Population Genetic Structure of *Tapesia acuformis* in Washington State

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ABSTRACT

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Eyespot of wheat is caused by *Tapesia yellundae* and *T. acuformis*. Historically, *T. yellundae* has been considered the more important causal agent of the disease in Washington state and consists of a large homogeneous population with a genetic structure consistent with both sexual and asexual reproduction. *T. acuformis* has increased significantly in Washington in the past 10 years and apothecia were found recently under natural field conditions, indicating that *T. acuformis* may have a more important role in eyespot of wheat than previously was thought. To determine the genetic structure of *T. acuformis* in Washington, 141 single conidial isolates were sampled from four subpopulations in the eastern wheat-growing region of the state. Isolates were scored for mating type and six amplified fragment length polymorphism markers. All markers

segregated in a 1:1 ratio and were determined to be unlinked based on genetic analysis of 24 progeny from an in vitro cross. No significant differences in allele frequencies ($0.127 < P < 0.809$) were found among individual loci across the four subpopulations and over all loci based on contingency table analysis of the log-likelihood ratio statistic G^2 . Likewise, no overall differences between subpopulations were detected using the population differentiation statistic θ ($\theta = -0.004$, $P = 0.537$). Random mating could not be rejected within each subpopulation or for the combined data using clone-corrected data sets based on (i) 1:1 ratio of mating-type, (ii) multilocus gametic disequilibrium analyses (index of association), (iii) phylogenetic analyses (parsimony tree length permutation test), and (iv) genotypic diversity analyses. *T. acuformis* has a genetic structure similar to that of sympatric populations of *T. yellundae* in Washington, with both sexual and asexual reproduction contributing to the structuring of this species.

Eyespot is an important fungal disease of cereals grown in the U.S. Pacific Northwest (PNW) wheat-growing regions of eastern Washington, northeastern Oregon, and northern Idaho (27,39). The cause of eyespot has been attributed to two pathotypes of the anamorphic species *Pseudocercospora herpotrichoides*, the wheat-type (W) and rye-type (R), which were described on the basis of cultural characteristics and host specificity (14,20,35). Apothecia belonging to the genus *Tapesia* were discovered in Australia in 1987 from wheat residue that yielded single ascospore isolates with W-type cultural characteristics (45). Apothecia were found later in Europe that yielded ascospore progeny with R-type cultural morphology, indicating that both pathotypes can sexually reproduce and establishing an anamorph-teleomorph connection (12). A two-allele heterothallic mating system has been described for both pathotypes in vitro, with isolates of complimentary mating type required for the sexual cycle to occur (11,13,26). Outcrossing under field conditions also has been demonstrated for both pathotypes based on DNA polymorphisms identified among progeny isolates from individual apothecia (21; P. S. Dyer, unpublished data). These pathotypes are now considered separate species, *T. yellundae* (W-type) and *T. acuformis* (R-type), based on differences in virulence (20,35), cultural characteristics (18,32), isozymes (19), ribosomal ITS region sequences (34), DNA markers (30,31,41,43,44), and their inability to interbreed (21,29). This evidence provides strong support for *T. yellundae* and *T. acuformis* as distinct morphological, biological, and

phylogenetic species despite the fact that both pathogens cause the same disease, often are found occurring sympatrically within the same wheat field, and often are isolated from the same individual diseased plants (6).

Both mating types of *T. yellundae* and *T. acuformis* are found in approximately equal frequency in many locations, indicating the possibility that sexual reproduction may occur (9). However, the extent of sexual reproduction and the role ascospores may play in the epidemiology of the disease and in the general ecology and life history of either species are not clear. An important aspect of sexual reproduction in outcrossing species is that unlinked genes will segregate independently during meiosis and result in offspring with recombined genotypes (25). Increased genotypic diversity resulting from recombination may allow sexual populations to respond more rapidly to selection imposed by fungicides or resistant cultivars than asexual populations (23,24), and ascospores could serve as a long-distance source of inoculum (21). In Europe, there is clear evidence of the natural occurrence of sexual reproduction in *T. yellundae*, with apothecia found in many countries where winter wheat is grown. Field surveys have demonstrated that apothecia may form on 1 to 5% of stems within fields (10,21) and that ascospores are capable of infecting wheat seedlings (21). Apothecia of *T. acuformis* have been found only rarely in Europe and the extent to which sexual reproduction occurs in this species is unknown (9,21).

