Genetic Structure of *Setosphaeria turcica* Populations in Tropical and Temperate Climates

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ABSTRACT

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Northern leaf blight, caused by *Setosphaeria turcica*, is a serious disease of maize in temperate and tropical environments. To examine the pathogen's population structure, we analyzed 264 isolates from four different continents with 70 random amplified polymorphic DNA markers and determined their mating types. Tropical populations (from Kenya, Mexico, and southern China) had an extremely high genotypic diversity, no or only weak gametic phase disequilibrium, and an even distribution of the two mating types, indicating frequent sexual recombination. Temperate populations (from Europe and northern China) had a much

lower genotypic diversity, strong gametic phase disequilibrium, and an uneven distribution of mating types, indicating that sexual recombination has been rare. Populations in different continents were genetically isolated. They shared no haplotypes and carried several "private" alleles. The number of migrants between continents and between regions (between northern and southern China, western and central Kenya, and Europe west and east of the Alps) was estimated to be less than one per generation. Multivariate statistics suggested a greater relatedness of populations from the same continents than from different continents. Within agroecological zones, migration must be extensive. The potential within populations of *S. turcica* for adaptation should be regarded as very high, especially in tropical climates.

The ascomycete *Setosphaeria turcica* (Luttrell) K. J. Leonard & E. G. Suggs, with its anamorph *Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs, causes northern corn leaf blight (NCLB), a severe and widespread foliar disease of maize (*Zea mays* L.). NCLB causes substantial crop losses in most of the major corn-producing areas of the world, particularly in midaltitude and highland areas of the tropics (15,17). Growing resistant cultivars is an effective (and the most widely used) means of NCLB control. *S. turcica* has a number of pathogenic races that are capable of overcoming the major resistance genes *Ht1*, *Ht2*, *Ht3*, or *Hm1* (15). Thus, in the United States (3) and probably in most developing countries, quantitative resistance is used more than qualitative, *Ht* gene, resistance.

Little is known about the genetic structure of *S. turcica* populations (15,30). The degree of diversity has not been fully examined, and the sexual stage of *S. turcica* has never been found in the field, although compatible mating types have been shown to coexist (1,26). However, the formation of pseudothecia can easily be induced in the laboratory (6,14). With population-structure information, a pathogen's potential to adapt to host resistance or new ecological niches may be indirectly assessed. Such information may strategically support resistance breeding programs (16), as was exemplified for the rice-*Magnaporthe grisea* pathosystem (41).

In this study, we used random amplified polymorphic DNA (RAPD) to examine basic parameters of population structure in *S. turcica*. Additionally, mating type frequencies of *S. turcica* were investigated. In particular, we investigated the level and distribution of genotypic and gene diversity and the significance of sexual recombination and migration. To provide a representative description of the *S. turcica* population in different maize-growing

regions of the world, samples were collected in four continents, including regions with tropical (Mexico, Kenya, and southern China) and temperate (northern China and Europe) climates.

MATERIALS AND METHODS

Sampling and culturing of fungal isolates. Lesions were sampled systematically from susceptible maize cultivars in farmers' fields (Table 1). The Kenyan samples contained six lesions per site and the Mexican samples two, and sites were 12 to 24 m apart. In China and Europe, only one lesion per site was collected, and sites were at least 3 m apart. Lesions were dried in a plant press and stored at -20°C until use. Surface-sterilized lesions (dipped for 30 s in 70% ethanol, 30 s in 0.5% hypochloride, and twice for 30 s in sterile distilled water) were incubated on moist filter paper in petri dishes for 2 days at 23°C with 12 h of fluorescent light. Individual conidia were picked with a needle under a dissecting microscope and plated on potato lactose agar (potatoes, 200 g per liter, were cut in half and cooked for 20 min; the cooking liquid was amended with 10 g of lactose per liter). A single *S. turcica* isolate per lesion was cultured. Mycelium for DNA extraction was grown in potato lactose broth in shake-cultures inoculated with conidia. After 5 to 7 days, the mycelium was filtered by means of suction, immediately frozen to -80°C , and later lyophilized. For long-term storage, isolates were subcultured for 8 to 10 days on potato lactose agar plus 5% glycerol. Mycelium with agar was punched into polypropylene cryostraws, put into cryovials, and stored at -80°C . Most isolates are still available.

Mating tests. Two sterile Johnson grass (*Sorghum halepense*) straw were embedded in modified Sachs agar (9) in the center of 9-cm petri dishes (6). First-generation subcultures (3-mm plugs) of the sample isolate and the tester isolate with known mating types (MAT1: Re $\times$ Ch54; MAT2: Re $\times$ Ch34) were placed on opposite sides of the straws. Every pairing of a sample isolate with the two tester isolates was replicated at least two times.

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Plates were kept at 25°C under low light conditions (12 h of mixed GroLux and white fluorescent light). Formation of fruiting bodies on the straws was recorded at 3 and 4 weeks after plating. Mating occurred exclusively with only one of the tester isolates. Production of asci and ascospores was not routinely recorded, except in check crosses.

