

Population genetic structure of the seed pathogen *Pyrenophora semeniperda* on *Bromus tectorum* in western North America

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Abstract: We examined genetic variation in the ascomycete pathogen *Pyrenophora semeniperda* cultured from seeds of the invasive grass *Bromus tectorum* in the Intermountain West of North America. We sequenced the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA genome in 417 monoconidial cultures collected from 20 sites in Washington, Idaho, Utah and Colorado, USA. ITS sequence diversity was surprisingly high; 12 unique haplotypes were identified, averaging 1.3% pairwise sequence divergence. All sites had at least two haplotypes present, and three sites had seven or more. One haplotype composed 60% of the isolates and occurred at all 20 locations; the remaining haplotypes generally occurred at low frequencies within sites but at multiple sites throughout the region. Sites in Washington and Idaho were more diverse than those in Utah and Colorado, averaging two more haplotypes and 67% more pairwise differences among haplotypes at a site. Analysis of molecular variance (AMOVA) indicated that more than 80% of the genetic variation was found within sampling locations, while 7–11% of the variation can be attributed to differences between northern (Washington and Idaho) and southern (Utah and Colorado) populations. The wide distribution of even uncommon haplotypes among sampling sites and weak correlations between genetic and geographic distances among populations (< 0.2) suggested that these populations recently were established from a common source. We hypothesize that the strains of *P. semeniperda* infecting *B. tectorum* in western North

America probably arrived with the invasive grass from its native Eurasian range.

Key words: analysis of molecular variance (AMOVA), ascomycete, *Drechslera campanulata*, genetic diversity, internal transcribed spacer (ITS), invasive species, ribosomal RNA

INTRODUCTION

Host-pathogen interactions can both influence and be influenced by the introduction of non-native species into a habitat. For example escape from natural enemies (including pathogens) in the native range has been posited as one factor letting exotic species become invasive in new habitats (Mitchell and Power 2003). On the other hand an introduced species might encounter novel pathogens in the new environment, which might limit the host's spread or drive evolutionary changes in both host and pathogen (Parker and Gilbert 2004, Mitchell et al. 2006). A third alternative is that introduced hosts might bring their own pathogens with them; these might or might not be new to the introduced range. Whether or not the pathogen is introduced with the exotic host it might alter pathogen interactions with other species in the new range, particularly if the introduced host becomes numerically abundant, geographically widespread or both (Beckstead et al. 2010).

We have been studying the interaction between *Pyrenophora semeniperda* (anamorph *Drechslera campanulata*), a generalist ascomycete seed pathogen of grasses, and *Bromus tectorum*, an exotic weedy annual grass that is highly invasive in the Intermountain West (IMW). In particular we have been examining genetic variation in *P. semeniperda* isolated from *B. tectorum* hosts to test hypotheses about the origins of this interaction and to support investigations of the potential for use of *P. semeniperda* as a mycoherbicide for biocontrol of *B. tectorum* (Meyer et al. 2008).

Pyrenophora semeniperda infects members of at least 36 genera of annual or perennial grasses, as well as some dicots (Medd et al. 2003). It is ubiquitous and sometimes abundant in the seed banks of *B. tectorum* in IMW (Meyer et al. 2007) and is readily encountered in the seed banks of other weedy annual bromes in the region (Meyer unpubl data). It is sometimes found at low to very low densities in the seed banks of native grasses in the region (Beckstead et al. 2010).

When we began our investigations *P. semeniperda* was not known definitely to occur in the native

Eurasian range of *B. tectorum*, having been confirmed only in Australia, New Zealand, North and South America and South Africa (Medd et al. 2003, Yonow et al. 2004). However Stewart et al. (2009) confirmed the presence of *P. semeniperda* on *B. tectorum* seeds in Turkey and Greece, indicating that it probably is not a novel pathogen on this host in North America. Instead it is likely that at least some of the strains of *P. semeniperda* infecting *B. tectorum* in IMW were introduced along with host seeds. Meyer et al. (2009) demonstrated that conidia of *P. semeniperda* can disperse into ripened inflorescences and onto undispersed *B. tectorum* seeds, allowing the pathogen to be dispersed with the host. In fact, given the low densities of *P. semeniperda* we observed in the seed banks of native grasses, the high densities we observed in *B. tectorum* seed banks and the extreme numerical dominance of *B. tectorum* in habitats it has invaded, it seems likely that the distribution and abundance of *P. semeniperda* in IMW is being driven primarily by that of *B. tectorum* (Beckstead et al. 2010).

Isozyme allele distributions indicated that IMW *B. tectorum* populations are descendents of multiple introductions from Europe (and possibly northern Africa and southwestern Asia at around the same time) in the late 1800s (Novak et al. 1991, 1993). The species was introduced much earlier in the eastern United States, but IMW populations are not closely related to these populations (Schachner et al. 2008). Three postulated centers of introduction are near Ritzville in eastern Washington, near Provo in northern Utah and near Reno in western Nevada. Populations in each of these areas have high frequencies of different and characteristic isozyme alleles, and some eastern Washington *B. tectorum* populations have higher isozyme diversity than Utah and Nevada populations (Novak et al. 1991). Subsequent work with microsatellites (short sequence repeats, SSRs) showed genetic differentiation in *B. tectorum* among these three regions (eastern Washington, western Nevada and Utah; Merrill and Meyer unpubl data). While several common SSR genotypes were found across IMW, each region also has high frequencies of SSR genotypes that are relatively uncommon to unknown in the other regions.

