

Characterization of Genetic Variability Among Natural Populations of Wheat Streak Mosaic Virus

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Cooperative investigation of the ARS, USDA, and the Nebraska Agricultural Experiment Station.

Published as paper 11497, Journal Series, Nebraska Agricultural Experiment Station.

This research was supported, in part, by USDA CSRS grant 94-34103-0140 to G. L. Hein and R. French.

Accepted for publication 7 August 1996.

ABSTRACT

McNeil, J. E., French, R., Hein, G. L., Baenziger, P. S., and Eskridge, K. M. 1996. Characterization of genetic variability among natural populations of wheat streak mosaic virus. *Phytopathology* 86:1222-1227.

A reverse transcriptase-polymerase chain reaction (RT-PCR) assay was developed to amplify cDNA from the coat protein coding region and 3'-noncoding region of the genome of wheat streak mosaic rymovirus (WSMV). *AluI* restriction fragment length polymorphisms (RFLPs) were found among RT-PCR products of four spatially or temporally distinct WSMV isolates. RT-PCR and RFLP analyses were then applied to examine intra- and interfield genetic variation in natural WSMV popula-

tions in 1994 and 1995. A total of 32 distinct RFLP types were found in five Nebraska counties, but most isolates could be grouped into three predominant genotypes. Chi-square analysis of the degree of sequence heterogeneity of isolates within fields, among fields in each county, and among counties indicated that there was as much variation within fields as among counties. There was, however, a significant difference ($P = 0.001$) in the frequencies of WSMV RFLP types between 1994 and 1995. Results of this study suggest that there are three main and many minor lineages of WSMV cocirculating in the region. The WSMV population structure is consistent with a quasispecies model. Isolates with distinctive RFLP patterns should facilitate future studies of WSMV dispersal.

Wheat streak mosaic (WSM) is a serious disease of wheat (*Triticum aestivum* L.), particularly in the Great Plains region of the United States. On average, WSM is estimated to reduce annual wheat yields about 2% per year in the region (2,10), but localized yield losses of up to 100% are not uncommon. Many factors involved in the epidemiology of WSM have been identified (6,10,36,37,41), but several unresolved questions remain. For example, the disease is often associated with the presence of volunteer wheat, which serves as an over-summering reservoir for both the etiologic agent, wheat streak mosaic virus (WSMV), and its eriophyid mite vector, the wheat curl mite (formerly identified as *Aceria tulipae* Keifer [27,36], but now considered to be *Aceria tosichella* Keifer [1]). In recent years, however, some outbreaks of WSM occurred in the absence of volunteer wheat, and it is possible that annual grass species influence WSM epidemiology (10). The ability to distinguish WSMV isolates would enable direct comparisons between those found in cultivated wheat and virus isolates prevalent in potential reservoir hosts.

WSMV has a relatively broad host range encompassing many plants in the grass family. It infects all varieties of wheat, barley (*Hordeum vulgare* L.), and oats (*Avena sativa* L.). Some varieties of maize (*Zea mays* L.) and millet (*Panicum* L., *Setaria* P. Beauv., and *Echinochloa* P. Beauv. spp.) are also susceptible to WSMV (5). It is the type member of the genus *Rymovirus* whose members are mite-transmitted viruses in the plant virus family Potyviridae (28,42). WSMV virions are flexuous rods roughly 13 nm in diameter and 700 nm in length (5) and are comprised of a 45-kDa

coat protein and a 8,500-nt, single-stranded, messenger sense RNA (7,26,42). The nucleotide sequence encoding the viral coat protein is known (26), but the majority of the WSMV genome has not yet been characterized.

Ever since the discovery of WSMV in 1932, it has been recognized that individual virus isolates vary widely in terms of symptom severity on wheat (9,21-23,37,38). WSMV isolates have also been classified into groups by differential virulence on maize varieties and inbred lines (4,9,25) or varieties of oats (4). However, tests based on symptoms often depend on local environmental conditions and have proven difficult to reproduce (9). WSMV isolates also vary in capsid protein properties, including relative molecular mass determined by polyacrylamide gel electrophoresis (7) and the presence or absence of an epitope recognized by a monoclonal antibody (24). WSMV occurs commonly in corn and grasses (6,10,37), but it is not known whether virus isolates differ depending on their host.