DNA extraction. Lyophilized mycelium was ground with a bullet mill (Retsch, Haan, Germany) for 30 s at 70 rpm. The DNA microextraction protocol of Möller et al. (23) was adjusted to 30 mg of mycelium and 700 µl of TES (100 mM Tris, pH 8.0; 40 mM EDTA; and 2% sodium dodecyl sulfate) buffer. Additionally, treatment with 50 µg of RNase was conducted under low-salt conditions in TE (Tris-EDTA) buffer followed by a second precipitation with isopropanol. DNA was quantified by visually comparing at least three different dilutions per isolate with λ-DNA standards in agarose gels stained with ethidium bromide.

RAPD polymerase chain reaction (PCR). The protocol of Schilling et al. (28) was slightly modified; only 1 ng of template DNA per PCR reaction was used. Some primers required higher annealing temperatures of 40, 45, or 50°C. Decamer primer kits 100/1 and 100/4 and simple sequence repeat targeted (ssrt) primer kit 100/9 were obtained from the University of British Columbia, Vancouver, Canada, and decamer primer kit T was obtained from Operon Technologies, Alameda, CA. All 320 primers were initially screened for clear amplification patterns with four *S. turcica* isolates of different origin. Primers showing clearly distinct banding patterns were then screened for detection of polymorphisms among 34 isolates. These included 31 *S. turcica* isolates from *Z. mays* from four continents, representing all virulences and both mating types, plus two isolates from *Sorghum bicolor* and one from *S. halepense*. Every polymorphism detected was confirmed for reproducibility by using 0.1, 1, and 10 ng of template DNA of one isolate with the respective fragment and one isolate without it. Nearly half of all bands classified as polymorphic in the first run were excluded from further analyses because their appearance depended on template DNA concentration. Routinely, we used 33 primers generating 87 markers that proved to be reproducibly polymorphic. Of these, 75 markers

differentiated among *S. turcica* isolates from maize, and 70 markers uncovered polymorphisms among isolates of at least one field population (Table 1). For every marker detecting polymorphism, two differential reference isolates were appointed, one generating the band and the other lacking the particular fragment. Two such reference isolates were used as checks in every PCR run and subsequent gel electrophoresis. PCR fragments were separated by 1.5% agarose gels in TAE (tris-acetate-EDTA) buffer, stained with ethidium bromide, visualized under UV light, and documented as thermoprints. In a sexual cross of two *S. turcica* isolates, 19 of 21 bands segregated 1:1 as single alleles. The remaining two bands segregated strictly together and therefore may be of extra-nuclear origin (D. S. Borchardt and H. G. Welz, unpublished data).

Data analysis. An amplified fragment detecting polymorphism between any two isolates is regarded as corresponding to one allele at one marker locus, the other allele or alleles being unknown. The absence of the concerning fragment cannot be regarded as indicative of a second allele. Multilocus haplotypes, in short haplotypes, are defined by the presence or absence of bands at the 70 marker loci.

The Dice similarity coefficient (33) of two isolates, x and y, was calculated as

$$S_d = 2n_{xy} / (2n_{xy} + n_x + n_y)$$

in which n_{xy} , n_x , and n_y are the number of bands formed by both isolates, only by x, and only by y, respectively (33). Principal-coordinates analysis was performed on the basis of Dice similarities with the procedures DCENTER (transformation into scalar products) and EIGEN (calculation of eigenvalues and eigenvectors) of NTSYS-pc (Exeter Software, Setauket, NY). The Dice similarity parameter S_d was transformed into a parameter of distance, $D = 1 - S_d$ (12), and subjected to an analysis of molecular variance (5). By this, a modification of Wright's (40) F_{ST} statistic, Φ_{ST} , was calculated from variance components (4,5,36). It estimates how the molecular variance of the total population is partitioned into subpopulations. The average number of migrants per

TABLE 1. Origin of *Setosphaeria turcica* isolates used in this study^a

Country	Location, region	Geographic position		Date	Sample size
Kenya	Embu, central	0°35' S	36°55' E	July 1994	42
	Kakamega, west	0°15' N	34°45' E	July 1994	44
	Kitale, west	1°0' N	35° E	July 1994	38
Mexico ^b	Chinalpa	19°30' N	99°W	January 1996	24
China ^c	Daxin, Guangxi, south	22°50' N	107°10' E	July 1992	27
	Harbin, Heilongjiang, north	45°30' N	127° E	July 1992	23
Germany	Eckartsweier, URV ^d	48°30' N	7°50' E	July 1994, August 1995	9
	Weisweil, URV	48°10' N	7°45' E	August 1995	4
	Kenzingen, URV	48°10' N	7°50' E	August 1995	5
	Emmendingen, URV	48°5' N	7°50' E	August 1995	5
	Lahr, ^e URV	48°20' N	7°50' E	November 1995	1
Switzerland	Hemishofen, URV	47°50' N	8°50' E	September 1995	3
	Cadenazzo, Tessin	46°10' N	8°55' E	July and August 1993	12
	Monda, ^f Tessin	46°10' N	8°55' E	September 1995	6
France ^g	Agen, southwest France	44°10' N	0°40' E	September 1990, September 1992	9
Austria ^h	Hörzendorf, Kärnten	46°50' N	14°20' E	September 1990	5
	Gleisdorf, Steiermark	47°10' N	15°45' E	September 1990	3
	Spielfeld, Steiermark	46°45' N	15°40' E	September 1990	2
	Wagerdorf, Steiermark	...	...	September 1990	1
	Feldbach, Steiermark	46°55' N	15°50' E	September 1990	1

^a The Kenyan, Chinese, and Mexican samples originated from one field each and are therefore designated field populations. The European isolates came from different locations with one to six isolates per field.

