DNA Restriction Fragment Length Polymorphisms Among *Mycosphaerella graminicola* (Anamorph *Septoria tritici*) Isolates Collected from a Single Wheat Field

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ABSTRACT

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Restriction fragment length polymorphism (RFLP) markers were used to measure the amount and distribution of genetic variation in a fungal pathogen population on a microgeographical scale. Ninety-three isolates of *Septoria tritici* sampled from a single wheat field were assayed for RFLP variation using eight probes that hybridized to single RFLP loci and one probe that hybridized to two RFLP loci in nuclear DNA. Single locus and multilocus analysis of RFLP data indicated that a high level of genetic variability was distributed on a fine scale within this population. In eight out of 31 comparisons, different pycnidial isolates collected from the same lesion had different alleles for at least two RFLP loci. Twenty-two different haplotypes were identified among the 93 isolates sampled. Identical haplotypes were clustered in the same location in the field. Data were consistent with the hypothesis that primary inoculum originated from a genetically diverse founding population (probably ascospores of *Mycosphaerella graminicola*), followed by clonal propagation via asexual pycnidiospores.

Additional keywords: population genetics, *Septoria tritici* leaf blotch.

Genetic variability in plant pathogens has often been studied on a macrogeographical scale but seldom at the level of individual populations. Knowledge of the amount and distribution of genetic variability in pathogen populations is prerequisite to studies of gene flow, natural selection, or host-pathogen coevolution in plant pathosystems (15). Most surveys to date have evaluated genetic variation from a limited number of isolates collected from a wide geographical area. Such surveys provide insight into the range of variability existing over large geographical areas, but the samples may have been collected from a large number of discrete pathogen populations, and thus they are probably inadequate to provide definitive data on the population genetics of plant

pathogens. An alternative sampling strategy is to collect large numbers of isolates from individual populations on a microgeographical scale as reported recently for the barley scald pathogen, *Rhynchosporium secalis* (Oudem.) J. J. Davis (12,14). In this case, sampling strategy and sample size were adequate to quantify genetic variation within pathogen populations from a single location and to demonstrate that genetic variation in this pathogen is distributed in a fine-scaled mosaic pattern on a microgeographical scale (14).

The most extensive surveys to date have utilized virulence and/or isozyme markers to quantify variation among geographically diverse groups of isolates (4,10,19,21). Isozyme data are more likely than virulence data to provide unbiased estimates of total genetic variability because they detect variation at loci that are not known to be under selection, while virulence loci are more likely to be under selection. Most isozyme surveys detected low levels of intraspecific variation (10,19,21), but Burdon and Roelfs (4) found high levels of variation in sexual *Puccinia graminis* f. sp. *tritici* populations, and McDonald, et al (14) found high levels of variation in two asexual *R. secalis* populations sampled from a single field. Recently, restriction fragment length polymorphisms (RFLPs), which reflect variation among homologous DNA sequences, have provided more precise tools for detecting and quantifying genetic variation (17). Though our knowledge of RFLP variation in pathogenic fungi has grown steadily (8,13,20), sample sizes generally have been small and inadequate to quantify variation within individual populations. In one case where a single *R. secalis* population was sampled repeatedly, a high level of variability was found using a ribosomal DNA probe (12), but more data are needed to establish a baseline level of variability for RFLP loci in pathogen populations.

Septoria tritici Roberge in Desmaz. (teleomorph *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn), which causes Septoria tritici leaf blotch on wheat, is a haploid fungus that has no naturally occurring morphological or known physiological markers such as vegetative incompatibility groups or auxotrophic markers, so it has been difficult to study genetically. Pathogenic specialization is thought to exist, but clearly defined virulence genes have not been identified (6). A survey of an international collection of isolates showed that the fungus is variable for virulence (5), but no study has measured genetic variability within populations. The goal of this research was to use a recently developed set of DNA probes that identify RFLPs (13) to estimate the amount and distribution of genetic variability in *S. tritici* populations.

