# Genetic Differentiation Between *Phaeosphaeria nodorum* and *P. avenaria* Using Restriction Fragment Length Polymorphisms

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## ABSTRACT

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Genetic variation among 14 isolates of *Phaeosphaeria nodorum* and 10 isolates of *P. avenaria* that originated from diverse geographic locations was assessed by the use of restriction fragment length polymorphisms (RFLPs). Genomic DNAs were digested with the restriction enzyme *Eco*RI and hybridized with 38 anonymous DNA probes. Twenty of the 38 probes were isolated from *P. nodorum,* and the other 18 probes were isolated from *P. avenaria.* Most of the probes hybridized with one or two DNA bands per isolate. Each isolate was assigned a RFLP genotype, which is a combination of the banding patterns with all 38 probes. Isolates of *P. nodorum* showed a significantly lower degree of genetic variation than the isolates of *P. avenaria.* There were seven genotypes among the 14 isolates of *P. nodorum,* whereas each isolate of *P. avenaria* had a different genotype. In addition, the minimum number of shared alleles was 34 out of a total of 38 RFLP loci among isolates of *P. nodorum* in pairwise comparisons, and the minimum number of shared alleles was as low as five among isolates of *P. avenaria.* In cluster analyses based on individual hybridizing bands (total 155 bands), isolates of *P. nodorum* formed a tight cluster with a minimum similarity value above 0.9. Bootstrap analysis strongly supported the separation of *P. nodorum* from *P. avenaria.* Considerable genetic variation was observed among isolates of *P. avenaria.* Several RFLP alleles were useful for differentiation of *P. nodorum* from *P. avenaria,* and some probes were specific for certain isolates. Those isolate-specific probes may be used as natural markers in epidemiological studies.

*Additional keywords*: *Leptosphaeria avenaria, L. nodorum,* oats, Septoria blotch, *Stagonospora avenae, S. nodorum,* wheat.

Glume blotch and Stagonospora avenae blotch of wheat are two symptomatically similar leaf diseases that are caused by two distinct fungal pathogens. *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (= *Leptosphaeria nodorum* E. Müller, anamorph *Stagonospora nodorum* (Berk.) Castellani & E. G. Germano, = *Septoria nodorum* (Berk.) Berk. in Berk. & Broome) causes the glume blotch disease of wheat (*Triticum aestivum* L. em. Thell.) and occurs widely around the world (4,37). It also infects cultivated barley (*Hordeum vulgare* L.) (4,10,35). *P. avenaria* (G. F. Weber) O. Eriksson (anamorph *Stagonospora avenae* (A. B. Frank) Bissett) causes Stagonospora avenae blotch disease. *P. avenaria* has two formae specialis, *P. avenaria* (G. F. Weber) O. Eriksson f. sp. *triticea* T. Johnson (anamorph *Stagonospora avenae* (A. B. Frank) Bissett f. sp. *triticea* T. Johnson) and *P. avenaria* f. sp. *avenaria* (anamorph *Stagonospora avenae* f. sp. *avenae*). *P. a. avenae* incites a leaf disease on oat and has been reported in eastern Canada (3), Germany (24), Finland (21,22), and the United States (11). *P. a. triticea* has a broader host range, infecting wheat (13,15), cultivated barley (33,34), wild barley (*Hordeum jubatum* L.) (32), rye (*Secale cereale* L.) (33), and several common grasses (37). The leaf blotch disease of wheat caused by *P. a. triticea* is severe and common in Canada (15), the northern United States (12,13,20,31,33), and northern Europe (19,21,22). *P. nodorum* and *P. avenaria* are morphologically similar and are distinguished on the basis of conidial size and host range (4,15,17,26).

*P. nodorum* and *P. a. triticea* produce identical disease symptoms (4,15,19,26). Identification relies on measurement of the conidia from infected tissues (15,26) or cultural morphology on

different media (19). *P. nodorum* is characterized as having smaller conidia (15–32 × 2–4 μm) compared with those of *P. a. triticea* (25–45 × 3–4 μm) (15,37). However, conidial size varies among single-spore cultures of the same isolate and is influenced by environmental conditions such as culture media and temperature (11,16). These features make spore size an unreliable characteristic for separating the two species (1,18). Considerable variation exists in colony morphology and/or pathogenicity among isolates within each of the two species (11,16,25). Little is known regarding intraspecific genetic variations in the two species or about the relationships between the two *Septoria* pathogens.

The objectives of this research were to examine the intraspecific genetic variation in *P. nodorum* and *P. avenaria* by use of restriction fragment length polymorphism (RFLP) with 38 anonymous DNA probes and to define the genetic differences between the two pathogens with a view to identifying species-specific probes or RFLP patterns that could facilitate the differentiation and identification of two important Septoria diseases in cereals.

## MATERIALS AND METHODS

**Fungal isolates, cultural conditions, and maintenance.** Fourteen isolates of *P. nodorum* isolated from wheat in Indiana, Florida, Georgia, Montana, New York, and Oregon were used (Table 1). Five of these isolates were used in a previous study (39). Two isolates of *P. a. avenaria* and eight isolates of *P. a. triticea* were also included (Table 1). Some isolates were used in pathogenicity tests of earlier studies (12,32). The fungal cultures were maintained on Czapek-Dox V8 agar supplemented with 5% complete supplement (25) at 21 C with constant near-ultraviolet illumination.

