

***Heterodera glycines* in Indiana: III. 2-D Protein Patterns of Geographical Isolates¹**

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Abstract: Protein patterns obtained by two-dimensional polyacrylamide gel electrophoresis for three isolates of *Heterodera glycines* from southern Indiana appear qualitatively similar and have higher pairwise Jaccard similarity coefficients with each other than with isolates from northern Indiana. Three isolates from three northern counties share proteins not present in the southern isolates, but as a group the northern isolates are less similar to each other than are the southern Indiana isolates.

Key words: soybean cyst nematode, polyacrylamide gel electrophoresis.

This paper continues our detailed comparative study of five isolates of *Heterodera glycines* Ichinohe from geographically sep-

arated areas of Indiana. The isolates were originally collected (3) from three counties in southern Indiana, where soybean cyst nematode (SCN) has been known to occur since about 1973, and from two counties in northern Indiana, where infestations were found more recently. We also include quantitative data about our previously analyzed isolate from Pulaski County, also in northern Indiana (5). Earlier we reported (3) that although all of the isolates except one (from White County in the north) are

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TABLE 1. Jaccard similarity matrix computed from 2-D PAGE data from eight isolates of *Heterodera glycines*. For the Indiana isolates the letter following the county name indicates whether the county is northern or southern.

	Posey (S)	Vander- burgh (S)	Vigo (S)	Pulaski (N)	Benton (N)	White (N)	Mississippi	Japan
Posey (S)	—							
Vanderburgh (S)	0.836	—						
Vigo (S)	0.841	0.817	—					
Pulaski (N)	0.766	0.800	0.781	—				
Benton (N)	0.792	0.782	0.762	0.840	—			
White (N)	0.776	0.713	0.747	0.766	0.805	—		
Mississippi	0.713	0.830	0.740	0.716	0.729	0.694	—	
Japan	0.625	0.661	0.648	0.640	0.659	0.730	0.712	—

classified as Race 3 isolates on the standard soybean differentials (6), differences occur in the ability of all our isolates to reproduce on selected soybean differentials, including resistant breeding lines. We further showed (4) from detailed morphological comparisons that although much morphometric overlap occurs, significant differences in means exist among the isolates for various morphometric comparisons of second-stage juveniles. By using various combinations of means, most of the isolates can be distinguished from the others. The protein data reported herein provide still another comparison of the isolates at a different level of analysis.

MATERIALS AND METHODS

Indiana isolates of SCN used for protein analysis were the same as those used previously (3–5). Counties from which the isolates were collected, plus a letter to indicate whether the county is in the north (N) or south (S) are as follows: Posey (S), Vanderburgh (S), Vigo (S), Benton (N), and White (N). All SCN isolates from which proteins were extracted were increased in plant growth chambers on the susceptible soybean *Glycine max* (L.) Merr. cv. Williams. Laboratory procedures were as described elsewhere (5). Proteins were obtained from 30 young female nematodes per sample as follows. Females were picked from roots, cleaned in tap water, homogenized in 0.2 M sodium borate at pH 9 in an ice bath, and centrifuged. The supernatant liquid containing the proteins was dialyzed and stored over liquid nitrogen until labeled *in vitro* by reductive methylation with for-

maldehyde and sodium [³H]borohydride (7), and subsequently stored at –80 C until gels were run. Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) was carried out essentially as described by O'Farrell (8). We ran proteins from each isolate in the same cell with proteins from other isolates to permit tracing of small variations in protein positions to unavoidable peculiarities of individual gel runs (5). Molecular weight standards were run and the pH gradient measured as discussed previously (5).