New technologies such as the polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLPs) have facilitated detection and analysis of sequence polymorphisms among virus isolates, strains, and populations (8,15,16,29). From the known sequence of the capsid protein coding region of WSMV RNA (26), a genomic region known to be taxonomically useful (34), primers were designed for use in the reverse transcriptase-polymerase chain reaction (RT-PCR) to specifically identify WSMV. In this study, isolates of WSMV were examined to determine the extent of capsid protein coding sequence variability in the virus population, whether such sequence variability differed from one geographical location to another, and whether the structure of WSMV populations varied over time. Knowledge of the predominant Nebraska WSMV isolates will enable plant breeders to use relevant virus isolates in screening wheat for WSMV resistance. It will also allow plant pathologists to monitor the rise and fall of particular isolates in Nebraska and study a range of factors in WSMV epidemiology.

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Publication no. P-1996-0918-01R

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MATERIALS AND METHODS

Virus isolates. Initially, four laboratory isolates were chosen for comparison, because of their diverse origins. Three isolates, originally from infected wheat, were obtained at different times and in different locations. The type isolate (WSMV-T; ATCC no. PV57) was collected in Kansas in 1932 (21), the Sidney 81 isolate (WSMV-S) (7) was collected in Nebraska in 1981, and the Wyoming isolate (WSMV-W) was collected in 1988. The fourth isolate (WSMV-C) was collected from WSMV-infected corn in 1978. These were propagated in wheat cv. Michigan Amber as described (7). Plants infected by WSMV-C were most chlorotic, while those infected with WSMV-T had a mild mosaic. WSMV-S and WSMV-W isolates induced symptoms intermediate to those induced by WSMV-C and WSMV-T. In June of 1993, wheat leaf samples showing WSMV-like symptoms were collected from fields near North Platte, Ogalalla, and Sidney, Nebraska. Samples were also collected in Colorado.

In 1994, a more intensive sampling study was conducted to examine WSMV populations in six counties spanning the major wheat production regions of Nebraska. In 1994, five to six fields each from Cheyenne, Box Butte, Keith, Lincoln, Red Willow, and Webster counties, Nebraska, were sampled. In 1995, four to five fields in Box Butte, Keith, Lincoln, Red Willow, and Webster counties, Nebraska, were sampled. A total of 20 leaves (one leaf per plant), showing apparent WSMV symptoms, were collected randomly from each field when possible. Therefore, up to 100 samples were collected per county. Samples were stored at 4°C until processed.

Total nucleic acid extractions. Approximately 0.2 grams of tissue was ground in 3 ml of distilled water and stored in centrifuge tubes at -20°C. For further processing, frozen samples were thawed in cold water and then mixed for 10 s. A 200- μ l aliquot of each sample was added to a tube containing 200 μ l of extraction buffer (0.2 M glycine [pH 9.5], 0.2 M NaCl, and 20 mM EDTA) and 20 μ l of 20% sodium dodecyl sulfate (Sigma Chemical Co., St. Louis), followed by 300 μ l of phenol (Fisher Scientific Co., Fair Lawn, NJ), and the sample was mixed for 30 s. Following centrifugation at 12,000 \times g for 5 min, 250 μ l of the upper aqueous layer was transferred to a fresh tube (on ice) containing 30 μ l of 3 M sodium acetate (pH 5.2) (on ice). Three volumes of 100% ethanol (750 μ l) was added, and the samples were centrifuged at 12,000 \times g for 5 min at 4°C. The supernatant was removed, and the pellet was washed with 70% ethanol (500 μ l). Samples were centrifuged at 12,000 \times g for 2 min at room temperature, and the supernatants discarded. The pellets were air-dried for 20 min and then resuspended in 25 μ l of sterile, distilled water.