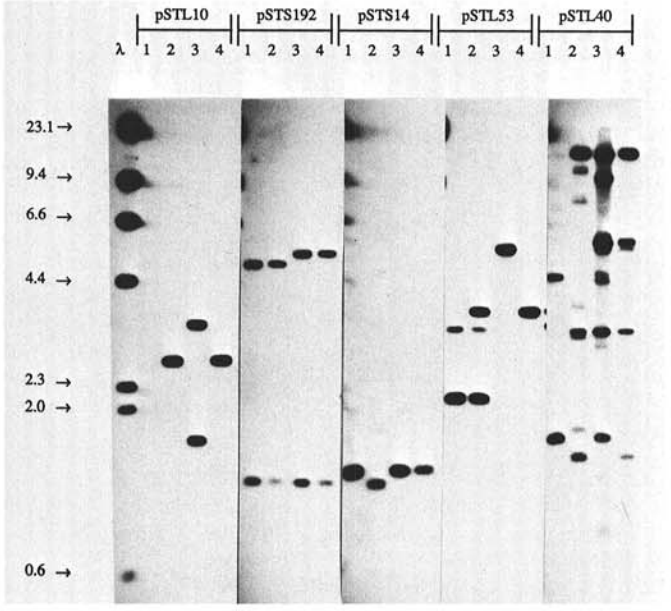

Fig. 1. Composite Southern blot showing different alleles at RFLP loci in *Septoria tritici* isolates sampled from a population in Davis, CA. DNA from isolates ST47, ST61, ST85, and ST96 (designated 1, 2, 3, and 4, respectively) was digested with *Pst*I and hybridized with plasmid probes pSTL10, pSTS192, pSTS14, pSTL53, and pSTL40. The first lane is a λ*Hin*dIII size standard. Isolate ST47 has a deletion at the locus homologous to pSTL10. Isolate ST61 is partially diploid at the locus homologous to pSTL53. pSTL40, which hybridized to several highly variable RFLP loci, can be used for DNA fingerprinting.

MATERIALS AND METHODS

Collection and maintenance of isolates. Leaf tissue infected with *S. tritici* was collected in April 1989 from a field planted to Yolo wheat in Davis, CA. Infected leaves were collected from seven different locations ca. 10 m apart in the field. The area of the collection measured approximately 40 m × 40 m. At each location, a single infected leaf was removed from each of three or four different plants. When available, leaves possessing more than one discrete lesion were collected. Leaf tissue from each location was placed in a paper envelope, allowed to air dry for 1 wk, and mailed to Texas A&M University. Dried leaf tissue was rewetted by immersion for 15 sec in 70% ethanol and surface sterilized by immersion for 90 sec in a 0.5% sodium hypochlorite solution. Sterilized tissue was placed on moistened filter paper in a petri dish overnight at room temperature to encourage extrusion of cirrhi from pycnidia. Three randomly chosen cirrhi from different pycnidia of each lesion were transferred to petri dishes containing potato-dextrose agar medium. Pycnidiospores were streaked onto fresh PDA petri plates to separate individual spores and single colonies were isolated for further analysis. Isolates were grown in yeast-sucrose broth and placed on silica gel for long-term storage (23).

In total, we sampled 93 isolates from 35 distinct lesions on 19 different leaves from the seven field locations. This collection strategy allowed us to compare genetic variation at several different levels, that is, among locations within a field, among plants within a location, among lesions within a leaf, and among pycnidia within a lesion. If we treat each sample from the population as a Bernoulli trial, we can use the binomial distribution to calculate the sample size n necessary to have probability P of detecting alleles present at frequency p or greater at any given locus in this population as:

$$n = \frac{\log (1 - P)}{\log (1 - p)} \quad (1)$$

Using our sample as an example, if we choose n = 35, which is the number of lesions sampled, and let P = 0.95, then we