Following separation in two dimensions, labeled proteins were located by fluorography. Six to twelve patterns were obtained for each isolate, and the transparent autoradiographs were overlaid and compared directly. As is usual with 2-D gels, internal "landmark" spots were used to align autoradiographs for comparison (9), and proteins or polypeptides with identical electrophoretic properties were assumed to be identical. We divided the patterns into sectors for analysis (5). Each reproducible spot in each sector was numbered and its presence or absence determined for autoradiographs of all gels of the isolates being compared. In two instances (one each in sectors 2 and 4), a spot consistently small and pale in autoradiographs of some isolates and larger and darker in patterns of other isolates was scored as different. Only spots consistently showing good resolution were scored; isolates averaged 132 spots.

To obtain a quantitative estimate of the phenetic similarity among the isolates, we used the data to compute two commonly used similarity coefficients (SC), the simple

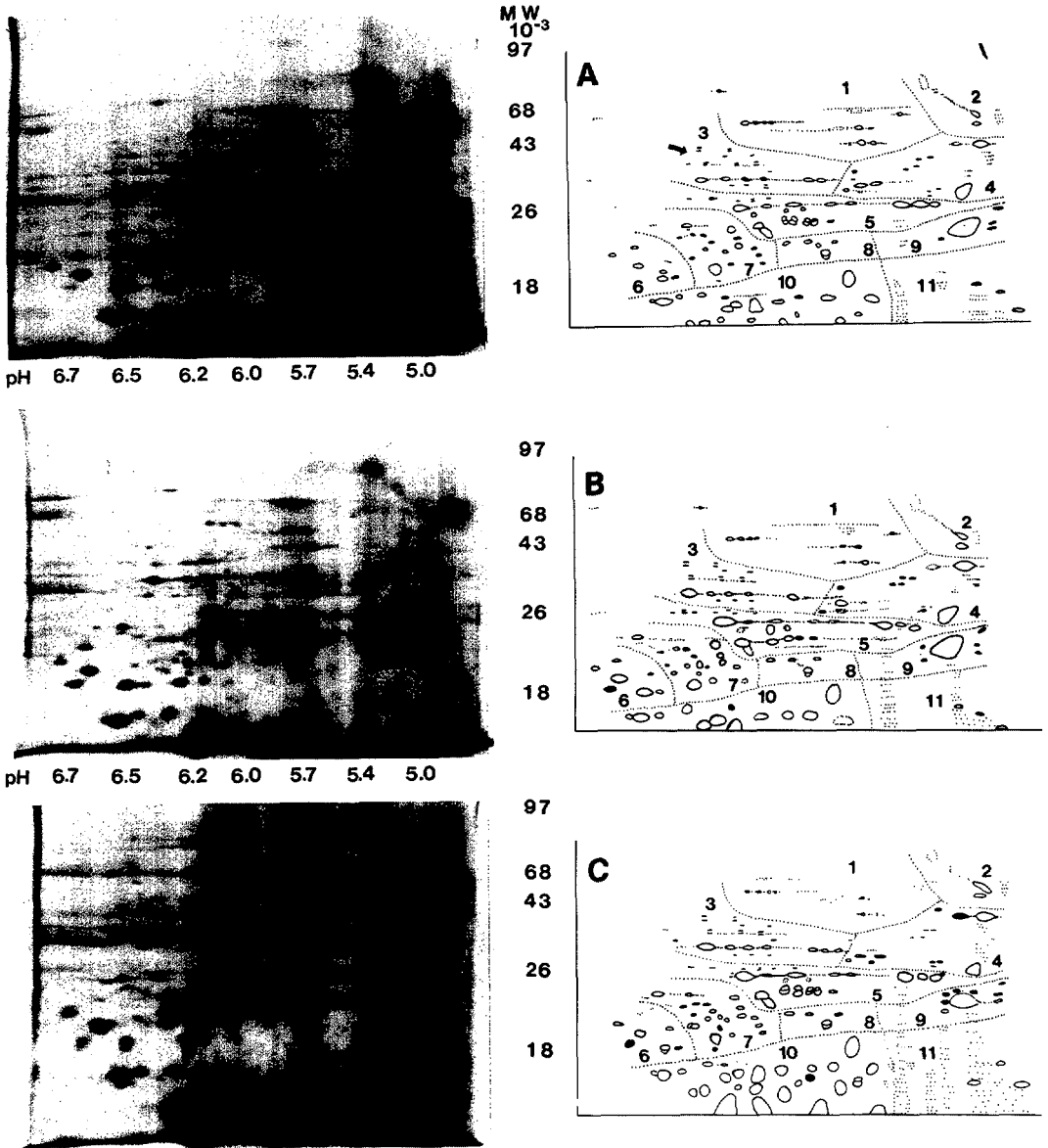


FIG. 1. 2-D PAGE protein patterns for *Heterodera glycines* isolates from three counties in southern Indiana. A) Posey. B) Vigo. C) Vanderburgh. Photographs of autoradiographs (left) and sketches of typical patterns for each isolate (right). For sketches B and C, protein spots also found in the pattern for the Posey isolate are shown in outline. Large new spots (i.e., not in Posey pattern) are solid black. Missing spots (i.e., spots that are in Posey pattern but not in pattern being compared) are omitted from the sketch. Arrow in Figure 1A indicates location of small paired spots of sector 3 discussed in text.

matching coefficient and the coefficient of Jaccard (1,10). The simple matching coefficient includes negative matches, whereas the Jaccard coefficient omits negative matches. We had no a priori reason for choosing one kind of analysis over the other. The aim of both analyses is to estimate

relative resemblance among the protein phenotypes by calculating the percentage of matching characters. For two identical patterns $SC = 1.00$. For comparative purposes we included data for two previously analyzed isolates, one from Pulaski County in northern Indiana (5) and one from Hok-

