting for yield losses in China of >5 million tons/annum (63). Like other bymoviruses, WYMV is soilborne, being transmitted to plant roots by a soil-inhabiting and root-infecting obligate parasite, *Polymyxa graminis* Led. (Plasmodiophorales, a fungoid protist) (30). The resting spores of *P. graminis* containing the virus can survive for decades in the soil and root debris (2,30), which makes it impossible to eliminate the virus from fields once infested by viruliferous *P. graminis*. The growth of resistant cultivars seems to be the only

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practical way to avoid crop losses caused by bymovirus infection in such fields (34,80).

Field studies involving the growth of different wheat cultivars in fields heavily infested with WYMV suggest that there may be differences in the virus populations between sites in Jiangsu and Sichuan provinces in China; however, these have not always been consistent between years and have proved difficult to characterize (13,15,76). Although this might be because the virus or vector are not uniformly distributed within the fields, resulting in poor reproducibility of disease responses, it is also possible that the virus populations are not genetically homogeneous; however, none of these possibilities has been investigated. Furthermore, evolutionary forces determining the population genetic structure of WYMV are unknown.

The aims of this study were to determine the evolutionary forces defining the population genetic structure of WYMV in China based on the CP- and VPg-coding regions of RNA 1 (Fig. 1). These genes were selected because their roles in the bymovirus lifecycle are likely to make them evolutionarily important determinants of the biological and genetic variability of virus populations. The CP is probably involved in bymovirus interactions with the host plant, cell-to-cell movement, and vector transmission (54), whereas the VPg is attached to the 5′ end of RNA during RNA synthesis and is involved in determining pathogenicity (29,35,81). The results provide evidence of a geographically structured population of WYMV in China and limited genetic variation in WYMV as a consequence of founder effects and purifying selection.

MATERIALS AND METHODS

Collection of samples. Isolates of WYMV characterized and used in this study were obtained from wheat plants with typical yellow mosaic virus symptoms that were tested by enzyme-linked immunosorbent assay as described previously (15) to confirm WYMV infection. The isolates were obtained from widely dispersed sites throughout the known geographic range of WYMV in China (Fig. 2). There were 12 sampling events (Table 1). Four of these used plants growing in experimental plots that had been established with WYMV-infested soil at the Zhejiang Academy of Agricultural Sciences (ZAAS), Hangzhou, China. Soil samples were collected from WYMV-infested fields in Yangzhou, Yantai,
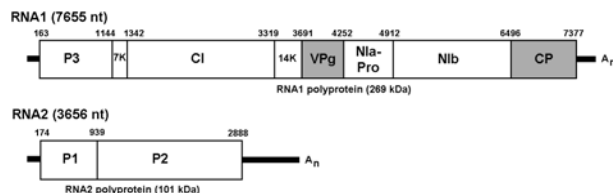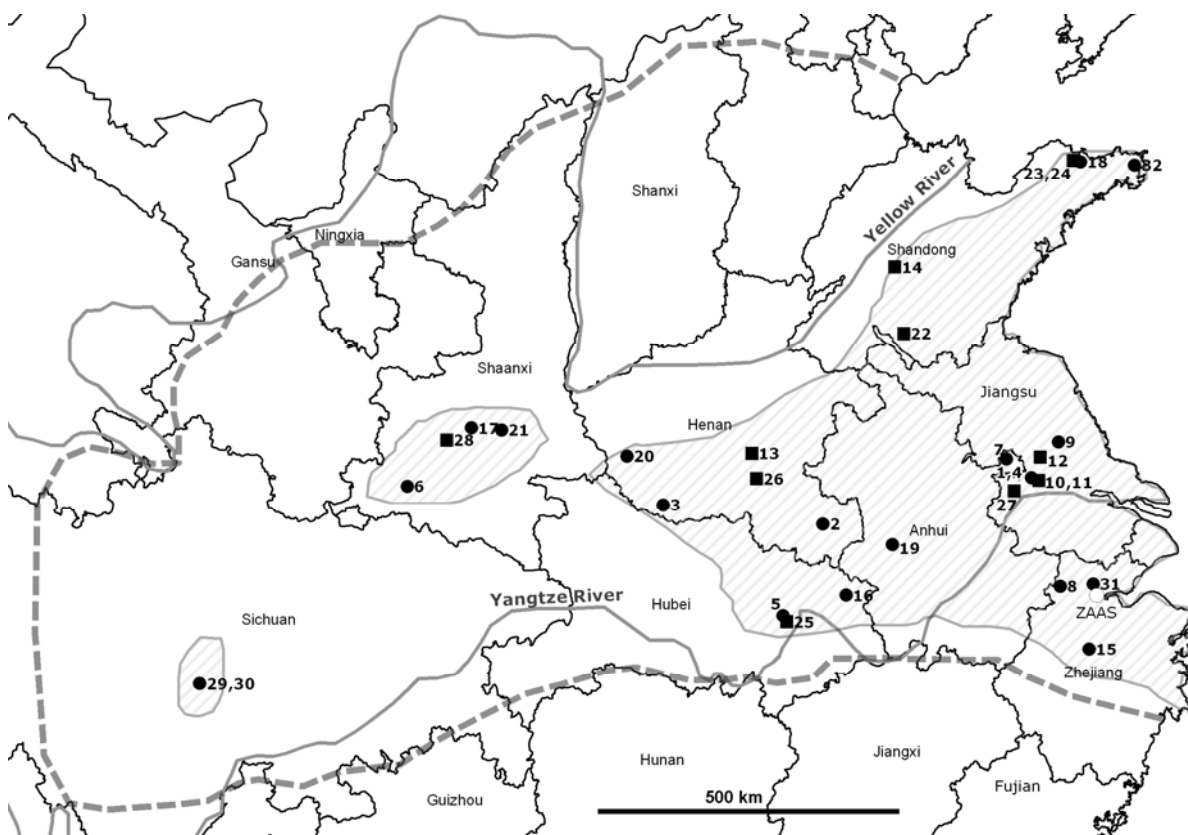
Fig. 1. Genome organization of *Wheat yellow mosaic virus*. A single polyprotein is translated from each RNA and processed into the functional proteins indicated. Nucleotide positions of the start of each cistron and of the 3′ untranslated region are indicated. Shading indicates the genome-linked virion protein (VPg) and coat protein (CP) regions that were used for this study.

Fig. 2. Map showing the location of wheat samples taken within the known distribution area of *Wheat yellow mosaic virus* (WYMV) in China. The dashed line shows the extent of winter wheat production and the shaded areas those where WYMV occurs. Samples taken for this study are represented by squares. Sources of coat protein gene sequences examined in an earlier study (14) are shown as circles and the accession numbers are provided here. 1: Yangzhou, Jiangsu, AJ131981; 2: Huangchuan, Henan, AJ239038; 3: Dengzhou, Henan, AJ240048; 4: Yangzhou, Jiangsu, AJ240051; 5: Wuhan, Hubei, AJ243981; 6: Hanzhong, Shaanxi, AJ243982; 7: Tianchang, Anhui, AJ243985; 8: Anji, Zhejiang, AJ243986; 9: Xinghua, Jiangsu, AJ243987; 10: Yangzhou, Jiangsu (JS1); 11: Yangzhou, Jiangsu (JS2); 12: Gaoyou, Jiangsu (JS3); 13: Xiping, Henan (HN2); 14: Taian, Shandong (SD1); 15: Pujiang, Zhejiang, AJ130983; 16: Luotian, Hubei, AJ240049; 17: Zhouzhi, Shaanxi, AJ240052; 18: Yantai, Shandong, AJ243953; 19: Lu'an, Anhui, AJ243984; 20: Xixia, Shanxi, AJ243988s; 21: Chang'an, Shaanxi, AJ243989; 22: Tengzhou, Shandong (SD2); 23: Yantai, Shandong (SD3); 24: Yantai, Shandong (SD4); 25: Wuhan, Hubei (HB3); 26: Zhumadian, Henan (HN1); 27: Yizheng, Jiangsu (JS7); 28: Zhouzhi, Shaanxi (SX2); 29: Yaan, Sichuan, AJ239039; 30: Yaan, Sichuan, AJ240050; 31: Hangzhou, Zhejiang, AJ243983; 32: Rongcheng, Shandong, AJ271840.