^b Samples provided by D. Jeffers, Mexico.

^c Samples provided by L. Guosheng and H. Wufang, China.

^d Upper Rhine Valley.

^e Samples provided by M. C. Tardin, France.

^f Samples provided by P. Bassetti, Switzerland.

^g Samples provided by J. Hock, France.

^h Samples provided by B. Zwatz, Austria.

generation was estimated according to Wright (31,39), by the formula

$$Nm = [(1/\Phi_{ST}) - 1]/2$$

in which N = local population size and m = average rate of migration, assuming neutral alleles and an “island” model of population structure; Φ_{ST} is used instead of F_{ST} in Wright’s original formula.

Average taxonomic distance (33), a geometric distance parameter, was calculated as

$$d_i = \sqrt[3]{[1/n \sum_l (p_{as} - p_{at})^2]}$$

in which two samples, s and t , are compared across l loci and p_{as} and p_{at} denote the relative frequency of the a th allele in samples s and t , respectively. Visualization of these distances between samples

was achieved by multidimensional scaling and a superimposed minimum-spanning tree (procedures MDSCALE, MST, and MOD3D of NTSYS-pc).

An estimate of genotypic diversity was obtained with the Shannon index, corrected for variable sample size according to Sheldon (29), as

$$H_S = \{-\sum_i [g_i \ln(g_i)]\} / \ln N$$

in which g_i is the frequency of the i th haplotype in one sample and N is the sample size. This measure accounts for both the richness and the evenness aspects of diversity (8). Different H_S values were compared by a t test (13), applying appropriate type I errors via a sequentially rejective Bonferroni procedure (11). In this paper, the term “clone” is used for a group of isolates with an identical haplotype.

TABLE 2. Frequencies of 70 random amplified polymorphic DNA bands considered in the *Setosphaeria turcica* samples^a

Primer ^b Band ^c	Kenya			Mexico	China		Germany	Switzerland	France	Austria
	Kakamega	Kitale	Embu		South	North				
23										
1.9	0	0	0	0	0	0	<u>0.33</u>	0	0	0
32										
1.02	1	1	1	0.96	1	0.96	1	1	1	1
1.68	0	0	0	0.96	0	0.48	1	1	0.89	1
49										
1.3	1	1	1	1	1	1	<u>0.71</u>	1	1	1
74										
0.8	0.02	0.11	0.9	0.96	0	0	0.67	0.96	1	0.33
0.85	1	1	0.14	0.04	1	1	0	0	0	0
76										
0.66	0.07	0.13	0.14	0	0.3	0	0	0	0	0
1.1	0	0	0	<u>0.08</u>	0	0	0	0	0	0
2.1	0	0	0	<u>0.08</u>	0	0	0	0	0	0
77										
1.2	1	1	1	1	1	1	1	1	1	<u>0.92</u>
1.5	1	1	1	<u>0.96</u>	1	1	1	1	1	1
86										
0.83	0	0	0.05	0	0	0.44	0	0	0	0
91										
1.8	<u>0.3</u>	<u>0.13</u>	<u>0.33</u>	0	0	0	0	0	0	0
92										
1.65	0.64	0.63	0.33	0	0	0	0.04	0	0.11	0
100										
2.0	0.89	0.82	0.67	0.92	0.39	0.81	1	1	1	1
403										
0.65	1	1	1	1	1	1	<u>0.67</u>	1	1	1
0.7	<u>0.32</u>	<u>0.37</u>	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	<u>0.33</u>	0	0	0
1.5	0	0	0	<u>0.25</u>	0	0	0	0	0	0
2.4	1	0.97	0.86	1	1	0.81	1	1	1	1
417										
0.72	0	0	0	0.25	0.04	0.48	0	0	0	0
0.9	0	0	<u>0.02</u>	0	0	0	0	0	0	0
1.45	0	0	0	0	<u>0.04</u>	<u>0.48</u>	0	0	0	0
1.55	1	0.97	1	0.71	0.91	1	1	0.9	0.89	1
425										
1.25	1	1	0.96	1	1	1	1	1	0.89	1
1.95	0.27	0.16	0	0	0	0	0	0	0.11	0
431										
0.8	<u>0.98</u>	<u>1</u>	<u>0.12</u>	0	0	0	0	0	0	0
433										
1.1	0	0	0	0.25	0	0	0	0	0	0.25
1.25	0.02	0	0	0.13	0	0.78	0.96	0.9	0.56	0
446										
0.72	0	0	0	<u>0.38</u>	0	0	0	0	0	0
2.1	1	1	0.36	0.33	1	0.78	0	0	0	0
447										
0.76	0	0	0	0.42	0	0	1	1	0.89	1
0.78	0.91	0.92	0.98	0.58	0	0	0	0	0.11	0

(continued on next page)

^a Frequencies of bands showing polymorphism in only one continent (“private” alleles) are underlined.

^b Simple numbers designate primers obtained from UBC, and T indicates primers from Operon kit T.

^c Fragment sizes compared with DNA size markers given in kilobases.

