The Frequencies and Spatial Distribution of Mating Types in *Stagonospora nodorum* Are Consistent with Recurring Sexual Reproduction

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ABSTRACT

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To test the hypothesis that *Stagonospora nodorum* undergoes regular cycles of sexual recombination, a total of 1,207 isolates sampled from 18 fields in 12 geographical regions in six countries on five continents were analyzed for mating type frequency and distribution using polymerase chain reaction amplification of the mating type locus. Restriction fragment length polymorphism and random amplified polymorphic DNA

fingerprints were used to clone-correct the data sets. Both mating types were often found on the smallest spatial scales tested, including within the same lesion, the same leaf, and the same 1-m² plot. In only one case out of the 18 fields tested was there a significant departure from the expected 1:1 ratio. Combining this result with previous data on the population structure of *S. nodorum*, we conclude that this pathogen undergoes regular cycles of sexual recombination in all regions we examined.

Additional keywords: epidemiology, hierarchical sampling, *Phaeosphaeria nodorum*, population genetic structure.

The heterothallic loculoascomycete *Stagonospora nodorum* (Berk.) Castellani and Germano (syn. *Septoria nodorum* Berk.), the anamorph form of *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (syn. *Leptosphaeria nodorum* E. Müller), causes *Stagonospora nodorum* glume blotch on wheat (*Triticum aestivum* L.), a major disease of wheat globally (19,20,25). It is able to infect all above-ground plant parts and overwinters in the field on infected stubble (44) where the sexual stage occurs (21,22). Teleomorph fruiting bodies have been detected in Australia (5), Europe (1,34), North Carolina (11), Oregon (C. Cowger, *personal communication*), Texas (B. McDonald, *unpublished data*), and western Canada (16), but have not been detected in Georgia (13) or New York (7) although both mating types were found in these locations. These observations suggest that the ability of the fungus to undergo sexual reproduction might vary among locations (45).

Though it is known that the fungus can undergo sexual reproduction, it is not clear what role sexual reproduction plays in the population genetic structure of the pathogen and the epidemiology of the disease. Results from Bathgate and Loughman (5), Keller et al. (23,24), and McDonald et al. (30) indicated that sexual reproduction plays an important role in the pathogen's population genetic structure, and the authors suggested that wind-borne ascospores are the main source of primary inoculum. Other studies argued against the importance of the sexual stage (7) and hypothesized that the seedborne pycnidiospores were the main source of primary inoculum (2,42). In either case, it is clear that following primary infection, asexually produced, rain-splashed pycnidiospores contribute to epidemic development during the remainder of the growing season, in particular under wet and warm weather conditions (1,34).

Knowledge of the extent of sexual reproduction and its contribution to primary inoculum is needed to understand the evolutionary potential and epidemiology of pathogens. Sexual populations may have evolutionary advantages over asexual populations because more fit genotypes can arise rapidly through recombination (8,9,33). If the sexual spores (ascospores in this case) are wind-dispersed, they have a greater potential for long-distance gene flow, and therefore can move selected characters such as virulence or fungicide resistance efficiently over large areas. Increased genetic variation combined with high gene flow potential increases the ability of a pathogen to evolve rapidly in response to changing environments, and therefore can reduce the useful life span of fungicides or cultivars carrying single major resistance genes. On the other hand, if the pathogen is limited to asexual reproduction and the main source of primary inoculum is seed-borne or splash-dispersed pycnidiospores, the risk of the spread of new pathotypes among neighboring populations would be low. Under this scenario, the increase of genetic diversity would be limited to mutations within a limited number of genetic backgrounds (clonal lineages), and gene flow over longer distances would have to be man-mediated. In this case, the spread of the pathogen could be more easily limited with appropriate control measures, such as using clean seeds and crop rotations.