**DNA isolation and genomic library construction.** Cultures were grown in a yeast-malt-sucrose liquid medium for 7–10 days at room temperature. For DNA isolation, mycelia were harvested by filtration and centrifugation, and total genomic DNA was isolated by a procedure described earlier (39), except that sodium dodecyl sulfate and sodium *N*-lauroylsarcosine (1% each) were used in the lysis buffer. Two DNA libraries were used. One was constructed from isolate SN209NY-88 of *P. nodorum* (39). The other DNA library was developed from isolate SAA001NY-85 of *P. a. avenaria*. Genomic DNA was digested with *Eco*RI enzyme (Promega, Madison, WI) and cloned into the *Eco*RI site of the plasmid pGEM3Z (Promega). Transformation, selection, and purification of cloned plasmid DNA were as described previously (39).

**Selection of DNA probes.** Twenty anonymous DNA probes originally developed from isolate SN209NY-88 (*P. nodorum*) were chosen (Table 2). These probes produced strong hybridization signals and hybridized to one or two DNA bands per isolate. The DNA bands showed no variation among 11 isolates of *P. nodorum* (39). A set of 18 anonymous DNA probes was randomly selected from a DNA library developed from isolate SAA001NY-85 (*P. a. avenaria*). Most of the randomly selected probes (17 of 18) also happened to hybridize to one or two DNA bands in each isolate (Fig. 1). These DNA probes ranged in size from 0.3 to 2.2 kb (Table 2).

**DNA hybridization.** Total genomic DNA was digested with the restriction enzyme *Eco*RI (3 units per microgram of DNA) at 37 C overnight. The digested DNA (2.5 μg per lane) was separated by 0.8% agarose gel electrophoresis at a constant voltage (20 V) for 17 h. Gel handling and blotting onto Nytran membranes (pore size 0.45 μm; Schleicher & Schuell, Keene, NH) were as described previously (36,39). *Eco*RI-digested plasmid DNA (0.5 μg from each isolate) containing the probes was labeled with $^{32}P$ by the random-priming technique (6,7). Membrane hybridization and washing conditions were the same as reported previously (39). X-ray film (X-Omat, Kodak, Rochester, NY) was exposed to the membranes for 1 wk at room temperature without an intensifying screen and developed according to the manufacturer's instructions.

**Data analysis.** Following to the convention of RFLP analysis, we considered the various banding patterns detected by each anonymous probe to be alleles of the putative RFLP locus. The assignment of the allele designation number was based on the frequency of the banding pattern; the most frequent banding pattern was assigned allele 1 and the next most frequent banding pattern allele 2 and so on. A zero was always assigned to a null allele. The allelic data were combined in the order presented in Table 2 to form RFLP genotypes (Table 3). Each isolate was assigned a genotype (Table 1). Pairwise measures of genetic

TABLE 1. Isolates of *Phaeosphaeria* species used in restriction fragment length polymorphism (RFLP) analysis and their assigned RFLP genotype

| Isolate designation | Geographic origin | Year of isolation | Host plant | RFLP genotype number[a] |
|---|---|---|---|---|
| *P. nodorum* | | | | |
| SN038NY-89[b] | Orleans County, New York | 1989 | Wheat | 1 |
| SN058NY-89[b] | Monroe County, New York | 1989 | Wheat | 1 |
| SN209NY-88[b,c] | Cayuga County, New York | 1988 | Wheat | 3 |
| SN91-X[b] | Tippecanoe County, Indiana | 1991 | Wheat | 2 |
| SNField 9011 | Pike County, Indiana | 1990 | Wheat | 4 |
| SNField 9035 | Posey County, Indiana | 1990 | Wheat | 2 |
| SNField 9066 | Sullivan County, Indiana | 1990 | Wheat | 2 |
| SN213MT-89[b] | Montana | 1989 | Wheat | 1 |
| SN GH-91 | Montana | 1991 | Wheat | 2 |
| SN 85-19 | Montana | 1985 | Wheat | 1 |
| S-80-504 | Washington County, Georgia | 1980 | Wheat | 5 |
| S-80-509 | Gadsden County, Florida | 1980 | Wheat | 6 |
| S-81-W12 | Marion County, Oregon | 1981 | Wheat | 1 |
| S-81-W14 | Benton County, Oregon | 1981 | Wheat | 7 |
| *P. avenaria* f. sp. *avenaria* | | | | |
| SAA001NY-85[c] | New York | 1985 | Oat | 8 |
| ATCC 12277 | United States | Unknown | Oat | 9 |
| *P. avenaria* f. sp. *triticea* | | | | |
| SAT001NY-84 (ATCC 58582) | New York | 1984 | Wheat | 10 |
| SAT002NY-84 (ATCC 58583) | New York | 1984 | Wheat | 11 |
| S-81-W10 | Washington | 1969 | Wheat | 12 |
| ATCC 18596 | North Dakota | Unknown | Wheat | 14 |
| ATCC 26370 | Minnesota | Unknown | Foxtail barley | 13 |
| ATCC 26371 | Minnesota | Unknown | Barley | 15 |
| ATCC 26373 | Minnesota | Unknown | Wheat | 16 |
| ATCC 26378 | Minnesota | Unknown | Rye | 17 |

