Population Genetic Structure of *Septoria passerinii* in Northern Great Plains Barley

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ABSTRACT

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The genetic structure of *Septoria passerinii* from nine field populations was examined at several scales (within lesions, among lesions in a leaf, among leaves in a field, and among fields in North Dakota and western Minnesota) by using amplified fragment length polymorphism (AFLP) markers. A total of 390 isolates were sampled from seven barley fields located in North Dakota and two barley fields located nearby in western Minnesota in 2003 and 2004. Based on 57 polymorphic AFLP markers, AFLP DNA fingerprints identified 176 different genotypes among 390 (non-clone-corrected) isolates in nine different fields. In two intensively sampled sites, ND16 (Williston, ND) and ND17 (Langdon, ND), only one to four different genotypes were found within a lesion. A higher level of genetic and genotypic diversity was found within a leaf in which six to

nine different genotypes were found from lesions on a leaf. The genetic diversity within a leaf was similar to the genetic diversity within a field. The average genetic diversity (H) within a field across all AFLP loci was approximately 0.3, except at site ND12 (Carrington, ND) where it was 0.16. Genotypic diversity was high in all populations, and with the exception of ND15 (Rothsay, MN), very low multilocus linkage disequilibrium values ($\bar{r}_d$) were found in all populations. The population differentiation, G_{ST} , was relatively high ($G_{ST} = 0.238$) among the nine populations due to the high G_{ST} in ND12, ND14 (Twin Valley, MN), and ND15. Population differentiation without those three populations was 0.09. A lack of correlation between geographical distance and genetic distance was found, suggesting the potential for a high level of gene flow between different geographical regions. The population genetic structure described in this study for *S. passerinii* in North Dakota and western Minnesota is consistent with that of a sexually reproducing fungus.

Septoria passerinii, the causal agent of Septoria speckled leaf blotch (SSLB) in barley, is one of the most common foliar diseases on *Hordeum vulgare* cultivated in the Upper Midwest region of the United States and the Prairie Provinces of Canada (9). Yield losses up to 38% were reported in barley infected with *S. passerinii* (29). To date, this pathogen has no known sexual stage in nature despite surveys of barley in several locations in North Dakota (ND) from 2002 to 2006 (S. M. Neate, *unpublished data*), and it is thought that the pathogen infects barley only through asexual spores (pycnidiospores), which are dispersed only over short distances by rain splash (6). Information on the type of reproduction (sexual or asexual) within a pathosystem is important to understand the genetic structure of the pathogen population and to develop pathogen management strategies.

Many sources of resistance to *S. passerinii* have been reported in different *Hordeum* accessions collected from around the world. However, all of the major six-rowed and two-rowed malting and feed cultivars grown in the Upper Midwest region of the United States are susceptible to *S. passerinii*. To date, three resistance genes, designated as *Rsp1*, *Rsp2*, and *Rsp3*, each identified as single dominant genes, have been found in the six-rowed barley accessions CIho 14300, CIho 4780, and CIho 10644, respectively (23). Recently, all three resistance loci were mapped on barley chromosomes (11). The pathogenicity of populations and races within *S. passerinii* has not been reported; thus, the population genetics of this pathogen may provide useful knowledge about the pathogen and host relationship.

The genetic structure of a plant pathogen population describes the amount and distribution of genetic variation within and among populations (13). Information about genetic structure in a plant pathogen population has direct implications for how fast genotypes with novel virulences can develop and spread between areas where a crop is cultivated (14). Information about genetic structure is also valuable for decisions about deployment of specific resistance genes for disease control. As a result, control strategies are best aimed at targeting a population instead of an individual for effective disease management. Information about genetic structure of the pathogen population can also be used as evidence of sexual reproduction (18). The genetic structures of populations with regular cycles of sexual reproduction have many more genotypes with consequently higher levels of genotypic diversity than those with asexual reproduction only. This type of genetic structure is found worldwide in most populations of *Mycosphaerella graminicola*, a pathogen that is genetically closely related to *S. passerinii* (8,16); however, there is no available information about the genetic structure of *S. passerinii* itself.

