High Genetic Similarity Among Populations of *Phaeosphaeria nodorum* Across Wheat Cultivars and Regions in Switzerland

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Accepted for publication 3 August 1997.

ABSTRACT

Keller, S. M., Wolfe, M. S., McDermott, J. M., and McDonald, B. A. 1997. High genetic similarity among populations of *Phaeosphaeria nodorum* across wheat cultivars and regions in Switzerland. *Phytopathology* 87:1134-1139.

Phaeosphaeria nodorum was sampled from nine wheat fields across a 30-km transect representing three geographical regions in Switzerland to determine the scale of genetic differentiation among subpopulations. Three different wheat cultivars were sampled three times to determine whether differences in host genotype correlated with differences among corresponding pathogen populations. Seven restriction fragment length polymorphism (RFLP) loci and one DNA fingerprint were assayed for each of the 432 isolates in the collection. DNA fingerprints differentiated 426

unique genotypes. Though absolute differences were small, five RFLP loci exhibited significant differences in allele frequencies across the nine subpopulations. Gene diversity within all subpopulations was high ($H_T = 0.51$), but only 3% of the total genetic variation was distributed among the nine subpopulations. When subpopulations were grouped according to geographical region or host cultivar, less than 1% of the genetic variation was distributed among groups, suggesting widespread gene flow and the absence of pathogen adaptation to specific wheat cultivars. Tests for gametic equilibrium within subpopulations and across the entire Swiss population supported the hypothesis of random mating.

Additional keywords: gametic disequilibrium, genetic structure, population genetics, *Septoria nodorum*, *Stagonospora nodorum*.

Glume blotch caused by *Phaeosphaeria nodorum* (E. Mueller) Hedjaroude is an important disease of wheat (*Triticum aestivum* L.) in humid temperate climates (6). Seedlings and adult plants of resistant wheat cultivars express different levels of partial resistance and disease tolerance (7). Resistance appears to be inherited as a quantitative character in the majority of cultivars (8,13,16). Resistance in adult plants is due to reduced infection frequency, longer latent period, and decreased spore production (17). Agronomic traits such as plant height, earliness, and winter growth habit also influence the progress of epidemics (6,19,21).

The complexity of resistance mechanisms has made it difficult to assess resistance in new wheat cultivars. Measurement of resistance is further complicated by differences in virulence among isolates of the fungus. While it is clear that isolates may differ for virulence (1,20), it is not certain whether *P. nodorum* populations differ according to geographical location or host cultivar. Because virulence data are largely quantitative rather than qualitative in this plant pathosystem, it is difficult to ascertain whether postulated differences among isolates sampled from different locations and hosts (20) are due to sampling bias or evolutionary processes. Our previous experiments showed that genetic variation within field populations of *P. nodorum* was high (5,10), suggesting that a significant number of isolates would be needed to determine if populations were subdivided as a result of geographical isolation or adaptation to particular hosts.

The onset of genetic engineering of wheat and the likely introduction of qualitative resistance genes increase the need for knowledge of the genetic structure of populations of *P. nodorum* and other pathogens. As new resistant cultivars are released, resistance may be overcome as a result of local adaptation by virulent mutants.

Virulent mutations may be recombined into many genetic backgrounds if the mating system includes sexual reproduction. Subsequent spread of virulence factors by gene flow may determine the durability of resistance across larger geographical areas.

This study was undertaken to investigate the regional genetic structure of populations of *P. nodorum* in Switzerland. The main objective of this experiment was to determine whether naturally infected field populations were subdivided as a result of either (i) geographical separation or (ii) selection according to host genotype. A secondary objective was to determine whether the genetic structure of Swiss populations was more consistent with sexual reproduction, asexual reproduction, or a mixed mating system.

MATERIALS AND METHODS

Hierarchical collection of isolates. The hierarchical sampling scheme included three spatial scales, region, field, and site within a field. Three fields were sampled from each of three geographical regions separated by ~10 to 20 km (Fig. 1). Distances between fields in each region ranged from 0.1 to 9.0 km. Fifty isolates were collected from each field (450 isolates total). Five infected wheat flag leaves were sampled from each of 10 sites within each field. Five sites were equally spaced along each of two parallel 50-m transects separated by 10 m. One single-spore isolation was made from each leaf. Leaves were collected at the end of the growing season between 27 June and 4 July 1994. All fields were infected naturally.