Nei's average gene diversity (25) was computed as

$$H_N = \sum_l h_l / L = \sum_l (1 - \sum_a p_a^2) / L$$

in which h_l is the gene diversity at the l th marker locus, L is the number of marker loci sampled, and p_a is the frequency of the a th allele at the locus considered. The estimate of gene diversity h_l in a homozygous autogamous population is equivalent to that of a haploid population. Note that a reduction of h_l results from artificially constructing a second allele for the absence of the concerning fragment in the case of more than two alleles per locus. The sampling variance of H_N was obtained by

$$V(H_N) = \sum_l (h_l - H_N)^2 / [(L - 1) * L].$$

Different H_N values were compared by a simple t test, applying the

sequentially rejective Bonferroni procedure (11), for two independent observations.

The index of multilocus association, I_A , suggested by Brown et al. (2), was used as a test for the importance of sexual reproduction. It is based on the variance of k , the number of loci at which two isolates differ, calculated over all $n(n - 1)/2$ possible pairs of isolates. It is calculated as $I_A = (V_O/V_E) - 1$, in which V_O and V_E are the observed and expected variances of k , respectively. The null hypothesis "no association" ($H_0: I_A = 0$) is tested by an upper confidence limit of V_O , which is calculated via the error variance of V_E . Only one isolate per clone was considered, as suggested by Maynard Smith et al. (18). Three ssr primers generated bands that were obviously allelic at their marker loci. Four of these bands were excluded from the data set for the calculation of I_A across marker loci because only one allele per marker locus should remain in the data set.

TABLE 2. (continued from preceding page)

Primer ^b Band ^c	Kenya			Mexico	China		Germany	Switzerland	France	Austria
	Kakamega	Kitale	Embu		South	North				
447 (continued)										
0.85	1	1	1	1	1	1	<u>0.04</u>	<u>0.05</u>	<u>0.33</u>	<u>1</u>
0.87	0	0	0	0	1	1	0.96	0.95	0.67	0
1.16	0.48	0.66	0.55	0.75	0.7	1	1	1	1	1
457										
0.78	0	0	0	<u>0.21</u>	0	0	0	0	0	0
1.4	0.07	0.08	0	<u>0.17</u>	0	0	0	0	0	0
2.3	0	0	0	0	0	0	0	0	<u>0.11</u>	0
2.4	0	0	0	<u>0.04</u>	0	0	0	0	0	0
460										
1.02	1	1	1	1	1	<u>0.74</u>	1	1	1	1
2.0	1	<u>0.95</u>	1	1	1	1	1	1	1	1
467										
1.16	0	0	0	1	0	0.15	1	0.95	0.89	0.25
2.3	0	0	0	0	0	0	0	<u>0.05</u>	0	<u>0.75</u>
487										
2.3	0	0	0	0.46	0	0.26	0.67	0.95	0.78	0.25
2.6	1	1	1	0.54	0.87	0.78	1	1	1	1
489										
0.55	0.95	0.71	0.86	0.25	1	0.96	0	0.05	0	0
497										
0.69	<u>0.05</u>	<u>0.11</u>	0	0	0	0	0	0	0	0
T7										
1.6	1	1	0.12	0	1	1	0	0	0	0
T14										
0.58	0.43	0.39	0.43	0.75	1	0.07	0.96	0.81	0.56	0
0.95	0	0	0	<u>0.4</u>	0	0	0	0	0	0
1.2	0	0	0	0	<u>0.26</u>	0	0	0	0	0
1.6	0.82	0.89	0.81	1	0.7	0.81	0	0	0	0
1.7	0	0	0	0	0	0	<u>0.5</u>	<u>0.52</u>	<u>0.67</u>	0
2.1	<u>0.68</u>	<u>0.66</u>	<u>0.5</u>	0	0	0	0	0	0	0
2.3	0	0	0	0	<u>0.26</u>	<u>0.19</u>	0	0	0	0
T15										
0.72	1	<u>0.97</u>	1	1	1	1	1	1	1	1
0.95	0.05	0.11	0.55	1	0.13	1	1	1	1	1
1.45	1	1	1	1	<u>0.78</u>	1	1	1	1	1
T17										
0.75	1	<u>0.97</u>	1	1	1	1	1	1	1	1
807										
0.63	0.41	0.63	0.5	0	0.26	0	0	0	0	0
0.69	0	0	0.02	0.08	0	0	1	0.81	0.56	0
0.71	0.45	0.29	0.48	0.58	0.74	1	0	0.19	0.44	1
0.75	<u>0.11</u>	<u>0.08</u>	0	0	0	0	0	0	0	0
811										
0.85	0.52	0.37	0.4	0.79	0.91	0.89	0.67	1	1	0.25
1.3	1	1	1	0.88	1	1	0.29	0.1	0.44	1
812										
1.33	1	1	1	0.04	1	0.52	0	0	0	0
841										
0.85	0.3	0.13	0.33	0	0	0.07	0	0	0	0
868										
0.88	<u>0.98</u>	<u>0.87</u>	<u>0.95</u>	1	1	1	1	1	1	1
0.9	<u>0.02</u>	<u>0.13</u>	<u>0.05</u>	0	0	0	0	0	0	0

Fig. 1. Agarose gel with random amplified polymorphic DNA patterns generated by primer UBC403 with *Setosphaeria turcica* isolates from four continents. Lanes 2 to 7 represent Kenyan isolates (Kakamega, lanes 2 and 3; Kitale, lanes 4 and 5; and Embu, lanes 6 and 7); lanes 8 to 11 Mexican isolates (Chinalpa); lanes 13 to 18 Chinese isolates (Daxin, lanes 13 to 15; and Harbin, lanes 16 to 18); and lanes 19 to 26 European isolates (Germany, lanes 19 and 20; Switzerland, lanes 21 and 22; France, lanes 23 and 24; and Austria, lanes 25 and 26). Reproducibly polymorphic bands are marked with arrows; fragment sizes are given in kilobases; and unscored (nonreproducible) bands are marked with a hyphen.