For more than 10 years, DNA-based genetic markers have been widely used to analyze the genetic structure of fungal populations. Molecular markers are versatile and highly informative tools for population genetic studies (13,17). The markers available include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs). Most fungal pathogen populations have been studied with RFLP, RAPD, and AFLP techniques. Choice of molecular marker depends on the research purpose, genetic diversity, gene flow, mating systems, and other factors for the organism of interest (4). For example, the codominant markers created by RFLP have been intensively applied in the study of the genetic structure of *M. graminicola* (12,14). Those studies showed that field popula-

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tions of *M. graminicola* in the United States and Europe have a high level of gene and genotype diversity, and that the sexual stage has a major impact on the genetic structure of the populations.

The population structure and genotypic diversity of *S. passerinii* is not clearly understood, nor is whether sexual recombination of *S. passerinii* occurs under field conditions. Determining the genetic structure of the pathogen population will greatly contribute to understanding the likelihood of sexual reproduction of the pathogen occurring in nature or reveal other sources of genetic variation and will allow the development of SSLB resistance breeding strategies and resistance gene deployment strategies for durable resistance.

The objective of this study is to characterize the current genetic structure of *S. passerinii* populations at various spatial scales including within/among lesions and fields in ND and western Minnesota (MN) using AFLP markers to determine whether the population structure is more consistent with sexual or asexual reproduction.

MATERIALS AND METHODS

Fungal isolates. At barley growth stage (GS) 51/54, inflorescence half emerged (31), barley leaves naturally infected by *S. passerinii* were collected from nine barley fields in ND and adjacent areas of western MN in 2003 and 2004 (Table 1).

Populations ND16 (from Williston, ND) and ND17 (from Langdon, ND), which are widely geographically separated and climatically different, were sampled using a hierarchical sampling method. The sample collections were made from 16 locations in each field in a grid pattern, each sampling point separated by 10 to 15 m. One leaf sample was collected at each sampling point. Isolations of *S. passerinii* were made from 15 locations in ND16 and 13 locations in ND17. The total areas sampled in the fields, ND16 and ND17, were approximately 2,000 and 3,000 m², respectively.

To determine the genetic variation within lesions in the same leaf, three non-overlapping lesions were chosen on a single leaf and a single pycnidiospore isolate was made from up to 20 pycnidia randomly chosen from within the lesion. This resulted in 9 to 17 single pycnidiospore isolates from each lesion from ND16, and 10 to 16 from ND17. To determine the genetic variation among lesions within the same leaf, all non-overlapping lesions were selected from four leaves from ND16 and three leaves from ND17 and a single pycnidiospore isolate was made from up to 20 single pycnidia within each lesion. To determine the genetic variation among leaves within the same field, three single non-overlapping lesions were selected from each leaf and a single pycnidiospore isolate was made from a single pycnidium within each lesion. A total of 134 isolates were obtained from 19 lesions in ND16 and 114 isolates from 17 lesions in ND17.

The seven populations from ND1, ND5, ND7, ND10, ND12, ND14, and ND15 were collected by a random stratified method

from randomly selected barley fields in ND and western MN. Starting at two points at the edge of a field and moving in a general diagonal direction, samples were randomly taken from infected plants every 5 m.

To understand the genetic variation among fields within ND and western MN, all single spore isolates from each field were separately pooled, including both stratified and hierarchically sampled fields.

The infected leaves were surface-sterilized in a 1% sodium hypochlorite solution or 70% ethanol for 30 s and washed with sterile distilled water. The leaf samples were placed in petri dishes containing a wet filter paper for 12 h. After the formation of cirri, pycnidiospores were streaked onto yeast malt agar (YMA) medium (4 g of yeast extract, 4 g of malt, 1.5 g of sucrose, 15 g of agar, and 1 liter of distilled water) in petri dishes to separate the individual spores and incubated for 48 h at 23°C. Colonies arising from a single spore were isolated and streaked onto YMA, and this process was repeated at least three times to ensure single spore isolates. Each single pycnidiospore isolate was suspended in yeast malt broth (YMB) (YMA without agar) containing 15% glycerol and stored at -80°C.