[a]The RFLP genotype number corresponds to the genotype definition in Table 3.
[b]Isolate used in a previous study (39).
[c]Isolate used to develop genomic DNA libraries from which DNA probes were selected.

relatedness were calculated on the basis of RFLP genotypes as numbers of shared alleles at the 38 putative loci. Data were also analyzed on the basis of the individual hybridization bands produced by the 38 DNA probes (total 155 bands), and Dice similarity ($S_d$) was calculated for each pair of isolates (27) as

$$S_d = 2N_{xy}/(N_x + N_y)$$

where $N_x$ and $N_y$ are the numbers of fragments in isolates x and y, respectively, and $N_{xy}$ is the number of fragments shared by the two isolates (27). This is mathematically the same as the formula for the fraction of DNA restriction fragment shared by two individuals (9). Cluster analysis with the unweighted pair group method with an arithmetic average (UPGMA) algorithm was performed to produce a phenogram (27). The correlation between the Dice similarity matrix based on 155 individual bands and the simple match similarity matrix based on genotypes of 38 putative RFLP loci was calculated by product-moment correlation. In the phylogenetic analysis, a hypothetical outgroup taxon with null characters was used, and the strength of internal branches was estimated by bootstrap analysis with 200 replications with the program PAUP (Phylogenetic Analysis Using Parsimony, version 3.1) (38).

TABLE 2. Anonymous DNA probes used in study and the number of alleles detected by each of the probes in isolates of *Phaeosphaeria* species

| Probe number[a] | Probe size (kb) | Number of alleles[b] | | |
|---|---|---|---|---|
| | | *P. nodorum* | *P. a. avenaria* | *P. a. triticea* |
| SNE3[c] | 1.3 | 1 | 1 | 5 |
| SNE9 | 1.2 | 1 | 2 | 3 |
| SNE10 | 2.2 | 1 | 1 | 3 |
| SNE20 | 1.2 | 1 | 1 | 3 |
| SNE26[c] | 1.4 | 1 | 1 | 4 |
| SNE30 | 1.4 | 1 | 2 | 2 |
| SNE36[c] | 1.4 | 1 | 1 | 3 |
| SNE40[c] | 1.7 | 1 | 2 | 3 |
| SNE48[c] | 1.1 | 1 | 2 | 2 |
| SNE62[c] | 1.2 | 1 | 2 | 4 |
| SNE64 | 1.4 | 1 | 1 | 3 |
| SNE71 | 1.1 | 1 | 1 | 3 |
| SNE72 | 1.3 | 1 | 1 | 2 |
| SNE85[c] | 1.4 | 1 | 2 | 3 |
| SNE111[c] | 1.5 | 1 | 2 | 4 |
| SNE116[c] | 1.0 | 1 | 2 | 4 |
| SNE120 | 1.6 | 1 | 2 | 4 |
| SNE122[c] | 1.1 | 1 | 2 | 3 |
| SNE123[c] | 2.2 | 1 | 1 | 3 |
| SNE165[c] | 1.2 | 1 | 2 | 4 |
| SAAE2 | 0.3 | 1 | 1 | 1 |
| SAAE7[c] | 0.5 | 1 | 1 | 2 |
| SAAE15[c] | 0.3 | 1 | 1 | 3 |
| SAAE19[c] | 0.8 | 1 | 1 | 4 |
| SAAE23[c] | 0.3 | 1 | 1 | 3 |
| SAAE24 | 0.4 | 1 | 1 | 2 |
| SAAE37 | 1.0 | 2 | 1 | 3 |
| SAAE38 | 0.3 | 2 | 2 | 1 |
| SAAE42 | 0.3 | 1 | 1 | 3 |
| SAAE47[c] | 1.0 | 1 | 1 | 2 |
| SAAE49 | 1.4 | 2 | 1 | 3 |
| SAAE52[c] | 0.9 | 1 | 1 | 4 |
| SAAE53 | 0.8 | 1 | 1 | 4 |
| SAAE71 | 0.5 | 1 | 1 | 4 |
| SAAE83 | 0.8 | 3 | 1 | 4 |
| SAAE84 | 0.4 | 2 | 2 | 4 |
| SAAE86[c] | 0.6 | 1 | 1 | 4 |
| SAAE90 | 1.3 | 1 | 1 | 3 |

[a] All DNA preparations were digested with *Eco*RI; each probe defines a putative restriction fragment length polymorphism (RFLP) locus. The SNE-series probes were obtained from isolate SN209NY-88 of *P. nodorum* and the SAAE-series probes from isolate SAA001NY-85 of *P. avenaria* f. sp. *avenaria*.

[b] Each restriction fragment or set of restriction fragments was assumed to be an allele for that putative RFLP locus. Values are numbers of alleles observed in isolates of *P. nodorum* ($n = 14$), *P. a.* f. sp. *avenaria* ($n = 2$), and *P. a.* f. sp. *triticea* ($n = 8$).

[c] Probe detected specific alleles for *P. nodorum*.