and Xiping in the Chinese provinces of Jiangsu, Shandong, and Henan, respectively, during spring 2008. Soil (≈250 kg) obtained from each field and used to establish experimental plots (8 m²) at ZAAS. A WYMV-susceptible wheat cultivar ('Yangmai 158') was sown in these plots in November 2008 and observed for yellow mosaic symptoms ≈3 months after sowing. In total, 20 to 30 individual plants were sampled at random from each plot. An additional sample was taken from the Yangzhou plot in April 2009. The remaining eight samples were taken directly from commercial fields distributed over five provinces that had previously been identified as WYMV infested (26,62). Leaf samples were collected from 20 to 30 randomly selected plants from each of the eight fields in spring 2009.

RNA extraction, reverse-transcription polymerase chain reaction, cloning, and sequencing. Total RNA was extracted from a pool of 10 randomly selected infected leaves (100 mg) from each sample by the Trizol method (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized from two independent RNA samples using M-MLV reverse transcriptase (Fermentas Ltd., Shanghai, China) according to the manufacturer's instructions, using an Oligo(dT)$_{18}$ primer. To amplify the entire CP (882 nucleotides [nt]) or VPg (561 nt) coding region, forward and reverse primers were designed based on the nucleotide sequence of RNA 1 of the WYMV Yangzhou isolate (accession number AJ131981). For the CP, the primers were CP-1, forward (5′-GAAGATCTACCATGGCAGCTGACACACAAACAG-3′) and CP-2, reverse (5′-ATTTGCGGCCGCTTAGGTTAGTTCTGGG TGTCC-3′); whereas, for the VPg, they were VPg-1, forward (5′-GAAGATCTACCATGGGAAAGGGAAACAAGTATCACC-3′), and VPg-2, reverse (5′-ATTTGCGGCCGCTTATTCAAGCAT GACTTCATCC-3′). The polymerase chain reaction (PCR) amplification mixture containing 2 µl of template cDNA was prepared as described for TransEco FastPfu DNA Polymerase (TransGen Biotech Co., Ltd., Beijing). The cDNA was first denatured at 94°C for 2 min; then amplified through 30 cycles of 94°C for 20 s, 56°C for 20 s, and 72°C for 1 min; with a final incubation at 72°C for 10 min. These cycling parameters were the same for both CP and VPg.

PCR products were purified by using a Sigma PCR extraction kit (Sigma-Aldrich, St. Louis), ligated into the pEASY-Blunt vector (TransGen Biotech Co.), and transformed into *Escherichia coli* strain DH5α. The sequencing of clones was carried out at the Shanghai Invitrogen Biotech Company using an ABI3100 DNA sequencer. At least three independent PCR reactions were run for each sample and at least 10 clones from each PCR product were sequenced. CP sequences were determined from all samples but VPg sequences were determined only from the ZAAS plots (Table 1).

Multiple sequence alignments and fitting the best nucleotide substitution model. Nucleotide sequences were aligned using CLUSTALX version 1.83 (66), examined visually, and translated into amino acid sequences using the EMBOSS web translation tool (http://www.ebi.ac.uk/emboss/transeq/index.html?). Percent nucleotide and amino acid identities between sequences were computed using the CLUSTALW procedure (67) as implemented in MEGALIGN program of the DNASTAR software package (DNASTAR Inc., Madison WI).

A maximum likelihood (ML) method implemented in MEGA5 (65) was used to find the best nucleotide substitution model explaining the mode of evolution. Models with the lowest Bayesian information criterion scores were considered to describe the substitution pattern the best.

Analysis of phylogenetic signal and test for recombination. Suitability of the nucleotide sequence data for molecular phylogenetic analyses was assessed by analysis of substitution saturation using the method of Xia et al. (75) as implemented in DAMBE, version 5.0.0.23. A plot of the transition and transversion rates (as estimated with DAMBE) versus the divergence based on the Kimura two-parameter nucleotide substitution model of pairwise comparisons between sequences (31) offered a visual display of substitution saturation.

Presence of recombination in the sequence data was tested using the pairwise homoplasy test (PHI) (10) as implemented in SPLITSTREE4 version 4.10. Parent-like sequences and approximation of recombination breakpoints were assessed using the methods RDP, GENECONV, BOOTSCAN, MAXIMUM CHI-SQUARE, CHIMAERA, and SISTER SCAN as implemented in the Recombination Detection Program (RDP3) package (43).

Analysis of phylogenetic relationships: nucleotide diversity and genetic differentiation. Phylogenetic trees based on the CP were constructed using the ML method and the Kimura two-parameter (K2) nucleotide substitution model (31) and a discrete gamma distribution (five categories; +G parameter = 0.4332) to model evolutionary rate differences among sites. For the VPg sequences, the Hasegawa-Kishino-Yano (HKY) nucleotide substitution model (27) was used. The bootstraped consensus tree was inferred from 500 and 1,000 replicates for the CP and VPg, respectively. Only the unique sequences (107 for the CP and 30 for the VPg) were considered for phylogenetic analysis. All phylogenetic analyses were implemented using MEGA5 (65).

For each of the CP (322 sequences) and VPg (94 sequences) regions, nucleotide diversity (π) was first calculated using a 100-nucleotide (nt) sliding window with 25-nt steps. Population genetics parameters with respect to the average number of nucleotide differences between two random sequences in a population (or nucleotide diversity index, π), the average number of nucleotide substitutions per nonsynonymous (π$_a$) and synonymous (π$_s$) sites, the statistic (θ) from the number of segregating sites (*S*), and the proportion of nonsynonymous mutations were computed. Synonymous codon usage bias was measured by quantifying the codon bias index (CBI) (9) and "effective" number of codons (ENC) (74) that are used in a gene. The value of CBI ranges from 0.0 (in

TABLE 1. Origins of isolates of *Wheat yellow mosaic virus* used in this study

Sample code	Total[a]	Plants sampled[b]	Origin: province in China
JS1	30*	Plot at ZAAS 'Yangmai 158' in February 2009	Soil from Yangzhou, Jiangsu
SD3	30*	Plot at ZAAS Yangmai 158 in February 2009	Soil from Yantai, Shandong
HN2	30*	Plot at ZAAS Yangmai 158 in February 2009	Soil from Xiping, Henan
HB3	32	Field plants 'Zengmai 88' in March 2009	Wuhan, Hubei
HN1	22	Field plants 'Fengkang 38' in March 2009	Zhumadian, Henan
JS3	29	Field plants Yangmai 14 in March 2009	Gaoyou, Jiangsu
JS7	13	Field plants Yangmai 158 in March 2009	Yizheng, Jiangsu
SD1	29	Field plants 'Taishan 23' in March 2009	Taian, Shandong
SD2	18	Field plants 'Linmai 4' in March 2009	Tengzhou, Shandong
SX2	22	Field plants 'Xinong 979' in March 2009	Zhouzhi, Shaanxi
SD4	10	Field plants 'Yanong 22' in April 2009	Yantai, Shandong
JS2	32	Same as JS1 but sampled in April 2009	Soil from Yangzhou, Jiangsu

[a] Total numbers of clones sequenced; asterisk (*) indicates coat protein (CP) and genome-linked virion protein (VPg) samples; others only CP.
[b] ZAAS = Zhejiang Academy of Agricultural Sciences, Hangzhou, China.

a gene with random codon usage) to 1.0 (in a gene with extreme codon bias). For the nuclear universal genetic code, the value of ENC ranges from 20 (if only one codon is used for each amino acid [i.e., the codon bias is maximum]) to 61 (if all synonymous codons for each amino acid are equally used [i.e., no codon bias]).