Fig. 2. Principal coordinates of random amplified polymorphic DNA haplotypes of all 264 *Setosphaeria turcica* isolates from four continents calculated on the basis of pairwise similarities according to Dice. The first two coordinates displayed account for 34% of the total variation.

RESULTS

Genetic distance between populations. The continental populations were clearly distinct from each other. Each haplotype was restricted to a single continent, and many bands were either present or lacking exclusively in samples from only one continent (Table 2). These specific bands are viewed as “private” alleles (24). Nearly half of all 70 marker alleles were private: 11 in Kenya, eight in Mexico, five in China, and nine in Europe. An example of an agarose gel with RAPD profiles generated by UBC primer 403 is shown in Figure 1. This primer detected several polymorphic markers. Four of the five reproducible bands were private (Fig. 1 and Table 2).

Principal-coordinates analysis of haplotype similarities revealed a clear grouping of isolates of each continental sample (Fig. 2). The different populations from a single continent (northern and southern China, western and central Kenya) were positioned in close neighborhood. The pronounced differentiation between populations from different continents is also reflected by the high value of $\Phi_{ST} = 0.528$ (calculated with only one isolate per haplotype: $\Phi_{ST} = 0.447$), with the four continental populations regarded as subpopulations. However, the Mexican and European isolates were not clearly separated (Fig. 2). Among the 19 nonprivate polymorphic bands present in the European sample, only three (92:1.65, 425:1.95, and 447:0.87) were absent in the Mexican sample (Table 2). One French isolate, represented in Figure 2 by the light triangle with coordinates of $-0.069, 0.196$, resembled African isolates. It bears alleles that were found otherwise only in African isolates (e.g., 425:1.25, 425:1.95 in Table 2 and 457:2.3 [D. S. Borchardt and H. G. Welz, unpublished data]).

In Europe, the samples from east (Austria) and west (Germany, Switzerland, and France) of the Alps were very different. In Figure 2, most of the light triangles (western Europe) are distinct from the dark triangles (Austria). The three light triangles close to the dark ones represent haplotypes from Germany and Switzerland resembling Austrian haplotypes.

The multidimensional scaling plot (Fig. 3) shows the Mexican sample in the center. The shortest distance links it to the European samples. The isolates of the populations from northern and southern China were clearly distinct from each other (Fig. 2). The linear distance was approximately 2,600 km. The smallest genetic

distance of the Chinese populations was to the populations from Kenya (Fig. 3). The two populations from western Kenya (Kakamega and Kitale) were not distinguishable. Isolates from these two populations were intermixed (Fig. 2) and showed an extremely small distance (Fig. 3). In contrast, Embu, situated 320 km from the western Kenya locations, housed a very different population (Fig. 3). This difference was expressed not only by the haplotypes (Fig. 2) and the frequencies of RAPD bands (Fig. 3), but also by unequal genotypic diversities (Table 3). In central Kenya, genotypic diversity was close to its theoretical maximum (40 haplotypes among 42 isolates), while it was significantly lower but still very high in western Kenya (Table 3).

Gene flow. Gene flow was never detected directly by shared haplotypes, neither between continents nor between regions within a continent. An indirect estimate of gene flow, Nm , indicated very rare migration between continents (Table 4). Between regions of a continent, Nm ranged from 0.76 to 0.96, which translates to a low level of migration. Within the agroecological region of western Kenya (Kakamega versus Kitale), migration appeared to be high ($Nm = 17$).

Genotypic diversity and recombination. There were general differences between *S. turcica* populations from tropical (Kenya, Mexico, and southern China) and temperate (northern China and Europe) regions in genotypic diversity and gametic phase equilibrium. The Shannon index of diversity, H_S , was extremely high in the populations from the tropics. In Mexico and Embu, central Kenya, there were no hints of clonal lineages. H_S was significantly lower in the populations of the temperate regions (Table 3). In western Europe, one-third of all isolates had identical haplotypes; i.e., they belonged to one clone dispersed over Germany, Switzerland, and France. The index of multilocus association, I_A , suggests that the tropical populations were near gametic phase equilibrium, while the populations from the temperate zones showed strong disequilibrium (Table 3). Moreover, in the tropical populations, both mating types occurred in every field population, mostly at equal frequencies ($\alpha \leq 0.05$) (Table 3). In contrast, mating types were very unevenly distributed in the samples from the temperate zones. In Europe, no field sample contained both mating types; however, only one to six isolates per field were tested. In Austria only MAT1 occurred, while all French isolates were MAT2. In Germany and Switzerland, most isolates were MAT2, but a few

TABLE 3. Genotypic diversity (Shannon index, H_S), gene diversity (Nei's average gene diversity, H_N), significance of recombination (Brown's index of multilocus association, I_A), and mating type frequencies in populations of *Setosphaeria turcica*

