# Genetic-Geographic Variation in *Peridermium harknessii* in the North-Central United States

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## ABSTRACT

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Starch gel electrophoresis was used to characterize the amount of isozyme variability found in 201 isolates of *Peridermium harknessii* collected from 13 disjunct geographic locations and three host species. Significant differences were detected among locations in allozyme frequencies for each of five putative polymorphic isozyme loci. Eight additional isozyme loci were monomorphic. Nei's $F_{ST}$ indicated that 51% of the total variation could be attributed to differences among locations. Cluster analysis, using estimates of genetic distances, grouped locations into two principal clusters. Geographic distribution and stand type appear to have influenced these clusters. Variations in host species did not substantially alter allozyme frequencies. The identification of genetic-geographic variation, in the absence of identified virulence patterns, has implications in selection of inoculum sources in studies of host-pathogen interactions.

*Additional keywords*: isozyme analysis, *Pinus banksiana*, *Pinus ponderosa*, *Pinus sylvestris*, western gall rust.

*Peridermium harknessii* J. P. Moore (≡ *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka) is an autoecious rust with repeating aeciospores that causes western gall rust in western and northern North America. Thirteen native and seven exotic pine species in the subgenus *Diploxylon* are known to be susceptible to *P. harknessii* (1,11). Due to these broad geographic and host ranges, genetic variability among isolates recovered from different geographic locations or host species would be expected. Genetic variability in pine stem rusts has been poorly quantified, even though phenotypic variability has been documented for a wide array of characters (7,14).

Isozyme analysis offers one method of quantifying levels of genetic variability within *P. harknessii*. For example, isozyme analysis has been used with several fungi to describe genetic variation among collections from different locations (6) and hosts (5,8,21). Tuskan and Walla (17) reported variability in isozyme frequencies among spore samples of *P. harknessii* collected from three sites in North Dakota. Vogler et al (19) reported that isolates of this fungus collected along the California coast were monomorphic whereas those from the inland mountains were polymorphic among locations and monomorphic within locations.

The characterization of genetic variability in a pathogen is a critical component in the study of host-pathogen interactions. Characterizing variability in virulence is a primary concern. To date, there is no measure of virulence in the pathosystem of *Pinus ponderosa* Doug. ex Laws.-*P. harknessii*, whereas isozyme analysis is available as a means of assessing variability. Direct assessment of virulence might not be possible through the use of isozyme data. However, isozyme markers could be used to identify genetically divergent inoculum sources in host screening studies. The primary objective of this study was to evaluate genetic variability based on isozyme analysis in isolates of *P. harknessii* collected from different geographic locations and from different host species in the north-central United States. A secondary objective was to identify markers that would allow the selection of genetically divergent inoculum sources.



## MATERIALS AND METHODS

**Collection and preparation of fungal spores.** In May and June of 1986, 1987, and 1988, 201 samples (aeciospores from individual galls) of *P. harknessii* were collected from 10 locations in North Dakota, two in Nebraska, and one in Minnesota (Table 1). Host species varied within and among locations. Twelve locations contained *Pinus ponderosa* var. *scopulorum* Engelm., three contained *Pinus sylvestris* L., and one contained *Pinus banksiana* Lamb. Stand type (an aggregate of trees of similar composition and history) varied with location. Five locations were typed as field windbreaks, three as native woodlands, three as forest plantations, and two as Christmas tree farms. Spores were collected from the surface of individual galls by means of a portable vacuum-powered cyclone spore collector (18). Locations with fewer than 10 galls were censused; otherwise, a minimum of 10 galls was sampled. Confirmation of rust species was based on spore germination characteristics: namely, germ tube length, branching habit, and tip lysis (2).