In the United States, *T. yellundae* has been considered the most important species causing eyespot in the PNW, but apothecia have not been found under natural field conditions (6,27,39). Abundant apothecia recently were found in an experimental field plot in Washington State where compatible mating types were used to inoculate winter wheat, demonstrating the potential for sexual reproduction to occur under field conditions (6). The population

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genetic structure of *T. yallundae* also is consistent with random mating and no significant population differentiation is found throughout the state of Washington, indicating that sexual reproduction in *T. yallundae* likely is occurring (7).

Apothecia of *T. acuformis* were found recently under natural field conditions in Washington State (6). Furthermore, isolates of *T. acuformis* accounted for 48% of 817 isolates recovered from diseased wheat in a recent survey (6). In contrast, Murray (27) conducted a survey in 1989 and 1990 where only 8% of 617 isolates recovered from diseased wheat were *T. acuformis*, indicating a significant increase in *T. acuformis* in the PNW over the past 10 years. Eyespot has been controlled in the PNW primarily by fungicides and the use of resistant cultivars (27). Fungicide application practices in the PNW have been specifically developed for *T. yallundae* and it is unclear if *T. acuformis* isolates respond similarly to these fungicides or if a greater potential for resistance development exists in this species. PNW isolates of *T. acuformis* have not been used to screen germ plasm for resistance to eyespot, and knowledge of the population genetic structure of this species would be useful for an effective breeding program because representative isolates could be used in the screening process.

The objectives of this research were to determine the population genetic structure of *T. acuformis* in Washington State. Specifically, we wanted to test the hypothesis of random mating and population differentiation among subpopulations of *T. acuformis*. A second objective was to compare the genetic structure of *T. acuformis* with sympatric populations of *T. yallundae*, which has been previously described (7).

MATERIALS AND METHODS

Sampling. *T. acuformis* was isolated frequently from four of five wheat fields sampled to determine the genetic structure of *T. yallundae* in Washington State in a previous study (7). For the purposes of this study, all isolates of *T. acuformis* collected from an individual wheat field were considered to represent a subpopulation of the pathogen and all isolates combined were considered to represent the overall population. A total of 27, 40, 53, and 21 *T. acuformis* isolates were obtained from subpopulations 98-1, 98-2, 99-1, and 00-14, respectively (Fig. 1). For subpopulations 98-1, 98-2, and 99-1, a single handful of diseased plants were sampled every 3 m along two parallel transects separated by 100 to 150 m. A total of 60 (2 × 30) samples were collected for subpopulations 98-1 and 98-2 and 100 (2 × 50) samples were collected for subpopulation 99-1. For subpopulation 00-14, diseased plants were collected every meter along two transects separated by 3 m for a total of 20 (2 × 10) samples to test the effect of a finer-scale sampling strategy on estimation of population structure. An attempt was made to recover a single isolate per sample from subpopulations 98-1, 98-2, and 99-1, whereas an attempt was made to recover up to three isolates per sample for subpopulation 00-14. Details of the isolation procedures, production of single conidial cultures, and DNA extraction have been described previously (7).

Marker development. Amplified fragment length polymorphisms (AFLPs) and mating-type markers were used in this study as previously described (7,9). Our first objective was to develop a set of genetically characterized markers to test for random mating and population differentiation among PNW field populations of *T. acuformis*. In order to find a set of markers that were unlinked and segregated in a 1:1 ratio, a set of single ascospore progeny ($n = 24$) derived from an in vitro cross between *T. acuformis* isolates originating from the United Kingdom (11) were used to develop the markers.