Sexual reproduction in heterothallic loculoascomycetes such as *S. nodorum* requires the presence of two genetically distinct fungal isolates carrying alternate forms of mating types, called *MAT1-1* and *MAT1-2*, at the same geographical locations. These alternate forms are termed idiomorphs instead of alleles because they lack significant sequence similarity (10,32,38,47). Each idiomorph carries a single regulatory gene that encodes proteins with confirmed or putative DNA binding motifs (38). The *MAT1-1* idiomorph of loculoascomycetes encodes a DNA binding protein containing an alpha (α) domain, while *MAT1-2* encodes a DNA binding protein containing a high mobility group (HMG) domain (10,38,47).

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The hypothesis that populations of *S. nodorum* undergo regular cycles of sexual reproduction could be tested by studying the frequencies and distributions of the two mating types in field populations. In populations where sexual reproduction is common, ascospores could be a significant source of primary inoculum. If the pathogen population within a field undergoes regular cycles of sexual reproduction and ascospores are a major source of primary inoculum, then both mating types should occur at equal frequencies within the field as a consequence of frequency-dependent selection operating on *MAT* genes (33). Skewed ratios, on the other hand, would be consistent with rarity of sexual reproduction, and in the case of *S. nodorum*, with a major role played by seedborne inoculum. This approach has been successfully applied to infer the importance of sexual recombination for several other fungi (3,15,17,29,49). The outcome of the test can be strengthened by combining the results with other population genetic data such as measures of genotypic diversity, measures of gametic equilibrium/disequilibrium, and direct observation of the teleomorph.

Previous reports on mating type frequencies of *S. nodorum* were inconsistent. Halama (21) found skewed mating type frequencies using in vitro crosses against known tester strains to assign the mating type. In Halama's test, 56 of 101 isolates were assigned to *MAT1-1*, 5 to *MAT1-2*, and the remaining 40 isolates could not be assigned to a specific mating type. Solomon et al. (46) found that frequencies of the two mating types in 23 isolates sampled from Western Australia did not deviate significantly from the 1:1 ratio, while Bennett et al. (7) found both skewed and equal mating type ratios in New York. Earlier studies on mating type frequencies of *S. nodorum* were either based on relatively small sample sizes or lacked genotype information. Small sample sizes lead to a low statistical confidence and lack of genotype information could lead to an overrepresentation of one or more common clonal genotypes, thus biasing the results (49).

The objectives in this experiment were to determine the frequencies and hierarchical distribution of mating types over spatial scales ranging from lesions to continents to test the hypothesis that *S. nodorum* populations undergo regular cycles of recombination and to determine if the same pattern would exist across a large number of fields and environments representing major wheat-production areas on five continents.

MATERIALS AND METHODS

Fungal isolates. A total of 1,207 *S. nodorum* isolates collected from 18 fields in 12 geographical regions in six countries on five continents were included in this study. Most collections were made following a hierarchical sampling strategy (31) from naturally infected flag leaves or heads at a single point in time (Table 1). With the exception of the Swiss populations, single or multiple pycnidial isolations were made from each of 10 infected leaves collected at each sampled site (plot) within the field. The Australia collection was made from a single field located north of

Norrogin following an eight-site hierarchical sampling strategy. The total area covered by this sampling strategy was ≈ 300 m². Swiss isolates were collected from nine fields in three locations, each of them planted with one of three wheat cultivars (Arina, Galaxy, and Tamaro) (23). Only five infected flag leaves were collected at each plot within these fields. The total sampling area covered for each Swiss field was approximately 400 m² and the geographic distances among these fields ranged from 0.1 to 9.0 km. The North Dakota collection was made in Fargo following a six-site hierarchical sampling scheme. The total area covered in this field was approximately 200 m². Hierarchical sampling strategies were also used for Oregon and Texas. The isolates from Oregon were collected from flag leaves and heads from three sites in a field in Hyslop. In Overton, Texas, collections were made at three different plots in two separate fields as previously described (30). The New York collection was made from naturally infected flag leaves sampled along parallel transects in a single field located at the Ithaca experiment station, covering an area of 400 m². The Chinese collection was made from a farmer's field located at Louyuan in the east part of China. The infected leaves were collected at 1 m intervals along eight 15-m-long transects, representing a total area of approximately 105 m². In Arkansas, isolations were made from seedlings grown from infected seed lots grown in a greenhouse or directly from infected seed. Mexican isolates were collected from the International Maize and Wheat Improvement Center (CIMMYT) disease nursery in Patzcuaro. The South Africa population was collected at random from an area of 10,000 m² located in the Gouda area (South-Western Cape).