Location	Genotypic diversity ^a			Gene diversity		Multilocus allele association ^d			Mating type ^e	
	<i>N</i>	<i>M</i>	H_S^b	b ^c	H_N^b	I_A	V_O	<i>L</i>	MAT1	MAT2
Kenya										
Embu	42	40	0.98 a	27	0.115 a	0.58	6.3	> 5.7	12	9
Kakamega	44	28	0.83 bc	26	0.095 b	0.11	3.8	< 5.2 ^f	5*	15*
Kitale	38	22	0.81 bc	28	0.104 ab	0.33	5.3	< 6.3 ^f	8	13
Mexico										
Chinalpa	24	22	0.96 ab	30	0.124 a	0.41	6.5	< 7.4 ^f	12	12
China										
Daxin	23	18	0.90 bc	15	0.065 c	0.07	2.3	< 4.2 ^f	5*	15*
Harbin	27	17	0.79 cd	20	0.091 b	3.47	19.8	>> 7.4	16**	1**
Europe ^g	66	20	0.56 de	26	0.092 b	2.10	12.8	>> 6.7		
Germany	24	7	0.49 e	14	0.063 c	...	...	...	7 ⁱ	15
Switzerland	21	6	0.48 e	14	0.037 d	...	...	...	2**	17**
France	9	6	0.76 de	18	0.084 bc	...	...	...	0**	8**
Austria	12	4	0.43 e	7	0.035 d	...	...	...	12**	0**

^a *N* = sample size, and *M* = number of multilocus haplotypes.

^b Values followed by the same letter are not significantly different at $\alpha = 0.05$, according to a sequentially rejected Bonferroni test.

^c Number of polymorphic bands.

^d V_O = observed variance (test statistic), and *L* = lower confidence limit.

^e Number of isolates. * and ** = H_O (equal distribution) rejected at $\alpha = 0.05$ and 0.01, respectively.

^f H_O ; $I_A = 0$ at $\alpha = 0.05$.

^g Values in this row are based on all isolates sampled from various locations in the four different countries.

^h *M* too small for calculation.

ⁱ All seven MAT1 isolates came from one field. The other fields had only MAT2.

were MAT1. In Harbin, northern China, both mating types were found in one field but at very different frequencies.

Gene diversity. Nei's average gene diversity, H_N , was numerically highest in the Mexican population. However, it was not significantly higher than the H_N value of the population in Embu, Kenya. The population from Daxin in southern China had the lowest H_N value, which is consistent with the small number of polymorphic bands observed in that sample (Table 3).

DISCUSSION

The general difference in genotypic diversity and gametic phase equilibrium found between *S. turcica* populations from tropical and temperate regions probably resulted from differences in the frequency of sexual reproduction. The degree of gametic phase disequilibrium as measured by the index of association is an appropriate and sensitive parameter for making inferences about the incidence of sexual reproduction in populations (2,18,22,38), even though it is not independent of allelic frequencies (10). Sexual recombination almost certainly occurs regularly in the tropics. This interpretation is consistent with the mating type frequencies, since in most tropical samples, they did not deviate from an equal distribution. The deviation in two of the samples (Table 3) could result from sample size or from unknown factors such as selection. Nothing is known about the fitness of the two mating alleles. Because the sexual stage has not been observed in the field, it is uncertain exactly where and when sexual reproduction would occur, although we assume it would be on the maturing or senescent host plant. This problem deserves further study.

Our data suggest that less sexual recombination has occurred in temperate climates (northern China and Europe), as indicated by the strong gametic phase disequilibrium and uneven frequencies of mating types in the pathogen population. The climatic requirements for induction and maturation of fertile pseudothecia may not be fulfilled or may be only seldom fulfilled. In Europe, where *S. turcica* epidemics are often less severe than in the tropics (32), pathogen population densities may be too low to allow frequent contact of sexually compatible isolates. However, when the severity of epidemics increases (37), the distribution of mating types within fields may become more random and enhance the chance for sexual reproduction. At least in years with favorable conditions (temperature, moisture, and appropriate sub-

strates from host tissue), sexual recombination may also occur in temperate zones.

Sexual recombination causes an increase in genotypic diversity (34). Consequently, it was extremely high in the tropical populations and significantly lower in the populations from temperate regions. However, the genotypic diversity in the temperate regions ($H_S = 0.43$ to 0.79) (Table 3) is of the same order of magnitude as that of other pathogen populations, such as *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*), $H_S = 0.82$ (20); *Rhynchosporium secalis*, $H_S = 0.52$ to 0.92 (7); *Mycosphaerella graminicola*, $H_S = 0.57$ (19); or *Pyrenophora teres*, $H_S = 0.34$ to 0.53 (27). Significant differences in genotypic diversity among tropical populations were also observed, e.g., between central and western Kenya. This might not result only from a slightly shifted ratio of sexual versus asexual spores. Diversifying selection may also play a role.