Reverse transcriptase reactions. A cDNA synthesis master mixture was made by combining 254 μ l of distilled water, 44 μ l of 5 \times reverse transcriptase buffer (250 mM Tris-HCl [pH 8.0]; 25 mM 2-mercaptoethanol; 50 mM MgCl₂; 350 mM KCl; 8.8 μ l of a mixture of dATP, dCTP, dGTP, and dTTP (10 mM each); 2 μ l of avian myeloblastosis virus (AMV) reverse transcriptase (25 units/ μ l; Boehringer Mannheim Biochemicals, Indianapolis, IN); and 11 μ l (10 pmole/ μ l) of primer RCF1 (5'-AGCTGGATCCCTTT-TTTTTTTTTT-3'; complementary to the viral poly A tail). Nine-microliter samples of this mixture were added to 0.5-ml centrifuge tubes with a 1- μ l nucleic acid sample. Samples were mixed, centrifuged briefly, and held at room temperature for 2 to 5 min. Samples were then incubated for 60 min at 42°C. Distilled water (10 μ l) was then added, and the samples were heated to 99°C for 5 min. cDNA samples were stored at -20°C until needed.

PCRs. A mixture of 453 μ l of distilled water, 60 μ l of 10 \times PCR buffer (Perkin-Elmer Cetus, Norwalk, CT), 12 μ l of dNTPs (10 mM), 3 μ l of *Taq* DNA polymerase (Perkin-Elmer Cetus), 12 μ l (10 pmole/ μ l) of the specific upstream primer WSMcp1a (5'-AGCTTATGAGCGCTATTATTGCAGCAT-3'; the underlined sequence is identical to bases 409-427 of the published WSMV sequence [26]), and 12 μ l (10 pmole/ μ l) of the downstream primer

WSMcp2 (5'-CGATTTTTTTTTTTTTTTGCCGTCGCCCT-3'; the underlined sequence is complementary to the WSMV RNA 3'-terminus) was assembled on ice. An aliquant (23 μ l) of this mixture was added to PCR tubes (Perkin-Elmer Cetus) with 2 μ l of cDNA sample. The tubes were mixed and centrifuged briefly. Samples were overlaid with approximately 100 μ l of mineral oil and incubated in a Perkin-Elmer thermal cycler (Perkin-Elmer Cetus) programmed for 35 cycles at 94°C (1 min), 50°C (1 min), and 72°C (2 min), held at 72°C for an additional 10 min after the last cycle, and then stored at 4°C. A second set of primers (14) served to test about 20% of the samples to confirm the presence or absence of WSMV. These samples were also tested for the presence of barley yellow dwarf virus (BYDV) by RT-PCR (29).

Agarose gel electrophoresis. PCR products were resolved on 1.0 or 1.5% agarose gels in 0.5 \times Tris-borate-EDTA (TBE) (33) containing 0.3 mg/ml of ethidium bromide. Gels were run for approximately 1 h and then photographed under ultraviolet illumination. The presence of WSMV was confirmed by the detection of a ~1.4-kbp PCR product.

***AluI* digestion and polyacrylamide gel electrophoresis.** Previous work with restriction nucleases revealed that *AluI* digestion detected more polymorphisms than other restriction enzymes. Therefore, positive samples were incubated with *AluI* restriction nuclease (Boehringer Mannheim Biochemicals) overnight at 37°C using the buffer supplied with the enzyme. Nuclease digestions were done by mixing 6 μ l of each PCR reaction with 6 μ l of 2 \times buffer containing two units of *AluI*. Digestion products were separated on a 10% polyacrylamide gel in 1 \times TBE (33) for 2.0 to 2.5 h, followed by staining with ethidium bromide (0.1 mg/ml) in water.