The extent of genetic differentiation or level of gene flow between subpopulations was evaluated by estimating F_{ST}. F_{ST} measures the degree of genetic differentiation between two putative subpopulations by comparing the agreement between two haplotypes drawn at random from each subpopulation with the agreement obtained when the haplotypes are taken from the same subpopulation. F_{ST} ranges from 0 to 1 for undifferentiated to fully differentiated populations, respectively, with $F_{ST} > 0.33$, suggesting infrequent gene flow (46,56). Population genetics parameters and gene flow estimates were calculated using DnaSP version 5 (39).

Analysis of selection pressures. The ratio of nonsynonymous (d_N) to synonymous (d_S) nucleotide substitution rate ($\omega = d_N/d_S$) provides a sensitive measure of selective constraints at the protein level. Values of $\omega < 1$, $\omega = 1$, and $\omega > 1$ indicate purifying (or negative) selection, neutral evolution, and diversifying (or positive) selection, respectively, according to which the direction and intensity of selection pressure on a functional protein can be predicted (25,47). The ML approach was applied on the CP (73 sequences with <97% nucleotide identity) and VPg (30 sequences used in phylogenetic analysis) of WYMV using six site models of codon evolution implemented in the CODEML program of the PAML4 package (77). The models employed include M0 (one-ratio), M1a (nearly neutral), M2a (positive selection), M3 (discrete), M7 (β), and M8 (β and ω) as described (73,78,79). Under the basic assumption of a codon as a unit of evolutionary change (25), site models treat ω for any codon in a protein-encoding nucleotide sequence as a random variable from a statistical distribution. The probability of observing data was computed as the log likelihood, which is the sum of probabilities over all codons in the sequence. Selection pressure was examined by assessing the value ω and comparing the log likelihoods of nested models (M0 versus M3, M1a versus M2a, and M7 versus M8) in likelihood ratio tests (LRTs), as described (73). Where LRTs were significant, a Bayes empirical Bayes (BEB) inference (79) was used to identify the amino acids under positive selection.

RESULTS

Nucleotide and amino acid differences in the CP and VPg of WYMV isolates. This study used 322 CP sequences (297 from this study and 25 previously published sequences from GenBank) and 94 VPg sequences (87 from this study and 7 from GenBank). The sequences determined in this study had 96.4 to 100% (CP) and 97.0 to 100% (VPg) nucleotide identity. They were submitted to the GenBank under accession numbers FR827946 to FR828239 (CP) and FR828240 to FR828326 (VPg).

All of the sequences of the CP and VPg characterized under this study were 879 and 561 nt long, respectively, translating into 293 and 187 amino acid residues. The Lys88-Leu101 central domain of the bymovirus VPg (50) was observed in all WYMV VPg sequences. However, the hydrophobic β-sheet between the RNA-bound Tyr-63 (49) and the Asp-77, associated with eIF4E-binding in the potyvirus VPg (37,55) was absent from WYMV VPg sequences.

There were no consistent amino acid sequence differences in the VPg associated with the provenance of the WYMV isolates. However, consistent and unique amino acid substitutions depending on the provenance of the isolates were identified in the CP. These substitutions were at nine positions (112, 115, 118, 146, 159, 174, 177, 211, and 264) located in the central and C-terminal regions of the CP (Table 2). For example, SX2 samples (from Shaanxi) had unique amino acid substitutions of Ala146Thr, Asn173Asp, and Ala177Val (Table 2); Ala146Thr and Ala177Val substitutions were also observed in two previously characterized isolates (accession numbers AJ240052 and AJ243989) from the same province (14). Similar observations apply to most of the other amino acid positions (see above) along the CP alignment (Table 2).

Although the amino acid substitutions are unique for isolates according to provenance, the amino acids involved for a given position are chemically similar, indicating a preservation of WYMV CP functional integrity. For example, Ile and Val (positions 112 and 118); Ala and Thr (position 146); and Ala, Thr, and Val (position 177) are hydrophobic, whereas Asp and Asn (positions 115, and 173) are polar residues (Table 2). Taken together, these observations suggest a population genetic structure that is dependent on provenance.

Phylogenetic signal and tests for recombination. The possibility that sequences in the two data-sets of the CP (322 sequences) and VPg (94 sequences) had lost phylogenetic information due to "substitution saturation" was investigated first before conducting phylogenetic estimations. No saturation was observed when transitions (s) and transversions (v) were plotted against evolutionary distance (Supplemental Figure 1) because, in all cases, the plots show linear increase of s and v with increasing divergence. Comparison of observed and critical substitution saturation indexes also showed no statistically significant evidence for substitution saturation (data not shown). Hence, the data contained enough phylogenetic information for testing appropriate evolutionary hypotheses.

No evidence for recombination was detected in the data when subjected to analysis using six programs included in the RDP3 package, and using the PHI ($P > 0.05$).

Phylogenetic relationships among WYMV isolates. Nucleotide substitution model fitting showed that K2 (31) with a discrete γ distribution (+G; K2+G) was the most suitable model for the CP data, whereas for the VPg it was the HKY model (27) (see above). Using these nucleotide substitution models, phylogenetic clustering of the 30 VPg sequences that were <100% identical showed phylogenetic congruence with their provenance (Fig. 3). Clustering of the 107 unique CP sequences also showed a relationship with their provenance (Fig. 4) but there were a few exceptions. For example, SD1-8, SD1-21, SD1-29, and SD2-9 (from Shandong), JS1-17 and JS1-28 (from Jiangsu), and HN1-29 (from

TABLE 2. Consensus amino acids at nine positions in the *Wheat yellow mosaic virus* coat protein amino acid sequence alignment for the virus isolates from various sampling areas in China

Position	JS1	JS2	JS3	JS7	SD1	SD2	SD3	SD4	HN1	HN2	HB3	SX2
112	I	I	I	I	I	V	V	V	V	I	V	V
115	N	N	N	N	N	D	D	D	D	N	D	D
118	I	I	I	I	I	I	V	V	V	I	I	V
146	A	A	A	A	A	A	A	A	A	A	A	T
159	A	A	A	A	A	S	A	A	A	A	A	A
173	N	N	N	N	N	N	N	N	N	N	N	D
177	A	A	T	T	A	A	A	A	A	A	A	V
211	L	L	I	I	L	L	L	L	L	L	L	L
264	H/Y	H/Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Henan) grouped with isolates from Henan, Shandong, and Jiangsu, respectively (Fig. 4; Table 1). Similar tree topologies were obtained using the neighbor-joining tree-building algorithm (57) (data not shown). These observations further suggest a spatial structuration of the WYMV population in China.