Embedded within the sampling scheme were the three Swiss wheat cultivars Arina (A), Galaxie (G), and Tamaro (T). Each cultivar was represented three times among the nine field populations, and field populations were named accordingly, e.g., A3 was the third replication of 'Arina'. All three cultivars exhibited average susceptibility to leaf infection by *P. nodorum* when compared with other Swiss cultivars, though 'Arina' was somewhat more resistant. All three cultivars showed above-average resistance to glume infection. In relative terms, 'Arina' was the most resistant in field

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Publication no. P-1997-0915-02R

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trials, followed by 'Galaxie' and 'Tamaro' (25). Though the different cultivars were planted randomly across the three regions (Fig. 1), the seeds for each cultivar came from the same seed lot. All wheat fields were low-input farmers' fields planted for seed production. No fungicides were used in any field.

Restriction fragment length polymorphism (RFLP) analysis. Isolates were grown for 5 to 7 days at room temperature on a rotary shaker in yeast sucrose broth (10 g of yeast extract and 10 g of sucrose per liter of water). Mycelium was harvested by filtration and lyophilized. DNA from each isolate was extracted as described previously (10). DNA was digested with *EcoRI*, size-fractionated by electrophoresis, and transferred to a nylon membrane as described previously (10). Eight anonymous DNA probes were used to detect RFLPs (10). Probe pSNS4, which hybridized to dispersed repetitive elements in the nuclear genome (10), was used to generate DNA fingerprints (5) to detect clones resulting from asexual reproduction in each population. The seven remaining probes hybridized to individual nuclear loci.

Data analysis. Genetic variation within populations. Allele frequencies for individual RFLP loci were calculated for each field population. DNA fingerprints based on hybridization to pSNS4 were used to identify clones. Keller et al. (5) showed previously that the probability that two unrelated individuals would share the same DNA fingerprint by chance was $\sim 1 \times 10^{-9}$ for these Swiss populations.

RFLP allele frequencies were used to calculate Nei's measure of gene diversity (15), which indicates the probability that two randomly chosen isolates from a subpopulation will have different alleles. Estimates of genotypic diversity within each field population were based on the genotype data provided by probe pSNS4. Isolates that showed partial digestions for one or more single RFLP loci were excluded from this analysis. Genotypic diversity was estimated using the method of Stoddart and Taylor (24) as described in McDonald et al. (10). The maximum value for this measure of genotypic diversity is the number of isolates in the sample. Stoddart's *G* can be better compared between subpopulations with different sample sizes by dividing by the sample size (10). *G* then ranges from $1/N$ to 1.

Gametic disequilibrium. Gametic disequilibrium between pairs of alleles and loci was calculated using the methods suggested by Weir (26). Comparisons were made for each subpopulation separately and also for the entire collection of Swiss isolates. Departures from gametic equilibrium were tested using chi-square statistics as described by McDonald et al. (10). These tests can result in significant values when one or more rare allele pairs are present at the two loci under consideration (4). This bias was avoided by pooling alleles present at frequencies lower than 10% into a single category for the Swiss-wide collection. In cases in which significant departures from gametic equilibrium existed in the subpopulations, allele-by-allele comparisons were used to determine the allele pair responsible for the overall gametic disequilibrium.

Genetic variation among populations. Contingency chi-square tests (27) were used to compare RFLP allele frequencies across all field populations. Measures of genetic identity (*I*) and average genetic identities across all loci were calculated according to Nei (14). The maximum value of *I* is 1 in the case of complete identity between populations, in which the same alleles are present at the same frequencies. *I* equals 0 when populations have no alleles in common.

The entire collection was screened for identical genotypes in different field populations using multilocus haplotypes and DNA fingerprints. Multilocus haplotypes were generated by combining alleles present at the seven individual RFLP loci into a seven-digit number for each isolate. Isolates showing the same multilocus haplotypes (i.e., having the same alleles for all RFLP loci) were then compared using DNA fingerprints. This simplified comparisons of DNA fingerprints on different Southern blots.