DNA extraction and polymerase chain reaction amplification of mating type idiomorphs. DNA extraction of the American populations was as described previously by McDonald et al. (30). DNA of Australian, Chinese, Swiss, and South African populations was extracted with DNeasy Plant Mini DNA extraction kit (Qiagen, GmbH, Hilden, Germany) following the manufacturer's instructions.

Mating types of the fungal strains were determined by polymerase chain reaction (PCR) amplification using four mating type-specific primers according to Bennett et al. (7) (Fig. 1). The combination of primers 5'-CTT CAC GAC CGG CCA GAT AGT-3' (F) and 5'-CAG AGG CTT GTC GGG TTC AT-3' (R) amplified an ≈ 360 -bp fragment in *MAT1-1* isolates. An ≈ 510 -bp fragment was amplified in *MAT1-2* isolates by using the combination of primers 5'-ACC CCG CCC CAT GAA CAA GTG-3' (F) and 5'-CTA GAC CGG CCC GAT CAA GAC CAA AGA AG-3' (R). PCR amplifications were performed using a mixture of all four primers (multiplex PCR) and were carried out in 96 well plates (Greiner) using a Biometra thermocycler (Goettingen, Germany). The total PCR reaction volume was 20 μ l per well, consisting of 1 μ l (10 μ M) of each primer, 1 μ l (2 mM) of dNTPs (dATP, dCTP, dTTP, and dGTP), 2 μ l of 10 $\times$ reaction buffer, 1 unit of *Taq* polymerase, and 5 μ l of resuspended *S. nodorum* genomic DNA (5 to 10 ng final DNA concentration). Thermal cycling conditions

TABLE 1. Description of *Stagonospora nodorum* populations used in this study

Population	Year of collection	Number of fields	Area sampled (m ²)	Collection method	Sample size	Clone-corrected sample size
Arkansas	1995	...	...	Greenhouse and seed lots	142	100
Australia	2001	1	≈ 300	Hierarchical	64	59
China	2001	1	≈ 105	Parallel transects	100	84
Mexico	1993	1	≈ 400	Random unordered	14	13
New York	1991	1	≈ 400	Parallel transects	21	15
North Dakota	1993	1	≈ 200	Hierarchical	18	9
Oregon	1993	1	≈ 60	Hierarchical	57	57
South Africa	1995	1	$\approx 10,000$	Random unordered	45	25
Switzerland	1994	9	$\approx 3,600$	Hierarchical	410	410
Texas	1992	2	≈ 100	Hierarchical	43	42
Total	...	18	...	...	914	814

were as follows: initial hold at 96°C for 2 min, 39 cycles of 96°C for 1 min, 59°C for 1 min, 72°C for 1 min. Finally, the products were incubated for 5 min at 72°C. Amplicons were separated by agarose gel electrophoresis (1% gel, 0.5× Tris-borate-EDTA) at 80 V for 3 h and visualized by staining the gels with ethidium bromide.

Genotype determination. All populations, apart from the Australian and the Chinese, were clone-corrected using restriction fragment length polymorphism (RFLP) markers. Seven single locus probes and one fingerprinting probe (pSNS4) were used for the clone-correction (24,30). Combined results from two random amplified polymorphic DNA (RAPD) primers (9442 and 9498) (14) were used to clone-correct populations from China and Australia because the RFLP fingerprinting probe pSNS4 was no longer available. The RAPD PCR conditions were as described by Czembor and Arseniuk (14). Isolates with the same multilocus haplotypes, RFLP fingerprints, and/or RAPD fingerprints were

considered clones. The clonal fraction for each sampling unit was calculated (49) by dividing the number of genotypes detected by the total number of isolates and subtracting it from one.