The hypothesis of Vavilov (35) states that a species has the highest degree of diversity in its center of origin. In regions of very ancient spread, diversity should be nearly as high. At the border of the worldwide spread of a species, diversity should be significantly lower. A center of origin of *S. turcica* may be Central America if the pathogen coevolved with its host *Zea mays* or East Africa if it coevolved with its host *Sorghum bicolor*. In our study, the multidimensional scaling plot shows the Mexican sample in the center (Fig. 3), and this field population possessed the highest number of polymorphic bands of all populations examined (Table 3). Its gene diversity, H_N , was numerically highest but not significantly higher than that of the Embu population. To fully resolve this problem, several samples of *S. turcica* from both Africa and Central America should be tested for host-specific pathogenicity and genotyped with molecular markers.

As expected, H_N was lower in the populations from northern China and Europe, representing the northern front of *S. turcica*'s worldwide distribution. However, H_N was lowest in the population from southern China. This population must have gone through a severe bottleneck (repeated reductions in population size or even local transient extinction), because a considerable number of the alleles were found to be fixed. On the other hand, NCLB is a well-known disease in the Guangxi province, where maize is grown during two seasons per year on more than 570,000 ha (J. Lothrop, *personal communication*). A sampling effect cannot be excluded.

In general, the genetic distances between populations from different continents were very large. About half the total variation detected in this study ($\Phi_{ST} = 0.53$) resulted from subdivision into continental populations. Genetic isolation is obviously very strong, even though the high number of private alleles must be interpreted with caution. Binomial probabilities of detecting an allele present at a frequency of $p_i = 0.1$ or 0.05 in a sample of $n = 40$ are 98.5 or 87.1%, respectively. Moreover, we did not directly detect gene flow between *S. turcica* populations in different continents. We tried to quantify gene flow at different scales by using Wright's (40) approach based on the Φ_{ST} statistic. According to Slatkin (31), $Nm < 1$ should be interpreted as little or no gene flow; the value ($Nm = 0.44$) observed between continents (Table 4) would indicate extremely rare migratory events. More gene flow was estimated to occur between regions within a continent. Between

Fig. 3. Multidimensional scaling of *Setosphaeria turcica* populations. The distances among all 10 populations plotted in three dimensions have a monotone relationship to the original distances calculated by the average taxonomic distance parameter. The dotted line indicates a superimposed minimum spanning tree.

TABLE 4. Migration between *Setosphaeria turcica* populations quantified according to Wright (40)

Migration	Populations considered	Φ_{ST}^a	Nm^b
Between continents	Africa, Asia, America, Europe	0.530	0.44
Between regions	Kenya, west and central	0.340	0.97
	China, north and south	0.395	0.76
	Europe, west and east	0.344	0.95
Within regions	Kenya, Kakamega and Kitale	0.028	17.23

^a Modification of Wright's F_{ST} statistic.

^b N = local population size, and m = average rate of migration.

northern and southern China, the large geographic distance of approximately 2,600 km is reflected through slightly less gene flow than between western and central Kenya and between Europe west and east of the Alps. Low rates of long-distance migration are consistent with the lack of seed transmission of *S. turcica* (21). In contrast, migration between populations of a contiguous agroecologic zone (western Kenya) appears to be extensive ($Nm > 10$). This was corroborated by the occurrence of identical haplotypes in Kitale and Kakamega. The *S. turcica* population from the Mexican field had medium genetic distances to all other samples of isolates investigated (Fig. 3). The relatively small genetic distance between the Mexican and the European isolates may indicate that the European populations were founded by isolates from Central America. Most RAPD bands present in the European sample were also present in the population of the Mexican field. The remaining bands that were present in the European sample but absent from the Mexican sample may be found somewhere else in Central America or perhaps in Africa. A single isolate from southwestern France bearing "African alleles" suggests migratory links to Africa. The similarity between Chinese and Kenyan populations (Figs. 2 and 3), however, may reflect traditional trade routes.

There is ample variability even in local *S. turcica* populations. Migration helps to maintain genetic variability inside agroecological zones, although it seems to be ineffective across large geographic distances. Sexual recombination may create new, possibly superior combinations of alleles, particularly in the tropics. Therefore, we conclude that the potential for adaptation of *S. turcica* should be high in those regions. How this relates to the potential for adaptation to host resistance remains to be investigated.