WYMV population structure in China. Analysis of genetic differentiation between WYMV subpopulations from the various provinces of China was then carried out for both VPg- and CP-encoding sequences. The F_{ST} values for the VPg (0.7981), and CP (0.6241) showed strong evidence of population genetic differentiation, implying that 79.8% (VPg) and 64.4% (CP) of total variance of the WYMV population is explained by the provenance of isolates. Based on the CP, the level of genetic differentiation was striking and highest between subpopulations SX2 and JS7 (F_{ST} = 0.959; spatially separated by 970 km) and lowest between subpopulations SD3 and SD4 (F_{ST} = 0.187; with a negligible spatial separation) (Table 3). Comparisons between subpopulations showed a high degree of genetic differentiation (F_{ST} values of 0.336 to 0.959) for nearly all subpopulations (Table 3). Only three subpopulation comparisons (SD3 versus SD4, F_{ST} = 0.187, negligible spatial separation; SD1 versus HN2, F_{ST} 0.209, spatially separated by ≈400 km; and HN1 versus SD3, F_{ST} = 0.223, spatially separated by ≈770 km) showed no evidence of genetic differentiation (Table 3). There was little evidence that the level of genetic differentiation is correlated with the distance between the sampling sites (Supplemental Figure 2).

Between-population diversity was usually larger than within-population diversity for both the VPg and CP (Tables 4 and 5), further implicating a differentiated population. For example, the subpopulation from Hubei (HB3) has a within-population diversity of 0.00139 ± 0.00033 but between-population diversities with other subpopulations were 4 to 10 times higher (Tables 4 and 5). Despite a few exceptions, phylogenetic clustering of isolates (Figs. 3 and 4), gene flow estimates of F_{ST} (Table 3), and within- and between-population diversity indices (Tables 4 and 5) all demonstrate genetic differentiation of WYMV in China.

Evolutionary processes affecting the CP and VPg of WYMV. Because of the small size and high replication speeds of viral RNA genomes, even synonymous mutations might be subjected to selective constraints due to codon usage bias or RNA structure. Hence, to provide insight into the evolutionary processes affecting VPg and CP genomic regions of WYMV, different population genetics parameters of WYMV subpopulations were examined. The nucleotide diversity (π) index and the statistic θ (S) from the number of segregating sites showed that genetic variation within the subpopulations was generally low. For the CP, values of π ranged from 0.00035 ± 0.00019 (JS7 subpopulation, S = 0.00073) to 0.01536 ± 0.00043 (SX2 subpopulation; S = 0.03375) (Table 6). For the VPg, they were between 0.00086 ± 0.00039 (HN2 subpopulation; S = 0.00318) and 0.00573 ± 0.00111 (JS1 subpopulation; S = 0.00908) (Table 6). Subsequently, the overall nucleotide diversities were 0.01536 ± 0.00043 and 0.00925 ± 0.00046 for the CP and VPg, respectively. Interestingly, the levels of nucleotide diversity along the studied genomic regions were not random: the highest diversity in the CP (≤3.75%) was observed in the N-terminal region (nucleotide positions 350 to 450) (Supplemental Figure 3), whereas diversity of ≤2.40% was observed in the C-terminal region (nucleotide positions 350 to 450) of the VPg, implying that different regions of a coding sequence may be subjected to different constraints.

There were no nonsynonymous mutations observed in the CP of subpopulations JS7 (n = 13) and SD4 (n = 10) (Table 6), possibly because of the small sample size. However, less than one-half (27.3 to 45.3%) of the mutations in the CP of most other subpopulations were nonsynonymous, and this percentage was higher for HN2 (52.8%) and HB3 (84.6%) subpopulations (Table 6), suggesting that HN2 and HB3 subpopulations were more flexible with regard to amino acid changes in the CP. Similar observations apply to the VPg, with nonsynonymous mutations constituting 28.6, 40.0, and 71.4% in the HN2, JS1, and SD3 subpopulations, respectively (Table 6).

Synonymous codon usage bias evaluated by the ENC showed that the values for the VPg (47.4 to 50.6) were lower than for the CP (52.0 to 54.4) (Table 6), suggesting that, although the coding regions have a "moderate" bias in codon usage (less than the 61 at no codon bias), the VPg has slightly more codon bias than the CP. This is consistent with the slightly smaller values of CBI (0.258 to 0.289) for the CP than for the VPg (0.360 to 0.378) (Table 6), suggesting a slightly more random codon usage in the CP than the VPg.

Evolutionary selection pressure imposed on the CP and VPg of WYMV. To further understand the evolutionary constraints imposed on the CP and VPg coding regions, the ratio of nonsynonymous diversity (π_a) to synonymous diversity (π_s) was estimated. The π_a/π_s ratios were <1.0, indicating that the CP and VPg were both subjected to negative selection and are strongly conserved (Table 6). The π_a/π_s ratio of some subpopulations (e.g., HN2, SD3 [VPg], HN2, JS3, SD1, and SD2 [CP]) are up to nine times higher than others (Table 6), suggesting that the VPg and CP of these subpopulations are considerably more tolerant to amino acid changes. An exception was the CP of HB3 subpopulations, with a π_a/π_s ratio of 2.724, which is also reflected in a high percentage (84.7%) of nonsynonymous mutations (Table 6), suggesting positive selection (adaptive evolution) of this subpopulation.

To compare in detail the selective constraints across the VPg and CP coding regions, a more explicit ML analysis of selection pressures on codons of the CP yielded log likelihood values of –3,117.495, –3,103.397, –3,102.279, –3,101.317, –3,103.675, and –3,101.456, under models M0, M1a, M2a, M3, M7, and M8, respectively (Table 7). For the VPg, the log likelihood values were –1,301.383 (M0), –1,301.264 (M1a), –1,301.253 (M2a), –1,301.256 (M3), –1,301.362 (M7), and –1,301.266 (M8) (Table 7). The estimated values of ω for the CP (≤0.261) and VPg (≤0.256) under all the six models were consistently low, suggesting strong purifying selective constraints on all 293 and 187 encoded amino acids of the CP and VPg, respectively. Indeed, parameter estimates under the different models showed most codons to be under purifying selection or nearly neutral evolution in both the CP and the VPg (Table 7).

Fig. 3. Maximum likelihood phylogenetic analysis of 30 *Wheat yellow mosaic virus* genome-linked virion protein (VPg)-encoding nucleotide sequences. The provenance of the sequences (sampling site: province in China) from where they were obtained are indicated on the right. Names of each of seven isolates characterized in previous studies are preceded by a black triangle. Numbers at branches represent bootstrap values of 1,000 replicates. Only bootstrap values ≥50% are shown. Scale indicates units in nucleotide substitutions per site.