Data analysis. Hierarchical pooling of the isolates enabled us to determine the frequency distribution of mating types across different spatial scales, including within lesions, leaves, field plots, fields, locations, and countries. Frequencies of mating types were calculated using both clone-corrected and uncorrected data. For clone-corrected data, only one representative haplotype per clone was included in the analysis. A χ^2 test (36) was applied to determine whether the frequencies of the two mating types departed from the null hypothesis of a 1:1 ratio within sampling units. Heterogeneity in mating type frequencies among sampling units was evaluated using a contingency χ^2 test (18). Only isolates with complete mating type and fingerprint data were included in the analysis.

RESULTS

Multiplex PCR. A total of 1,207 fungal isolates were assayed for mating type frequencies, following PCR amplifications of part of the α -domain and HMG-box with specific primers. Among these isolates, 1,080 produced a single amplicon of $\approx$ 510 or $\approx$ 360 bp corresponding either to the *MAT1-2* or the *MAT1-1* idiomorph (Table 2). Four isolates produced a 100-bp amplicon in addition to the *MAT1-2* amplicon. Eight isolates produced both *MAT1-1* and *MAT1-2* amplicons, possibly due to mixed cultures or contamination during preparation of microtiter plates. These latter 12 isolates were excluded from the analysis of mating type fre-

Fig. 1. Polymerase chain reaction amplification with mating type-specific primers of eight randomly chosen Swiss isolates of *Stagonospora nodorum*. The $\approx$ 510-bp amplicon corresponds to the *MAT1-2* idiomorph, while the $\approx$ 360-bp amplicon corresponds to the *MAT1-1* idiomorph. First and last lanes are a 100-bp size ladder.

TABLE 2. Summary of results from polymerase chain reaction (PCR) amplification of the mating type idiomorphs in a global collection of the wheat pathogen *Stagonospora nodorum*

PCR products	Number of isolates	Proportions
<i>MAT1-1</i>	446	0.37
<i>MAT1-2</i>	468	0.39
Both <i>MAT1-1</i> and <i>MAT1-2</i>	8	0.01
<i>MAT1-2</i> and 100-bp amplicon	4	0.00
No product	115	0.10
No fingerprint	166	0.14
Total	1,207	1.00

TABLE 4. Distribution of *Stagonospora nodorum* mating types among 1 m² plots

Population	Number of plots	Plots with a missing mating type	Average clonal fraction
Australia	8	0	0.01
Switzerland 1	9	0	0.00
Switzerland 2	9	3	0.00
Switzerland 3	9	1	0.00
Switzerland 4	9	1	0.00
Switzerland 5	9	1	0.00
Switzerland 6	9	1	0.00
Switzerland 7	9	1	0.00
Switzerland 8	9	3	0.00
Switzerland 9	9	1	0.00
Oregon	6	0	0.00
Texas 1	2	0	0.00
Texas 2	2	0	0.00
Texas 3	5	1	0.03
Total	104	13	...

TABLE 3. Mating type frequencies of *Stagonospora nodorum* in populations from Arkansas, Australia, China, Mexico, New York, North Dakota, Oregon, South Africa, Switzerland, and Texas

States/countries	Clone-corrected sample size	Clonal fraction	Frequencies		χ^2 values ^a		
			<i>MAT1-1</i>	<i>MAT1-2</i>	Within regions clone-corrected data	Within regions uncorrected data	Among regions
Arkansas	100	0.30	0.49	0.51	0.04	4.06*	...
Australia	59	0.08	0.37	0.63	3.82	4.00*	...
China	84	0.16	0.56	0.44	1.19	2.56	...
Mexico	13	0.07	0.31	0.69	1.92	2.57	...
New York	15	0.29	0.47	0.53	0.06	0.43	...
North Dakota	9	0.50	0.56	0.44	0.11	5.56*	...
Oregon	57	0.00	0.63	0.37	3.95*	3.95*	...
South Africa	25	0.44	0.44	0.56	0.36	6.42*	...
Switzerland	410	0.00	0.51	0.49	0.04	0.04	...
Texas	42	0.02	0.43	0.57	0.86	1.14	...
Total	814	0.11	0.49	0.51	0.01	0.53	12.34 (9) ^b