For AFLP, a total of 12 primer combinations with two selective nucleotides at the 3' ends were used to score polymorphic markers between the parental and progeny isolates. Three randomly chosen isolates from each of the Washington subpopulations were in-

cluded in the screening process to ensure that all markers scored also were found in the Washington subpopulations, because the parental isolates were from the United Kingdom. Putative alleles at each polymorphic AFLP locus and idiomorphs at the mating-type (*MAT*) locus were scored using a binary code (1 or 0) corresponding to positive and null alleles, respectively, and to *MAT1-1* and *MAT1-2*, respectively. For the selection of AFLP markers, only polymorphic loci that were reproducible and easily identifiable between the parents and progeny were chosen (Fig. 2). Each putative locus was named using a letter code corresponding to the primer combination used followed by the size of the fragment.

Segregation of alleles among the progeny isolates was tested for each putative AFLP locus and mating-type idiomorph using χ^2 analysis. Segregation ratios were tested against the null hypothesis of 1:1 segregation ($P = 0.05$), which is the ratio expected for the segregation of alleles at a single genetic locus in a haploid organism (25). To determine that the markers were in random association (not linked), progeny data sets were created randomly using the mating-type locus and six to nine additional AFLP markers. These data sets were analyzed using the four approaches described below to test for random mating until a set of markers was found where random mating could not be rejected based on all four tests. The genetically characterized markers were then scored for all field isolates of *T. acuformis*.

Data analysis. Genetic diversity statistics and tests for random mating were conducted on clone-corrected data sets due to the presence of multiple clonal genotypes within each subpopulation, whereas genotypic diversity was estimated from complete data sets (25). Allele frequencies at each locus were estimated from each subpopulation and used to calculate standard population genetic statistics using the software POPGENE (PC version 1.31; Molecular Biology and Biotechnology Centre, University of Alberta, Canada), FSTAT (PC version 2.9.1; Institute of Ecology—Laboratory for Zoology University of Lausanne, Switzerland), and MULTILOCUS (version 1.21; Department of Biology, Imperial College at Silwood Park, United Kingdom). Genetic diversity at each locus was estimated using Nei's genetic diversity as $h = 1 - p_i^2$, where p_i is the frequency of the i th allele at each locus, and averaged over all loci (28). Genotypic diversity was calculated as $(n/n - 1)(1 - \Sigma p_i^2)$, where p_i is the frequency of the

Fig. 1. Four subpopulations of *Tapesia acuformis* were collected in the winter-wheat-growing region of eastern Washington State between 1998 and 2000. Sample sizes were 27, 40, 53, and 21 isolates for populations 98-1, 98-2, 99-1, and 00-14, respectively. The first two numbers in the code correspond to the year the isolates were collected and the second number designates individual fields.

i th genotype and n is the number of individuals sampled, which is the probability that two individuals taken at random have unique genotypes (1). This value is 0 if every individual is the same and 1 if every individual is different.

Fig. 2. Partial amplified fragment length polymorphism fingerprints of the U.K. parental and progeny isolates and Washington field isolates of *Tapesia acuformis*. Parental isolates are in lanes 1 and 2 followed by representative progeny isolates in lanes 3 to 9. Field isolates of *T. acuformis* are represented in lanes 10 to 18. Only two polymorphisms are visible between the parental and progeny isolates (>) with only one of these polymorphisms found within the Washington field isolates. In contrast, many polymorphic markers are present among the *T. acuformis* field isolates (<) with many more polymorphisms out of the range of this figure. L = 10-bp ladder (Invitrogen, Carlsbad, CA).

Population differentiation was tested among subpopulations by comparing allele frequencies using the log-likelihood statistic G^2 (4) and Weir and Cockerham's population differentiation statistic θ (46). The estimated G^2 and θ values were tested under the null hypothesis of nondifferentiation among populations ($\theta = 0$, $G^2 = 0$) by comparing the observed values of G^2 and θ to values estimated for data sets in which alleles were resampled without replacement 10,000 times using the programs FSTAT and MULTILOCUS, respectively.