An indirect measure of gene flow was estimated as the number of migrating isolates (*N_m*) between populations each generation need-

ed to maintain the observed level of genetic similarity among populations. Calculations of *N_m* were based on *G_{ST}* (the amount of genetic variation distributed between subpopulations) as described previously (5). Population differentiation and gene flow expressed by *G_{ST}* and *N_m*, respectively, were calculated for each pair of field populations and across all field populations in this study.

Isolation by distance and host. Populations at equilibrium between gene flow and genetic drift can be tested for isolation by distance because of restricted dispersal among subpopulations. Under conditions of restricted dispersal, the logarithm of the geographical distance between each pair of subpopulations is expected to be negatively correlated with the average number of migrants per generation (*N_m*) between the pairs of subpopulations in question (23). This analysis was conducted using the average *N_m* across all seven single RFLP loci for all 36 pairs of subpopulations. To compare the effects of geographical distance and host on population subdivision, the nine field populations were grouped into three subpopulations according to either hosts or geographical location. Allele frequencies for each group were compared using a contingency chi-square test. *G_{ST}* values were calculated for each set of subpopulations. For further comparison, the range and distribution of *G_{ST}* in this set of subpopulations were obtained by calculating *G_{ST}* for all 280 possible combinations of three groups of three subpopulations.

RESULTS

Only 432 of the 450 isolates were included in the analysis. The 18 missing isolates were due to partial digestions, broken gel lanes, or insufficient DNA to make Southern blots. Forty-two alleles

Fig. 1. Map showing locations of nine low-input wheat fields that were sampled for Swiss populations of *Phaeosphaeria nodorum*. Three replications of the wheat cultivars Arina (A), Galaxie (G), and Tamaro (T) were distributed across three regions (GEO 1, GEO 2, and GEO 3) separated by ~10 to 20 km.

were found among the seven RFLP loci. Fourteen of the alleles were not detected previously in Texas or Oregon (5,10). Seven of these were private alleles present in only one of the nine subpopulations. No private alleles were found in the subpopulations originating from the cultivar Arina. Since the hierarchical sampling was balanced and only six clones were detected, allele frequencies were not clone-corrected as reported in a previous study (5).

Genetic variation within populations. Final sample sizes (N) for all subpopulations, allele frequencies, and measures of gene diversity (H_i) for locus pJSN73 are shown in Table 1. Other loci gave similar results. Total gene diversity (H_T) was generally high across all loci. On average, $H_T = 0.51$ across all seven RFLP loci, with a maximum gene diversity of 0.60 at locus pJSN15 (Table 2). Genotype diversity ($\hat{G}$) based on DNA fingerprints was high in all field populations (Table 1). The genotypic diversity was at 100% of its theoretical maximum ($\hat{G} = N$, all isolates had a different DNA fingerprint) in five of the nine subpopulations. The lowest genotype diversity ($\hat{G} = 88\%$ of maximum value) was due to the presence of two pairs of clones in populations A2 and A3. Replicate clones were always found in the same sampling site within a field. No clones were shared by different populations.

Gametic equilibrium. Thirty-seven out of 189 (20%) locus-by-locus comparisons within field populations resulted in rejection of the null hypothesis of random associations among loci at $P < 0.05$.

Rare allele combinations were involved in all of these departures from equilibrium. Ninety-four of the 2,314 allele-by-allele comparisons (4%) within subpopulations resulted in significant departures from gametic equilibrium ($P < 0.05$ [51], $P < 0.01$ [43]). Subpopulations A1, G2, G3, and T3 exhibited no departures from gametic equilibrium in the locus-by-locus comparisons. The results of the locus-by-locus comparisons across the entire Swiss population ($N = 432$) are summarized in Table 3. After pooling alleles with a frequency lower than 0.1 at each locus into one category, only 19 alleles or allele pools were available for the chi-square tests. Four of the 21 locus-by-locus comparisons showed significant departures from gametic equilibrium.