^a * Indicates mating-type frequencies that are significantly different at *P* = 0.05.
^b Number in parenthesis indicates the degrees of freedom.

quencies. A total of 115 isolates did not produce any amplicons, probably due to low DNA concentrations or DNA degradation. Of the 1,080 isolates that produced single amplicons of the expected size, DNA fingerprint (RFLP or RAPD) and multilocus haplotype data were available for 914 (75.7%) of these isolates. A total of 814 distinct genotypes were identified among these 914 isolates. The clonal fractions for most populations were low (Table 3).

Spatial distribution of mating types within and among leaves. It was common to find both mating types among isolates sampled from the smallest spatial scales, including different pycnidia within the same lesion, different lesions on a leaf or different kernels on the same head. Both mating types were found in 4 of 24 cases where isolations were made from different pycnidia within the same lesion. In 55 cases across five geographical regions (China, Mexico, North Dakota, Oregon, and Texas) where isolations were made from different lesions on the same leaf, 23% (12 out of 55) were occupied by both mating types and about 98% (54 out of 55 leaves) were occupied by different genotypes. In the population sampled from Oregon, 20% (3 of 15) of heads were infected by both mating types.

Spatial distribution of mating types among plots within fields. In the 104 1-m² plots sampled from four countries or states across 14 fields, both mating types were found in all but 13 of the 1-m² plots (Table 4). In the Australian population, both mating types were found in all plots. Both mating types were found in 87% (78 of 90) of the plots in Swiss populations. In Oregon, both mating types were found in all six plots. In Texas, eight of nine sites, located in three fields at two locations, had both mating types (Table 4).

Spatial distribution of mating types among fields and regions within countries. Different fields from the same geographical regions in Switzerland and Texas were analyzed for mating type frequencies and tested for deviation from the null hypothesis of a 1:1 ratio. In Switzerland, χ^2 analysis revealed no significant differences in the frequencies of two mating types within and among fields (Table 5) and locations (Table 6). In Texas, both mating types were identified in the fields at both locations and χ^2 tests revealed no difference in mating type frequencies within and among locations.

Spatial distribution of mating types among countries and continents. With the exception of Oregon, the null hypothesis of a 1:1 ratio between the two mating types was not rejected at regional and continental scales when clone-corrected data were used (Table 3). In Oregon, the frequencies of the two mating types deviated significantly from the expected 1:1 ratio ($\chi^2 = 3.95$, $P < 0.05$). The null hypothesis was rejected in 5 out of 11 populations when data were not clone-corrected (Table 3).

DISCUSSION

The primary objective of this study was to analyze the frequency and distribution of *S. nodorum* mating types in order to gain insight into the role of sexual reproduction in the population genetics and epidemiology of this pathogen. We achieved this objective by using a large collection of genotyped strains sampled hierarchically from 18 wheat fields on five continents. We found that both mating types were present at all spatial scales and were at similar frequencies in the clone-corrected data sets. It was common to find both mating types in the same lesion and the same leaf when multiple isolations were made, suggesting that close physical proximity of the two mating types is common in agricultural fields. The close proximity of opposite mating types increases the likelihood that the teleomorph will form. Most of the sampled plots and fields did not depart significantly from the null hypothesis of 1:1 ratio. The finding of skewed ratios between the two mating types in five populations when data were not clone-corrected does not contradict our null hypothesis. Rather, this finding illustrates the importance of genotyping the strains to identify clones that would otherwise distort the analysis. Most of the samples with skewed ratios in the uncorrected data sets had the highest clonal fractions.