RFLP analysis. Polymorphisms were scored by visual analysis of the banding pattern. Beginning with the 1994 data, distinct RFLP types were arbitrarily assigned a number. The sizes of the *AluI* digestion products were estimated by comparison with DNA size standards separated on the same gels.

Chi-square analysis. To determine whether WSMV RFLP types differed among fields within a county, among counties, and between 1994 and 1995, a chi-square analysis (13) was performed using the frequency command in the SAS statistical package (SAS Institute, Cary, NC).

Types 1, 3, and 4 (Table 1) were found in the highest proportions, while types 2 and 5 to 32 were found infrequently. For this reason, types 2 and 5 to 32 were grouped together, labeled as 'all other,' and collectively analyzed with types 1, 3, and 4. In 1994, the proportions of occurrence of types 1, 3, 4, and all other types were compared by chi-square analysis of samples within each field, and with other fields in Cheyenne County. Fields were similarly compared within each of Box Butte, Red Willow, and Webster counties. Then, the proportions of the four WSMV types within each county were compared with the proportions of types in other counties. Lincoln and Keith counties could not be analyzed statistically in 1994, because there were very few positive samples, due to a low incidence of WSMV in these counties. In 1995, the chi-square analysis was repeated for fields within each of Box Butte, Red Willow, Webster, and Keith counties, as well as among these counties. To determine if WSMV population distributions varied with time, frequencies of the four types were summed over Box Butte, Red Willow, and Webster counties. The chi-square was then used to compare proportions of WSMV types in 1994 with those of 1995. Lincoln, Keith, and Cheyenne counties could not be included in this analysis, because of the low incidence of WSMV in both 1994 and 1995.

RESULTS

RFLPs can distinguish WSMV isolates. Four laboratory isolates of WSMV that differed in year of collection and geographical origins were chosen to examine the degree of potential sequence heterogeneity among isolates. Using RT-PCR with primers

WSMcp1a and WSMcp2, a 1.4-kbp cDNA product encompassing the coat protein coding region and 3'-noncoding region of the viral genome could be amplified from each isolate (Fig. 1). Several restriction nucleases such as *Sau3AI* gave identical digestion patterns after gel electrophoresis. However, restriction fragment patterns following digestion with *AluI* were unique for each isolate (Fig. 1). Similarly, *AluI* digestion of RT-PCR products of total nucleic acid extracts of wheat samples collected in 1993 from several locations in Colorado and Nebraska revealed distinctive patterns. Two patterns or types occurred most frequently (Fig. 2). Five of the eight samples shown had *AluI* digestion patterns indistinguishable from that of the WSMV-S isolate. There were no PCR products produced when no cDNA was added to the reaction or when cDNA from total nucleic acid extracts of uninfected wheat was added (Fig. 2, lanes 1 and 2, respectively).

One-week-old seedlings of 'Michigan Amber' wheat were inoculated with WSMV-C, WSMV-S, WSMV-T, and WSMV-W, as well as with eight field isolates. Sap from infected plants was extracted 6 weeks after inoculation and used as inoculum for a second set of plants. After 6 weeks, a third set of plants was inoculated with sap from the second set of plants. Two weeks later,

TABLE 1. Frequency of occurrence and banding patterns of wheat streak mosaic rymovirus (WSMV) *AluI* restriction fragment length polymorphism types