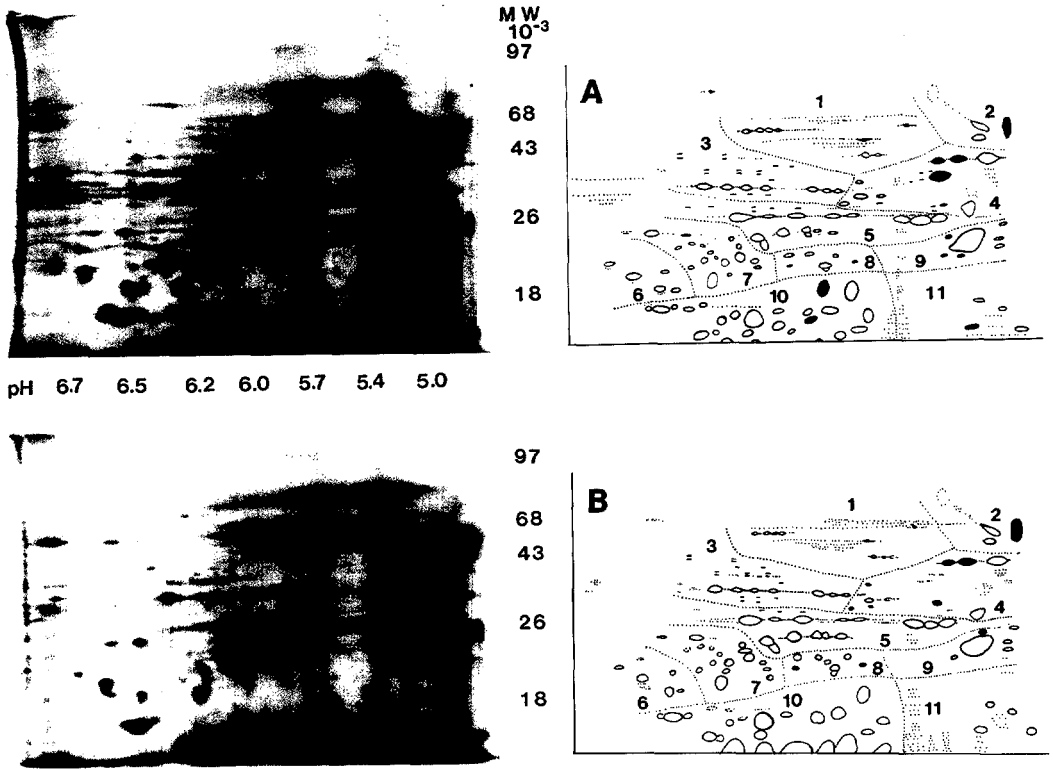


FIG. 2. 2-D PAGE protein patterns for *Heterodera glycines* isolates from two counties in northern Indiana. A) Benton. B) White. Photographs of autoradiographs (left) and sketches of typical patterns for each isolate (right). Shading of spots indicates comparison with pattern of Posey isolate as indicated for Figure 1.

kaido, Japan (5), plus data for an isolate from Lake Cormorant, Mississippi.

RESULTS

The coefficients in the similarity matrices obtained by the two methods were jointly monotonic with minor exceptions. Therefore, only the Jaccard matrix is shown (Table 1). The protein patterns for the five Indiana isolates are shown in Figures 1 and 2, with the pattern for the Posey County isolate (Posey) used as the basis for comparing all others. In each sketch accompanying a photograph of an autoradiograph, larger protein spots that were not also present in Posey (or in two cases were consistently larger or darker than in Posey) are solid black for emphasis. Spots missing from the other patterns, but present in the Posey pattern, were omitted from the sketches of patterns in which they were missing. The patterns were highly reproducible, but not every spot showed equally well in every autoradiograph (particularly