**Electrophoresis and isozyme analysis.** Isozyme analysis was conducted using techniques described by Tuskan and Walla (18). Each sample of spores was kept separate by gall, passed through a 90-μm sieve, desiccated, and stored at −60 C until required for isozyme analysis. A 20-mg subsample was used for each spore sample. Eleven enzymes representing 13 putative loci (Table 2) were resolved using three electrophoretic buffer systems and 12% starch gels. Tuskan and Walla (18) indicated that eight of the isozyme loci were monomorphic and five were polymorphic. An isozyme locus was considered polymorphic when the most common isozyme allele (that is, allozyme) occurred in fewer than 99% of the samples. Isozymes were numbered sequentially from the anodal end of the gel, and allozymes were numbered, with one being the most frequent, two being the next most frequent, and so on. Allelic mobility designations have been presented (18).

Isozyme data were summarized by geographic location for percent polymorphism, average number of allozymes per isozyme locus, and total number of electrophoretic types. An isozyme locus was considered polymorphic per location when at least two allozymes were detected in the geographic population. Electrophoretic type was based on the composite phenotype of the five polymorphic loci. Allozyme frequencies were calculated for each locus at each location and for each host species. Nei's

(10) F-statistic ($F_{ST}$), as modified by Weir and Cockerman (20) for small, unequal sample sizes, was calculated based on differences among locations. $F_{ST}$ also was calculated based on differences among host species for those locations that contained multiple host species. Genetic distance between pairwise combinations of locations was estimated with the data from both the polymorphic and monomorphic loci (9). Genetic distance ($D$) was calculated as:

$$D = \sum_{k=1}^{l} -\log_e (\bar{P}_{12k} (\bar{P}_{1k} \cdot \bar{P}_{2k})^{-0.5})$$

where, $\bar{P}_{12k}$ = the mean probability that two alleles from locus $k$ chosen at random from within populations 1 and 2 are identical; $\bar{P}_{1k}$ and $\bar{P}_{2k}$ = the mean probability that two alleles chosen from locus $k$ at random within population 1 and population 2, respectively, are identical; and $l$ = the number of loci.

**Statistical analysis.** Contingency chi-square analyses were conducted for each polymorphic locus to detect differences among isolates recovered from different locations or host species with the use of "Genestat" statistical software (4). Chi-square was calculated as:

$$\chi^2 = 2N \sum_{i=1}^{n} (\sigma^2_{p_i} / \bar{p}_i)$$

where, $N$ = total number of samples, $\sigma^2_{p_i}$ = the variance of allozyme frequencies across $i$ populations, and $\bar{p}_i$ = the weighted mean allozyme frequency across $i$ populations (means were weighted by sample size per population), and $n$ = the number of populations.

A single-linkage hierarchical cluster analysis was used to group locations based on genetic distances among locations (15). Locations with fewer than 10 samples were not included in the estimates of genetic distance, contingency chi-square, or the cluster analysis.

## RESULTS

Five isozyme loci were polymorphic: *Acp*, *Cat*, *Est*, *Got*, and *Pgm2*. The frequency of polymorphic loci per location varied from 0% at locations 2 and 12 to 38.5% at locations 1, 9, and 11 (Table 3). The remaining eight isozyme loci were monomorphic. The average number of allozymes per locus per location varied in accordance with percent polymorphic loci and ranged from an average of one allozyme per locus to 1.46 allozymes per locus. Based on a composite score of the allozymes at each polymorphic locus, one to 10 electrophoretic types were identified per location (Table 4). At several locations the majority of the sampled isolates was represented by electrophoretic type A. Locations 1, 3, 5, 7, 8, 9, and 11 each contained electrophoretic types that were not found at any other location (Table 4). These descriptive parameters indicate that there are similarities and variabilities in the genetic backgrounds of *P. harknessii* among the sampled locations, as well as genetic variability from gall to gall in some locations.