## RESULTS

**RFLPs between *P. nodorum* and *P. avenaria*.** There were distinct polymorphisms between *P. nodorum* and *P. avenaria* (Table 2). No isolates from the two species shared the same genotype (Table 1), and the maximum number of shared alleles between the two species is eight of 38 putative loci (Table 3). Of the 38 probes used, 19 detected no variation among isolates of *P. nodorum* and unambiguously differentiated *P. nodorum* from *P. avenaria* (Tables 2 and 3). The putative locus defined by probe SAAE2 was monomorphic among all 24 isolates used in this study (Table 2). Cluster analysis of Dice coefficients clearly separated isolates of *P. nodorum* from those of *P. avenaria* on the basis of the individual hybridizing bands (Fig. 2). Cluster analysis based on the RFLP genotypes showed essentially the same relationships among the isolates (not shown). The product-moment correlation between the similarity matrix based on RFLP genotypes and the similarity of individual hybridizing bands was 0.996. The distinction between the two species was also supported by phylogenetic analysis. A high level of confidence (100%) was placed in the internal branch that separated the two species in 200 bootstrap replications (Fig. 3).

**RFLPs within *P. nodorum*.** There was very little genetic variation among isolates of *P. nodorum*. The 20 anonymous DNA probes selected from *P. nodorum* detected no variation among the 14 isolates. The hybridization of probe SNE72 with 13 isolates of *P. nodorum* is shown in Figure 1A. Additionally, 13 of the 18 randomly selected probes from *P. avenaria* did not detect any polymorphisms among isolates of *P. nodorum*. Only five probes (SAAE37, SAAE38, SAAE49, SAAE83, and SAAE84) from *P. avenaria* detected variable RFLP loci among the 14 isolates of *P. nodorum* (Tables 2 and 3). Seven genotypes were detected among the 14 isolates (Table 1). Five isolates (SN038NY-89, SN058NY-89, SN213MT-89, S-81-W12, and SN85-19) from New York, Montana, and Oregon shared one genotype (genotype 1) (Table 1 and Fig. 2). Four other isolates (SNField 9035, SNField 9066, SN91-X, and SN GH-91) from Indiana and Montana belonged to another genotype (genotype 2) (Table 1 and Fig. 2). These two genotypes shared the same banding patterns at 36 of the 38 putative RFLP loci (Table 4). Each of the remaining five isolates of *P. nodorum* had a different genotype. The minimum number of putative loci with shared alleles in pairwise comparisons among the isolates of *P. nodorum* was 34 of 38 (Table 4). The 14 isolates of *P. nodorum* formed a very tight cluster (Fig. 2) with an average similarity value above 0.9. Bootstrap analysis strongly supported the formation of a clade by the 14 isolates of *P. nodorum* (Fig. 3).

**RFLPs within *P. avenaria*.** Considerable genetic variation was detected among 10 isolates of *P. avenaria*. Thirty-seven of the 38 DNA probes detected polymorphisms, and each isolate had a different genotype (Table 1). Two isolates of *P. a. avenaria* were genetically similar (Figs. 2 and 3). Two of the eight isolates of *P. a. triticea* showed a close relationship with *P. a. avenaria*, but the other six isolates showed various relationships with the *P. a. avenaria* cluster. The ATCC cultures from Minnesota and North Dakota, except isolate ATCC 26370, formed a tight cluster with a minimum similarity value 0.96 (Fig. 2). Isolate ATCC 26370, isolated from wild barley and identified as *P. a. triticea*, is genetically more similar to *P. nodorum* than to the other isolates of *P. avenaria* used in this study. Of 20 probes that hybridized to DNA of this isolate, seven detected the same alleles as in *P. nodorum* (Table 3). However, this isolate is still quite distinct from *P. nodorum*, since eight of the *P. nodorum* probes (SNE3, SNE26, SNE40, SNE48, SNE62, SNE71, SNE85, and SNE111) did not hybridize to any DNA bands in this isolate (Table 3).

Isolate S-81-W10 from Washington showed a closer relationship to isolates of *P. avenaria* from New York than to the isolates from Minnesota and isolate ATCC 18596 from North Dakota in the cluster analysis (Fig. 2). The similarity between the isolates

of *P. avenaria* from New York and those ATCC cultures from Minnesota and North Dakota was very low (0.21) (Fig. 2). Only three of the 38 probes (SAAE2, SAAE7, and SAAE24) detected the same RFLP alleles in these two groups of *P. avenaria* isolates.

**Specificity of DNA probes.** Several probes showed various degrees of specificity in the hybridization studies and will be useful for future epidemiological and taxonomical studies. DNA probe SAAE7 detected two alleles that differentiated *P. nodorum* from *P. avenaria,* except that it did not detect any DNA bands in isolate ATCC 26370, an unusual isolate of *P. avenaria* (Table 2). Probe SAAE24 also detected two alleles that differentiated *P. nodorum* from *P. avenaria,* except in isolate ATCC 26370 (Table 2). Nineteen probes produced specific alleles for isolates of *P. nodorum* (Table 2), whereas some other probes produced specific alleles for only certain isolates (Table 2 and Fig. 1). We have selected a combination of six probes that can be used to differentiate four major groups of isolates used in this study (Table 5). These probes had either a + or − reaction in the Southern blot hybridization and thus could be adopted for dot blot applications. Depending on the objectives, as few as two probes will be sufficient for differentiation of groups of isolates. For example, a combination of probes SNE48 and SNE85 could be used to differentiate the two groups of isolates in *P. a. triticea* (Table 5).