LRTs comparing the log likelihoods of M0 versus M3, M1a versus M2a, and M7 versus M8 on the VPg were not significant, which further implicates homogeneous purifying selective constraints on the encoded VPg amino acids (data not shown). Therefore, the deduced weak positive selection for a VPg amino acid residue 47K under M2a, M3, and M8 ($\omega \le 1.505$) (Table 7) is quite an unlikely event in the evolutionary history of WYMV, because the nested log likelihood values are nearly identical and their LRTs are nonsignificant (Table 7). However, for the CP, the LRT of M0 versus M3 was significant ($P < 0.0001$), which suggested heterogeneous selection pressures, with 55.4% of the codons experiencing strong purifying selection ($\omega = 0.018$), 43.9% experiencing weak purifying selection ($\omega = 0.497$), and only a tiny proportion (0.66%) under positive selection ($\omega = 3.387$). Naïve empirical Bayes inference (79) implemented under M3 deduced amino acid 115D to be under positive selection ($\omega =$
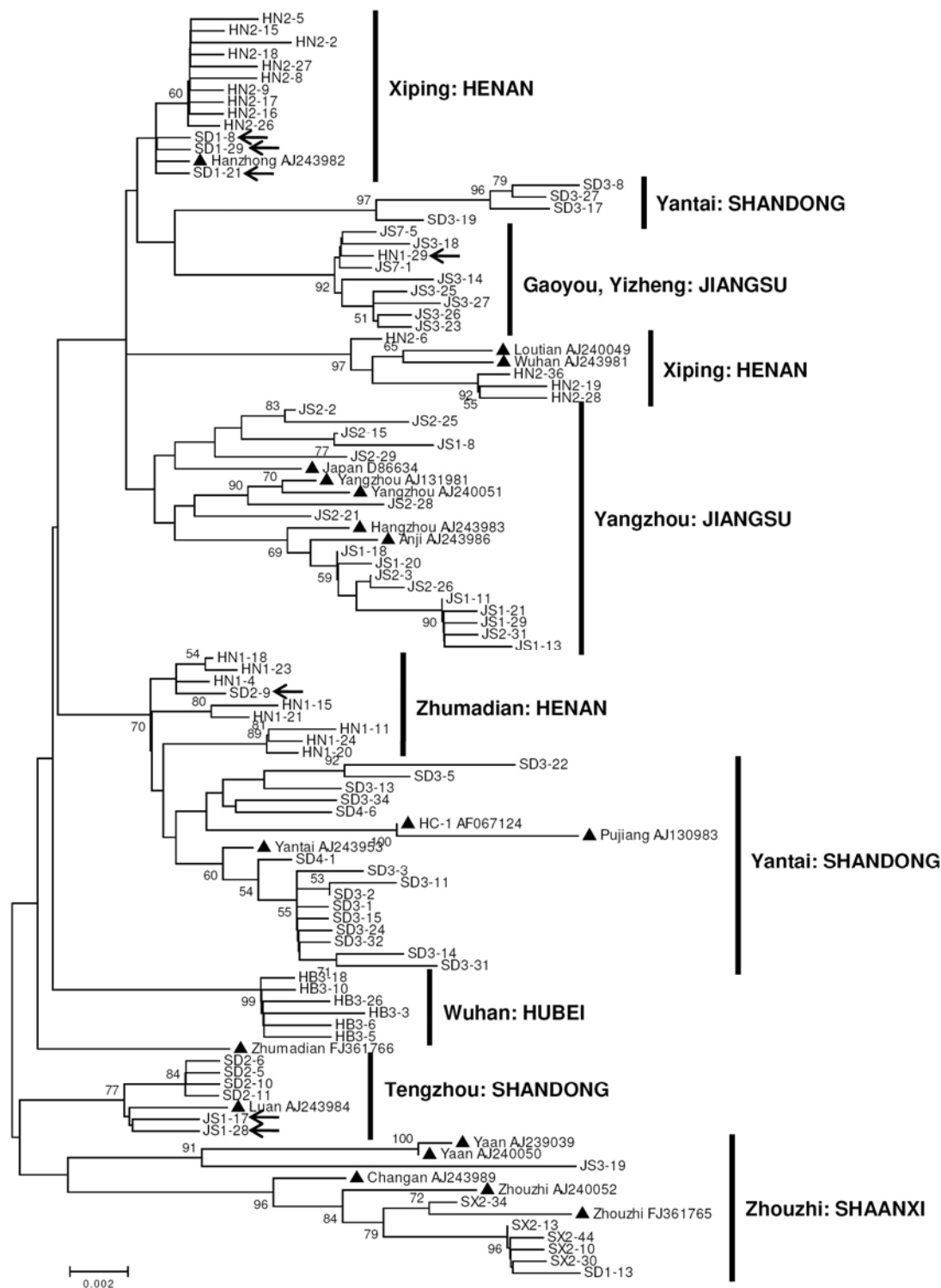

Fig. 4. Maximum likelihood phylogenetic analysis of 107 *Wheat yellow mosaic virus* coat protein (CP)-encoding nucleotide sequences. The provenance of the sequences from this study (sampling site: province in China) from where they were obtained are indicated on the right. Eight sequences (indicated by arrows) from various provinces grouped with others from different provinces. Names of each of 18 isolates characterized in previous studies are preceded by a black triangle. Numbers at branches represent bootstrap values of 500 replicates. Only bootstrap values ≥50% are shown. Scale indicates units in nucleotide substitutions per site.

3.375), with a 99.6% posterior probability (Table 7). Furthermore, although the LRTs of M1a versus M2a and M7 versus M8 were not significant, BEB inference implemented under M2a and M8 also deduced amino acid 115D and two additional amino acids (146A and 177A) of the CP of WYMV to be under positive selection (Table 7). In particular, positive selection for 115D is significant, with associated high posterior probabilities of 89.3% (M2a), 96.0% (M8), and 99.6% (M3). It is noteworthy that the amino acid positions predicted to be experiencing positive selection contain unique amino acid substitutions in the various groups of WYMV isolates from various sampling sites (see above).

Taken together, the amino acids of WYMV VPg and CP are largely (or exclusively) under purifying selection, while a tiny proportion of the CP codons are undergoing positive selection.

DISCUSSION

This article reports the first comprehensive analysis of the genetic variability, population genetic structure, and forces driving the evolution of WYMV in China. Although recombination is a major force driving the evolution of several groups of plant RNA viruses, including members of family *Potyviridae*

TABLE 3. Measure of genetic differentiation (F_{ST}) (based on the coat protein (CP) nucleotide sequences) between subpopulations of *Wheat yellow mosaic virus* isolates collected from different sites spatially separated from each other (distance in kilometers) in China[a]

	SD3	HN2	HB3	HN1	JS3	JS7	SD1	SD2	SX2	SD4
JS1/JS2	0.401 (550)	0.394 (490)	0.700 (490)	0.516 (480)	0.595 (30)	0.627 (50)	0.448 (430)	0.694 (340)	0.801 (1,010)	0.608 (550)
SD3	…	0.369 (740)	0.579 (930)	0.223 (770)	0.583 (510)	0.609 (570)	0.404 (350)	0.514 (410)	0.700 (1,180)	0.187 (0)
HN2	…	…	0.707 (300)	0.477 (40)	0.625 (490)	0.670 (450)	0.209 (400)	0.696 (330)	0.817 (520)	0.595 (740)
HB3	…	…	…	0.720 (260)	0.891 (520)	0.948 (450)	0.846 (630)	0.868 (530)	0.936 (670)	0.803 (930)
HN1	…	…	…	…	0.716 (490)	0.762 (440)	0.548 (440)	0.652 (350)	0.815 (540)	0.336 (770)
JS3	…	…	…	…	…	0.415 (80)	0.766 (410)	0.871 (320)	0.919 (1,010)	0.805 (510)
JS7	…	…	…	…	…	…	0.859 (440)	0.926 (330)	0.959 (970)	0.852 (570)
SD1	…	…	…	…	…	…	…	0.817 (110)	0.905 (820)	0.699 (350)
SD2	…	…	…	…	…	…	…	…	0.906 (810)	0.748 (410)
SX2	…	…	…	…	…	…	…	…	…	0.861 (1,180)

[a] Distances to the nearest 10 km, estimated from a large-scale map. With the exception of SD3 versus SD4, SD1 versus HN2, and HN1 versus SD3 comparisons, whose F_{ST} values were 0.187, 0.209, and 0.223, respectively, indicating no genetic differentiation, all other subpopulations were genetically differentiated from each other ($F_{ST} > 0.33$).