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LITERATURE CITED

- Abadi, R., Levy, R. S., and Levy, Y. 1993. Mating types of *Exserohilum turcicum* in Israel. *Phytoparasitica* 21:315-321.
- Brown, A. H. D., Feldman, M. W., and Nevo, E. 1980. Multilocus structure of natural populations of *Hordeum spontaneum*. *Genetics* 96:523-536.
- Carson, M. L. 1995. Inheritance of latent period length in maize infected with *Exserohilum turcicum*. *Plant Dis.* 79:581-585.
- Cockerham, C. C. 1973. Analyses of gene frequencies. *Genetics* 74:679-700.
- Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA data. *Genetics* 131:479-491.
- Fallah Moghaddam, P., and Pataky, J. K. 1994. Reactions of isolates from matings of races 1 and 23N of *Exserohilum turcicum*. *Plant Dis.* 78:767-771.
- Goodwin, S. B., Saghai Maroof, M. A., Allard, R. W., and Webster, R. K. 1993. Isozyme variation within and among populations of *Rhynchosporium secalis* in Europe, Australia and the United States. *Mycol. Res.* 97:49-58.
- Groth, J. V., and Roelfs, A. P. 1987. The concept and measurement of phenotypic diversity in *Puccinia graminis* on wheat. *Phytopathology* 77:1395-1399.
- Hebert, T. T. 1971. The perfect stage of *Pyricularia grisea*. *Phytopathology* 61:83-87.
- Hedrick, P. W. 1987. Gametic disequilibrium measures: Proceed with caution. *Genetics* 117:331-341.
- Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6:65-70.
- Huff, D. R., Peakall, R., and Smouse, P. E. 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. *Theor. Appl. Genet.* 86:927-934.
- Hutcheson, K. 1970. A test for computing diversities based on the Shannon formula. *J. Theor. Biol.* 29:151-154.
- Leonard, K. J. 1988. *Setosphaeria turcica*, cause of northern corn leaf blight, and other *Setosphaeria* spp. Pages 241-248 in: *Advances in Plant Pathology*. Vol. 6, Genetics of Plant Pathogenic Fungi. G. S. Sidhu, ed. Academic Press, London.
- Leonard, K. J., Levy, Y., and Smith, D. R. 1989. Proposed nomenclature for pathogenic races of *Exserohilum turcicum* on corn. *Plant Dis.* 73:776-777.
- Leung, H., Nelson, R. J., and Leach, J. E. 1993. Population structure of plant pathogenic fungi and bacteria. *Adv. Plant Pathol.* 10:157-205.
- Lothrop, J. E. 1990. The CIMMYT headquarters highland maize program. Pages 75-92 in: *Maize Improvement, Production and Protection in Eastern and Southern Africa*. B. Gebredikan, ed. Regional Maize Workshop, Nairobi, Kenya.
- Maynard Smith, J., Smith, N. H., O'Rourke, M., and Spratt, B. G. 1993. How clonal are bacteria? *Proc. Natl. Acad. Sci. USA* 90:4384-4388.
- McDonald, B. A., and Martinez, J. P. 1990. DNA restriction fragment length polymorphisms among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. *Phytopathology* 80:1368-1373.
- McDonald, B. A., Miles, J., Nelson, L. R., and Pettway, R. E. 1994. Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. *Phytopathology* 84:250-255.
- McGee, D. C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*. American Phytopathological Society, St. Paul, MN.
- Milgroom, M. G. 1996. Recombination and the multilocus structure of fungal populations. *Annu. Rev. Phytopathol.* 34:457-477.
- Möller, E. M., Bahnweg, G., Sandermann, H., and Geiger, H. H. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Res.* 20:6115-6116.
- Neel, J. V. 1973. "Private" genetic variants and the frequency of mutation among South American Indians. *Proc. Natl. Acad. Sci. USA* 70:3311-3315.
- Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Pedersen, W. L., and Brandenburg, L. J. 1986. Mating type, virulence, and cultural characteristics of *Exserohilum turcicum* race 2. *Plant Dis.* 70:290-292.
- Peever, T. L., and Milgroom, M. G. 1994. Genetic structure of *Pyrenophora teres* populations determined with random amplified polymorphic DNA markers. *Can. J. Bot.* 72:915-923.
- Schilling, A. G., Möller, E. M., and Geiger, H. H. 1993. RAPDs of *Fusarium culmorum* and *F. graminearum*: Application for genotyping and species identification. Pages 47-56 in: *Modern Detection Assays for Plant Pathogenic Fungi*. A. Schots, M. Dewey, and R. Oliver, eds. CAB International, Oxford.
- Sheldon, A. L. 1969. Equitability indices: Dependence on the species count. *Ecology* 50:466-467.
- Simcox, K. D., Pedersen, K. D., and Nickrent, D. L. 1993. Isozyme diversity in *Setosphaeria turcica*. *Can. J. Plant Pathol.* 15:91-96.
- Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science* 236:787-792.
- Smith, I. M., Dunez, J., Lelliott, R. A., Phillips, D. H., and Archer, S. A. 1988. *European Handbook of Plant Diseases*. Blackwell Scientific, Oxford.
- Sneth, P. H. A., and Sokal, R. R. 1973. *Numerical Taxonomy*. W. H. Freeman, San Francisco.
- Stoddart, J. A., and Taylor, J. F. 1988. Genotypic diversity: Estimation and prediction in samples. *Genetics* 118:705-711.
- Vavilov, N. I. 1928. Geographic centers of our cultivated plants. Pages 342-369 in: *Proc. Int. Congr. Genet.*, 5th.
- Weir, B., and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358-1370.
- Welz, H. G., Bassetti, P., and Geiger, H. H. 1996. *Turcicum-Blattdürre und Aleppohirse: Zwei Schaderreger auf dem Vormarsch*. *Mais* 24:66-68.
- Welz, H. G., and Leonard, K. J. 1995. Gametic phase disequilibria in populations of race 2 and race 3 of *Cochliobolus carbonum*. *Eur. J. Plant Pathol.* 101:301-310.
- Wright, S. 1931. Evolution in Mendelian populations. *Genetics* 16:97-159.
- Wright, S. 1951. The genetical structure of populations. *Ann. Eugen.* 15:323-354.
- Zeigler, R. S., Tohme, J., Nelson, R., Levy, M., and Correa, F. 1994. Lineage exclusion: A proposal for linking blast population analysis to resistance breeding. Pages 267-292 in: *Linking Blast Population Analysis to Resistance Breeding: A Proposed Strategy for Durable Resistance*. R. S. Zeigler, S. Leong, and P. S. Teng, eds. CAB International, Wallingford, England.