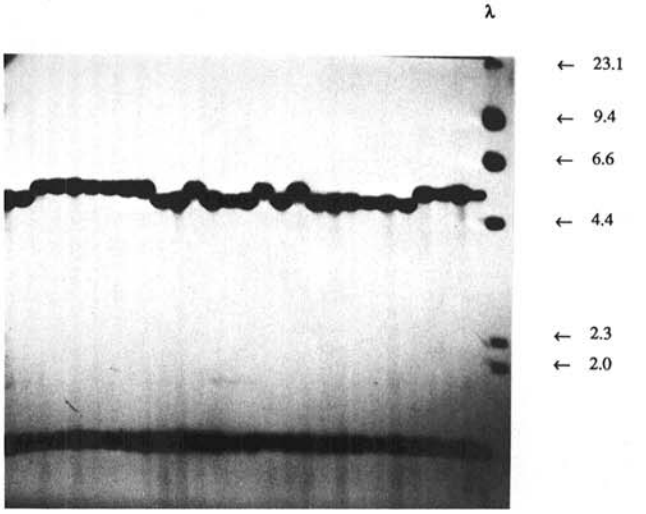

Fig. 2. Southern hybridization of *Pst*I-digested DNA from 28 *Septoria tritici* isolates sampled from a population in Davis, CA. Plasmid probe pSTS192 hybridized to one RFLP locus and one locus which showed no length polymorphism. The last lane is a λ-*Hin*dIII size standard.

have 95% confidence that our sample included all alleles present at a frequency of 0.08 or greater. If we choose n = 93, which is the total number of isolates in the sample, then we have 95% confidence that our sample included all alleles present at a frequency of 0.03 or greater.

DNA isolation and choice of probes. DNA was extracted as described previously (13), but with the following modifications: tissue frozen in liquid nitrogen was ground into a powder, dispersed in 5 ml of CTAB extraction buffer in a 15-ml centrifuge tube, and placed in a 60 C water bath for 30–45 min. An equal volume of chloroform:isoamyl alcohol (24:1, v:v) was added and the tube mixed by inversion to form an emulsion. The emulsion was spun 10 min in a centrifuge at 12,900 × g, and the upper aqueous phase was transferred to a fresh 15-ml tube. Nucleic acids were precipitated by adding an equal volume of isopropanol and centrifuging for 5 min at 10,400 × g. The nucleic acid pellet was dissolved in 2 ml TE (10 mM Tris, 1 mM EDTA, pH 8.0); it was then precipitated by adding 1 ml of 7.5 M NH_4OAc and 5 ml of 95% ethanol and centrifuging for 5 min at 10,400 × g. The pellet was dissolved in 0.4 ml of TE and transferred to a 1.5-ml microfuge tube, and nucleic acids were precipitated by adding 0.8 ml of 95% ethanol and centrifuging 2 min at 16,000 × g. Nucleic acid pellets were dried in a desiccator and then dissolved in 40–80 μl of TE.

Development of probes used to detect RFLPs was described previously (13). Probes for this study were selected using three criteria: 1) probes detected RFLPs among a geographically diverse group of six *S. tritici* isolates, 2) probes produced a strong hybridization signal and hybridized to only one or two fragments, and 3) probes hybridized to fragments between 0.5–6.0 kb to insure adequate resolution of all restriction fragments present in our sample.

Enzyme digestion, electrophoresis, and Southern blotting. Three μg DNA from each isolate were digested separately with the restriction enzymes *Pst*I and *Xho*I. Digestions were for 16 hr with 15 units of enzyme according to the manufacturer's instructions. DNA electrophoresis, Southern blotting, prehybridization, and hybridization conditions were as described previously (13). Probes pSTS192, pSTL10, and pSTL53 were hybridized to both *Pst*I- and *Xho*I-digested DNA of each isolate. Probes pSTS2, pSTS14, and pSTS199 were hybridized only to *Pst*I-digested DNA and probes pSTS196, pSTS197, and pSTL2 were hybridized only to *Xho*I-digested DNA.