TABLE 4. Within- and between-population nucleotide diversities in the genome-linked virion protein (VPg) of *Wheat yellow mosaic virus* isolates collected from different regions of China[a]

Subpopulation	HN1 ($n = 29$)	SD3 ($n = 29$)	JS1 ($n = 29$)
HN1 ($n = 29$)	**0.00086 (±0.00039)**	0.00727 (±0.00029)	0.01000 (±0.00064)
SD3 ($n = 29$)	…	**0.00097 (±0.00027)**	0.00553 (±0.00060)[b]
JS1 ($n = 29$)	…	…	**0.00573 (±0.00111)**

[a] Values in parenthesis are standard errors. Values indicated in bold represent the within-population nucleotide diversities, most of which are lower than between-population nucleotide diversities.
[b] Values of between-population nucleotide diversities that are slightly lower than within-population nucleotide diversity.

TABLE 5. Within- and between-population nucleotide diversities in the coat protein (CP) of *Wheat yellow mosaic virus* isolates collected from different regions of China[a]

Sub[b]	HB3 ($n = 32$)	SX2 ($n = 22$)	HN1 ($n = 22$)	HN2 ($n = 30$)	JS1 ($n = 30$)	JS2 ($n = 32$)	JS3 ($n = 29$)	JS7 ($n = 13$)	SD1 ($n = 29$)	SD2 ($n = 18$)	SD3 ($n = 30$)	SD4 ($n = 10$)
HB3 ($n = 32$)	**0.00139 (± 0.00033)**	0.01332 (± 0.00073)	0.00853 (± 0.00070)	0.00894 (± 0.00081)	0.01225 (± 0.00068)	0.01255 (± 0.00060)	0.01034 (± 0.00064)	0.00779 (± 0.00090)	0.00617 (± 0.00067)	0.00730 (± 0.00061)	0.01271 (± 0.00100)[c]	0.00685 (± 0.00112)
SX2 ($n = 22$)	…	**0.00190 (± 0.00062)**	0.01378 (± 0.00064)	0.01419 (± 0.00074)	0.01799 (± 0.00048)	0.01836 (± 0.00041)	0.01540 (± 0.00081)	0.01389 (± 0.00112)	0.01087 (± 0.00049)	0.01214 (± 0.00059)	0.01785 (± 0.00080)	0.01201 (± 0.00159)
HN1 ($n = 22$)	…	…	**0.00658 (± 0.00104)**	0.00965 (± 0.00088)	0.01292 (± 0.00054)	0.01282 (± 0.00041)	0.0117 (± 0.00091)	0.00962 (± 0.00042)	0.01536 (± 0.00043)	0.00881 (± 0.00055)	0.01220 (± 0.00107)[c]	0.00730 (± 0.00072)
HN2 ($n = 30$)	…	…	…	**0.00668 (± 0.00136)**	0.01106 (± 0.00079)	0.01102 (± 0.00072)	0.00865 (± 0.00093)	0.00786 (± 0.00093)	0.00489 (± 0.00111)[c]	0.00990 (± 0.00091)	0.01328 (± 0.00097)[c]	0.00944 (± 0.00112)
JS1 ($n = 30$)	…	…	…	…	**0.00995 (± 0.00144)**	0.01016 (± 0.00090)	0.01108 (± 0.00091)	0.01080 (± 0.00085)	0.00852 (± 0.00094)[c]	0.01290 (± 0.00047)	0.01591 (± 0.00065)	0.01306 (± 0.00091)
JS2 ($n = 32$)	…	…	…	…	…	**0.00989 (± 0.00088)**	0.01131 (± 0.00077)	0.01091 (± 0.00054)	0.00852 (± 0.00088)[c]	0.01362 (± 0.00035)	0.01608 (± 0.00061)	0.01300 (± 0.00069)
JS3 ($n = 29$)	…	…	…	…	…	…	**0.00265 (± 0.00158)**	0.00240 (± 0.00112)[c]	0.00603 (± 0.00096)	0.01045 (± 0.00095)	0.01416 (± 0.00099)	0.00919 (± 0.00158)
JS7 ($n = 13$)	…	…	…	…	…	…	…	**0.00035 (± 0.00019)**	0.00439 (± 0.00105)	0.00959 (± 0.00059)	0.01448 (± 0.00081)	0.00987 (± 0.00096)
SD1 ($n = 29$)	…	…	…	…	…	…	…	…	**0.00188 (± 0.00143)**	0.00645 (± 0.00086)	0.01063 (± 0.00110)[c]	0.00565 (± 0.00124)
SD2 ($n = 18$)	…	…	…	…	…	…	…	…	…	**0.00223 (± 0.00118)**	0.01351 (± 0.00093)[c]	0.00809 (± 0.00089)
SD3 ($n = 30$)	…	…	…	…	…	…	…	…	…	…	**0.01379 (± 0.00148)**	0.01236 (± 0.00139)[c]
SD4 ($n = 10$)	…	…	…	…	…	…	…	…	…	…	…	**0.00478 (± 0.00086)**

[a] Values in parenthesis are standard errors. Values indicated in bold represent the within-population nucleotide diversities, most of which are lower than between-population nucleotide diversities.
[b] Subpopulation.
[c] Values of between-population nucleotide diversities that are slightly lower than within-population nucleotide diversity.

(12,64), it was not detected in the CP and VPg between any two isolates of WYMV from the same or different geographical regions in China. Lack of detectable recombination may be because (i) there are few opportunities for co-infection of plants by WYMV isolates from different regions in China, (ii) recombination occurs between two closely related isolates and is difficult to detect by methods employed here, or (iii) recombination occurs and the recombinants are selected against, although this seems unlikely because the isolates are probably not sufficiently divergent for recombinants to incur fitness deficits (19,36,44). In earlier studies, recombination was absent or rare between closely related sympatric populations of a soilborne multipartite RNA virus, *Beet necrotic yellow vein virus* (BNYVV, genus *Benyvirus*), based on the p25 encoding region, despite high potentials of mixed infection (33,58); however, later, extended analyses provided evidence for recombination in the CP-, p25-, and p31-encoding regions of BNYVV (16). It has been argued that recombination in RNA viruses may be a mechanistic byproduct of RNA polymerase processivity and not necessarily a trait that has been optimized by natural selection for its own selective value, although it may occasionally generate beneficial genotypes (60). It is possible that recombination in WYMV occurs in other genomic regions beyond the scope of this study.