The hypothesis of random mating was tested using four approaches. First, a χ^2 test was used to test the segregation of mating type, which should be 1:1 for a randomly mating haploid fungus (25). Second, multilocus gametic disequilibrium was tested using the index of association (I_A) statistic (3,22) as implemented in the program MULTILOCUS (1). I_A has an expected value of zero if there is no association of alleles at unlinked loci, as expected in a randomly mating population (3,22). The significance of I_A was tested by randomization (1,000 times) procedures by comparing the observed value of I_A to that expected under the null hypothesis of random mating (1,5). Third, genotypic diversity was estimated as $G = 1/\sum p_i^2$, where p_i is the observed frequency of the i th genotype (17,24,40). The significance of G was tested by randomization (1,000) procedures by comparing the observed G to that expected under the null hypothesis of random mating using a computer program provided by Michael Milgroom, (Department of Plant Pathology, Cornell University, NY) in which only allele frequencies between 0.1 and 0.9 were included in the analyses. Fourth, the parsimony tree length permutation test (PTLPT) was used to determine reproductive mode (5,42,43). PTLPT was analyzed using PAUP (version 4.0b10; Sinauer Associates, Sunderland, MA) and is a phylogenetic approach based on the concept that a clonal population should evolve like a parsimonious tree with little homoplasy, whereas recombining populations will have little phylogenetic consistency due to the reshuffling of alleles during meiosis (4,42,43). The significance of PTLPT was determined using randomization procedures by comparing the observed tree lengths to the lengths of 1,000 artificially recombined trees in PAUP from an input file generated in MULTILOCUS.

RESULTS

A total of 35 segregating polymorphic markers were scored among the *T. acuformis* U.K. parental and progeny isolates. Of the 12 primer combinations used, one to six polymorphic markers were identified, with an average of approximately three polymorphisms per primer combination. All AFLP and mating-type markers segregated in a 1:1 ratio ($P < 0.05$) in the progeny isolates. Only 15 of the 35 markers were polymorphic among the Washington *T. acuformis* field isolates used in the screening process and the remaining 20 markers were either monomorphic or absent (Fig. 2). Randomized data sets were generated and analyzed using subsets of the 15 AFLP markers along with mating-type until a data set was found where random mating could not be rejected based on all four statistical tests, as described above. The final AFLP markers used to score field isolates were E198, K250, L224, L342, L355, and F192.

Positive alleles were present among all Washington subpopulations with the exception of L342 in subpopulation 99-1 (Table 1). The estimated genetic diversity among the four subpopulations ranged from 0.399 to 0.411, with an overall estimate of 0.424. No significant differences in allele frequencies ($0.127 < P < 0.809$) were found among individual loci across the four subpopulations and over all loci based on contingency table analysis of the log-likelihood ratio statistic G^2 . Likewise, no overall differences between subpopulations were detected using the population differentiation statistic θ ($\theta = -0.004$, $P = 0.537$) (Table 1).

Thirty-five unique multilocus genotypes (MLG) of *T. acuformis* were found among the four Washington subpopulations using

mating-type and six AFLP markers to identify individual genotypes (Table 2). The estimated genotypic diversity ranged from 0.795 to 0.963, with an overall estimate of 0.930. The two most common MLG were found among all four subpopulations and accounted for 28.5% of the total sampled isolates. The remaining MLG accounted for 10% or less of the total isolates collected,

with most MLG representing approximately 2% or less of all isolates.

The mating-type ratios for each subpopulation and the combined data were not significantly different from 1:1 ($0.840 < P < 0.933$) (Table 3). The null hypothesis of random mating could not be rejected for all subpopulations and the combined data based on

TABLE 1. Allele frequencies, population differentiation, and genetic diversity among four subpopulations and the overall population of *Tapesia acuformis* in Washington

Marker	Allele frequency					G^2 ^a	θ ^b
	98-1 ($n = 17$)	98-2 ($n = 18$)	99-1 ($n = 17$)	00-14 ($n = 9$)	Overall ($n = 35$)		
E198	0.470	0.333	0.529	0.333	0.426	1.843	-0.028
K250	0.412	0.500	0.353	0.556	0.443	1.331	-0.04
L224	0.882	0.556	0.589	0.778	0.689	6.059	0.058
L342	0.118	0.056	0.000	0.111	0.066	3.210	-0.018
L355	0.647	0.556	0.706	0.444	0.607	2.005	-0.024
F192	0.412	0.778	0.647	0.778	0.639	6.050	0.068
MAT	0.353	0.444	0.471	0.556	0.443	1.080	-0.046
Overall	...	...	...	...	...	21.578	-0.004
h^c	0.399	0.411	0.401	0.402	0.424	...	...