The contingency chi-square test for allozyme frequency heterogeneity per locus detected significant differences among locations for each polymorphic locus, indicating that at least one of the locations differs from the rest (Table 5). The only significant difference among isolates from different host species occurred with *Cat*. Nei's $F_{ST}$ for locations was 0.513 ± 0.026 standard

TABLE 1. Site descriptions, host species, and stand type for spore samples of *Peridermium harknessii* used in starch gel electrophoresis

| Location number | County and state | Host species (*Pinus*) | Number of samples[a] | Stand type |
|---|---|---|---|---|
| 1 | Stutsman/ND | *P. ponderosa* | 11 | Windbreak |
| 2 | McIntosh/ND | *P. ponderosa* | 2 | Windbreak |
| 3 | Slope/ND | *P. ponderosa* | 22 | Native |
| 4 | Slope/ND | *P. ponderosa* | 21 | Native |
| 5 | Bowman/ND | *P. ponderosa* | 2 | Windbreak |
| 6 | Oliver/ND | *P. ponderosa* | 10 | Windbreak |
| 7 | McHenry/ND | *P. ponderosa* | 16 | Plantation |
| | | *P. sylvestris* | 10 | |
| 8 | Pembina/ND | *P. ponderosa* | 10 | Christmas trees |
| | | *P. sylvestris* | 10 | |
| 9 | Pembina/ND | *P. ponderosa* | 12 | Christmas trees |
| | | *P. sylvestris* | 10 | |
| 10 | Beltrami/MN | *P. banksiana* | 10 | Native |
| 11 | Traill/ND | *P. ponderosa* | 5 | Windbreak |
| 12 | Cass/NE | *P. ponderosa* | 25+ | Plantation |
| 13 | Adams/NE | *P. ponderosa* | 25+ | Plantation |

[a] A sample refers to all spores collected from a single gall. In locations with fewer than 10 samples, all galls within those locations were censused.

TABLE 2. Enzyme name, E.C. number, enzyme abbreviation, putative isozyme locus, and the number of electromorphs used in electrophoretic studies of *Peridermium harknessii*

| Enzyme name, E.C. number, and abbreviation[a] | Putative isozyme locus | Electromorphs identified[b] |
|---|---|---|
| Acid phosphatase (3.1.3.2) (ACP) | *Acp* | 2 |
| Catalase (1.11.1.6) (CAT) | *Cat* | 3 |
| Diaphorase (1.6.4.3) (DIA) | *Dia1* | 1 |
| | *Dia2* | 1 |
| Esterase (3.1.1.1) (EST) | *Est* | 2 |
| Glutamic oxaloacetic transaminase (2.6.1.1) (GOT) | *Got* | 3 |
| Malate dehydrogenase (1.1.1.37) (MDH) | *Mdh* | 1 |
| Mannose phosphate isomerase (5.3.1.8) (MPI) | *Mpi* | 1 |
| Menadione reductase (1.6.99.2) (MNR) | *Mnr* | 1 |
| Phosphoglucomutase (2.7.1.5) (PGM) | *Pgm1* | 1 |
| | *Pgm2* | 3 |
| 6-phosphogluconate dehydrogenase (1.1.1.44) (6PGD) | *6pgd* | 1 |
| Phosphoglucose isomerase (5.3.1.9) (PGI) | *Pgi* | 1 |

[a] Nomenclature Committee of the International Union of Biochemistry (12).

[b] Buffer systems and staining formulas are as described in Tuskan and Walla (18).

TABLE 3. Descriptive isozyme data for spore samples of *Peridermium harknessii* from 13 geographic locations

| Location number | Percent polymorphic loci[a] | Average number of allozymes/locus |
|---|---|---|
| 1 | 38.5 | 1.46 |
| 2 | 0.0 | 1.00 |
| 3 | 23.1 | 1.38 |
| 4 | 15.4 | 1.15 |
| 5 | 23.1 | 1.23 |
| 6 | 15.4 | 1.15 |
| 7 | 23.1 | 1.31 |
| 8 | 30.8 | 1.46 |
| 9 | 38.5 | 1.46 |
| 10 | 23.1 | 1.23 |
| 11 | 38.5 | 1.38 |
| 12 | 0.0 | 1.00 |
| 13 | 7.6 | 1.08 |

[a] A locus was considered polymorphic when at least two allozymes occurred in the geographic population.