Type	1994 ^a	1995 ^b	Size of <i>AluI</i> restriction fragments (bp) ^c
1	67	28	450, 330, 300 ^d , 230, 200, 100, 45
2	9	0	500, 410 ^d , 380, 230, 200
3	33	33	450, 260, 230, 200, 100, 45
4	96	118	450, 410 ^d , 380, 230, 200, 45
5	4	0	450, 330, 300, 230, 100
6	5	2	450, 330, 230, 200, 90, 55, 45
7	3	0	500, 330, 300 ^d , 230, 200, 45
8	8	0	900, 230, 200, 45
9	1	1	475, 330, 230, 200, 110, 75
10	3	3	450, 410, 230, 200
11	1	1	450, 260, 230, 200, 75, 45
12	1	1	410, 330, 300, 100
13	2	0	450, 230, 215, 200, 100, 75
14	1	5	450, 380, 100, 45
15	1	0	450, 410, 380, 45
16	1	0	450, 320, 310, 100, 45
17	2	0	410, 240, 230, 100, 55, 45
18	1	0	450, 410, 340, 90, 55, 45
19	1	2	450, 410, 380, 330
20	1	0	380, 340, 230, 200, 75, 45
21	1	2	410, 240, 230, 100, 75
22	1	0	450, 260, 230, 190, 115, 100, 45
23	1	6	450, 410, 380, 55
24	1	0	1,200, 200
25	1	0	450, 410, 250, 100, 75, 45
26	2	0	410, 330 ^d , 300 ^d , 230, 200, 100, 45
27	2	0	600, 410 ^d , 380, 230, 200
28	1	0	450, 230, 200, 190, 45
29	1	4	450, 330, 300 ^d , 230, 200, 100
30	0	1	450 ^d , 380, 330, 300, 290, 100, 45
31	0	1	450, 380, 200, 100, 45
32	0	1	450, 410 ^d , 380, 230, 140, 90, 60, 45
Total	252	209	
WSMV-S ^e			450, 410 ^d , 380, 230, 200, 45
WSMV-C ^f			450, 410, 330, 300, 45
WSMV-T ^g			380, 300, 250, 230, 200, 45
WSMV-W ^h			450, 410, 300 ^d , 230, 200, 100, 45

^a Number of samples of each type found in 1994.

^b Number of samples of each type found in 1995.

^c Estimated sizes in base pairs; several fragments of undetermined sizes less than 45 bp were also present.

^d Intensity of stained fragment at less than equimolar ratio.

^e The Sidney 81 isolate of WSMV.

^f The isolate collected from WSMV-infected corn.

^g The type isolate of WSMV.

^h The Wyoming isolate of WSMV.

total nucleic acid extracts were prepared from the last set of plants before performing RT-PCR assays and *AluI* digestions. In all cases, the *AluI* restriction patterns were identical to those of the original isolates (data not shown). Thus, the genotypes of these isolates were stably maintained over at least three passages.

Analysis of WSMV populations. To better evaluate the sequence heterogeneity of WSMV and the geographical distribution of that genetic diversity, fields were more extensively sampled in 1994 and 1995. Six Nebraska counties in the south-central, southwest, and panhandle regions of the state were chosen for sampling and, when possible, 20 symptomatic plants were sampled from each of five fields per county. The incidence of WSMV in Nebraska was visually estimated to be 10% or less during both 1994 and 1995. Because of the negligible incidence of WSMV in Lincoln and Keith counties in 1994 and Lincoln and Cheyenne counties in 1995, we were unable to collect a full complement of 20 samples per field or five fields per county at those locations. In other locations, some samples were negative for WSMV by our PCR assay, which further reduced the sample size. About 20% of the negative samples were tested for BYDV by PCR, as well as with another set of WSMV-specific primers. The lack of a PCR product with two sets of primers indicated an absence of WSMV infection, but many of these samples contained BYDV (data not shown).

PCR products from individual isolates collected in 1994 and 1995 could also be differentiated by *AluI* restriction analysis. In fact, WSMV isolates from within individual fields proved to be highly polymorphic with respect to *AluI* restriction patterns. Figure 3 shows the *AluI* RFLP patterns from wheat samples collected in Cheyenne County, field 1. Five distinct RFLP types were identified in a total of 17 samples. Assays of samples from other fields