DNA preparation. The stored single spore isolates were transferred to fresh YMA in petri plates and incubated for 72 h at 23°C in an alternating day/night cycle under cool-white fluorescent tubes. Then, a single colony of each isolate was transferred into a 15-ml tube containing YMB and allowed to germinate and grow for 2 weeks at 23°C in a rotary shaker (150 rpm). Cultured pycnidia (about 1 g) were centrifuged at 5,000 rpm for 10 min, and the pellet was washed twice with sterilized water. The pycnidia were transferred into 15-ml tubes and kept at -80°C for further DNA extraction. Alternatively, single spore isolates were grown on YMA as described above, and spores were harvested by flooding the agar surface with sterile distilled water and scraping the agar surface with a rubber policeman when pycnidia and sporulation had developed 4 to 5 days after incubation. Spores were placed in sterile 15-ml tubes and kept at -80°C.

DNA was extracted by a CTAB extraction protocol (25) with modifications. The stored pycnidia or spores were ground in liquid nitrogen with white quartz sand (-50 to 70 mesh; Sigma-Aldrich Co., St. Louis, MO) in a micropestle at 35,000 rpm powered by a Dremel Rotary Tool (Robert Bosch Tool Corp., Racine, WI). Two volumes of prewarmed 2× CTAB extraction buffer were added to the tube and mixed gently. The sample was incubated for 1 h at 65°C with occasional mixing. An equal volume of the DNA was added to chloroform/isoamyl alcohol (24:1, vol/vol), and the solution was gently emulsified and centrifuged for 10 min at 12,000 × g at room temperature for 10 min. The resultant aqueous phase was removed and incubated with 10 µl of RNaseA (20 mg/ml) at 37°C for 10 min. After another chloroform/isoamyl alcohol extraction, the DNA was precipitated by addition of 7.5 M ammonium acetate (pH 7.7) to a final

TABLE 1. Details of *Septoria passerinii* populations used in this study

Population ^a	Location			Sampling method	Area sampled (m ²)	No. of isolates
	Latitude °N	Longitude °W	Place name			
ND1	47.8058	-103.6465	Alexander	Random stratified	≈150	16
ND5	48.2970	-101.1795	Minot	Random stratified	≈400	24
ND7	48.4920	-100.5787	Bantry	Random stratified	≈400	12
ND10	48.0278	-97.9736	Petersburg	Random stratified	≈500	30
ND12	47.5090	-99.1320	Carrington	Random stratified	≈900	39
ND14	47.2408	-96.3096	Twin Valley	Random stratified	≈300	11
ND15	46.5436	-96.3201	Rothsay	Random stratified	≈300	10
ND16	48.1130	-103.7390	Williston	Hierarchy	≈2,000	134
ND17	48.7595	-98.3401	Langdon	Hierarchy	≈3,000	114
Total					≈7,950	390

^a Seven populations from ND1 to ND12 and ND16 and ND17 were sampled in barley fields in North Dakota and from ND14 and ND15 were from barley fields in western Minnesota.

concentration of 2.5 M and two-thirds volume of ice-cold 100% isopropanol. The DNA was pelleted by centrifuging at 10,000 × g at room temperature for 10 min. The DNA pellet was washed with 70% ethanol and dissolved in 100 µl of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). The DNA was quantified by UV-spectrophotometry.

AFLP analysis. AFLP analysis was performed according to the method of Vos et al. (30) with the following modifications. Genomic DNA samples (500 ng) were digested for 3 h at 37°C with 5 U of *MseI* (New England Biolabs Inc., Ipswich, MA) and 5 U of *EcoRI* (Promega Corp., Madison, WI) followed by inactivation at 70°C for 15 min. The 5-µl ligation mixture containing 50 pmol *MseI* adapters, 5 pmol *EcoRI* adapters, and 1 U of T4 DNA ligase (Promega Corp.) was added to 25 µl of digested DNA, and the reaction was incubated for 3 h at room temperature. A 20-µl aliquot of the adapter-ligated DNA was diluted 10-fold with distilled water, and the remaining 10-µl portion was used to verify complete digestion. The preamplification reaction contained 5 µl of adapter-ligated DNA and was carried out with the primer combination *EcoRI*-A/*MseI*-C as follows: 30 cycles at 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min followed by 10 min at 72°C. The polymerase chain reaction (PCR) product was diluted 1:10, and 2 µl of the dilution was used for the selective amplification. The PCR reaction that followed was one cycle for 2 min at 94°C, 12 cycles of 30 s at 94°C, 30 s at 65°C (decreasing annealing temperature by 0.7°C each cycle), and 1 min at 72°C. The remaining 25 cycles were carried out for 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C, with the final extension step at 72°C for 2 min. Six microliters of amplified product was separated on a 5% polyacrylamide denaturing sequencing gel in 1× TBE buffer (0.089 M Tris, 0.089 M boric acid, and 0.002 M Na-EDTA in distilled water), and the gel was run for 3 h at 75 W. The gel was stained with silver stain using the Silver Sequence DNA Sequence System (Promega Corp.) according to the supplied protocols. For AFLP analysis, only polymorphic fragments that produced repeatable bands between 100 and 700 bp were scored. Monomorphic markers were not scored. The AFLP fragments were coded as binary strings for presence (1) and absence (0) of a polymorphic band. The combined allelic state for all AFLP primer pairs was designated as the multilocus haplotype of an isolate. Unclear bands due to weak PCR amplification in a few isolates were scored as missing data.