If *P. semeniperda* came to North America along with *B. tectorum*, we might expect to see the regional differentiation of *B. tectorum* genotypes mirrored in the genetic structure of *P. semeniperda* strains isolated from *B. tectorum* hosts. Thus we would expect regional differentiation between the centers of introduction and greater genetic diversity of *P. semeniperda* isolates from Washington compared to other regions of IMW. On the other hand *P. semeniperda* strains already present in these habitats

might readily colonize *B. tectorum* once it arrives because of broad pathogen host range and rapid *B. tectorum* population increase, high densities and displacement of native hosts. In that case we would not expect the genetic variation of *P. semeniperda* to correlate strongly with that of *B. tectorum*.

To test the hypothesis that genetic variation of *P. semeniperda* reflects the variation in its *B. tectorum* host we sequenced the internal transcribed spacer region (ITS1 and ITS2) and the intervening 5.8s coding region of the ribosomal RNA gene in more than 400 isolates from 20 locations in IMW. We used phylogenetic approaches to determine evolutionary relationships among the observed sequences and population genetic analyses to test for genetic differentiation between the geographic regions within which *B. tectorum* was introduced to the western United States.

MATERIALS AND METHODS

Killed *B. tectorum* seeds with *P. semeniperda* stromata were extracted by hand from soil cores (6–10 cm diam and 2–4 cm deep mineral soil) and bulk litter samples collected Jun 2007–Jun 2008 at 20 locations in Washington, Idaho, Utah and Colorado (TABLE I). Locations of all populations were recorded with handheld global positioning system (GPS) units. Seeds with attached stromata were stored at room temperature up to 2 y before isolates were cultured.

To prepare fungal cultures a sterile dissecting needle was used to lift conidia from a single stromatum, which were washed off in a small vial of sterile deionized water. The vial was shaken briefly and the contents poured onto a Petri dish of water agar. After a few hours a single germinating conidium was transferred with a flamed hyphal tipping needle to V-8 agar. If there were no conidia on any stromata on a seed, a single stromatum would be plated onto water agar. After 2–4 d a piece of agar with mycelium was transferred to V-8 agar. When the V-8 cultures reached ca. 2 cm diam, a flamed transfer needle was used to slice the culture and the agar into small pieces, which were transferred to 20 mL potato dextrose broth in a 50 mL flask and shaken 5–6 d. The contents of the flask were centrifuged and the liquid decanted, and the mycelium was emptied onto autoclaved filter paper in a Petri dish to dry at room temperature for several days.

DNA was isolated from the mycelium with the QIAGEN Plant Mini Kit (QIAGEN Inc., Valencia, California) following the manufacturer's protocol. Final elutions were performed with $2 \times 50 \mu\text{L}$ buffer, instead of $2 \times 100 \mu\text{L}$, to increase final DNA concentration. The ITS1 and ITS2 regions and the intervening 5.8s coding region of the ribosomal RNA genome were amplified as a single unit with the polymerase chain reaction (PCR) and standard primers (ITS 4: 5'-TCCTCCGCTTATTGATATGC-3'; ITS 5: 5'-GGAAGTAAAGTCGTAACAAGG-3'; White et al. 1990). Cycling conditions included an initial denaturation at 94 C for 30 s followed by 45 cycles of 20 s at 92 C, 90 s at 60 C, and 120 s at 72 C, and a final extension step of 7 min at 72 C.

TABLE I. Sampling locations, number of isolates, number of haplotypes (S), Shannon diversity indices (H'), evenness (E) and mean pairwise sequence differences among haplotypes (π) from 20 locations where *P. semeniperda* isolates were collected from *B. tectorum* seeds

Site	State	Latitude (°)	Longitude (°)	Number of isolates	S	H'	E	π
Dr Lefcort's	Washington	47.62007	-117.30855	28	7	1.57	0.81	3.347
Fishtrap	Washington	47.39115	-117.83991	11	4	1.03	0.75	2.582
Kahlotus	Washington	46.69635	-118.55418	15	5	1.40	0.87	3.200
Marcellus	Washington	47.22832	-118.40722	21	8	1.86	0.89	3.648
Packer Creek	Washington	47.09808	-117.82301	15	3	0.76	0.70	2.419
Saddle Mountain	Washington	46.76523	-119.47222	16	3	0.46	0.42	0.933
Cinder Cone	Idaho	43.22113	-115.99278	44	7	1.56	0.80	3.161
Confusion Range	Utah	39.07274	-113.66500	18	3	0.98	0.89	2.092
Cricket Mountain	Utah	39.04076	-112.89785	17	3	0.81	0.74	1.941
Dog Valley	Utah	39.71638	-111.95585	22	3	0.90	0.82	1.939
Dutch John	Utah	40.93316	-109.41565	20	2	0.50	0.72	1.347
Gusher	Utah	40.30317	-109.77281	18	3	0.90	0.82	2.013
House Range	Utah	39.23060	-113.28781	19	3	0.66	0.60	1.404
Milk Ranch	Utah	37.66594	-109.72000	20	3	0.52	0.47	1.074
Santaquin Canyon	Utah	39.94079	-111.76920	23	4	1.10	0.80	2.087
Strawberry	Utah	40.22723	-111.12358	19	3	0.92	0.84	2.199
Tenmile Creek	Utah	41.86488	-113.13594	20	4	0.59	0.42	0.858
Whiterocks	Utah	40.32818	-112.77816	22	3	0.69	0.63	1.472
White's Valley	Utah	41.80767	-112.30322	22	6	1.47	0.82	2.429
Dinosaur	Colorado	40.38707	-108.99555	27	3	0.31	0.29	0.575
Mean				21	4.0	0.95	0.70	2.0
Standard deviation				6.7	1.7	0.43	0.18	0.9
Maximum				44	8	1.86	0.89	3.648
Minimum				11	2	0.31	0.29	0.575