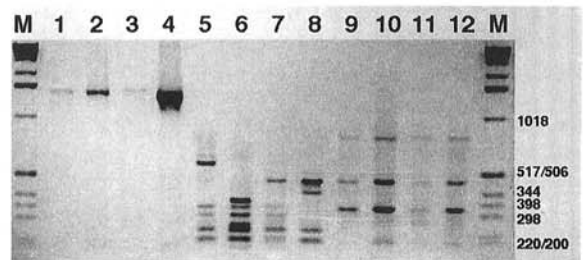


Fig. 1. Characterization of reverse transcriptase-polymerase chain reaction (RT-PCR) products of wheat streak mosaic rymovirus (WSMV) isolates on a 1.5% agarose gel stained with ethidium bromide. Lanes 1, 5, and 9, isolate collected from WSMV-infected corn (WSMV-C); lanes 2, 6, and 10, type isolate of WSMV (WSMV-T); lanes 3, 7, and 11, Wyoming isolate of WSMV (WSMV-W); and lanes 4, 8, and 12, Sidney 81 isolate of WSMV (WSMV-S). Lanes 1 to 4 are uncut PCR products; lanes 5 to 8, *AluI* digestion products; and lanes 9 to 12, *Sau3AI* digestion products. Lanes at extreme left and right are DNA size markers with sizes in nucleotide base pairs shown in the right margin. A negative gel image was digitally scanned using an Apple OneScanner (Apple Computer, Inc., Cupertino, CA) and labeled using provided software before printing.

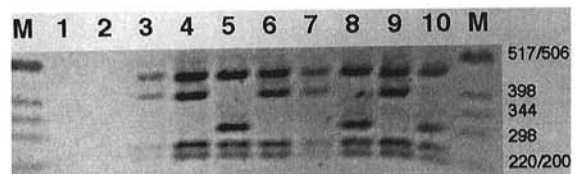


Fig. 2. Negative image of *AluI* digestion patterns of 1993 wheat streak mosaic rymovirus (WSMV) field samples separated on a 1.5% agarose gel stained with ethidium bromide. Lane 1, no RNA reverse transcriptase-polymerase chain reaction (RT-PCR) control; lane 2, RT-PCR product from healthy wheat; lanes 3 to 10, *AluI* digestion products of field isolates from western Nebraska (3 to 7) or eastern Colorado (8 to 10). Lanes marked M are DNA size markers with sizes in nucleotide base pairs shown in the right margin.

revealed similar polymorphisms. In 1994, 29 distinct RFLP types were found. In 1995, three additional types were found and identified as types 30, 31, and 32. All types were different from the WSMV-C, WSMV-T, and WSMV-W isolates (Table 1). Type 4 closely resembled the WSMV-S isolate. Some bands were present in less than equimolar amounts and, while they were always present, the band intensity varied from experiment to experiment. These may represent fragments with *AluI* cleavage sites that are poorer substrates for the enzyme or could be indicative of sequence heterogeneity within individual samples. Only 11 isolates (out of a total of 472 samples) were clearly a mixture of two or more RFLP types. These mixed isolates were not included in the analysis, because it was not possible to unambiguously determine the type components of each mixture.

A total of 32 distinct RFLP types were found, but most types were infrequent (Table 1). Three types (types 1, 3, and 4) were more frequent than other types. To facilitate analysis, all infrequent types were grouped into a single class designated 'all other.' A chi-square analysis comparing the proportions of the four WSMV types among individual fields in Cheyenne, Box Butte, Red Willow, and Webster counties in 1994 showed no significant differences ($P > 0.05$). This result confirmed the conclusion that the distribution of WSMV types was similar across individual fields within each county. The proportions of WSMV types did not significantly differ among counties in 1994 (Table 2). In 1995, samples from fields in Box Butte, Red Willow, Webster and Keith counties were analyzed (Table 2). As in 1994, the relative incidence of WSMV RFLP types within a single field paralleled the type frequencies for the entire region.

Proportions of RFLP types significantly differed between 1994 and 1995, however (Table 3). Type 4 was more prevalent in 1995, while type 1 decreased in frequency (Table 1). The occurrence of type 3 was similar for both years.