## DISCUSSION

*P. nodorum* and *P. avenaria* are morphologically similar and produce identical symptoms on host plants. Differentiation of the two species is mainly based on conidial size, degree of pycnidia formation, development of the teleomorph, and mycelial color (19,26). The variability in conidial size, colony morphology, and pathogenicity of these two species is the cause of controversy in identification (18). Our data show that *P. nodorum* and *P. avenaria* can be unambiguously differentiated from each other on the basis of RFLPs. Nineteen of 38 anonymous DNA probes produced consistent RFLP bands in isolates of *P. nodorum* that were different from those in *P. avenaria* (Table 2). Five additional probes (SNE9, SNE10, SNE20, SAAE24, and SAAE42) also differentiated the two species, except for isolate ATCC 26370 of *P. avenaria* (Table 2). Both cluster and phylogenetic analyses supported the conclusion that *P. nodorum* and *P. avenaria* represent genetically distinct populations. Isolate ATCC 26370 of *P. avenaria* is unusual in that it is genetically more similar to isolates of *P. nodorum* than to the other isolates of *P. avenaria* (Figs. 2 and 3). This isolate was also unusual in pathogenicity tests (32). It is the only isolate of *P. a. triticea* from Minnesota that parasitized and caused appreciable disease on wheat, barley, foxtail barley, and a range of uncultivated *Hordeum* spp. (32). It produced pycnidia more readily on host plants, whereas the other isolates from Minnesota produced appreciable disease only on barley (32). The taxonomic identity of this isolate (ATCC 26370) cannot be determined until more isolates of *P. avenaria* and other morphologically similar species are compared. In the following discussion, isolate ATCC 26370 is not considered to be *P. avenaria.*

Because the biological meanings of the anonymous RFLP probes as well as the precise mechanisms of variation they detected are undetermined, we believe the approach of the single enzyme-multiple probe combination is more conservative than multiple enzyme-multiple probe combinations in estimating genetic diversity. Given a set of DNA probes, it is less likely that the same DNA variation will be counted more than once in the single-enzyme approach. For instance, a two-enzyme approach would