Presence of a single PCR product was confirmed with agarose gel electrophoresis, and the remaining product was sequenced with the BigDye Terminator Kit (Perkin-Elmer). Sequenced fragments were detected and analyzed on an ABI 3730xl DNA analyzer. Fragments were sequenced simultaneously from both directions, and the opposing reads were aligned to confirm the sequence.

The sequences were aligned with AlignIR™ software with the default parameters, with further alignment by eye. The result was an aligned region of 462 nucleotides and six insertion/deletion locations (indels). Indels can be problematic in phylogenetic and population genetic analysis because the evolutionary mechanisms that lead to them might be different from those causing single-nucleotide substitutions. Therefore they often are ignored in phylogenetic analyses. The six indels in our sample represent a significant amount of the variation in this region, so we wished to include them to the extent possible in the analysis. Simmons and Ochoterena (2000) discuss the value and challenges presented by indels and propose a series of rules for coding indels. Following those rules we coded five indel locations as presence/absence characters and one as a non-directional three-state character. The alignment with the original indel locations was uploaded to TreeBase and can be accessed at <http://purl.org/phylo/treebase/phyloids/study/TB2:S10386>.

We used Arlequin 3.1 (Excoffier et al. 2005) to generate a pairwise difference matrix among the 12 haplotypes based

on nucleotide sites only. Differences among the haplotypes based on the recoded indels were counted by hand and added to the differences based on nucleotide sites. The resulting total pairwise difference matrix was used in subsequent analyses.

We used PAUP 4.10 Beta (Swofford 2003) to create gene trees among the 12 haplotypes with distance (neighbor joining), parsimony and maximum likelihood methods. Neighbor joining was based on the total pairwise difference matrix with ties broken randomly. Parsimony analysis was based on the 468-character matrix that included both nucleotide positions and recoded indels. We used a heuristic search with stepwise addition and TBR branch swapping, collapsing branches with length zero. Maximum likelihood analysis was based on the character matrix of nucleotide changes alone. Nucleotide frequency and substitution rates were estimated from the data with a gamma distribution of rates across sites and a rate parameter (alpha) estimated from the data. Starting branch lengths were based on the Rogers-Swofford approximation method, and branch lengths were optimized with the one-dimensional Newton-Raphson method. Trees from each analysis were bootstrapped with 1000 replicates, and majority rule consensus trees were saved along with the bootstrap values (the percentage of trees in which the observed clade occurred.)

We calculated the genetic diversity of each sampled population in three ways: as the number of haplotypes

present at the site (S); as the Shannon diversity index ($H' = -\sum p_i \ln(p_i)$, where p_i = the relative abundance of haplotype i at the site); and as the mean pairwise difference between isolates within each site (π). The Shannon index captures differences in diversity due to both the number and relative abundances of haplotypes, while the mean pairwise difference measures diversity due to sequence variation among haplotypes. If all haplotypes were equally abundant at a location, the Shannon index would be equal to the natural logarithm of S , the number of haplotypes present. Thus we calculated evenness as $E = H' / \ln(S)$, which has a maximum of 1. We used t -tests to determine differences in genetic diversity between geographic regions. For these tests sites in Washington were designated “north” and those in Utah and Colorado were designated “south”. Because the Cinder Cone, Idaho, population is roughly midway between these groupings we ran separate analyses with it included in the north and south groups and we report the results from both analyses.

To test for genetic differentiation among populations and between geographic regions we performed analyses of molecular variation (AMOVA) in Arlequin 3.1 (Excoffier et al. 1992, Weir 1996, Weir and Cockerham 1984). These analyses were based on haplotype frequencies in each of the sampled locations and the pairwise difference matrix among haplotypes that included both nucleotide variation and indels. Significance of the tests for differentiation was based on 1023 permutations of haplotypes among populations (Excoffier et al. 2005).

We evaluated the degree to which populations are isolated by distance by examining the correlation between geographic distance and genetic distance among all pairs of populations with a Mantel test (Smouse et al. 1986) as executed in Arlequin 3.1. Genetic distances were calculated three ways: mean pairwise differences among haplotypes between populations; pairwise F_{ST} values based on population haplotype frequencies and pairwise differences; and Slatkin's (1995) linearized F_{ST} . The latter statistic, calculated as $F_{ST} / (1 - F_{ST})$, is proportional to the divergence time between populations.