Genetic variation among populations. RFLP loci pJSN121, pSNL15, pJSN35, pJSN27, and pSNL13 exhibited significant differences in allele frequencies across the nine subpopulations (Table 2). Only 3% of the gene diversity was distributed among the nine subpopulations on average ($G_{ST} = 0.03$). The average G_{ST} was used to calculate the average number of migrants (N_m) that would need to be exchanged across populations each generation to maintain the observed level of genetic similarity. The average N_m was estimated as 19 migrants across all nine populations. Nei's measure of genetic identity for pairs of subpopulations across all loci suggested the same low population differentiation as Wright's G_{ST} (Table 4, above diagonal). Population differentiation between pairs of subpopulations as measured by G_{ST} ranged between 0.01 and

TABLE 1. Frequencies of alleles 1 to 8 at restriction fragment length polymorphism locus pJSN73-EcoRI for nine subpopulations of *Phaeosphaeria nodorum* originating from Swiss wheat cultivars Arina (A), Galaxie (G), and Tamaro (T)

Allele	A1	A2	A3	G1	G2	G3	T1	T2	T3
1 ^a	0.62	0.66	0.74	0.56	0.47	0.59	0.57	0.50	0.65
2	0.21	0.15	0.17	0.23	0.40	0.32	0.19	0.23	0.27
3	0.06	0.15	0.02	0.08	0.12	0.10	0.17	0.09	0.06
4	0.02	0.02	0.02	...	...	...	0.02	0.02	0.02
5	0.09	0.02	0.02	0.04	0.02	...	...	0.14	...
6	...	...	0.02	0.04	...	...	0.04	0.02	...
7	...	...	...	0.02	...	...	...	...	...
8	...	...	...	0.02	...	...	...	...	...
N^b	49	48	47	48	46	48	48	48	50
$\hat{G}^c$	100	88	88	100	100	94	100	100	94
H_i^d	0.56	0.52	0.42	0.62	0.61	0.55	0.60	0.67	0.51

^a Restriction fragments as defined in McDonald et al. (10).

^b Sample size for subpopulation.

^c Stoddart's (24) genotype diversities presented as a percentage of the maximum possible value for each subpopulation.

^d Nei's gene diversity (15).

TABLE 2. Contingency chi-square tests for differences in allele frequencies for seven restriction fragment length polymorphism loci across nine Swiss subpopulations of *Phaeosphaeria nodorum*

	pJSN121	pJSN73	pSNL15	pJSN35	pJSN3	pJSN27	pSNL13
Chi-square	54.7***	69.0	56.9**	71.5**	37.4	83.1**	65.2*
Degrees of freedom	32	56	32	40	32	40	48
H_T^b	0.55	0.58	0.60	0.50	0.33	0.47	0.55
G_{ST}^c	0.03	0.03	0.02	0.02	0.03	0.04	0.03

^a Level of significance: * = $P < 0.05$, ** = $P < 0.01$.

^b Total gene diversities across the nine subpopulations. Average $H_T = 0.51$.

^c The fraction of genetic variation distributed between subpopulations. Average $G_{ST} = 0.03$.

TABLE 3. Pairwise tests for gametic equilibrium among different restriction fragment length polymorphism loci in the entire sample ($N = 432$) of Swiss *Phaeosphaeria nodorum* isolates

	pJSN73 ^a	pSNL15	pJSN35	pJSN3	pJSN27	pSNL13
pJSN121	3.58	5.31	3.09	2.84	4.19	9.61 ^b
pJSN73		3.54	1.35	0.55	3.63	4.76
pSNL15			3.50	4.22	9.90*	2.18
pJSN35				1.04	12.56**	2.60
pJSN3					0.05	6.59*
pJSN27						5.97

^a Alleles present at frequencies lower than 0.10 were pooled into a single category.

^b Level of significance: * = $P < 0.05$, ** = $P < 0.01$.

0.03 (Table 4, below diagonal). These values translate into Nm values ranging from 37 to 56 between pairs of populations.