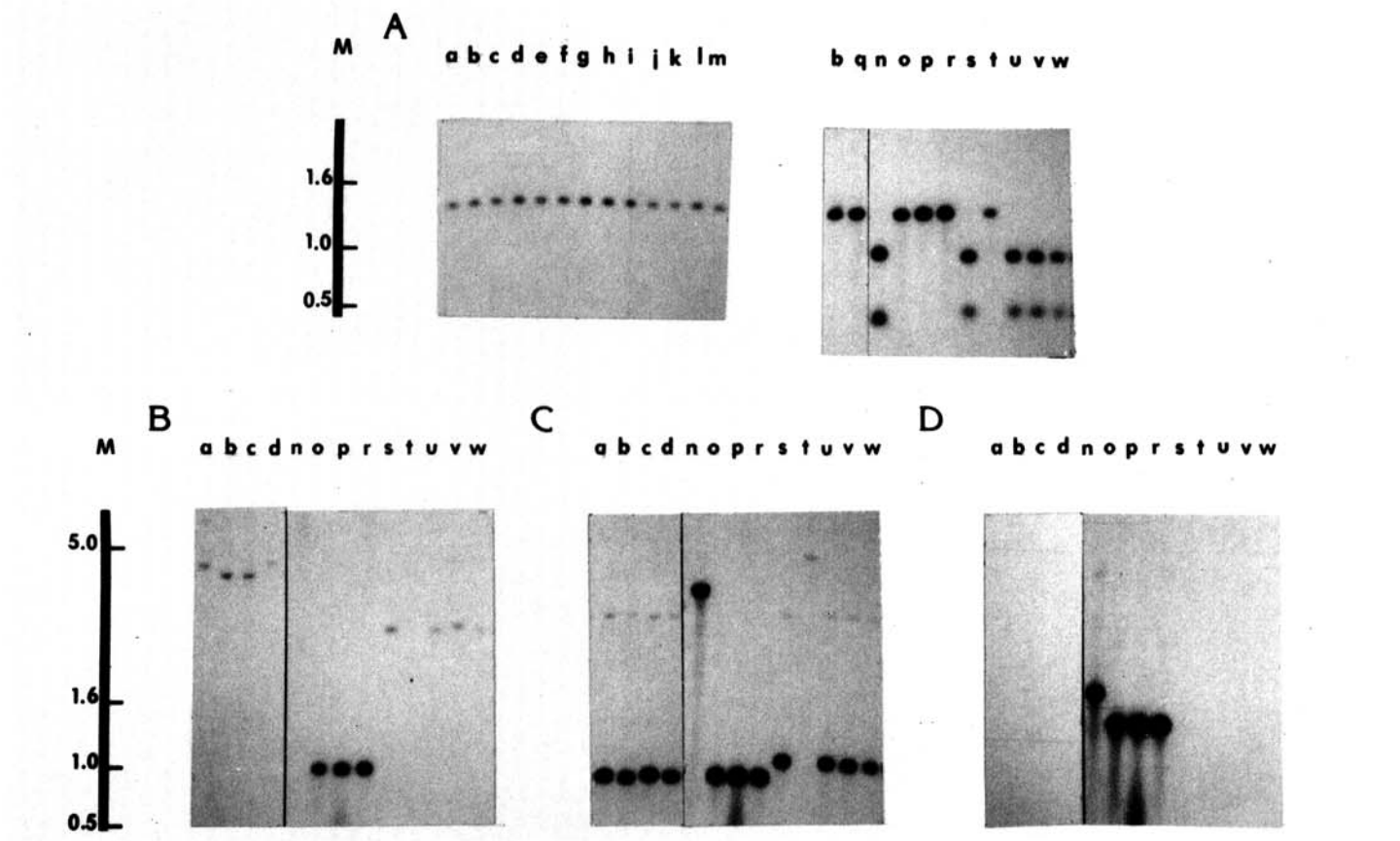


**Fig. 1.** Southern blot hybridization analyses of isolates of *Phaeosphaeria nodorum* and *P. avenaria* with probes **A,** SNE72, **B,** SAAE37, **C,** SAAE53, and **D,** SAAE90. Isolates: a = SN058NY-89; b = SN209NY-88; c = SN91-X; d = SNField 9011; e = SNField 9035; f = SNField 9066; g = SN GH-91; h = SN85-19; i = SN213MT-89; j = S-80-504; k = S-80-509; l = S-81-W12; m = S-81-W14; n = S-81-W10; o = SAA001NY-85; p = SAT001NY-84; q = SAT002NY-84; r = ATCC 12277; s = ATCC 18596; t = ATCC 26370; u = ATCC 26371; v = ATCC 26373; and w = ATCC 26378.

count a length mutation twice (2). Nevertheless, considerable genetic variation was detected among isolates of *P. avenaria* with the single-enzyme approach.

The selection of 20 probes from a previous study that did not detect variation among 11 isolates of *P. nodorum* had certainly biased the estimate of genetic diversity in *P. nodorum* relative to *P. avenaria*. However, considering only the variation detected by the 18 probes randomly selected from *P. avenaria, P. nodorum* still exhibited a lower degree of genetic variation relative to *P. avenaria*. Five of the 18 probes detected polymorphisms in *P. nodorum*, whereas 17 of the 18 probes (15 of 18 excluding isolate ATCC 26370) detected polymorphisms in *P. avenaria*. Admittedly, the sample sizes were too small to estimate intraspecific genetic variation. Nevertheless, the trend in differences in intraspecific variation between the two species is clear. We have additional evidence that indicates a low degree of genetic variation within *P. nodorum*. Hybridization with 33 anonymous DNA probes from *P. nodorum* detected no polymorphisms in a sample of 22 isolates from various geographic locations of the United States (P. P. Ueng, *unpublished*). It is not clear why such a drastic difference exists in intraspecific variation between the two species. We can only speculate about some possible explanations that could be