RESULTS

A total of 417 isolates were sequenced from 20 locations (21 ± 6.7 isolates per population, range: 11–44; TABLE I). The 417 isolates produced 12 unique haplotypes based on the aligned ITS region sequences. Sequences are available at GenBank under accession numbers GQ168724, GQ168726, GQ168727, GQ168729, GQ168733–GQ168735 and GQ168737–GQ168741. Ten of the 462 nucleotide sites were variable (2.2%), with five transversions and five transitions. Including the six recoded insertion/deletion sites the mean number of pairwise differences among haplotypes was 5.9 ± 2.9 (range: 1–13; 0.21–2.8%). The distribution of haplotypes among isolates was strongly skewed. One haplotype (A) composed 63% of isolates and was found in all sampling locations. The next most

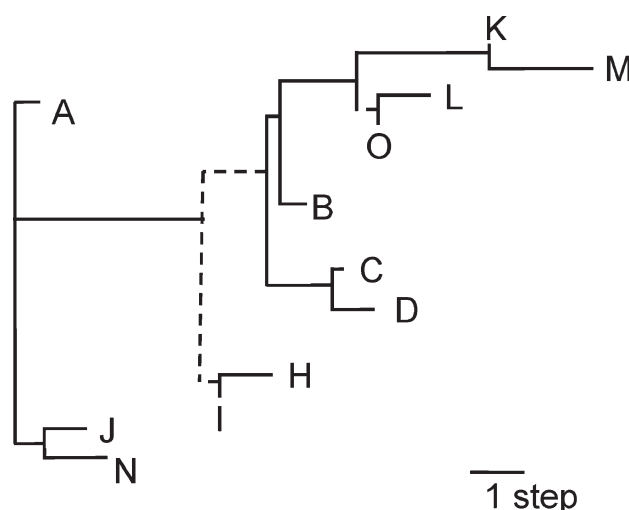


FIG. 1. Phylogram of the hypothesized relationships among 12 ITS haplotypes (A–O) of *P. seminiperda* isolated from *B. tectorum* seeds. The tree is based on a neighbor joining analysis with branch lengths proportional to the number of steps between haplotypes. Similar topologies were produced by parsimony and maximum likelihood analyses. Branches that did not appear in the majority rule consensus trees of all three analyses are dashed lines.

common haplotype (H) was found in only 10% of isolates, although it occurred at 85% of the sampled locations. Distribution of haplotypes among sites was much more even than among isolates. Three haplotypes (J, N and O) each were found in only a single isolate, and four were found at only a single location.

Phylogenetic analysis of the haplotypes by neighbor joining, parsimony and maximum likelihood methods showed clear, consistent differentiation among haplotypes (FIG. 1) with support depending on the method used. Parsimony analysis resolved fewer relationships than did neighbor joining, and maximum likelihood resolved the fewest. Consensus trees from all three methods show a deep split with high bootstrap support (82–92%) separating haplotypes A, N and J from the others (FIG. 1). In all cases N and J are sisters, although bootstrap support for that partition is weaker (53–62%). Two other clades appeared consistently in all three trees. Haplotypes C and D grouped together with bootstrap support of 54–72%. Haplotypes K, M, L and O also form a clade with moderate to high support (66.5–78%), with K and M forming a clade sister of the other two (FIG. 1).

All sites had at least two haplotypes present and three sites had seven or more (TABLE I). The mean number of haplotypes per site was four, with the median and mode equal to three. Shannon diversity closely mirrored haplotype richness, indicating relatively little difference among sites in the proportional abundance of haplotypes. Half the locations sampled

TABLE II. Analysis of molecular variance (AMOVA) testing for genetic differentiation between northern and southern populations of *P. semeniperda* collected from *B. tectorum* hosts. A. Analysis with the Cinder Cone, Idaho, population grouped with the Washington sites in the northern region. B. Analysis with the Cinder Cone population grouped with Utah and Colorado sites in the southern region

A. ^a Source of variation	df	Percent variation	P
Northern vs. southern populations	1	11.51	0.000
Among populations within regions	18	6.46	0.000
Within populations	397	82.03	
Total	416		
B. ^b Source of variation	df	Percent variation	P
Northern vs. southern populations	1	7.44	0.000
Among populations within regions	18	9.27	0.000
Within populations	397	83.29	
Total	416		

^a $F_{ST} = 0.18$.

^b $F_{ST} = 0.17$.

had evenness values of 0.8 or greater, and all but four had values greater than 0.6. Locations also differed in the sequence diversity of haplotypes present. The mean number of pairwise differences among haplotypes within a site was 2.0 ± 0.9 but ranged from a low of 0.6 to a high of 3.6 (TABLE I).

Analysis of molecular variance showed significant differentiation between northern and southern populations (TABLE IIA, B). As mentioned haplotype A was found in all locations sampled. Haplotypes H and I were more abundant in Utah, while haplotypes B and K were more common in Washington. Most rare haplotypes were found in Washington (FIG. 2).

The classification of the Idaho population as “northern” or “southern” altered the outcome of the analysis quantitatively but not qualitatively. With Cinder Cone classified as a “northern” population 11.5% of the total genetic variation was attributed to differences between regions (TABLE IIA). When it was grouped with the southern populations in Utah and Colorado that percentage dropped to 7.4% with a concomitant increase in variation among populations within regions (TABLE IIb). In both cases more than 80% of the genetic variation among *P. semeniperda* isolates was found within populations (TABLE IIa, b).