DISCUSSION

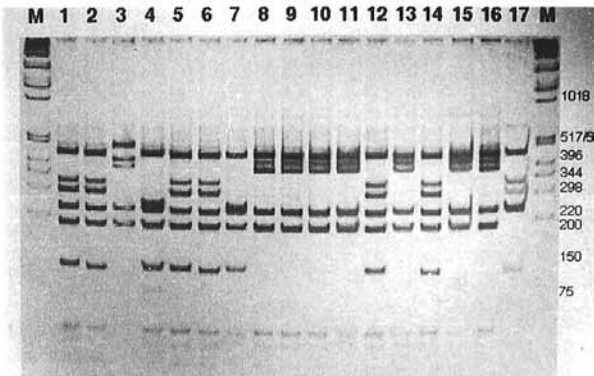
We found that cDNA from field samples could be readily amplified by RT-PCR from total nucleic acid extracts and differentiated by *AluI* polymorphisms indicating WSMV genetic diversity in natural field populations. Although RFLP analysis detects only a subset of potential sequence differences among isolates, a total of 32 distinct RFLP types appeared in field collections over 2 years (Table 1). The three most common RFLP types accounted for 78% of the total isolates collected in 1994, and 86% of the total in 1995. The other 29 types occurred only sporadically. Inter-

estingly, the predominant type in both years (type 4) was indistinguishable from WSMV-S, which had been collected in the region more than a decade earlier.

Genetic diversity within WSMV subpopulations of individual fields was as great as that found among fields in a single county, which, in turn, paralleled the genetic diversity among all counties. Thus, based on our data, there was no geographical bias in the composition of the virus populations, even though some were over 600 km apart. In 1994, the distributions of RFLP types in fields throughout Cheyenne, Box Butte, Webster, and Red Willow counties were similar as well (Table 2). Also, the distributions of WSMV types in 1995 throughout fields in Box Butte, Webster, Red Willow, and Keith counties were similar (Table 2). This similarity is most apparent when looking at the distribution of types among counties in both years. For both years, counties were indistinguishable by type frequency. Temporal variation in WSMV subpopulations in Box Butte, Red Willow, and Webster counties was significant between 1994 and 1995 (Table 3). This mostly reflected a decrease in the proportion of RFLP type 1 and an increase in the frequency of type 4 between 1994 and 1995. The genetic diversity of WSMV populations changed over time, but was geographically homogeneous for each of the 2 years, suggesting extensive yearly dispersal of WSMV isolates in the region.

Only 11 of 472 Nebraska samples were obvious mixtures of two or more WSMV polymorphisms. The vast majority of the isolates consisted of a single *AluI* RFLP type. This could reflect cross-protection in which infection by an initial genotype prevents subsequent establishment of other WSMV genotypes. In fact, McKinney (23) demonstrated that prior inoculation of wheat with a mild isolate of WSMV protected it from later infection by a severe isolate.

How can the observed variability of WSMV isolates be explained? RNA replicons are generally considered to have high mutation rates, and an RNA viral genome may be viewed as a



Type: 1 1 2 3 1 1 3 4 4 4 4 1 4 1 4 4 5

Fig. 3. Negative image of *AluI* digestion patterns of wheat streak mosaic rymovirus (WSMV) field samples from the same field in Cheyenne County, NE, collected in 1994. After reverse transcriptase-polymerase chain reaction (RT-PCR) and *AluI* digestion the DNA fragments were separated on a 10% polyacrylamide gel stained with ethidium bromide. Lanes marked M are DNA size markers with sizes in nucleotide base pairs shown in the right margin.

TABLE 2. Frequencies, proportions, and chi-square for comparing the distribution of wheat streak mosaic types 1, 3, 4, and all others among counties in 1994 and in 1995

Year	County	Type			
		1	3	4	All others
1994	Cheyenne	15 (0.27) ^a	8 (0.15)	19 (0.35)	13 (0.23)
	Box Butte	12 (0.31)	1 (0.03)	13 (0.33)	13 (0.33)
	Red Willow	20 (0.26)	8 (0.11)	35 (0.46)	13 (0.17)
	Webster	18 (0.26)	12 (0.17)	24 (0.35)	15 (0.22)
Chi-square = 8.90 ^b					
1995	Box Butte	11 (0.18)	5 (0.08)	32 (0.53)	12 (0.20)
	Red Willow	4 (0.09)	7 (0.16)	28 (0.65)	4 (0.09)
	Webster	4 (0.10)	9 (0.23)	22 (0.55)	5 (0.12)
	Keith	9 (0.16)	11 (0.19)	30 (0.51)	8 (0.14)
Chi-square = 8.75 ^b					

^a Proportions are in parentheses.