^a Log-likelihood statistic (4). No significant differences in allele frequencies were found under the null hypothesis of $G^2 = 0$ ($0.127 < P < 0.809$). The observed values of G^2 were compared to values estimated for datasets in which alleles were resampled without replacement 10,000 times.

^b Population differentiation estimator of Weir and Cockerham (46). No significant overall population differentiation among the five subpopulations was found under the null hypothesis of $\theta = 0$ ($\theta = -0.004$, $P = 0.5367$) determined using randomization procedures as described previously.

^c Nei's genetic diversity (28).

TABLE 2. Occurrence, genotypic diversity, and frequency of multilocus genotypes among four subpopulations and the overall population of *Tapesia acuformis* in Washington

Multilocus genotypes	Population					Frequency
	98-1	98-2	99-1	00-14	Total	
0010110	2	8	10	4	24	0.171
1110001	2	1	4	9	16	0.114
0000100	4	3	3	...	10	0.100
0010111	...	6	6	1	13	0.093
1100111	...	5	8	1	14	0.071
0100111	1	2	1	...	4	0.057
0111010	1	1	...	1	3	0.036
0010011	...	1	1	1	3	0.029
1010100	1	...	7	...	8	0.021
1010110	2	...	3	...	5	0.021
1010010	1	...	1	...	2	0.021
0110111	1	...	1	...	2	0.021
0110110	2	1	...	...	3	0.021
1010000	2	1	...	...	3	0.014
1000100	...	2	1	...	3	0.014
1000110	...	...	1	1	2	0.014
0011100	2	...	...	...	2	0.014
1010101	2	...	...	...	2	0.014
1110010	...	2	...	...	2	0.014
0100110	...	2	...	...	2	0.014
0100101	...	...	2	...	2	0.014
0110011	...	...	...	2	2	0.014
1110000	1	...	...	...	1	0.007
1010001	1	...	...	...	1	0.007
0010100	1	...	...	...	1	0.007
0110101	1	...	...	...	1	0.007
1010111	...	1	...	...	1	0.007
0100010	...	1	...	...	1	0.007
0010010	...	1	...	...	1	0.007
0000111	...	1	...	...	1	0.007
0100011	...	1	...	...	1	0.007
1110100	...	...	1	...	1	0.007
1010011	...	...	1	...	1	0.007
0000010	...	...	1	...	1	0.007
0110000	...	...	...	1	1	0.007
N^a	27	40	53	21	141	...
Genotypes ^b	17	18	17	9	35	...
D^c	0.963	0.923	0.904	0.795	0.930	...

^a Total number of isolates included in the analyses for each (sub)population.

^b Number of unique genotypes in each (sub)population.

^c Genotypic diversity calculated as $(n/n - 1)(1 - \sum p_i^2)$, where p_i is the frequency of the i th genotype and n is the number of individuals sampled, which is the probability that two individuals taken at random have unique genotypes (1).

the I_A ($0.537 < P < 0.974$), G ($P = 1.00$), and PTLPT ($0.558 < P < 0.998$) tests (Table 3).

DISCUSSION

The null hypothesis of random mating could not be rejected for any subpopulation of *T. acuformis* based on our analyses, which suggests that sexual reproduction occurs in this species in the PNW. Identical results were found when the analyses were conducted on the combined data set for all subpopulations, which was justified because no evidence of population differentiation was found. Our inferences based on multilocus disequilibrium analyses also are supported by the recent findings of apothecia of *T. acuformis* under natural field conditions and the widespread distribution of both mating types under field conditions in the PNW (6). Moreover, apothecia recently have been found in experimental field plots inoculated with compatible mating types (S. Tsai, G. W. Douhan, and T. D. Murray, unpublished data).