Washington and Idaho sites were more diverse than those in Utah and Colorado, averaging two more haplotypes and 67% more pairwise differences among haplotypes at a site (FIG. 3). If the Idaho site was grouped with Utah and Colorado populations, the regional difference in diversity persisted but was not as strong ($P = 0.082$ for number of haplotypes; $P = 0.114$ for Shannon index; $P = 0.024$ for mean pairwise differences; results not shown). Because both haplotype counts and the Shannon index are confounded by sample size we used *t*-tests to

determine differences in number of isolates per population between the regions. The northern populations had slightly and nonsignificantly smaller sample sizes than the southern ones, indicating that the differences in diversity were not due to differences in sampling intensity.

The correlation between geographic and genetic distances among populations was significant but weak. Mantel tests produced correlation coefficients of 0.196 ($P = 0.041$), 0.146 ($P = 0.053$) and 0.160 ($P = 0.045$) for respectively mean pairwise differences among haplotypes, pairwise F_{ST} values and Slatkin's linearized F_{ST} .

DISCUSSION

Our results represent the first survey of genetic diversity in *P. semeniperda* on any host plant. Based on sequence data from the ITS region of the ribosomal RNA genome, we found 12 unique haplotypes that differed by 0.2–2.8% sequence divergence. Phylogenetic analyses detected well supported structure among the haplotypes (FIG. 1). Examination of the ITS region in other *Pyrenophora* species has shown much less variation. Friesen et al. (2005) sequenced 97 isolates of *P. tritici-repentis* from wheat, durum, winter barley and eight non-crop grasses from North America, South America and Europe. A maximum likelihood analysis of the haplotypes showed no strongly supported differentiation among isolates, despite this wide geographic and host range. The analysis also failed to separate *P. tritici-repentis* isolates from an isolate of *P. bromi* from smooth brome (*B. inermis*). Stevens et al. (1998) found a maximum of 0.9% sequence divergence in the ITS region among four *Pyrenophora* species infecting

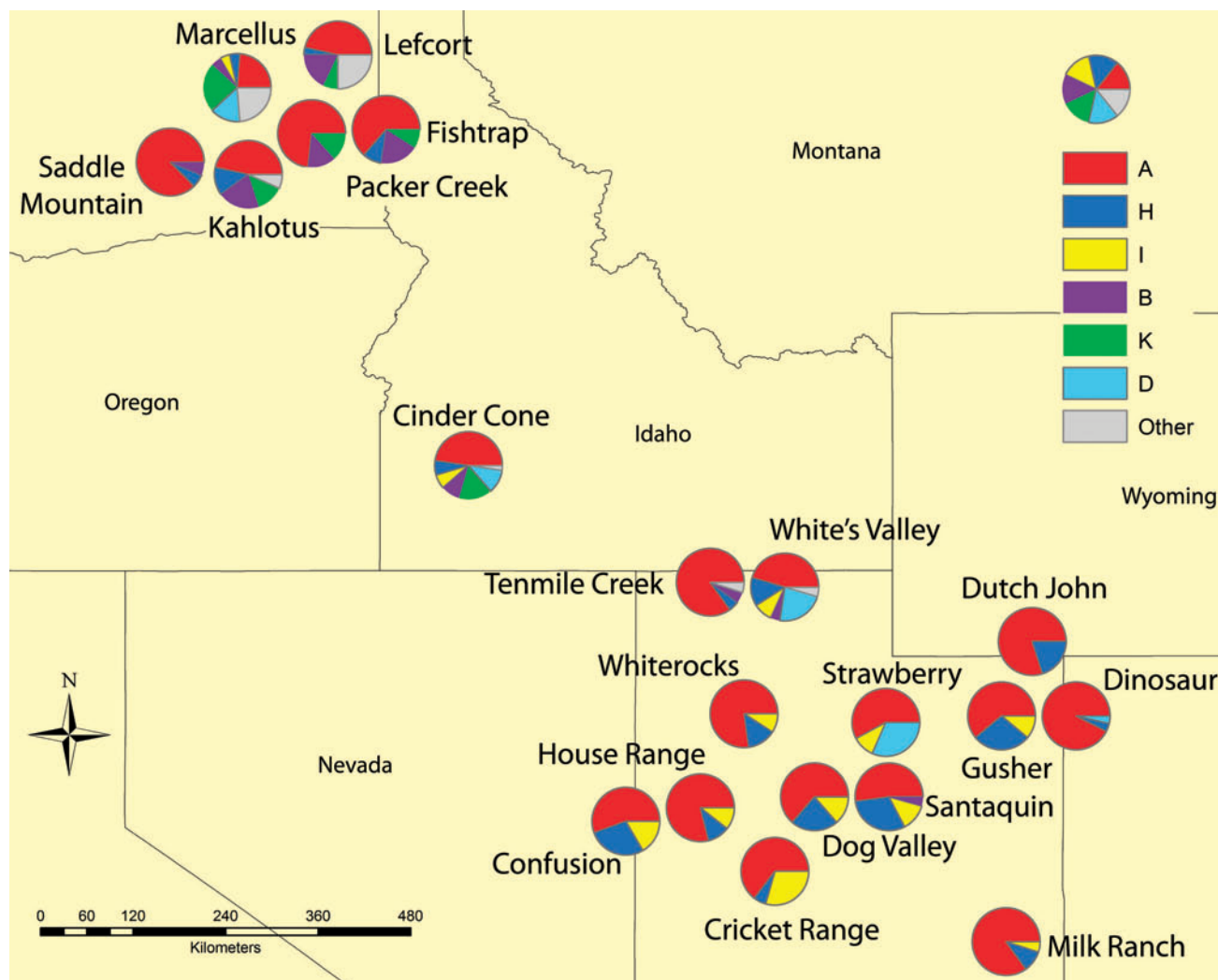


FIG. 2. Map of *P. semeniperda* ITS haplotype frequencies at 20 locations in the western United States. Locations of some diagrams have been adjusted slightly to avoid overlap.

barley (*P. graminea*, *P. teres* f. sp. *teres*, *P. teres* f. sp. *maculata* and *P. hordei*).