TABLE 4. Electromorphs of the 24 electrophoretic types of *Peridermium harknessii* found among 201 isolates collected in north-central United States

| Electrophoretic type[a] | Allozymes per isozyme locus[b] | | | | | Composite frequency (%) | Frequency per location (%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Acp* | *Cat* | *Est* | *Got* | *Pgm2* | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| A | 1[c] | 1 | 1 | 1 | 1 | 56 | 0 | 0 | 71 | 90 | 0 | 50 | 73 | 27 | 0 | 0 | 0 | 100 | 91 |
| B | 1 | 1 | 1 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 17 | 8 | 7 | 0 | 0 | 0 | 0 | 9 |
| C* | 1 | 1 | 2 | 1 | 2 | 1 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 1 | 2 | 2 | 2 | 2 | 5 | 63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 20 | 0 | 0 |
| E* | 1 | 2 | 2 | 1 | 2 | <1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 |
| F* | 1 | 3 | 1 | 2 | 1 | <1 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 1 | 3 | 1 | 1 | 1 | 5 | 0 | 100 | 14 | 0 | 50 | 17 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| H | 1 | 3 | 1 | 1 | 3 | 3 | 0 | 0 | 5 | 10 | 0 | 16 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| I* | 1 | 3 | 2 | 2 | 1 | <1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 |
| J* | 2 | 3 | 1 | 1 | 2 | <1 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K* | 1 | 1 | 1 | 2 | 1 | <1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 |
| L | 1 | 1 | 2 | 2 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 20 | 20 | 0 | 0 | 0 |
| M | 1 | 2 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 20 | 0 | 0 |
| N* | 1 | 1 | 2 | 3 | 1 | <1 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| O* | 2 | 1 | 1 | 1 | 1 | <1 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| P* | 2 | 1 | 2 | 1 | 1 | <1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 |
| Q | 1 | 2 | 1 | 2 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 20 | 0 | 0 | 0 |
| R | 1 | 2 | 1 | 1 | 1 | 2 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 7 | 0 | 20 | 0 | 0 | 0 |
| S | 1 | 2 | 2 | 3 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 20 | 40 | 0 | 0 | 0 |
| T* | 1 | 2 | 2 | 1 | 1 | <1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| U* | 2 | 2 | 2 | 2 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 |
| V* | 1 | 2 | 1 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 |
| W | 1 | 2 | 1 | 2 | 3 | 2 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 40 | 0 | 0 |
| X* | 2 | 2 | 2 | 2 | 3 | 1 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] * designates electrophoretic types that are found only at single locations.
[b] Eight additional putative isozyme loci were monomorphic for all locations and therefore are not included in the table.
[c] Allozyme designations represent homozygous pairings per isozyme locus in dikaryotic spores of *Peridermium harknessii*. Allozyme nomenclature is as described in Tuskan and Walla (18).

TABLE 5. Contingency chi-square analysis for allozyme frequency heterogeneity per locus for 10 geographic locations and two host species

| Isozyme (locus) | Heterogeneity chi-square | |
|---|---|---|
| | Among locations[a] | Among host species[a] |
| Acid phosphatase, 3.1.3.2 (*Acp*) | 67.41** | 6.59 |
| Catalase, 1.11.1.6 (*Cat*) | 307.60** | 13.55** |
| Esterase, 3.1.1.2 (*Est*) | 249.32** | 3.33 |
| Glutamate oxaloacetate transaminase, 2.6.1.1 (*Got*) | 278.46** | 11.52 |
| Phosphoglucomutase, 2.7.1.5 (*Pgm2*) | 212.19** | 9.67 |
| $F_{ST}$[b] (± standard error) | 0.513 (± 0.026) | 0.039 (± 0.008) |

[a] ** indicates significant differences among locations or host species based on chi-square values at $\alpha \leq 0.01$.
[b] The proportion of total gene diversity attributed to differences among populations.