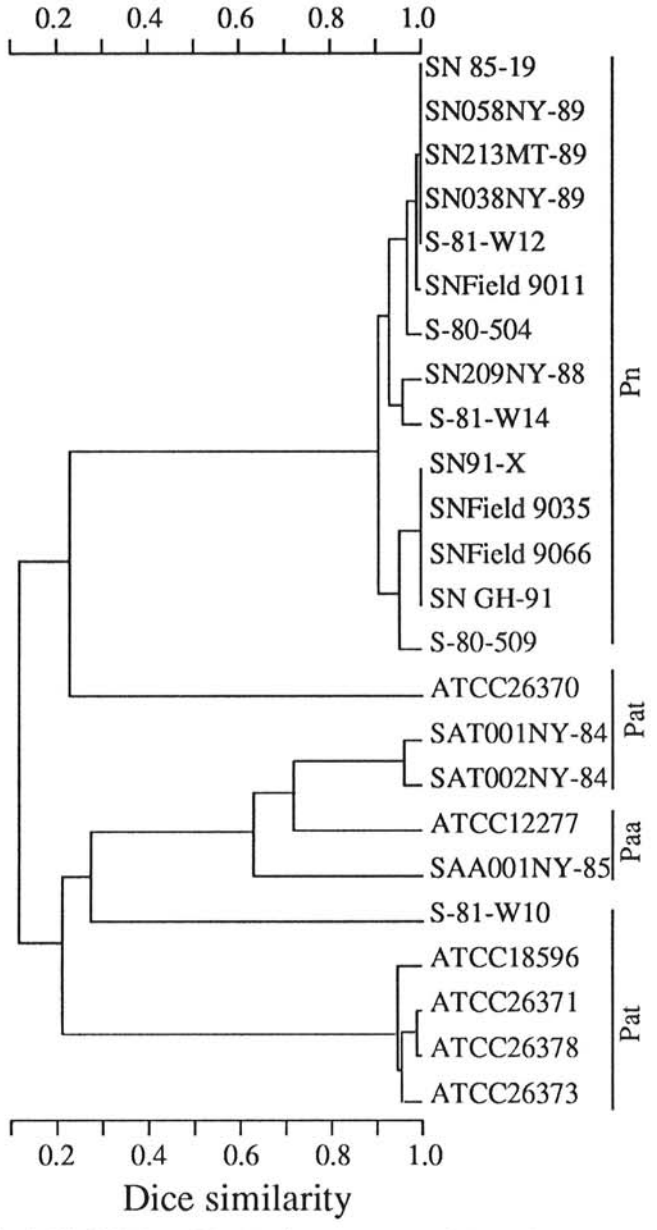


**Fig. 2.** UPGMA (unweighted pair group method with an arithmetic average) phenogram of 24 isolates of *Phaeosphaeria nodorum* and *P. avenaria* based on the Dice similarity coefficients ($S_d$) of 155 individual hybridizing DNA fragments. The isolates with $S_d = 1$ had the identical genotype on the basis of restriction fragment length polymorphisms. Cophenetic correlation was 0.993. Pn = *P. nodorum; Paa* = *P. avenaria* f. sp. *avenaria;* and Pat = *P. avenaria* f. sp. *triticea*.

TABLE 3. Seventeen restriction fragment length polymorphism (RFLP) genotypes found in 24 isolates of *Phaeosphaeria nodorum* and *P. avenaria*

| RFLP genotype number[a] | RFLP genotype[b] | N[c] |
|---|---|---|
| *P. nodorum* | | |
| 1 | 11111111111111111111111111111111111110 | 5 |
| 2 | 11111111111111111111111121111112110 | 4 |
| 3 | 11111111111111111111111121110111 5110 | 1 |
| 4 | 11111111111111111111111111111111111010 | 1 |
| 5 | 11111111111111111111111121111111110 | 1 |
| 6 | 11111111111111111111111121111112110 | 1 |
| 7 | 11111111111111111111111111111101115010 | 1 |
| *P. a.* f. sp. *avenaria* | | |
| 8 | 22232332351113555425122332433222114431 | 1 |
| 9 | 23232134441110641226122332403222114231 | 1 |
| *P. a.* f. sp. *triticea* | | |
| 10 | 23222122031112333223122332402222114231 | 1 |
| 11 | 23222122031112333323122332402222114431 | 1 |
| 12 | 42244143041123474234122422001234326042 | 1 |
| 13 | 01110150003010066547100501004205430000 | 1 |
| 14 | 32323220222220222222123222302003223220 | 1 |
| 15 | 32323220222220222222123222302003203320 | 1 |
| 16 | 52323220222220222222123222302003223320 | 1 |
| 17 | 32323220222220222222123222302003223320 | 1 |

[a]The RFLP genotype number corresponds to the number assigned to each isolate in Table 1.
[b]The RFLP genotypes are defined as combinations of allelic data for 38 RFLP loci in the order presented in Table 2.
[c]Number of isolates that shared the same RFLP genotype.

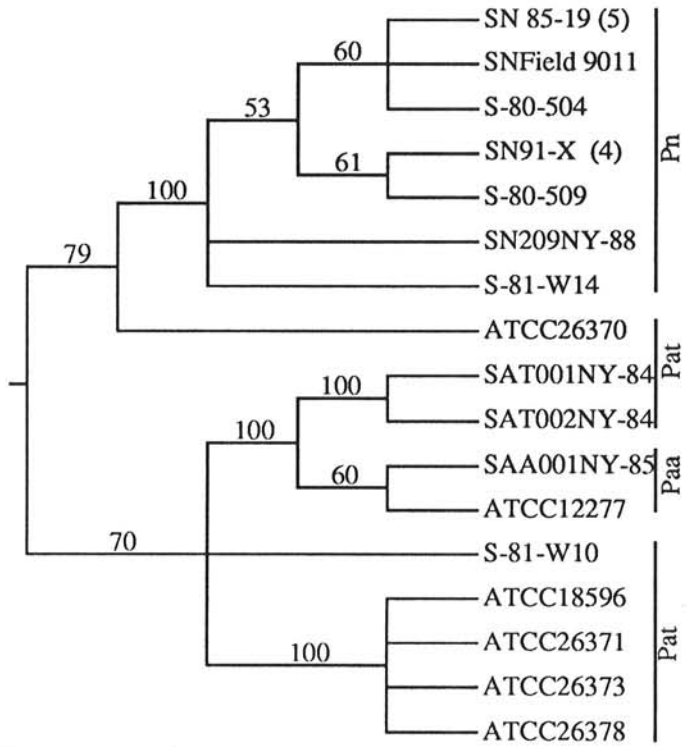


**Fig. 3.** Phylogenetic relationship based on restriction fragment length polymorphisms (RFLPs) of 17 genotypes representing 24 isolates of *Phaeosphaeria nodorum* and *P. avenaria* generated by PAUP (Phylogenetic Analysis Using Parsimony) and based on 155 individual hybridizing DNA fragments. Bootstrap values (of 200 replications) of the internal branches are indicated. Branches supported by less than 50% were collapsed to yield polytomies. The numbers of isolates that had an identical RFLP genotype (Dice similarity = 1) are indicated in parentheses after the isolate number. Pn = *P. nodorum;* Paa = *P. avenaria* f. sp. *avenaria;* and Pat = *P. avenaria* f. sp. *triticea*.