Absence of recombination presupposes that the genetic variation and relationships based on the studied genomic regions could be best explained by a model of evolution dominated by mutations. Hence, the population exhibited overall low genetic diversity, averaging 0.01536 ± 0.00043 (CP) and 0.00925 ± 0.00046 (VPg). Similarly, low genetic diversity has been found in several other plant RNA viruses; for example, *Cucumber mosaic virus* (CMV) (40), *Cucurbit yellow stunting disorder virus* (42), *Citrus psorosis virus* (45), *Cucumber vein yellowing virus* (28), and *Squash vein yellowing virus* (72). Moreover, phylogenetic analysis revealed that WYMV isolates were closely related (Figs. 3 and 4). Together with the low genetic diversity, these data suggest that WYMV populations are highly genetically stable as a function of strong purifying (negative) selection, or that the population may have arisen relatively recently from founder effects or genetic bottleneck events. Bottlenecks reduce genetic variation of a population and may occur at different points during the life cycle of the virus (7,38). For example, genetic bottlenecks during systemic movement of *Wheat streak mosaic virus* within the plant (24), and horizontal transmission of CMV by vectors (6) constrain genetic variation in these viruses. Bottlenecks result in a limited number of randomly selected individuals creating a small founding population. It is plausible that bottlenecks during horizontal transmission of WYMV by a largely immobile vector (*P. graminis*) have constrained genetic variation of the WYMV population.

Interestingly, unique and consistent amino acid substitutions (fixed mutations) corresponding to the provenance of the isolates were observed at nine positions along the CP of WYMV, further suggesting founder effects. Therefore, singletons (mutations found solely in one clone of the entire subpopulation) accounted for most of the low diversity in the subpopulations, while fixed mutations accounted for the variation found between subpopulations. Although the fixed mutations may reflect the degree of subpopulation isolation, nearly all the amino acids at the substitution sites were chemically similar (presumably to preserve CP functional integrity), indicating that no particular selective advantage is possessed by one subpopulation over the other. Therefore, founder effects alone may not sufficiently account for the limited genetic variation observed.

Our analysis revealed an excess of synonymous over nonsynonymous diversity, indicating strong purifying (negative) selection as an additional mechanism constraining genetic variation, which was confirmed by more explicit models of codon evolution. Purifying selection on the VPg and CP is a common feature of functional proteins whose values of ω are mostly <1 and the majority of amino acids are largely invariable due to strong structural and functional constraints (32,59). Similar observations were

TABLE 6. Population genetics parameters based on the genome-linked virion protein (VPg) and coat protein (CP) of different subpopulations of *Wheat yellow mosaic virus* from China

Subpopulation[a]	Nucleotide diversity (π)[b]	S (θ per site)[c]	Nonsynonymous mutations (%)[d]	π_a/π_s Ratio[e]	ENC[f]	CBI[g]
VPg						
HN2 (*n* = 29)	0.00086 (± 0.00039)	0.00318	28.6 (2/7)	0.111 (0.00031/0.00282)	47.399	0.378
SD3 (*n* = 29)	0.00097 (± 0.00027)	0.00318	71.4 (5/7)	0.476 (0.00079/0.00165)	50.577	0.360
JS1 (*n* = 29)	0.00573 (± 0.00111)	0.00908	40.0 (8/20)	0.057 (0.00126/0.02178)	50.324	0.370
CP						
HB3 (*n* = 32)	0.00139 (± 0.00033)	0.00367	84.6 (11/13)	2.724 (0.00164/0.00060)	53.498	0.258
SX2 (*n* = 22)	0.00190 (± 0.00062)	0.03375	44.4 (4/9)	0.069 (0.00159/0.02257)	53.245	0.287
HN (*n* = 52)	**0.00965 (± 0.00088)**	**0.01636**	**40.0 (26/65)**	**0.138 (0.00393/0.02797)**	**53.127**	**0.282**
HN1 (*n* = 22)	0.00658 (± 0.00104)	0.01030	27.3 (9/33)	0.069 (0.00159/0.02257)	53.163	0.281
HN2 (*n* = 30)	0.00668 (± 0.00136)	0.01034	52.8 (19/36)	0.150 (0.00286/0.01891)	53.160	0.285
JS (*n* = 104)	**0.01135 (± 0.00056)**	**0.01636**	**41.3 (31/75)**	**0.086 (0.00328/0.03733)**	**53.436**	**0.282**
JS1 (*n* = 30)	0.00995 (± 0.00144)	0.01063	40.5 (15/35)	0.088 (0.00292/0.03258)	54.371	0.278
JS2 (*n* = 32)	0.00989 (± 0.00088)	0.00876	32.2 (10/31)	0.054 (0.00195/0.03546)	54.152	0.280
JS3 (*n* = 29)	0.00265 (± 0.00158)	0.00898	35.5 (11/31)	0.149 (0.00113/0.00755)	52.020	0.288
JS7 (*n* = 13)	0.00035 (± 0.00019)	0.00073	0.0 (0/2)	0.000 (0.00000/0.00148)	52.673	0.279
SD (*n* = 87)	**0.01140 (± 0.00072)**	**0.01807**	**43.7 (35/80)**	**0.108 (0.00391/0.03538)**	**52.714**	**0.286**
SD1 (*n* = 29)	0.00188 (± 0.00143)	0.00660	39.1 (9/23)	0.220 (0.00102/0.00463)	52.811	0.284
SD2 (*n* = 18)	0.00223 (± 0.00118)	0.00529	37.5 (6/16)	0.239 (0.00127/0.00530)	52.096	0.289
SD3 (*n* = 30)	0.01379 (± 0.00148)	0.01522	45.3 (24/53)	0.092 (0.00421/0.04445)	52.520	0.288
SD4 (*n* = 10)	0.00478 (± 0.00086)	0.00322	0.0 (0/8)	0.000 (0.00000/0.02005)	54.128	0.279

[a] Genomic region and subpopulations. Names of the subpopulations are according to provenance in China: HN = Henan, SD = Shandong, JS = Jiangsu, HB = Hubei, and SX = Shaanxi. For the CP, where the subpopulation is further subdivided according to collection sites (e.g., HN, JS, and SD subpopulations), data representing the overall subpopulation are indicated in bold.

[b] Average number of nucleotide differences between two random sequences in a population (π). Values in parentheses represent the standard deviation for the nucleotide diversity index.

[c] Statistic θ from the number of segregating sites (S).

[d] Percentage of total mutations that are nonsynonymous. Values in parentheses are the number of nonsynonymous mutations/total number of mutations.

[e] Values in parentheses are π_a/π_s (average number of nucleotide substitutions per nonsynonymous site/average number of nucleotide substitutions per synonymous site).

[f] Effective number of codons.

[g] Codon bias index.

previously made for the VPg of a potyvirus, *Sweet potato feathery mottle virus* (70). VPg is covalently linked to the 5′ end of the viral RNA and is required for potyvirus, sobemovirus, and bymovirus infectivity (53). Potyviral VPg can be an important virus strain-specific determinant of host response and symptom induction (18) and host-specific long-distance movement in plants (51,52). In contrast, positive selection in six and five codons within the central and C-terminal regions of the VPg of potyviruses *Potato virus Y* and *Potato virus A*, respectively, has been attributed to selective events in host plant adaptation (8,48). The VPg of bymoviruses is also known to interact with the eukaryotic translation initiation factor 4E (eIF4E) for infection (29,61,81). Consequently, the ability to overcome *rym4*-mediated resistance in European isolates of the bymovirus *Barley yellow mosaic virus* is due to substitution of the basic amino acid Lys-132 for the uncharged Asn-132, which possibly alters VPg's biochemical properties (35,81). Because WYMV is only known to infect wheat, purifying selection may be viewed as a mechanism for maintaining structural and functional integrity of the VPg in wheat hosts.