Data analysis. Only fragments that could be scored unambiguously were included in each AFLP analysis. Any fragments that did not show clear presence or absence patterns in all the populations were excluded in each analysis. Each unique multilocus haplotype was considered as representing one individual or genotype, and isolates with the same multilocus genotype and the same DNA fingerprint were regarded as clones. Clone-corrected data were used for all analyses in this study to prevent a bias in the statistical analysis of the genetic structure of *S. passerinii* populations resulting from repeat sampling of the same clone in any population.

TABLE 2. Total number of amplified fragment length polymorphism (AFLP) bands scored and number of polymorphisms with five primer combinations used in AFLP analysis of 390 *Septoria passerinii* isolates from North Dakota and western Minnesota sampled in 2003 and 2004

Primer combinations	No. of bands ^a	No. of polymorphic bands	Polymorphism (%)
<i>EcoRI</i> -AG/ <i>MseI</i> -CG	26	3	11.5
<i>EcoRI</i> -AG/ <i>MseI</i> -CA	25	8	32.0
<i>EcoRI</i> -AA/ <i>MseI</i> -CT	22	7	31.8
<i>EcoRI</i> -AT/ <i>MseI</i> -C	42	22	52.4
<i>EcoRI</i> -AC/ <i>MseI</i> -C	39	17	43.6
Total	154	57	37.0

^a Only bands between 100 and 700 bp were included.

To examine the genetic variation within a lesion on a leaf in ND16 and ND17, the multiple isolates from a single discrete lesion on each leaf were assessed for number of genotypes and Nei's (20) genetic diversity statistic was calculated. In the two sites, ND16 and ND17, the isolates sampled from two to three pycnidia per lesion in a leaf were used to analyze the genetic structure of *S. passerinii* populations within the field.

The analysis of hierarchical gene diversity was performed with three spatial categories: within leaves, among leaves within fields, and among fields within ND and western MN (2,13). Only the populations from sites ND16 and ND17 that were sampled using the hierarchical method were included in the analysis of the gene diversity, which was partitioned into within leaves and among leaves within the field. All nine populations were included for analysis at the macrogeographical level.

The population differentiation (G_{ST}) across five AFLP loci was measured between fields (20), and the estimate of gene flow (Nm) between populations was calculated from average G_{ST} across the five AFLP primer combinations, where $Nm = 0.5(1 - G_{ST})/G_{ST}$ (13). To examine whether genetic differences had occurred as a result of geographical isolation, the correlation between geographical distance and genetic distance between all populations was examined (28). The significance of correlations was assessed by the Mantel test, with 999 permutations. The genetic identity (I) between field populations was calculated by using unbiased Nei's measures (21). The genetic relationship among the nine field populations was investigated by the unweighted pair-group method with arithmetic average (UPGMA) cluster analysis with Dice's coefficient (1,000 replications) using WinBoot (provided online by the International Rice Research Institute, Los Baños, Philippines), and Nei's (21) unbiased distance was calculated using Tools for Population Genetic Analysis version 1.3 (TFPGA is provided online by M. P. Miller at Utah State University). Genotypic diversity (GD), the probability that two individuals taken at random have different genotypes and calculated by

$$\frac{n}{n-1} \left(1 - \sum_i p_i^2 \right)$$

as well as $\bar{r}_d$, an index of multilocus linkage disequilibrium and sample-size-independent alternative to I_A , were calculated using MultiLocus 1.3 software (1). Tests of significance for both statistics were calculated by using 1,000 randomizations for each of the nine populations.