Isolation by distance and host. Estimates of gene flow were high and equally distributed between pairs of subpopulations. In this case, population subdivision was too low to merit an analysis of the correlation between log(Nm) and log(geographical distance). Instead, subpopulations were grouped according to region and host. Both geographically grouped subpopulations (Fig. 1, GEO) and the host-specified grouping (HOST) had significant allele frequency differences for locus pJSN27 at a significance level of $P < 0.01$. The GEO and HOST groups also exhibited significant differences in allele frequencies ($P < 0.05$) at the pSNL15 and pJSN73 loci, respectively. To make a more meaningful comparison of the genetic differentiation among HOST and GEO groupings, G_{ST} was calculated for all 280 possible three-way combinations of the nine subpopulations taken three at a time. These G_{ST} values are plotted in Figure 2. In comparison to the HOST groups ($G_{ST} = 0.006$), the GEO groups were closer to the middle of the G_{ST} range ($G_{ST} = 0.007$). It should be pointed out that the range of G_{ST} values for the comparisons of subpopulation groups was extremely narrow (0.004 to 0.011), reflecting the low degree of genetic differentiation among these subpopulations.

DISCUSSION

The genetic structures of the Swiss populations of *P. nodorum* assayed in this experiment were consistent with a fungus that exhibited regular sexual reproduction, limited dispersal of conidia, and significant gene flow on a regional scale. We found no evidence that host genotype or seed source influenced the genetic structure of these populations.

Evidence for sexual reproduction. We have three lines of evidence to support the hypothesis that the sexual stage plays a major role in the population genetics of *P. nodorum* in Switzerland: (i) a high degree of genotype diversity was found in each subpopulation; (ii) when clones were detected, they were clustered in a small area within a field; and (iii) alleles at individual RFLP loci were mostly at gametic equilibrium for individual fields and across the entire Swiss population.

Wheat fields in Switzerland appear to be infected initially by a genetically diverse, moderately large population of spores. Though these spores could originate from asexual or sexual reproduction, we believe that the primary inoculum is more likely to be ascospores. If pycnidiospores were the major source of primary and secondary inoculum, we would expect the fungal population to exhibit a more clonal population structure by the end of the growing season, characterized by fewer genotypes and more widely distributed clones within a field. In the absence of sexual reproduction, clonal lineages would develop. Within each clonal lineage, new genotypes could arise by neutral mutations at individual RFLP loci, but these mutations would be at disequilibrium with alleles at other RFLP loci within the clonal lineage. We did not detect evidence of clonal lineages or mean-

ingful gametic disequilibrium within any of the Swiss subpopulations.

Gametic disequilibrium can result from many factors including asexual reproduction or nonrandom mating, population subdivision, and selection. Moderate departures from gametic equilibrium are difficult to detect with sample sizes of only 50 per population. Since there was little evidence for population subdivision ($G_{ST} = 0.03$ on average across all loci), we pooled the nine subpopulations to make a more powerful test for disequilibrium across the entire collection of Swiss isolates (432 isolates total). Seventeen of 21 locus-by-locus comparisons supported the hypothesis of random mating when all isolates were included in the analysis. One locus pair, pJSN35/pJSN27, exhibited a highly significant ($P < 0.01$) departure from gametic equilibrium. In this case, we believe the disequilibrium was due to population subdivision rather than asexual reproduction. Both of these RFLP loci showed significant differences ($P < 0.01$) in allele frequencies when compared across all populations. In addition, pJSN27 was the only RFLP locus with a significant difference ($P < 0.01$) in allele frequency across both geographical and cultivar-specific groups of subpopulations. Given that five of the seven RFLP loci exhibited significant differences in allele frequencies across the nine populations, it may seem surprising that greater disequilibrium was not detected, because of the potential for population subdivision to generate disequilibrium. However, the absolute differences in allele frequencies among populations were too small to generate detectable disequilibrium among most pairs of RFLP loci.

The sexual stage of *P. nodorum* has been identified in wheat fields in North America, Europe, and Australia (2,12,18). If asco-

Fig. 2. The distribution of population differentiation (G_{ST}) values (averaged across seven restriction fragment length polymorphism loci) for all 280 possible three-way combinations of the nine Swiss *Phaeosphaeria nodorum* subpopulations taken three at a time. Grouping subpopulations according to host cultivar (Arina, Galaxie, and Tamaro) yielded a G_{ST} of 0.006. Grouping subpopulations according to the three geographical regions shown in Figure 1 produced a G_{ST} of 0.007.