Our finding of significant sequence variation and well supported phylogenetic structure in ITS sequences within *P. semeniperda* is surprising, and could suggest the presence of cryptic speciation in this taxon. The role and frequency of sexual reproduction in *P. semeniperda* is currently unknown because the sexual state is rarely observed in nature and difficult to obtain in culture (Paul 1969). An assessment of recombination rates in this organism awaits the development of more sensitive molecular markers.

More than 80% of the variation in ITS haplotypes in our study was found within populations (TABLE II). Other *Pyrenophora* species appear to show a similar distribution of genetic variation within and among populations. In *P. tritici-repentis* isolates from wheat Mironenko et al. (2007) found 72–75% of the

variation in random amplified polymorphic DNA (RAPD) phenotypes was within populations collected in Germany, Russia and the Czech Republic. While overall differentiation among the five populations was significant, they found no differentiation between eastern and western European regions. Peever and Milgroom (1994) used RAPD markers to examine genetic variation in *P. teres* collected from commercial barley fields at five locations in the United States, Canada and Germany. At this scale they found only 54% of the variation to be within populations, with 46% due to variation among populations. However, when only the four North American populations were compared, 66% of the variation was found to be within populations and 33% due to among-population differences. Serenius et al. (2005) found significant differentiation among *P. teres* isolates from two fields 400 km apart in Finland. With AFLP markers

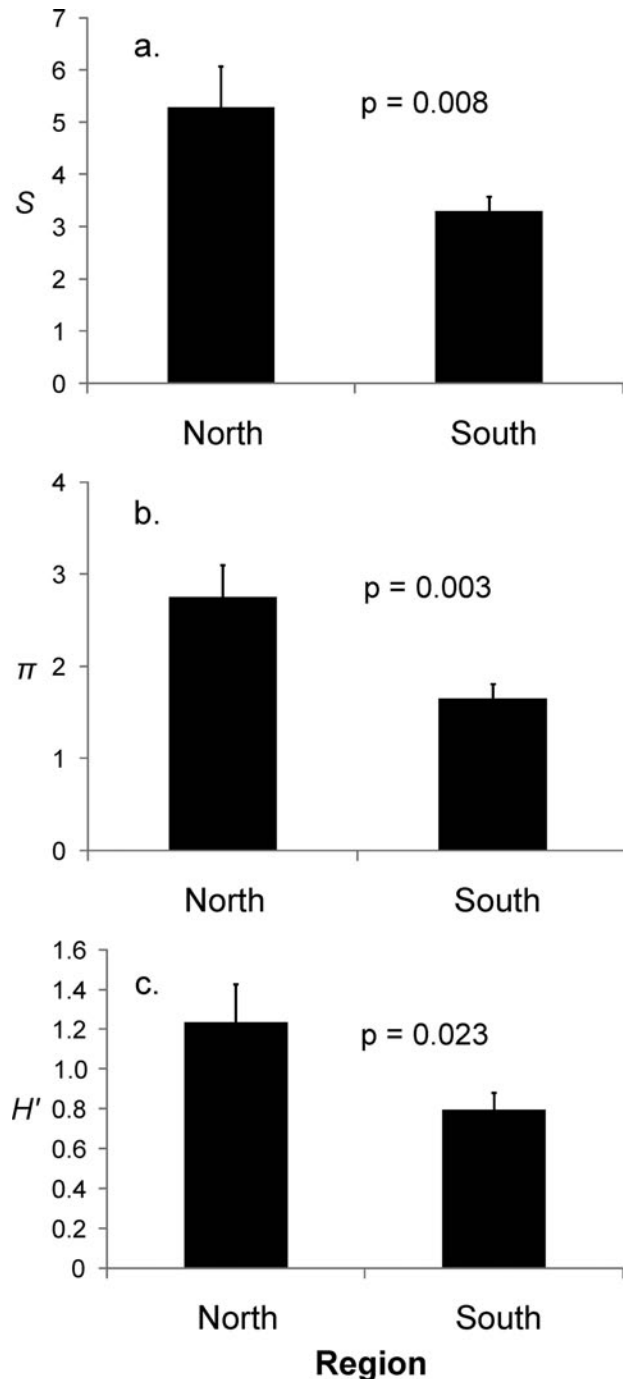


FIG. 3. Mean number of *P. semeniperda* haplotypes (S), mean pairwise sequence differences among haplotypes (π) and Shannon index of diversity (H') in sites from northern and southern regions in western USA. Bars are standard errors of the mean; p-values are from a t -test of the null hypothesis that the means are equal.

they examined 72 isolates and found 68.5% of the genetic variation within populations and 30.3% between. With only one exception a neighbor joining tree grouped isolates with others from the same field.