RESULTS

AFLP analysis. Sixty-four primer pair combinations randomly selected from *EcoRI*-1/*MseI*-1 and *EcoRI*-2/*MseI*-2 were first tested on four isolates, and the combinations that produced the highest number of polymorphic bands were subsequently used. A total of five primer pair combinations (*EcoRI*-AG/*MseI*-CG, *EcoRI*-AG/*MseI*-CA, *EcoRI*-AA/*MseI*-CT, *EcoRI*-AT/*MseI*-C, and *EcoRI*-AC/*MseI*-C) were used to analyze the 390 *S. passerinii* isolates. The AFLP primer pairs produced 22 to 42 bands, ranging from 100 to 700 bp, which were clear, identical, and reproducible over all *S. passerinii* isolates, and 57 (37.0%) of them were polymorphic markers for the 390 isolates tested. Of the five primer combinations used, primer pairs *EcoRI*-AT/*MseI*-C and *EcoRI*-AC/*MseI*-C generated the greatest degree of polymorphism (52.4 and 43.6%, respectively) (Table 2).

Genetic variation within lesions and leaves. In the population ND16, a total of 20 different genotypes originating from seven lesions were found among 101 isolates, and the average number of genotypes per lesion was 2.9 (Table 3). In the population ND17, 13 unique genotypes were identified among 83 *S. passerinii* isolates, and the average number of genotypes was 2.3

per lesion. One to four genotypes were found within each lesion in both populations, and one or two clones dominated most lesions. The highest genetic diversity (H) within a lesion was found in ND16-4 (0.197), ND17-2 (0.134), ND17-4 (0.211), and ND17-6 (0.228). Only one genotype was found in three of six lesions in ND16. The average genetic diversity within a lesion was 0.043 in ND16 and 0.096 in ND17 (Table 3). However, in both populations, there was a wide variation between leaves with a standard deviation of 0.068 for ND16 and 0.109 for ND17.

TABLE 3. Distribution of genetic variation within a lesion in seven *Septoria passerinii* lesions from sample site ND16, and six *S. passerinii* lesions from sample site ND17 in 2004

Population Sampling location	Lesion	Sample size ^a	G^b	H^c
ND16 Williston, ND	ND16-1	17	4	0.034
	ND16-2	16	3	0.019
	ND16-3	19	3	0.019
	ND16-4	17	4	0.197
	ND16-5	9	2	0.017
	ND16-6	11	2	0.009
	ND16-7	12	2	0.009
	Total	101	20	0.043
ND17 Langdon, ND	ND17-1	16	1	0.000
	ND17-2	12	3	0.134
	ND17-3	16	1	0.000
	ND17-4	12	6	0.211
	ND17-5	11	1	0.000
	ND17-6	16	2	0.228
	Total	83	13	0.096

^a Sample size before clone-correction.

^b Number of genotypes.

^c Nei's (1973) gene diversity.

Genetic variation within fields. Across all nine populations sampled, the average number of polymorphic loci was 42.4 and the average percentage of polymorphic loci within a population was 74.5%. An average of 15.9 genotypes per population were identified in the populations sampled from ND and western MN. In populations ND1, ND7, and ND15, no clones were found (Table 4). Genotypic diversity within populations was very high in all fields (Table 4); being 1.0 and nonsignificant ($P = 1.00$) in most populations, with the exception of ND12, ND16, and ND17 where it was 0.99 and significant (Table 4).

Population differentiation. The average gene diversity (H) across all loci ranged from 0.16 to 0.40 (Table 4). ND16 showed the highest value of gene diversity ($H = 0.40$), and the lowest gene diversity was found in ND12 ($H = 0.16$). The population differentiation (G_{ST}) average across all AFLP loci was 0.24 (SE = 0.02) among all populations.

The proportion of average gene diversity within leaves was 0.908 across all AFLP primer pairs (Table 5). The total gene diversity among leaves within fields and among fields within ND and western MN was 0.081 and 0.011, respectively.