TABLE 6. Genetic distances among geographic sources of *Peridermium harknessii* based on 13 isozyme loci

| Geographic location | Geographic location | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 6 | 7 | 8 | 9 | 10 | 12 | 13 |
| 1 | 0.212 | 0.226 | 0.218 | 0.217 | 0.080 | 0.023 | 0.055 | 0.235 | 0.230 |
| 3 | | 0.004 | 0.012 | 0.002 | 0.043 | 0.192 | 0.104 | 0.007 | 0.007 |
| 4 | | | 0.022 | 0.001 | 0.049 | 0.209 | 0.117 | 0.001 | 0.001 |
| 6 | | | | 0.017 | 0.050 | 0.195 | 0.107 | 0.033 | 0.030 |
| 7 | | | | | 0.045 | 0.198 | 0.110 | 0.003 | 0.002 |
| 8 | | | | | | 0.055 | 0.017 | 0.056 | 0.052 |
| 9 | | | | | | | 0.026 | 0.220 | 0.214 |
| 10 | | | | | | | | 0.127 | 0.122 |
| 12 | | | | | | | | | 0.001 |

error, indicating that 51.3% of the total variation in allozyme frequency could be attributed to differences among locations. Nei's $F_{ST}$ indicated that less than 4% of the total variation in allozyme frequency within locations 7, 8, and 9 could be attributed to differences among host species (Table 5).

Estimates of genetic distances between pairwise combinations of locations ranged from 0.001 between locations 4 and 7, 4 and 12, 4 and 13, and 12 and 13 to 0.235 between locations 1 and 12 (Table 6). Genetic distance provides an estimate of the number of allelic differences per 100 loci that occur between two populations. Cluster analysis of the genetic distances grouped locations into two principal clusters (Fig. 1). Locations 3, 4, 6, 7, 12, and 13 were grouped into a cluster segregated from locations 1, 8, 9, and 10. Less distinct clusters also existed, yet the minimum distance between clusters was greatest for the two principal clusters.

## DISCUSSION

Isozyme variability within locations was indicated by three measures: percent polymorphic loci (Table 3), average number of allozymes per locus (Table 3), and total number of electrophoretic types (Table 4). These measures also indicated that the amount of variability within locations differed by locations. Differences in host species, geographic location, and stand type may have contributed to this variability. Chi-square analysis and the $F_{ST}$ values, however, indicated that there was little difference in isozyme data among isolates collected from different host species. Alternatively, geographic location accounted for half of the total variability. The variability we

observed among and within locations contrasts with data for coastal and inland populations of *P. harknessii* in California (19). Location differences in our study are apparently due to differences in geographic distribution and in stand type. The majority of the coniferous plantings in the sampled region was not native. Isozyme variability among isolates collected at different locations appears to have been influenced by stand type through the distribution of *P. harknessii* during the establishment of windbreaks, plantations, and Christmas tree farms.

The cluster analysis of genetic distances among locations provided some insight into the current genetic distribution of *P. harknessii* in the sampled region. The two principal clusters, clusters I and II, appear to have a geographical and biological basis which the less distinct clusters lack. Clusters I and II separated North Dakota populations into western and eastern locations, respectively. The Nebraska locations were clustered with the western North Dakota samples, and the Minnesota location was clustered with the eastern North Dakota samples. Cluster I was dominated by electrophoretic type A and had greater affinity than did cluster II. This would be expected because the locations in cluster II were comprised of many diverse electrophoretic types, not dominated by any one type.

Stand type was confounded within locations associated with clusters I and II. Locations 8 and 9 in cluster II are Christmas tree farms where seedlings have been planted over many consecutive years. Many of these seedlings came from nurseries in Minnesota, Wisconsin, and Michigan. *P. harknessii* could have been introduced into locations 8 and 9 from origins east of North Dakota through such seedlings. The eastern origin of *P. harknessii* in these locations would account for the cluster with location 10, a native stand of *P. banksiana*. Notably, the number of electrophoretic types per location was highest for locations 8 and 9, which supports the hypothesis that annual planting in the Christmas tree farms introduced new genotypes of *P. harknessii* into the sampled populations.