Data analysis. RFLP loci were detected by hybridization of particular DNA probes to complementary DNA restriction fragments present on a Southern blot. Alleles were defined as specific DNA restriction fragments identified by particular probe-enzyme combinations. Frequencies of alleles at individual RFLP loci were tabulated for each location in the field and for the total population. Two measures of genetic diversity were used to assess total genetic variability. Nei's measure of genetic diversity (18) is given by the formula,

$$h = 1 - \sum_{j=1}^{k} x_j^2 \qquad (2)$$

where x_j is the frequency of the j-th allele. The proportion of polymorphic loci is given by the formula $\hat{P} = x/r$ where x is the number of polymorphic loci and r is the total number of loci examined. Differences in allele frequencies between locations were tested using a χ^2 test for heterogeneity (26). Since *S. tritici* is haploid, we calculated the χ^2 values for each RFLP locus using the formula

$$\chi^2 = N \sum_{j=1}^{k} \sigma^2 p_j / \bar{p}_j \qquad (3)$$

where N is the number of isolates and $\bar{p}_j$ and $\sigma^2 p_j$ are the mean and weighted variance of the frequencies of the j-th allele. Hierarchical gene diversity analysis (2) was used to partition genetic variation into components among and within locations in the field. Multilocus genotypes (hereafter referred to as haplotypes) of individual isolates were constructed by combining allelic data from different probe-enzyme combinations in the following manner: 1) each allele was assigned a number in order of decreasing frequency for each probe-enzyme combination, e.g., allele 1 was the most common, followed by allele 2, etc.; 2) numbers were combined in the order pSTS192-*Xho*I, pSTS192-*Pst*I, pSTS14-*Pst*I, pSTS196-*Xho*I, pSTS197-*Xho*I, pSTS2-*Pst*I, pSTL2-*Xho*I, pSTL10-*Pst*I, pSTL10-*Xho*I, pSTL53-*Xho*I, pST153-*Pst*I, and pSTS199-*Pst*I. As an example, the haplotype 211111211113 possessed the second most common allele identified by probe-enzyme pSTS192-*Xho*I, the most common alleles identified by the probe-enzymes pSTS192-*Pst*I, pSTS14-*Pst*I, pSTS196-*Xho*I, pSTS197-*Xho*I, etc.

RESULTS

Examples of the different alleles present at different RFLP loci are shown in Figure 1 and an example data set is shown in Figure 2. Polymorphisms existed within the population for each of the 12 probe-enzyme combinations tested. If polymorphic RFLP loci are defined as those loci at which the most common allele makes up less than 95% of the sample, the proportion of polymorphic RFLP loci was P = 0.923. On average, we observed 3.3 alleles per RFLP locus in this population. Nei's genetic diversity index ranged from h = 0.06 to h = 0.75 per locus and averaged 0.45 ± 0.01 over the 12 RFLP loci (Table 1). Frequencies of the different alleles at each RFLP locus at each location in the field are summarized in Table 1. A $\chi 2$ test for heterogeneity among locations within the field showed that allele frequencies were significantly different among locations for all probe-enzyme combinations except pSTS197-*Xho*I (Table 1). Hierarchical gene diversity analysis indicated that the majority of variability was distributed within locations for 9 of the 12 probe-enzyme combinations (Table 2).

Twenty-two different haplotypes were identified among the 93 isolates screened, with no haplotype present at a frequency greater than 15% (Table 3). With one exception, identical haplotypes originated from the same location in the field. The exception was probably a migrant from a neighboring location. Assuming that identical haplotypes are clones of the same individual (this assumption is supported by data from DNA-fingerprinting probes, manuscript in preparation), then a crude measure of clone diversity can be calculated as the number of clones divided by the number of isolates sampled, in this case 22/93 = 0.24. Six haplotypes (27%) occurred only once in our sample. The haplotype data allowed us to determine the level at which clonal variation first became apparent. One of the seven locations (location E) had only a single haplotype; all other locations possessed at least two different haplotypes. Unlike haplotypes occurred on different lesions for 11 of the 12 comparisons where isolates were sampled from different lesions on the same leaf. There were 31 cases in which multiple pycnidia were sampled from a single lesion, and, in 8 (26%) of these lesions, isolates derived from different pycnidia within the lesion had different haplotypes.