In only one case (Oregon) was there a significant departure from a 1:1 ratio of the two mating types in a clone-corrected data set. Several processes can explain this finding. Selection (e.g., due to a fungicide application or a resistant cultivar) that favors one or the other idiomorph may affect the frequency and distribution of mating types. It has been reported that mating type genes may be associated with other functions, such as cellular processes (27),

TABLE 5. *Stagonospora nodorum* mating type distribution in field collections from Switzerland and Texas

Population	Clone-corrected sample size	Clonal fraction	Frequencies		χ^2 values ^a	
			<i>MAT1-1</i>	<i>MAT1-2</i>	Within fields	Among fields
Swiss 1	47	0.00	0.51	0.49	0.02 (1) ^b	10.16 (8)
Swiss 2	45	0.00	0.56	0.43	0.82 (1)	...
Swiss 3	46	0.00	0.53	0.47	0.20 (1)	...
Swiss 4	45	0.00	0.42	0.58	1.09 (1)	...
Swiss 5	46	0.00	0.59	0.41	1.39 (1)	...
Swiss 6	45	0.00	0.42	0.58	1.09 (1)	...
Swiss 7	50	0.00	0.48	0.52	0.08 (1)	...
Swiss 8	44	0.00	0.63	0.37	3.13 (1)	...
Swiss 9	43	0.00	0.38	0.62	2.38 (1)	...
Texas 1	10	0.00	0.30	0.70	1.60 (1)	1.45 (2)
Texas 2	5	0.00	0.40	0.60	-- ^c	...
Texas 3	25	0.04	0.52	0.48	0.04 (1)	...

^a Test on clone-corrected data.
^b Number in parenthesis indicates the degrees of freedom.
^c -- Indicates sample size too small to perform χ^2 analysis.

TABLE 6. *Stagonospora nodorum* mating type distribution among three Swiss locations separated by less than 10 km

Location	Clone-corrected sample size	Clonal fraction	Frequencies		χ^2 values within fields ^a	Among fields ^a
			<i>MAT1-1</i>	<i>MAT1-2</i>		
Location CH1	136	0.00	0.54	0.46	0.74 (1) ^b	0.39 (2)
Location CH2	136	0.00	0.48	0.52	0.26 (1)	...
Location CH3	138	0.00	0.50	0.50	0.00 (1)	...

^a Test on clone-corrected data.
^b Number in parenthesis indicates the degrees of freedom.

maintenance of cell wall integrity (48), and virulence (26,28). It is possible that the products of the mating-type genes may affect pathways influencing ecological functions, such as fungicide resistance or that these genes are linked with other genes that confer fungicide resistance. Another possibility is that the significant differences found in Oregon were due to a false rejection of the null hypothesis (type I error, 36). As the probability of the type I error for tests conducted at the $P = 0.05$ level is 0.05, the likely number of falsely rejected null hypotheses in our 25 tests (Tables 3, 5, and 6) is 1.3, which is similar to what we obtained.

Our results are consistent with findings made by Solomon et al. (46) who found 9 *MAT1-1* and 14 *MAT1-2* strains in a sample of 23 isolates, and partially in agreement with findings made in New York (7) where clone-corrected field samples from Niagara County (22 single haplotypes) and Cayuga County (32 single haplotypes) were analyzed. A χ^2 test showed that frequencies did not deviate significantly from 1:1 mating type ratio in Cayuga County ($\chi^2 = 0.32$, $P > 0.05$) but were different in Niagara County ($\chi^2 = 8.91$, $P < 0.05$). Deviation from the expected 1:1 ratio in this case could be due to selection (e.g., due to a fungicide application) or to nonrepresentative sampling.