^b Proportions of types not significantly different ($P > 0.05$) among counties.

TABLE 3. Frequencies, proportions, and chi-square for comparing 1994 and 1995 distribution of wheat streak mosaic types 1, 3, 4, and all others in 1994 and 1995 in Box Butte, Red Willow, and Webster counties

Year	Type			
	1	3	4	All others
1994	67 (0.27) ^a	33 (0.13)	96 (0.38)	56 (0.22)
1995	28 (0.13)	33 (0.16)	118 (0.56)	30 (0.14)
Chi-square = 16.33 ^b				

^a Proportions are in parentheses.

^b Proportions of types differ significantly ($P < 0.01$) across years.

population of sequence variants (a quasispecies) distributed around one or more master sequences (11,12). Sampling of this inherently variable population at random would result in a diverse distribution of virus genotypes. Such random sampling could occur by restricted virus acquisition and transmission by vectors. A severe genetic bottleneck imposed by virus transmission by individual mites, for example, would randomly establish WSMV sequence variants in individual plants. The variation observed, therefore, would reflect genetic drift rather than selection. Since the RFLP patterns of eight isolates remained the same after three passages in wheat, there does not seem to be an obvious difference in the replicational fitness among those WSMV isolates.

On the other hand, we cannot rule out that diversity in WSMV genotypes is driven by selection. Adaptation to various alternative host species, which has been documented for other plant viruses (20,40), is one such potential selective factor. Note that the quasispecies distribution hypothesis mentioned above is a model of population structure. In this model, the composition and proportional abundance of the variant sequences are a function of mutation rate and the relative biological (e.g., replicational) fitness of the component viral genomes (12). This concept was originally applied to populations of RNA genomes within single virus clones, but it is equally applicable to virus populations in the field (11). In the latter case, biological selection includes both replicational fitness of the virus, as well as complex interactions of virus with host and vector populations, among many other factors. Viewed in this light, the WSMV population sampled in this study could be characterized as a quasispecies comprised of three master sequences with a number of other much less frequent variants.

Genetic variation has also been detected among spatially or temporally diverse isolates of other viruses (3,8,15-19,29-32,35,39,43), but virus variation from plant to plant within a single field has rarely been reported. Rodriguez-Alvarado et al. (31) found seven different genotypes among 19 cucumber mosaic virus isolates from the same field, which resembles the within-field genetic variation of WSMV. In addition, there were two main RNase protection patterns found, with the rest occurring only once or twice. It will be of interest to see if other natural plant virus populations vary as much within fields. The genetic diversity found with WSMV may facilitate epidemiological studies. Because none of the WSMV isolates were identical to the type isolate, the latter may be a molecularly distinct isolate useful for the examination of intra- and interfield dispersal.

This is the first study to examine genomic differences of WSMV isolates within and among fields. Our results underscore the complex nature of WSMV epidemiology. Based on the portion of the viral genome analyzed in this study, there are three main and many minor lineages of WSMV cocirculating in the region with multiple chains of virus transmission. This is consistent with the endemic nature of WSMV and known multiple reservoirs of mite and virus in wheat, corn, and several grass species. Two implications of this are that (i) single isolates from a given location will not necessarily be representative of, or unique to, that location; and (ii) virus populations need to be monitored frequently to ensure that genetic resistance screening employs appropriate virus isolates. Further work is needed to search for correlations between RFLP patterns and symptomology, serological properties, or host adaptation.

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