The molecular evolution of the WYMV CP seems to be parallel to that of the VPg, as suggested by similar molecular phylogenetic tree topologies and strong purifying selection. Selection pressures imposed on capsid genes of plant RNA viruses usually reflect the mode of transmission, in which the need to retain specific interactions with their vectors greatly constrains the genetic variability of virus populations (11). As such, positive selection has been documented in the CP of some potyviruses (70), with positively selected amino acids located in the CP N-terminal region known to be important in vector transmission, virus–host interactions, and several other functions (71). The role of the bymovirus CP in transmission by the plasmodiophorid vector is uncertain and remains a topic for future study. Although the CP appears not to be the sole determinant of plasmodiophorid vector transmission (4), some role seems intuitively likely and cannot be excluded. Therefore, the positive selection demonstrated on unique amino acid positions in the CP of WYMV may indicate evolving subpopulations of the virus in China, with potential to fix selectively advantageous genotypes and enhance species diversification in the future.

Diversification of Chinese WYMV populations in the future is, indeed, likely because there was phylogenetic congruence of isolates with their provenance (province) in China, suggesting a structured population of WYMV. This was further supported by a population genetics approach in which subpopulations were strongly genetically differentiated from each other, with limited gene flow. Although on a much wider geographical scale than in this study, geographical structuring in plant viruses has been reported for the global populations of many viruses, including the potyviruses *Turnip mosaic virus* (68,69) and *Sweet potato feathery mottle virus* (70). Certainly, the current geographical structuring observed in WYMV in China may not be explained by virus adaptation in response to new hosts or transmission modalities because there was no clear evidence of a correlation between positively selected codons (see above) and the provenance of the isolates, nor is there evidence that "geographical variants" differ in their biology.

The limited mobility of the vector (*P. graminis*) transmitting WYMV (2) implies that virus subpopulations in different provinces are effectively isolated from each other, which distinguishes WYMV (and other *P. graminis*-transmitted viruses) from viruses transmitted by highly itinerant vectors. Dispersal of the resting spores may occur through soil movement on farm machinery but usually is assumed to occur only over relatively short distances (2). However, although this argument may be reasonable for subpopulations that are widely spatially separated (e.g., ≥300 km), it may not sufficiently account for high genetic differentiation between subpopulations at close distances from each other (e.g., ≤50 km). Although population genetic structures of many soil-borne viruses are still unknown, isolates of BNYVV (transmitted by a related soilborne vector species, *P. betae*), largely from Imperial Valley of California, clustered according to plant cultivar–virus interaction (1), whereas isolates from broad geographical areas in Asia and Europe show phylogeographic clustering according to provenance (16). It seems likely that the wheat cultivars (and the resistance genes they employ) grown in a given

TABLE 7. Parameter estimates, d_N/d_S (ω) values, log-likelihood (lnL) values, likelihood ratio test (LRT) statistics, and positively selected amino acid sites under six maximum likelihood (ML) models of codon substitution used to investigate selection pressures on the genome-linked virion protein (VPg) and coat protein (CP) of *Wheat yellow mosaic virus* in this study

Protein, ML[a]	Parameter estimates[b]	ω Ratio	lnL	LRT statistic[c]	Positive sites[d]
VPg					
M0	$\omega = 0.255$	0.255	–1301.383		None
M3	$p_0 = 0.983$, $p_1 = 0.017$	0.256	–1301.256	M0 vs. M3:	47K
	$\omega_0 = 0.238$, $\omega_1 = 1.338$			$P > 0.975$	
M1a	$p_0 = 0.971$ ($p_1 = 0.029$), $\omega_0 = 0.232$ ($\omega_1 = 1.000$)	0.255	–1301.264		Not allowed
M2a	$p_0 = 0.983$, $p_1 = 0.000$ ($p_2 = 0.017$)	0.256	–1301.253	M1a vs. M2a:	47K
	$\omega_0 = 0.0236$, $\omega_1 = 1.000$, $\omega_2 = 1.338$			$P > 0.975$	
M7	$p = 7.166$, $q = 20.795$	0.256	–1301.362		Not allowed
M8	$p_0 = 0.984$ ($p_1 = 0.016$)	0.256	–1301.267	M7 vs. M8:	47K
	$p = 31.032$, $q = 99.000$, $\omega_s = 1.343$			$P > 0.900$	
CP					
M0	$\omega = 0.246$	0.246	–3117.495		None
M3	$p_0 = 0.554$, $p_1 = 0.439$ ($p_2 = 0.0066$)	0.251	–3101.317	M0 vs. M3:	115D**
	$\omega_0 = 0.018$, $\omega_1 = 0.497$, $\omega_2 = 3.387$			$P < 0.0001$	
M1a	$p_0 = 0.849$ ($p_1 = 0.150$), $\omega_0 = 0.115$ ($\omega_1 = 1.000$)	0.248	–3103.397		Not allowed
M2a	$p_0 = 0.860$, $p_1 = 0.135$ ($p_2 = 0.005$)	0.261	–3102.279	M1a vs. M2a:	115D, 146A, 177A
	$\omega_0 = 0.125$, $\omega_1 = 1.000$, $\omega_2 = 3.578$			$P > 0.100$	
M7	$p = 0.264$, $q = 0.793$	0.249	–3103.675		Not allowed
M8	$p_0 = 0.994$ ($p_1 = 0.006$)	0.251	–3101.456	M7 vs. M8:	115D*, 146A, 177A
	$p = 0.400$, $q = 1.318$, $\omega_s = 3.433$			$P > 0.100$	

[a] ML model descriptions are according to Yang et al. (78) (M0, M3, M7, and M8), Wong et al. (73), and Yang et al. (79) (M1a and M2a).

[b] Numbers of parameters for the different models were 1 (M0), 2 (M1a), 4 (M2a), 5 (M3), 2 (M7), or 4 (M8).

[c] LRT statistics of M3 versus M0 are tests of heterogeneity of selection pressures among codons, while M2a versus M1a and M8 versus M7 are tests of positive selection, all of which assess LRT statistics ($2 \times \delta ln$L) against a χ^2 distribution with the degrees of freedom (df) equal to the difference in the number of parameters between the nested models under comparison.

[d] Positively selected amino acid sites at posterior probabilities $P > 95.0$ (*) or $P > 99.0$ (**) are shown. Identification of amino acids under positive selection is based on Näive empirical Bayes (under M3) or Bayes empirical Bayes inference (under M2a or M8).

field also may have drastic effects on the WYMV population genetic structure; however, this remains a subject for future study.

Despite being known for a long time, a number of soilborne plant viruses, including WYMV, are still a major decimating factor of cereal crops (30,34). WYMV is geographically restricted to Asia but the evolutionary forces determining its population structure were hitherto unknown. To our knowledge, the current study is the first comprehensive study examining the population genetic structure and forces driving the evolution of a bymovirus. In particular, we demonstrated that founder effects and selection are not mutually exclusive forces and may act synergistically to limit genetic variation (1,7) although, in some virus populations, one may predominate over the other (17,72). Founding effects are more pronounced in smaller founding populations by increasing rates of fixation and extinction. In contrast, selection is a directional process by which the fittest variants increase in the population (positive selection) or the less fit variants decrease in the population (negative or purifying selection). The combined effects of these forces (and inadvertent genetic drift) acting simultaneously on the same or different genomic regions will ultimately determine divergence within a viral species. Future studies will be directed at genome-wide analyses of evolutionary constraints and bottlenecks limiting genetic variation in WYMV.

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