TABLE 4. Pairwise comparisons of gene identity (I) and population differentiation (G_{ST}) in nine populations of *Phaeosphaeria nodorum* sampled from Swiss wheat cultivars Arina (A), Galaxie (G), and Tamaro (T)

	A1 ^a	A2	A3	G1	G2	G3	T1	T2	T3
A1	...	0.97 ^b	0.98	0.98	0.97	0.97	0.96	0.97	0.97
A2	0.02	...	0.98	0.98	0.97	0.96	0.97	0.97	0.98
A3	0.01	0.02	...	0.98	0.98	0.98	0.98	0.97	0.98
G1	0.01	0.01	0.02	...	0.98	0.96	0.97	0.96	0.98
G2	0.02	0.02	0.01	0.01	...	0.98	0.97	0.96	0.98
G3	0.01	0.03	0.01	0.03	0.02	...	0.97	0.99	0.99
T1	0.02	0.01	0.01	0.02	0.01	0.02	...	0.97	0.97
T2	0.02	0.02	0.02	0.02	0.03	0.01	0.02	...	0.97
T3	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.02	...

^a Measures of I are shown above the diagonal and G_{ST} is shown below the diagonal.
^b Maximum value of 1.0 occurs when the same alleles occur at the same frequencies for each pair of populations.

spores are the main source of primary inoculum and pycnidiospores are the main source of secondary inoculum, we predict a population structure in which there are many different genotypes (originating from air-dispersed ascospores) arranged as small clusters of clones (resulting from splash-dispersed pycnidiospores) by the end of the growing season. Our results are consistent with this hypothesis. Genotype diversity was at, or near, its theoretical maximum in each subpopulation. The sample size of 50 isolates per field provided 95% confidence of detecting genotypes present at a frequency greater than 6% (9). Thus, our sample size was only adequate to detect clones present at moderately high frequencies within field populations. Altogether, six pairs of clones were detected among the nine wheat fields, demonstrating that asexual reproduction occurred during the growing season. Clones always originated from the same site within a field, suggesting that conidia did not move far over the course of the growing season. No clones were shared between fields, though seed for each cultivar came from the same source. Earlier studies in Germany showed that airborne ascospores were present during the early growth stages for both winter and spring wheat (12). When these data are considered together with the evidence for a high degree of gene flow among populations, we consider it unlikely that the pycnidiospores play a major role in determining the genetic structure of populations of this fungus in Switzerland. Ascospores appear to be the primary determinant of genetic structure.

Evidence for gene flow. The genetic structures of the *P. nodorum* populations were extremely similar across all field populations. Though the high gene diversity at individual RFLP loci (average $H_T = 0.51$) provided the potential for unequal distributions of alleles that would lead to population subdivision, it appeared that the genetic composition of the primary inoculum for each field was nearly identical, as reflected by the small value for G_{ST} .

Milgroom and Lipari (11) pointed out that quantitative estimates of average gene flow per generation (Nm) were valid only under conditions of equilibrium between genetic drift and gene flow. They found no correlation between Nm and geographical distance as expected with subpopulations of *Cryphonectria parasitica* at equilibrium between genetic drift and gene flow.

We did not apply this reasoning to our study. Genetic distance need not be correlated with geographical distance for pathogens that are dispersed over long distances by wind or as a result of human-mediated dispersal. In this experiment, the distances between populations were within the potential dispersal distances for ascospores. Though it is possible that these populations were not at a drift-migration equilibrium, estimates of gene flow remain useful, because they reflect the current population subdivision as expressed by the G_{ST} . We do not consider the estimates of Nm to reflect exactly the number of migrants that are exchanged between pairs of populations each generation. Rather, Nm estimates provide an indication of the relative degree of gene flow that likely occurred between populations over time. In this case, the average Nm across all subpopulations was found to be 19. This suggests that there have been few restrictions to gene flow in the past among these populations.