Stand type also appeared to have influenced the grouping of cluster I. Locations 6, 7, 12, and 13 contain *P. ponderosa* which had been planted in association with windbreak programs. Planting records indicated that the geographic origins of these plantings were in the Rocky Mountain region of western North America. Locations 3 and 4, also in cluster I, are native stands of *P. ponderosa* and most likely contain sources of *P. harknessii* endemic to western North America. Again, all of these locations were dominated by electrophoretic type A.

The homogeneity of the isozyme data among isolates from different host species suggests that selective pressure for host specificity in *P. harknessii* in the sampled population has been minimal. Complete host specificity associated with electrophoretic phenotypes, as in *Leptographium wageneri* (Kendrick) M. J. Wingfield (21), has not occurred in the sampled population of *P. harknessii*. In fact, electrophoretic type S occurred on all three host species. Variability in virulence among electrophoretic types may exist, but the sample size used in our study may not have been large enough to detect these host-specific differences. Furthermore, the isozyme loci we examined may not be linked to host specificity genes. A larger number of samples, collected from locations containing multiple-host species, will have to be analyzed before the relationship between host specificity in *P. harknessii* and specific isozyme loci can be determined.

Historically, the taxonomic classification of *P. harknessii* into one or more taxa has been debated (13). Gall rust initially described as *P. cerebroides* Meinecke and the Woodgate *Peridermium* have been incorporated into *P. harknessii*. Our data suggest that there may have been selective pressure for the development of *P. harknessii* into at least two geographic groupings. This geographic variability may reflect an earlier premise that *P. harknessii* is a western rust (3) and that the Woodgate *Peridermium* is an eastern rust (16), or it may reflect divergent evolution between eastern and western populations. Further study is merited before separate racial or species classifications can be proposed. Until then, the genetic-geographic differences among isolates of *P. harknessii* have implications in the study of host-pathogen interactions. Because no other parameters are available, these genetic-geographic populations of *P. harknessii* must be recognized as potentially diverse in virulence and thus should be considered during examinations of host resistance.

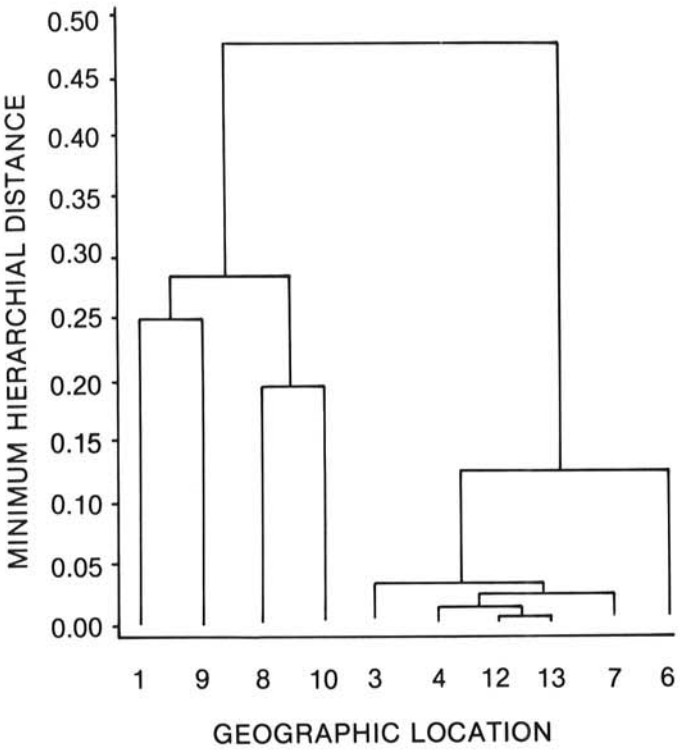


**Fig. 1.** Single-linkage hierarchical cluster of genetic distances among 10 geographic sources of *Peridermium harknessii* from the north-central United States.

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