DISCUSSION

Our goal in this survey was to assess the ability of RFLPs to characterize the amount and distribution of genetic variation in a pathogen population. The data show that the *S. tritici* population sampled contained a large amount of genetic variation distributed on a very fine scale. This distribution was similar to that found in two populations of *R. secalis* sampled from a single field (14). Measurements of genetic variation were consistent with high levels of diversity for 11 of the 12 RFLP loci. The averaged gene diversity over all 12 RFLP loci (0.45) was higher than that found at seven isozyme loci by Burdon and Roelfs (4) in sexual populations of *P. graminis* (0.37) and was generally higher than that found in a number of animal species where large numbers of isozyme loci were studied (18). It is likely

that measurements of genetic variation based on anonymous RFLP loci will be greater than at isozyme or virulence loci due to the greater sensitivity of RFLP analysis, but more data are needed to test this hypothesis. It is notable that 22 different haplotypes were identified in a sample of only 93 isolates collected from a single field. The advent of DNA fingerprinting techniques using multilocus RFLP analysis or single hypervariable probes such as pSTL40 (Fig. 1) (13) may allow identification of individual clones in populations of plant pathogens.

Analysis of single-locus and multilocus data shows that genetic variability in this *S. tritici* population was distributed on a fine scale. Hierarchical gene diversity analysis allows total genetic variation (as measured using Nei's measure of diversity) to be partitioned into components reflecting any level of subdivision, in a manner analogous to the partitioning of variance using analysis of variance. Due to the limited number of isolates available for within-leaf comparisons, we chose to divide total variation into two components: within locations in the field and among locations in the field. As shown in Table 2, the majority of RFLP variation was distributed within locations for 9 of the 12 probe-enzyme combinations. χ^2 tests showed that significant differences in allele frequencies existed among locations within the field for

TABLE 1. Allele frequencies and measure of gene diversity in *Septoria tritici* for 12 variable RFLP loci in field locations A–G

Probe-enzyme combination	Allele[a]	A P = 3[b] L = 6[c] N = 16[d]	B P = 2 L = 3 N = 5	C P = 3 L = 7 N = 20	D P = 2 L = 4 N = 10	E P = 3 L = 3 N = 8	F P = 3 L = 4 N = 12	G P = 3 L = 8 N = 22	Total P = 19 L = 35 N = 93	χ^2[e] d.f. = 6	h[f]
pSTS192 *Xho*I	1	1.00	1.00	0.55	1.00	0.00	0.17	1.00	0.71	61***[h]	0.41
	2	0.00	0.00	0.45	0.00	1.00	0.83	0.00	0.29		
pSTS192 *Pst*I	1	0.94	0.20	1.00	0.40	0.00	0.58	0.00	0.51	65***	0.50
	2	0.06	0.80	0.00	0.60	1.00	0.42	1.00	0.49		
pSTS14 *Pst*I	1	1.00	1.00	1.00	1.00	1.00	1.00	0.32	0.84	58***	0.27
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.68	0.16		
pSTS196 *Xho*I	1	0.75	1.00	1.00	0.70	1.00	0.58	1.00	0.87	22***	0.23
	2	0.25	0.00	0.00	0.30	0.00	0.42	0.00	0.13		
pSTS197 *Xho*I	1[g]	0.63	0.80	0.55	0.70	1.00	0.83	0.73	0.71	8	0.41
	2	0.37	0.20	0.45	0.30	0.00	0.17	0.27	0.29		
pSTS2 *Pst*I	1	0.94	1.00	1.00	1.00	1.00	0.83	1.00	0.97	23***	0.06
	2	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.02		
	3	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.01		
pSTL2 *Xho*I	1	0.31	0.80	0.45	1.00	1.00	0.17	1.00	0.65	74***	0.50
	2	0.31	0.20	0.55	0.00	0.00	0.83	0.00	0.29		
	3	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.06		
pSTL10 *Pst*I	1	0.63	1.00	1.00	1.00	1.00	0.58	1.00	0.88	65***	0.22
	2	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.07		
	3[g]	0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.05		
pSTL10 *Xho*I	1	0.19	0.80	0.55	0.60	1.00	0.42	0.32	0.48	159***	0.71
	2	0.00	0.00	0.00	0.40	0.00	0.00	0.68	0.20		
	3	0.06	0.20	0.45	0.00	0.00	0.08	0.00	0.13		
	4	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.09		
	5	0.25	0.00	0.00	0.00	0.00	0.08	0.00	0.05		
	6[g]	0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.05		
pSTL53 *Xho*I	1	0.19	0.20	0.55	0.60	1.00	0.58	0.05	0.40	155***	0.75
	2	0.00	0.80	0.00	0.00	0.00	0.42	0.27	0.16		
	3	0.25	0.00	0.45	0.00	0.00	0.00	0.05	0.15		
	4	0.00	0.00	0.00	0.00	0.00	0.00	0.64	0.15		
	5	0.56	0.00	0.00	0.30	0.00	0.00	0.00	0.13		
	6	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.01		
pSTL53 *Pst*I	1	0.13	0.20	1.00	0.10	1.00	0.33	0.05	0.40	170***	0.75
	2	0.56	0.80	0.00	0.00	0.00	0.00	0.27	0.20		
	3	0.25	0.00	0.00	0.60	0.00	0.25	0.05	0.15		
	4	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.15		
	5	0.00	0.00	0.00	0.30	0.00	0.42	0.00	0.09		
	6	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.01		
pSTS199 *Pst*I	1	0.19	0.80	0.00	0.70	1.00	0.58	0.73	0.48	63***	0.62
	2	0.81	0.20	0.55	0.30	0.00	0.17	0.00	0.32		
	3	0.00	0.00	0.45	0.00	0.00	0.25	0.27	0.20		