Among all nine populations sampled, pairwise comparisons of genetic identity (I) ranged from 0.642 (ND12 and ND14) to 0.960 (ND17 and ND1) (Table 6) and pairwise measures of genetic distance (D) ranged from 0.041 (ND1 and ND17) to 0.444 (ND14 and ND10) (data not shown). Using UPGMA cluster analysis based on Nei's (19) original genetic distance, and bootstrapping, there was strong support for a single clade containing ND1, ND5, ND7, ND10, ND14, ND16, and ND17, with ND12 and ND15 separate from this clade (Fig. 1).

Linkage disequilibrium. $\bar{r}_d$ was low to very low in all populations, with the exception of ND15. In ND15, it was 0.249, and in the remaining populations it ranged from 0.032 to 0.097. In all cases, the values were significant at $P < 0.001$ (Table 4).

The Mantel test showed that the correlation between geographic distance and gene flow (Nm) among the nine populations

TABLE 4. Population diversity of all *Septoria passerinii* isolates sampled from North Dakota and western Minnesota in 2003 and 2004, calculated using five polymorphic amplified fragment length polymorphism primer pairs

Population	Sample size ^a	No. of poly- morphic loci	P (%) ^b	G^c	G/N^d	Genotypic diversity ^e	H^f	$\bar{r}_d^g$
ND1	16	48	84.2	16	100.0	1.00	0.38	0.050***
ND5	24	48	84.2	20	83.3	1.00	0.36	0.039
ND7	12	44	77.2	12	100.0	1.00	0.31	0.032
ND10	30	44	77.2	18	60.0	1.00	0.29	0.066***
ND12	39	23	40.4	19	48.7	0.99*	0.16	0.097***
ND14	11	38	66.7	9	81.8	1.00	0.33	0.086***
ND15	10	35	61.3	10	100.0	1.00	0.30	0.249***
ND16	33	49	86.0	17	51.5	0.99***	0.40	0.054***
ND17	31	53	93.0	23	71.0	0.99***	0.37	0.042***
Average		42.4	74.5	16	77.4		0.32	

^a Clone-corrected data set.

^b Percentage of polymorphic loci within a population.

^c Total number of genotypes.

^d Percentage of genotype.

^e Significant results for test indicated by *** ($P < 0.001$) and * ($P < 0.05$).

^f Nei's gene (1973) diversity within population.

^g Multilocus linkage disequilibrium: significant results for test indicated by *** ($P < 0.001$).

TABLE 5. Proportion of gene diversity (H)^a at different spatial scales across all *Septoria passerinii* isolates collected from North Dakota and western Minnesota in 2003 and 2004

Gene diversity	Amplified fragment length polymorphism primer pairs ^b					Average
	<i>E-AG/M-CG</i>	<i>E-AG/M-CA</i>	<i>E-AA/M-CT</i>	<i>E-AT/M-C</i>	<i>E-AC/M-C</i>	
Within leaves	0.842	0.929	0.960	0.958	0.922	0.908
Among leaves within fields	0.148	0.053	0.038	0.025	0.073	0.081
Among fields within the region	0.010	0.018	0.002	0.018	0.005	0.011

^a Gene diversity was calculated according to the method of Beckwitt and Chakraborty (2).

^b $E = EcoRI$, $M = MseI$.

was not significant, indicating that no decrease in gene flow occurred as geographical distance increased ($r = 0.017$, $P > 0.05$).

DISCUSSION

In this study, the genetic structure of *S. passerinii* populations in ND and western MN was examined at several scales: within lesions, within leaves, among leaves, and among fields. Based on 57 polymorphic AFLP markers, high levels of gene and genotypic variation were observed in the nine populations sampled. Moreover, multiple clones in a lesion and multiple haplotypes were determined with five AFLP primer combinations.