Compared with earlier studies (7,21,45), we used larger sample sizes and many more field collections to increase the statistical confidence of the results. We also clone-corrected the data set to eliminate the possible overrepresentation of any clones present in pycnidial collections. The use of the PCR technique allowed us to avoid the use of in vitro crosses to determine the mating types. The results from in vitro crosses may be affected by unsuccessful mating due to isolate-specific infertility and/or media selection. Finally, the hierarchical sampling method we employed enabled us to analyze the geographical distribution of mating types over spatial scales ranging from 1-m² plots to continents, allowing us to show that the 1:1 ratio existed at all spatial scales and across many different environments and regions on five continents.

The finding of both mating types at all spatial scales coupled with an even distribution among all field populations suggest this fungus undergoes regular cycles of sexual reproduction. If the sexual cycle is occurring regularly, it is likely that the ascospores are making a significant contribution as a source of primary inoculum in epidemics of *Stagonospora nodorum* glume blotch. It has been hypothesized that equal mating type frequencies are mediated by frequency-dependent selection, which is a type of balancing selection that favors rare genotypes (39). Under frequency-dependent selection, the fitness of a phenotype is dependent on the relative frequency of other phenotypes in the population. In the case of skewed mating type ratios, frequency-dependent selection will decrease the frequency of the dominant idiomorph as it will be less likely to find a mate and will increase the frequency of the less frequent idiomorph until the probability of finding a compatible mate is maximized. The 1:1 ratio of mating types observed throughout the sampled locations clearly demonstrates the likelihood of recurrent sexual reproduction. Based on our data, we hypothesize that sexual reproduction occurs at least once a year in most locations. But we recognize that the absence of functional mating types or favorable environmental conditions may preclude recurrent sexual reproduction in some areas.

The hypothesis of recurrent sexual reproduction in *S. nodorum* also is consistent with high levels of genotypic diversity found in this fungus both in current and previous studies (23,30,35). The finding of gametic equilibrium among neutral loci (23) is a further indication that sexual reproduction is a major factor influencing the population genetic structure and epidemiology of *S. nodorum*. Two previous investigations (23,43) showed that when clones were found, they were clustered in small areas within the field. These findings are also consistent with the hypothesis that the primary inoculum of *S. nodorum* is sexually produced ascospores. Under this scenario, the ascospores land on wheat and initiate the infection and then conidia are produced as secondary inoculum that is

dispersed locally (onto the upper leaves and spikes) by rain splash, leading to clusters of the same genotypes in a small spatial area. If the fungus lacked sexual reproduction and the main source of primary inoculum were asexual pycnidiospores and/or infected seeds (7,12,40,41), we would be more likely to find the two mating types at unequal frequencies and a higher degree of clonality within field populations due to a combination of recurrent genetic drift and selection over seasons. Bennett et al. (6) found that some *S. nodorum* populations isolated from wheat spikes had high genotypic variation and were in gametic equilibrium. However, it was not clear whether the populations assayed originated from infected seeds, ascospore infection, or a combination of these possible sources of inoculum. It was proposed that mutations in other genes or environmental factors that limit sexual reproduction could explain the inability to find the teleomorph in some locations (7). Other possible explanations for not finding the teleomorph in these locations include inappropriate sampling methodology, examining too little plant material, or collecting plant material at the wrong point in the disease cycle.

Upon combining our findings of a 1:1 ratio of the two mating types at all spatial scales and in all locations surveyed, high genotypic diversity, random association among alleles among neutral loci, and the identification of the teleomorph (4,11,37), we conclude that *S. nodorum* populations undergo regular cycles of sexual reproduction. We acknowledge that seedborne inoculum can contribute to the primary inoculum, and it is likely to be especially important as a mechanism for long-distance movement (gene flow) of the pathogen among regions and continents. But given our findings, we consider it more likely that the main primary inoculum of *S. nodorum* disease is airborne ascospores. If this is the case, clean seed and crop rotation may not be sufficient to prevent primary inoculum from entering a wheat field. We are presently conducting field experiments with mark-release-recapture techniques to quantify directly the amount of primary inoculum coming from the different possible sources.

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