[a] Allele designation is in order of decreasing frequency in the total population.
[b] P is the number of plants sampled.
[c] L is the number of lesions sampled.
[d] N is the number of pycnidia sampled.
[e] χ^2 test is for heterogeneity of allele frequencies among locations in the field.
[f] Nei's measure of gene diversity (18).
[g] Allele was a deletion indicated by lack of hybridization with DNA probe.
[h] *** = $P < 0.001$.

11 of 12 probe-enzyme combinations. On average, 3.4 different haplotypes were found in each location, but, with one exception, identical haplotypes were always collected from the same location. Taken together, these data suggest that this population was highly subdivided into a mosaic of independent clones without significant migration between different locations in the field. Different lesions from the same leaf possessed different haplotypes in 92% (11/12) of the comparisons. This distribution of genetic variation is consistent with the hypothesis that a genetically diverse founding population (i.e. *M. graminicola* ascospores) provided the initial inoculum, and reproduction via asexual pycnidiospores resulted in localized clusters of clones in the field. *M. graminicola* has been identified in several countries (5), but the importance of the sexual stage in the population genetics of this fungus is not known. Shaw and Royle (22) presented evidence that ascospores are the source of primary inoculum for infection in Great Britain. *M. graminicola* was discovered recently in Davis, CA (11), and our results suggest that it may be an important source of primary inoculum and genetic diversity in California.

Haplotype variation between lesions within leaves was higher in *S. tritici* (92% of comparisons different) than in *R. secalis* (50% of comparisons different; J. M. McDermott and B. A. McDonald, *unpublished data*). We did not expect to find a high degree of within-leaf variation because we assumed that most lesions on the same leaf resulted from secondary infections caused by spores from primary lesions on the same leaf. However, the results indicated that different lesions on the same leaf usually resulted from independent infection events involving different haplotypes. Variation within lesions was much higher than in *R. secalis*, whereas no variation within lesions was detected (J. M. McDermott and B. A. McDonald, *unpublished data*). In *S. tritici*, different pycnidia from the same lesion produced unlike haplotypes in 26% (8/31) of the comparisons. In each case where variation within lesions was detected, unlike haplotypes had different alleles at between 2 and 9 RFLP loci, suggesting that these lesions resulted from coinfection by different haplotypes.

We believe that deletion and insertion events are common in *S. tritici* nuclear DNA. Approximately 9% of a collection of random probes detected deletions (noted by absence of probe hybridization) among a geographically diverse group of isolates during an initial screening for RFLP variation (13). Among the isolates sampled from this single field, DNA sequences homologous to probes pSTS197 and pSTL10 were absent in 71% and 5.4% of the isolates, respectively. In several cases, deletion polymorphisms were found among isolates from the same leaf and the same lesion. The initial finding of differences among geographically distinct populations that presumably evolved in isolation was not surprising, but we were surprised to find deletion polymorphisms among isolates from a single population. We have other evidence (manuscript in preparation) that genome rearrangements occur at a high frequency in *S. tritici*.