Distribution of *S. passerinii* genotypes within seven naturally infected leaf lesions in population ND16 and six lesions in population ND17 indicated that the number of genotypes within a lesion was generally low in both field populations. The locations of ND16 and ND17 are geographically and climatically different and separated by a distance of 406 km. The 30-year minimum and maximum average temperature in the barley-growing period of April to September is 8 and 23°C in ND16 and 6 and 20°C in ND17 (available online by the North Dakota Agricultural Weather Network [NDAWN], verified 7 August 2006). The 30-year average precipitation from April to September at ND16 is 49 mm and at ND17 is 60.3 mm (available online by NDAWN, verified 7 August 2006). Despite the geographic and climatic differences between the two sites, overall a single lesion was occupied by one to four genotypes in both populations and the average haplotype variation between different pycnidia within the same lesion was about 20% in ND16 and about 16% in ND17 (Table 3). This finding is similar to the results previously reported in the wheat pathogen *M. graminicola* (anamorph; *S. tritici*) (12,15,26), which is genetically closely related to *S. passerinii* (8). Identical genotypes were not found between populations ND16 and ND17, even though the two populations were genetically very similar ($I = 0.957$). The same AFLP fingerprint haplotypes were mostly collected from the same lesion on a leaf. The finding of multiple clones within a lesion and higher genotype variation within leaves has implications for interpretation of the potential life cycle of *S. passerinii* under field conditions and supports the hypothesis that in barley in the field the initial inoculum is sexual spores, with asexual spores as the secondary inoculum.

In *M. graminicola*, the distinct spatial clusters of clones within a lesion are thought to result from reproduction by asexual pycnidiospores over the growing season. However, the occurrence of unique multiple genotypes within the same lesions and leaves are thought to be mainly caused by the initial inoculum, ascospores of *M. graminicola* that are sexually derived (3,5,32). In our populations, we found that the pattern of genetic variation of *S. passerinii* within and among lesions on a leaf was very similar to the observed genetic diversity in *M. graminicola*, which is known to have regular cycles of sexual reproduction in fields.

Very low multilocus linkage disequilibrium values, high genotypic diversity, and moderately high gene diversity were found in most of the nine populations, which supports the hypothesis that frequent sexual recombination is occurring in the field. The

highest linkage disequilibrium was observed in ND15 and the second highest value was observed in ND12, which agrees with the result of the genetic distance cluster analysis, where ND15 and ND12 were distinctly separate from a cluster containing seven of the populations. However, ND15 also had moderately high gene diversity and high genotypic diversity. It is possible that if the telomorph exists under field conditions, that it is not common in the ND15 population. The ND12 population, which was also separate from the cluster containing seven of the populations, had high genotypic diversity and low multilocus linkage disequilibrium but also had relatively low gene diversity. In a related study, Lee and Neate (*unpublished data*) showed that only one *S. passerinii* mating type, *MAT-1*, existed in the ND12 population among the 39 clone-corrected isolates tested, and in the ND15 population, the ratio of the two mating types was 1:9 *MAT-1*:*MAT-2* in the 10 clone-corrected isolates studied. Thus, if the sexual stage exists in *S. passerinii*, regular cycles of sexual reproduction may not be common in these two populations. Further studies using more intensively collected field populations and increased sample size from ND12 and ND15 will allow us to determine whether these two populations are truly different from the others studied.

Recently, microsatellites or SSR primers have been developed for *M. graminicola* (8,22). SSR markers are codominant and have the advantage of being able to predict heterozygosity in the population. SSRs have been effectively used to examine the genetic structure of a Saskatchewan (Canada) population of *M. graminicola* (24). That study also supported the hypothesis that sexual reproduction of the pathogen in Saskatchewan was the primary source of genetic variability within and among locations. In our study, we tested SSR primers developed from *M. graminicola* (8). Among 35 *M. graminicola* SSR primers tested, only two were polymorphic and reproducible in a random selection of 10 *S. passerinii* isolates (data not shown) so we were not able to use these markers in our populations.

Fig. 1. Unweighted pair-group method with arithmetic average dendrogram analysis of nine *Septoria passerinii* field populations in North Dakota and western Minnesota in 2003 and 2004 using Nei's (1978) unbiased distance. Bootstrap P values are indicated at the corresponding node for each cluster.