The discovery of *P. semeniperda* on *B. tectorum* seeds in Greece and Turkey (Stewart et al. 2009) indicated that this host-pathogen interaction might predate the arrival of *B. tectorum* in North America. All ITS haplotypes found in Turkey and Greece also were found in IMW populations. These included the common haplotype A and the rare but closely similar haplotype J in isolates from Turkey and haplotype C in the single isolate from Greece. If the host and pathogen arrived together and are tightly linked ecologically, then we would expect the distribution of genetic variation in the pathogen to reflect the pattern of introduction and spread of the host.

The pattern of genetic variation we observed in *P. semeniperda* seems to fit this hypothesis better than the alternative hypothesis of local shifts onto *B. tectorum* from native hosts. The common ITS haplotype A was found at all locations and in more than 60% of isolates; this haplotype was also the most common haplotype in the collection from Turkey. Several less common haplotypes also were widely distributed geographically; the five next most abundant haplotypes were found in only 5–11% of isolates but occurred at 2–85% of sampled locations. Most sites had three or more haplotypes present, and co-occurring haplotypes were not necessarily genetically similar (TABLE I). Despite this generally widespread distribution of haplotypes, we were able to detect regional differences between populations in Washington and those in Utah and Colorado (TABLE II). Washington populations also had greater diversity of ITS haplotypes than Utah and Colorado populations, primarily due to a larger number of rare haplotypes (FIGS. 2, 3). Both weak but detectable regional differentiation and a trend for higher allelic diversity in Washington also are seen in *B. tectorum* and could reflect a common introduction history.

The populations of *P. semeniperda* we sampled showed minimal evidence of genetic isolation by distance. While correlations between genetic and geographic distance among populations were significant, they were small, explaining only 2.1–3.8% of total genetic variance. The fact that even uncommon haplotypes were widely distributed among sampling sites suggested either significant current gene flow among locations or that the populations were recently established from a common source. *Bromus tectorum* was introduced into western USA around 1890 and reached its present distribution by approximately 1930 (Mack 1981). This rapid expansion also would have led to a wide distribution of those *P. semeniperda* haplotypes associated with the invader.

The extent of current gene flow among these pathogen populations is unknown. Conidia of *P. semeniperda* can colonize undispersed *B. tectorum*

seeds (Meyer et al. 2009), and the host seeds can be transported widely by animal vectors (Hulbert 1955). Even if an infected *B. tectorum* seed fails to establish, the *P. semeniperda* strain it brings might be successful, leading to less differentiation in pathogen strains than in hosts. Conidia also may be transported directly by wind, although the extent of such dispersal is unknown in *P. semeniperda*. De Wolf et al. (1998) indicated that wind transport of *P. tritici-repentis* conidiospores from wheat is generally limited but that transport distances of 10–200 km are possible if spores reach moderate heights in the atmosphere (ca. 100 m). Given prevailing wind patterns (west to east) and the mostly north-south orientation of our sampling locations, it seems unlikely that wind transport of conidia is responsible for the observed pattern of genetic variation.

Our results, while intriguing, do not conclusively demonstrate the origin of *P. semeniperda* populations on *B. tectorum* in IMW of North America. Our sample populations are limited to Utah, Idaho and Washington. It would be interesting to look at pathogen populations in western Nevada to see whether their genetic makeup would further support the hypothesis of independent introductions along with the host. It also would be helpful to obtain pathogen population samples from a wider array of Eurasian *B. tectorum* sites. A more sensitive marker system, such as SSRs or single nucleotide polymorphisms (SNPs), would let us examine relationships among populations in more detail as well as permitting examination of outcrossing rates. And finally examination of the genetic composition of pathogen populations on native hosts would help us to determine whether there are strains on native species in IMW that are different from the strains on *B. tectorum*.