Several researchers have utilized isozymes and RFLPs to assign taxonomic groupings or infer genetic relatedness among geographically diverse collections of isolates (1,3,7,9,16,20,24), but almost all have ignored variation in local populations. In most cases, sample sizes were inadequate to measure within-taxon or within-population variability. We calculate from equation 1 that a sample of 58 isolates from one population is necessary to have a 95% probability of detecting all alleles present at a frequency of 0.05 or greater in that population, while a sample of five isolates from the same population will detect only those alleles present at frequency 0.45 or greater with 95% confidence. Many previous surveys sampled fewer than five isolates from the different populations or species being compared, and results from these surveys may have been biased by sampling error. Accurate measurements of within-population or within-species variation are necessary to gauge the relevance of between-population or between-species variation, especially when sensitive electrophoretic markers are used for measuring variation.

Our data suggest that the majority of genetic variation in the fungus *S. tritici* may be distributed on a local scale, rather than on a macrogeographical scale. We found 14 new alleles in the California population that were not identified in our original screening of six isolates from Texas, Montana, and Israel (13). Though adequate sampling of other populations is needed to test this hypothesis, we consider the amount of variation to be significant, given that a total of only 19 leaves and 35 separate lesions were sampled from a 40- × 40-m section of a single field. Assuming that these RFLP data accurately reflect the amount of genetic variability in *S. tritici* populations, they indicate that these populations probably contain sufficient genetic variability to allow for rapid selection of clones that are resistant to fungicides or virulent on wheat varieties having pathotype-specific resistance. The best method to counter this diversity may be to deploy resistance genes or fungicides on a similar fine scale, that is, by using mixtures of fungicides for chemical control and/or deploying resistance genes in variety mixtures or multilines (25). We are currently sampling additional *S. tritici* populations to make more precise estimates of within-, between-, and among-population variability and to estimate rates of gene flow between populations.

TABLE 2. Gene diversity analysis of the California population of *Septoria tritici* by RFLP locus. Total diversity was partitioned into components between and within locations in a single field using the method of Beckwitt and Chakraborty (2)

Probe-enzyme combination	H_T[a]	G_L[b]	G_F[c]
pSTS192 *Xho*I	0.41	0.27	0.73
pSTS192 *Pst*I	0.50	0.40	0.60
pSTS14 *Pst*I	0.27	0.22	0.78
pSTS196 *Xho*I	0.22	0.82	0.18
pSTS197 *Xho*I	0.41	0.83	0.17
pSTS2 *Pst*I	0.06	0.95	0.05
pSTL2 *Xho*I	0.50	0.52	0.48
pSTL10 *Pst*I	0.21	0.62	0.38
pSTL10 *Xho*I	0.70	0.61	0.39
pSTL53 *Xho*I	0.75	0.56	0.44
pSTL53 *Pst*I	0.75	0.51	0.49
pSTS199 *Pst*I	0.62	0.56	0.44

[a] H_T is total diversity.
[b] G_L is the proportion of total diversity within locations.
[c] G_F is the proportion of total diversity among locations.

TABLE 3. Frequencies and distribution of the 22 multilocus haplotypes found in the California population of *Septoria tritici*

Haplotype[a]	n	Location(s)[b]
111111112611	1	D
111111114111	2	A
111111211112	2	C
111111215332	4	A
111121113312	9	C
111121211111	2	F
111121213112	1	B
111121324522	6	A
111211111522	3	A
111221112552	3	D
121111111131	7	D,G
121111111221	4	B
121121111223	6	G
121213213161	1	A
122111112311	1	G
122111112441	14	G
211111211113	9	C
211111211133	3	F
211112113112	1	F
211112115112	1	F
221111111111	8	E
221211236251	5	F

[a] Each digit in the haplotype corresponds to the allele present at each of the 12 RFLP loci identified by a specific probe-enzyme combination in the order shown in Table 1.
[b] Locations corresponds to the seven field locations shown in Table 1.

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