TABLE 6. Pairwise comparisons of Nei's unbiased measure of genetic identity (I) in nine *Septoria passerinii* populations from North Dakota and western Minnesota in 2003 and 2004

	ND5	ND7	ND10	ND12	ND14	ND15	ND16	ND17
ND1	0.939	0.909	0.905	0.729	0.819	0.736	0.950	0.960
ND5	...	0.930	0.905	0.730	0.781	0.719	0.926	0.932
ND7	...	...	0.887	0.711	0.730	0.687	0.905	0.907
ND10	...	...	...	0.658	0.691	0.685	0.787	0.900
ND12	...	...	...	...	0.642	0.669	0.719	0.766
ND14	...	...	...	...	...	0.763	0.778	0.792
ND15	...	...	...	...	...	...	0.915	0.750
ND16	...	...	...	...	...	...	...	0.957

High levels of genetic diversity were found within leaves across all populations, and almost the same magnitude of genetic diversity was found within a field. This indicates that the majority of gene diversity is distributed on a small spatial scale in *S. passerinii* (Table 5). Even though a high level of genetic diversity was found, no shared multilocus genotypes were observed. Such unique genotypes may be the result of newly created genotypes by recombination and long-distance migration. According to analysis of spatial distribution of gene diversity across all *S. passerinii* isolates, approximately 89% of total genetic diversity was distributed within leaves that were sampled 10 m apart within fields ND16 and ND17. About 9% of genetic diversity was distributed among leaves within fields and 1.5% among fields within the ND and western MN region sampled.

The G_{ST} values ranged from 0.215 to 0.241 among all nine populations, and the overall average of G_{ST} was 0.238. This value for population differentiation in *S. passerinii* is relatively higher than the G_{ST} found in *M. graminicola* (12,26). However, the average G_{ST} for the six populations (ND1, ND5, ND7, ND10, ND16, and ND17) was very small ($G_{ST} = 0.09$) compared with the G_{ST} commonly found in other ascomycete fungi with active sexual cycles. The G_{ST} values indicate a low level of population differentiation with very little differentiation between the sampling sites and are consistent with substantial gene flow between these geographically separated populations. However, in this study, it is not clear whether the lower G_{ST} value in the three populations (ND12, ND14, and ND15) is a result of independent evolution or the small sample size. Further sampling of these areas is recommended to resolve this problem.

To facilitate gene flow between populations, short- or long-distance ascospore migration is considered to be the most significant factor in preventing isolation by geographical distance in *M. graminicola* (3,27). In another wheat leaf pathogen, *Phaeosphaeria nodorum*, the long-distance movement of infected seed is considered to be the major mechanism for intercontinental dispersal (10). Gene flow between *S. passerinii* populations may occur by human-mediated movement such as infected stubble and residues on machinery of contract harvesting companies or as feed bought for animal consumption. However, the high genetic similarity found in the widely geographically spread populations (ND1, ND16, and ND17) cannot be explained by human-mediated movement alone (3). In a study by Boeger et al. (3), Oregon and California *M. graminicola* populations that are in different geographic regions showed very high genetic uniformity between two populations due to a high level of gene flow. A similar result was found for the three populations (ND1, ND16, and ND17). ND17 is 450 km from ND1 and ND16, which are geographically close together, but ND17 showed greater genetic uniformity with ND1 and ND16 than with other populations that were geographically closer. A similar result was also found with ND5 and ND14, which are separated by 350 km. It is unlikely that there was movement of machinery or plant materials between these two sites. Thus, gene flow due to natural dispersal mechanisms such as wind-blown spores rather than human activity may exist between geographically distant populations in the region.

In the UPGMA cluster analysis, one large clade was found containing ND1, ND5, ND7, ND10, ND14, ND16, and ND17. This result supports the hypothesis of active gene flow among populations. In contrast, there were considerable genetic differences between populations ND12 and ND15, and the other populations. Populations ND12 and ND15 showed a low level of gene diversity (H), high level of multilocus linkage disequilibrium, and high level of population differentiation (G_{ST}) compared with other *S. passerinii* populations. Assuming that these differences are not an artifact of the small population sizes, the migration of new genes or genotypes may be limited in these populations or the populations have not yet reached equilibrium between gene flow and genetic drift.

The previous finding of approximate equal frequencies of the two mating types in a population of less than 20 isolates from ND (7) supports our conclusion of possible sexual reproduction with random mating under field conditions. The occurrence of gene flow between the geographic populations in ND may indicate the potential for widespread and long-distance dispersal of *S. passerinii*. This information on current genetic structure of *S. passerinii* is valuable in understanding the potential for existence of sexual reproduction of *S. passerinii* in fields and to develop breeding strategies for durable disease resistance in barley where the only available sources of resistance are single dominant genes that have not been tested for their durability.

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