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LITERATURE CITED

- Beckstead J, Meyer SE, Connolly BM, Huck BM, Street LE. 2010. Cheatgrass facilitates spillover of a seed bank pathogen onto native grass species. *J Ecol* 98:168–177, doi:10.1111/j.1365-2745.2009.01599.x
- , Molder CJ, Smith C. 2007. A race for survival: Can *Bromus tectorum* seeds escape *Pyrenophora semeniperda*-caused mortality by germinating quickly? *Ann Bot* 99:907–914, doi:10.1093/aob/mcm028
- de Wolf ED, Effertz RJ, Ali S, Franc L. 1998. Vistas of tan spot research. *Can J Plant Pathol* 20:349–370, doi:10.1080/07060669809500404
- Excoffier L, Laval G, Schneider S. 2005. Arlequin version 3.0: an integrated software package for population genetics data analysis. *Evol Bioinf Online* 1:47–50.
- , Smouse P, Quattro J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491.
- Friesen TL, Ali S, Klein KK, Rasmussen JB. 2005. Population genetic analysis of a global collection of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. *Phytopathology* 95:1144–1150, doi:10.1094/PHYTO-95-1144
- Hulbert LC. 1955. Ecological studies of *Bromus tectorum* and other annual brome grasses. *Ecol Monogr* 25(2):181–213, doi:10.2307/1943550
- Mack RN. 1981. Invasion of *Bromus tectorum* into western North America: an ecological chronicle. *Agroecosystems* 7:145–165.
- Medd RW, Murray GM, Pickering DI. 2003. Review of the epidemiology and economic importance of *Pyrenophora semeniperda*. *Australas Plant Pathol* 32:539–550, doi:10.1071/AP03059
- Meyer SE, Beckstead J, Allen PS, Smith DC. 2009. A seed bank pathogen causes seedborne disease: *Pyrenophora semeniperda* on undispersed grass seeds in western North America. *Can J Plant Pathol* 30:525–533, doi:10.1080/07060660809507552
- , Nelson DL, Clement S, Beckstead J. 2008. Cheatgrass (*Bromus tectorum*) biocontrol using indigenous fungal pathogens. *Shrublands under fire: disturbance and recovery in a changing world*; 6–8 Jun 2006; Cedar City, Utah. Fort Collins, Colorado: USDA Forest Serv., Rocky Mountain Research Station. 61 p.
- , Quinney D, Nelson DL, Weaver J. 2007. Impact of the pathogen *Pyrenophora semeniperda* on *Bromus tectorum* seedbank dynamics in North American cold deserts. *Weed Res* 47:54–62, doi:10.1111/j.1365-3180.2007.00537.x
- Mironenko N, Timopheeva E, Mikhailova L, Kopahnke D, Krämer I, Ordon F. 2007. Intraspecific genetic diversity of *Pyrenophora tritici-repentis* (Died.) Drechs. (*Drechslera tritici-repentis* [Died.] Shoem.) detected by random amplified polymorphic DNA assays. *Arch Phytopathol Plant Protect* 40:431–440, doi:10.1080/03235400600627965
- Mitchell CE, Agrawal AA, Bever JD, Gilbert GS, Hufbauer RA, Klironomos JN, Maron JL, Morris WF, Parker IM, Power AG, et al. 2006. Biotic interactions and plant invasions. *Ecol Lett* 9:726–740, doi:10.1111/j.1461-0248.2006.00908.x

- , Power AG. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* 421:626–627, doi:[10.1038/nature01317](https://doi.org/10.1038/nature01317)
- Novak SJ, Mack RN, Soltis DE. 1991. Genetic variation in *Bromus tectorum* (Poaceae): population differentiation in its North American range. *Am J Bot* 78:1150–1161, doi:[10.2307/2444902](https://doi.org/10.2307/2444902)
- , ———, ———. 1993. Genetic variation in *Bromus tectorum* (Poaceae): introduction dynamics in North America. *Can J Bot* 71:1441–1448.
- Parker IM, Gilbert GS. 2004. The evolutionary ecology of novel plant-pathogen interactions. *Annu Rev Ecol Evol* 35(1): 675–700, doi:[10.1146/annurev.ecolsys.34.011802.132339](https://doi.org/10.1146/annurev.ecolsys.34.011802.132339)
- Paul AR. 1969. The production of *Pyrenophora semeniperda* in culture. *Trans Brit Mycol Soc* 52:373–379, doi:[10.1016/S0007-1536\(69\)80121-X](https://doi.org/10.1016/S0007-1536(69)80121-X)
- Peever TL, Milgroom MG. 1994. Genetic structure of *Pyrenophora teres* populations determined with random amplified polymorphic DNA markers. *Can J Bot* 72: 915–923, doi:[10.1139/b94-116](https://doi.org/10.1139/b94-116)
- Schachner LJ, Mack RN, Novak SJ. 2008. *Bromus tectorum* (Poaceae) in midcontinental United States: Population genetic analysis of an ongoing invasion. *Am J Bot* 95: 1584–1595, doi:[10.3732/ajb.0800225](https://doi.org/10.3732/ajb.0800225)
- Serenius M, Mironenko N, Manninen O. 2005. Genetic variation, occurrence of mating types and different forms of *Pyrenophora teres* causing net blotch of barley in Finland. *Mycol Res* 109:809–817, doi:[10.1017/S0953756205002856](https://doi.org/10.1017/S0953756205002856)
- Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Syst Biol* 49: 369–381, doi:[10.1093/sysbio/49.2.369](https://doi.org/10.1093/sysbio/49.2.369)
- Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139:457–462.
- Smouse PE, Long JC, Sokal RR. 1986. Multiple regression and correlation extensions of the mantel test of matrix correspondence. *Syst Zool* 35:627–632, doi:[10.2307/2413122](https://doi.org/10.2307/2413122)
- Stevens EA, Blakemore EJA, Reeves JC. 1998. Relationships amongst barley and oat infecting isolates of *Pyrenophora* spp. based on sequences of the internal transcribed spacer regions of ribosomal DNA. <http://www.bspp.org.uk/mppol/1998/1111stevens>
- Stewart TE, Allen PS, Meyer SE. 2009. First report of *Pyrenophora seminiperda* in Turkey and Greece. *Plant Dis* 93:1351, doi:[10.1094/PDIS-93-12-1351B](https://doi.org/10.1094/PDIS-93-12-1351B)
- Swofford DL. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.
- Weir BS. 1996. Genetic data analysis II: methods for discrete population genetic data. Sunderland, Massachusetts: Sinauer Associates.
- , Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370, doi:[10.2307/2408641](https://doi.org/10.2307/2408641)
- White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. New York: Academic Press. 315 p.
- Yonow T, Kriticos DJ, Medd RW. 2004. The potential geographic range of *Pyrenophora semeniperda*. *Phytopathology* 94:805–812, doi:[10.1094/PHYTO.2004.94.8.805](https://doi.org/10.1094/PHYTO.2004.94.8.805)