All evidence indicates that sexual reproduction is likely to occur in *T. acuformis* in Washington, but it is not possible to know how often or how important sexual reproduction is for this pathogen. The statistical tests used in this study to test for recombination (I_A , G , and PTLPT) are very sensitive and it may only take a few individuals reproducing sexually per generation to create enough recombination to accept the null hypothesis (5,25). Additionally, the underlying recombining genetic structure may be due to past or parasexual events (15,42,43), which may be a reason why recombining population structures have been identified for supposedly asexual fungi using some of these tests (5). Moreover, random mating also may be inferred from these tests due to hyper-variable loci (3,42), rapid population expansion (38), or the lack of statistical power due to small sample sizes (25). Only two alleles at each locus were scored in this study; therefore, hyper-variable markers are not a likely reason for accepting random mating. The latter two causes for inferring recombination are potential criticisms of this study because *T. acuformis* apparently has increased dramatically in the PNW during the past 10 years (6) and the sample sizes were small after correcting for clonal genotypes. However, apothecia have been found experimentally and under natural field conditions in Washington, with both mating types widely distributed throughout the PNW (6), which indicates that sexual reproduction occurs in *T. acuformis* to some extent in the life history of this pathogen.

Apothecia of *T. acuformis* have been collected only four times under natural field conditions; one time each from Germany, England, Belgium, and recently from the PNW (6,21), despite the widespread occurrence of both mating types where the disease occurs (6,9,21). In Europe, apothecia appear to be rare (21), have not been detected in field plots inoculated with compatible iso-

lates (8), and in vitro crosses with known mating types rarely produce apothecia under laboratory conditions (21). These observations have been used to suggest low fertility or sterility between field isolates of *T. acuformis*, evolution toward asexuality, or that requirements for specific environmental conditions exist which are not understood (8,9). In the PNW, by contrast, apothecia of *T. acuformis* were detected the first time a systematic approach was used to try to find apothecia (6), abundant apothecia developed in experimental field plots the first year the experiment was conducted (S. Tsai, G. W. Douhan, and T. D. Murray, unpublished data), and all of our analyses are consistent with random mating. It also is interesting to note that the *T. acuformis* isolates from the United States were much more diverse than the U.K. parental isolates used to screen the markers (Fig. 2). All AFLP primer combinations detected only one to six polymorphisms between the U.K. parental isolates, whereas there were typically over 15 polymorphic markers detected between any two randomly chosen PNW *T. acuformis* isolates (data not shown). This may suggest that sexual reproduction occurs more often in the United States compared with other parts of the world, but this hypothesis would have to be tested.

Previous reports indicate that *T. acuformis* is genotypically less diverse than *T. yallundae* using many different types of genetic markers (21). This evidence has been used to suggest that sexual reproduction is more common in *T. yallundae* than in *T. acuformis*. However, these previous studies have used isolates pooled from diverse geographic areas in Europe. In this study, genetic diversity estimates for *T. acuformis* ($h = 0.427$) were similar to sympatric *T. yallundae* ($h = 0.425$) populations and genotypic diversity was only slightly less for *T. acuformis* (35 genotypes from 141; $D = 0.930$) compared with *T. yallundae* (79 genotypes from 175 isolates; $D = 0.971$) (7). It is possible that the genetic structure of *T. acuformis* in the PNW is fundamentally different than in Europe or it may be that the population genetic structure of *T. acuformis* in Europe has not been thoroughly tested.