Population differentiation by distance. Under drift-migration equilibrium, populations with restricted dispersal are expected to show isolation by distance (11,23). Epidemiology studies suggest that pycnidiospores are not naturally dispersed over long distances via splash dispersal (3). If we assume that *P. nodorum* did not recently colonize Switzerland, isolation by distance will be expected when (i) pycnidiospores are dominant in all phases of the life cycle (limited dispersal) and (ii) pycnidiospores originate from wheat debris that remains in individual fields. Isolation by distance is not as likely if (i) airborne ascospores contribute significantly to the infection of wheat fields or (ii) infected seed from a common source are a major source of primary inoculum, i.e., human-mediated dispersal (22). If infected seed were involved, we would expect to find shared clones or clonal lineages among the fields derived from

the same seed source. We found no evidence for shared clones among these fields.

Although population differentiation was very small (average G_{ST} of 0.03), some allele frequencies differed significantly among subpopulations according to a contingency chi-square test. This result shows that sample sizes were adequate to detect small differences among subpopulations. However, the degree of population subdivision between pairs of subpopulations was too small to measure isolation by distance by Slatkin's measure (23). When subpopulations were grouped in three-way combinations according to region, differences among subpopulations became even smaller (Fig. 2). The lack of population subdivision according to geography supports the important role(s) of either airborne ascospores or human-mediated dispersal through infected seed or wheat debris distribution. Since little evidence was found for clones or clonal lineages among isolates taken from the same seed lot and since wheat seed is commonly treated with a fungicide in Switzerland, we consider it more likely that ascospores are the primary agent for unifying geographically separate populations on a regional scale.

Host specialization. Because wheat cultivars show quantitative resistance to *P. nodorum*, population differentiation by host selection is not expected to be as strong as in systems with qualitative resistance mechanisms. If the individual RFLP loci are neutral and not linked to loci that are under selection, then the individual RFLP loci will not be useful to detect pathogen adaptation. Our data support the neutrality of the individual RFLP markers. G_{ST} for individual RFLP loci across host-specified groups ranged from 0.001 for pJSN3 to 0.012 for pJSN73, suggesting little evidence for host differentiation across the seven loci. Since individual RFLP loci were not useful for detecting pathogen adaptation, we relied on the DNA fingerprints to consider the possibility that particular clones or clonal lineages would increase in frequency on specific host genotypes. We found no evidence for host-specific clones or clonal lineages, suggesting that host specialization does not occur by selection for host-specialized clones in Swiss populations of *P. nodorum*.

Restricted dispersal of pycnidiospores in combination with seed-transmitted primary inoculum could lead to genetic differentiation of fungal populations on different wheat cultivars. The seed for each cultivar in this experiment came from the same seed lot, but we found no evidence for higher genetic similarities among subpopulations sampled from the same wheat cultivar (Fig. 2). Clones were not shared between any of the field populations. The sample size of 432 isolates across populations provided 95% confidence of detecting genotypes present at a frequency of greater than 0.7% (9). While it remains possible that genotypes present at lower frequencies were shared between populations, this result suggests that seedborne inoculum contributes only a small percentage to the primary inoculum. There was no evidence for seed transmission of isolates among subpopulations.

Overall, the genetic structure of Swiss populations of *P. nodorum* is best explained by assuming that the primary inoculum is composed of airborne ascospores. Dispersal of ascospores on a regional scale distributes genes evenly among field populations, leading to the lack of population subdivision. Regular sexual reproduction shuffles genes regularly, generating many new genotypes and maintaining random associations among neutral RFLP loci. Under this scenario, resistance gene pyramids are not likely to last long, because the pathogen is likely to recombine and disseminate virulence genes as quickly as plant breeders can recombine and disseminate resistance genes.

ACKNOWLEDGMENTS

This work was supported by the Swiss National Fund (grant 97-888-2383) and the USDA National Research Initiative Competitive Grants Program (grant 93-37303-9039). We thank U. Brändle, M. Finckh, U. Haemmerli, and M. Ruckstuhl for their help during the collection of isolates around Zürich. We also thank the Swiss farmers who kindly allowed and promoted our investigation in their fields.

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