TABLE 4. The number of shared alleles in 38 restriction fragment length polymorphism (RFLP) loci in pairwise comparisons among 17 RFLP genotypes in *Phaeosphaeria nodorum* and *P. avenaria*

| Species | RFLP genotype number | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype number[a] | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| *P. nodorum* | | | | | | | | | | | | | | | | | |
| 1 | | | | | | | | | | | | | | | | | |
| 2 | 36 | | | | | | | | | | | | | | | | |
| 3 | 35 | 36 | | | | | | | | | | | | | | | |
| 4 | 37 | 35 | 34 | | | | | | | | | | | | | | |
| 5 | 37 | 35 | 34 | 36 | | | | | | | | | | | | | |
| 6 | 36 | 36 | 34 | 35 | 37 | | | | | | | | | | | | |
| 7 | 35 | 34 | 36 | 36 | 34 | 34 | | | | | | | | | | | |
| *P. avenaria* | | | | | | | | | | | | | | | | | |
| 8 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | | | | | | | | | | |
| 9 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 25 | | | | | | | | | |
| 10 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 23 | 27 | | | | | | | | |
| 11 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 24 | 25 | 36 | | | | | | | |
| 12 | 5 | 5 | 5 | 6 | 5 | 5 | 6 | 10 | 12 | 12 | 11 | | | | | | |
| 13 | 7 | 7 | 8 | 8 | 7 | 7 | 9 | 3 | 6 | 6 | 6 | 7 | | | | | |
| 14 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 5 | 8 | 10 | 8 | 9 | 6 | | | | |
| 15 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 5 | 7 | 9 | 8 | 8 | 6 | 36 | | | |
| 16 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 5 | 7 | 9 | 8 | 9 | 6 | 36 | 36 | | |
| 17 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 5 | 7 | 9 | 8 | 9 | 7 | 37 | 37 | 37 | |

[a]Isolates represented by the RFLP genotypes are given in Table 1, and the combination of allelic data of 38 putative RFLP loci for each genotype is presented in Table 3.

TABLE 5. Combination of DNA probes that can be used to differentiate and identify groups of isolates in *Phaeosphaeria nodorum* and *P. avenaria*

| Probe number | *P. nodorum*[a] | *P. a.* f. sp. *avenaria*[a] | *P. a.* f. sp. *triticea* | |
|---|---|---|---|---|
| | | | SAT001NY-84[b] | ATCC 18596[c] |
| SNE40 | +[d] | + | + | − |
| SNE48 | + | + | − | + |
| SNE85 | + | + | + | − |
| SAAE47 | + | + | + | − |
| SAAE90 | − | + | + | − |

[a]Isolates included in these groups are given in Table 1.
[b]Includes isolate SAT002NY-84.
[c]Includes isolates ATCC 26371, ATCC 26373, and ATCC 2637.
[d]+ = Hybridizing DNA fragment(s) detected, and − = no hybridizing DNA fragments detected in Southern blots.

tested in future investigations. One is that *P. avenaria* consists of more than one biological species (or isolated populations) and that what we consider to be intraspecific variation could actually be interspecific. Another is that the introduction of *P. nodorum* into North America is a more recent historical event than that of *P. avenaria,* and *P. nodorum* in North America has not had enough time to accumulate a high degree of genetic variation relative to *P. avenaria.* Still another explanation is that sexual reproduction occurs more frequently in *P. avenaria* than in *P. nodorum.* Sexual reproduction of *P. avenaria* readily occurs in North America (13–15,29,30), whereas sexual stages of *P. nodorum,* a heterothallic species (8), is rarely observed in North America (28). Sexual reproduction of *P. nodorum* may not readily occur in the regions where the isolates were obtained. The last explanation, however, is inconsistent with a recent study by McDonald et al (23). They observed a high level of genetic variation among field populations of *P. nodorum* and suggested that ascospores were a significant source of primary inoculum (23).

Several probes developed in this study will aid in the identification and differentiation of the two species and certain isolates. Nineteen RFLP alleles were identified that were specific for *P. nodorum* (Table 2) and could be used to facilitate identification of isolates in this species. DNA probes SAAE7 and SAAE24 each detected a consistent allele in *P. nodorum* and a different but consistent allele in *P. avenaria* (except in isolate ATCC 26370) and could be used to differentiate the two species. The DNA probes that had either a + or − reaction in Southern hybridization for the groups of isolates (Table 5) will be useful in developing dot blot applications. Some alleles are specific for certain isolates and could be used as natural markers to follow the fate of introduced isolates in epidemiological studies. For instance, probe SAAE90 hybridized to one DNA band each of only five isolates of *P. avenaria* (Fig. 1D).

This study is an extension of previous work on 11 isolates of *P. nodorum* (39). Five of the 11 isolates were included in this study and were shown to be consistent in DNA banding patterns. Genetic variation is low but does exist among isolates of *P. nodorum,* as detected by five DNA probes in this study (Table 3) and as published previously (39). *P. nodorum* can attack both wheat and barley (10,18), and there is evidence that some isolates are adapted to wheat and others are adapted to barley (5,17,35). Further investigation may enable us to differentiate and identify the host-adapted isolates in *P. nodorum* (40).

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