No single genotype of *T. acuformis* predominated in this study, but many isolates with identical genotypes were detected within and among each subpopulation, demonstrating the importance of asexual reproduction. Similar results also were found within the sympatric *T. yallundae* subpopulations (7). The importance of asexual reproduction in the dissemination of *T. acuformis* also is evident by isolating the same genotype from plants collected over 200 km apart; however, more clonal genotypes, on average, were found within fields than among fields, which is consistent with localized dissemination of asexual spores. This same general trend also was found for sympatric populations of *T. yallundae* (7) and is likely the result of successful clonal genotypes. However, we cannot be sure that individuals with the same genotype were exact clones without further studies because identical MLG sometimes

TABLE 3. Segregation of mating type and tests for random mating among four subpopulations and the entire sampled population of *Tapesia acuformis* in Washington

Population	<i>N</i> ^a	Mating type segregation ^b			Index of association ^c		Genotypic diversity ^d		PTLPT ^e	
		<i>Mat1-1:Mat1-2</i>	χ^2	<i>P</i>	<i>I</i> _A	<i>P</i>	<i>G</i>	<i>P</i>	<i>L</i>	<i>P</i>
98-2	18	8:10	0.025	0.874	0.085	0.797	13.6	1.00	20	0.937
98-1	17	6:11	0.173	0.677	0.021	0.537	12.5	1.00	17	0.558
99-1	17	8:9	0.007	0.933	-0.065	0.746	12.9	1.00	18	0.849
00-14	9	5:4	0.025	0.874	-0.068	0.618	7.7	1.00	12	0.781
Total	35	15:20	0.041	0.839	-0.088	0.974	22.2	1.00	35	0.998

^a Total number of isolates used in the analysis.
^b Segregation ratio of mating type determined by multiplex polymerase chain reaction (10). χ^2 value based on a 1:1 ratio with 1 degree of freedom and the probability (*P*) of a greater χ^2 value under the null hypothesis of 1:1 segregation.
^c Index of association (*I*_A) statistic (3,22). The significance of *I*_A was tested with randomization (1,000 times) procedures by comparing the observed value of *I*_A to that expected under the null hypothesis of complete panmixis (1,5).
^d Expected genotypic diversity (*G*) (40). The significance of the test was determined with randomization (1,000 times) procedures by comparing the observed genotypic diversity to that expected under the null hypothesis of random mating (17,40,24).
^e PTLPT = parsimony tree length permutation test. The significance of the test was determined with randomization (1,000 times) procedures by comparing the observed tree length (*L*) to tree lengths of 1,000 artificially recombined data sets (5,42,43).

can be differentiated using additional methods, as has been demonstrated in *Mycosphaerella graminicola* (2).

No evidence of population differentiation was found among the four subpopulations analyzed ($\theta = -0.004$, $P = 0.537$). This is consistent with the hypothesis that enough migration and gene flow between subpopulations is occurring to homogenize the allele frequencies (37), which could be the result of ascospore dissemination as described for other ascomycete plant pathogens (2,33). However, the extent of migration or gene flow cannot be determined from the data because populations of pathogenic fungi probably are not at an evolutionary equilibrium (25), which is one of the theoretical assumptions when determining gene flow from F statistics. Plant-pathogenic fungi are likely to experience population crashes due to agricultural practices that make it impossible to distinguish current gene flow from past gene flow because not enough time has elapsed for the effects of drift and migration to reach equilibrium (36,37). Lack of differentiation could be explained alternatively by subpopulations of *T. acuformis* being founded by a common source population, with limited migration among subpopulations to cause genetic differentiation due to genetic drift (16). However, this hypothesis is not likely, given the amount of genetic diversity found within the Washington population of *T. acuformis*. Lack of population differentiation also was found among sympatric subpopulations of *T. yallundae* in Washington (7).

The recombined population structure and the lack of population differentiation among subpopulations of *T. acuformis* in Washington suggests that sexual reproduction, followed by extensive dispersal of ascospores or conidia or mycelial inoculum, is adequate to genetically homogenize the entire population, which also was found among the sympatric subpopulations of *T. yallundae*. Overall, these results suggest that sexual reproduction in both pathogen species is responsible for maintaining the observed high level of genetic diversity and in the creation and dissemination of recombined genotypes throughout the state. However, the role of ascospore inoculum in the disease cycle of either species in the PNW is not known and should be determined in order to better understand the biology of these important pathogens.

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