for photographic reproduction). The exposure time necessary to produce good contrast of small, pale spots on the autoradiographs resulted in over-development of larger darker spots. The lowest molecular weight proteins of every isolate (which show best along the bottom of the autoradiograph and sketch of Fig. 1C, Vanderburgh) were well separated for only a brief period near the end of a plate gel run, after which they dropped sufficiently to be no longer visible on the autoradiograph. The sketches of Figures 1 and 2 include proteins scored after examination and comparison of all autoradiographs. They are intended to represent typical patterns of the accompanying photographs, not to replicate them.

The protein patterns for isolates from the three southern Indiana counties (Fig. 1) appeared qualitatively similar, with no conspicuously different large dense protein spots to distinguish the pattern of any isolate. Differences among the patterns in

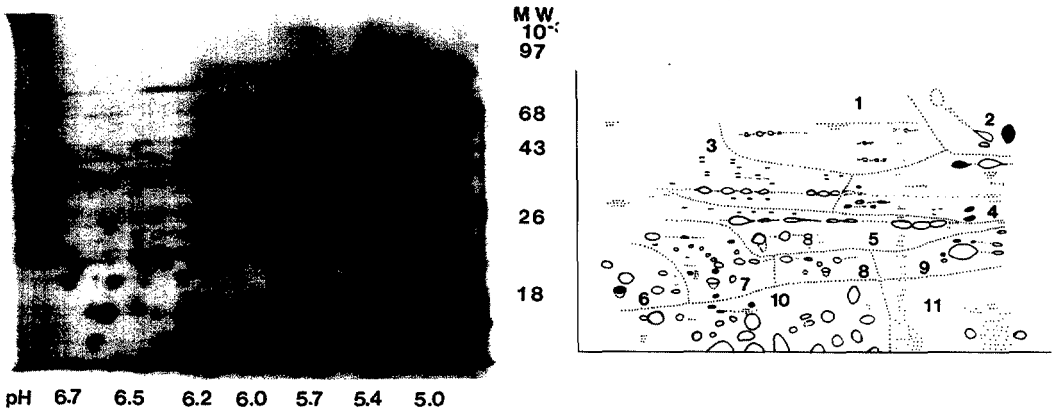


FIG. 3. 2-D PAGE protein pattern for *Heterodera glycines* isolate from Mississippi. Photograph of autoradiograph (left) and sketch of typical pattern (right). Shading of spots indicates comparison with pattern of Posey isolate as indicated for Figure 1.

small protein spots existed, however, in all sectors except 1 and 2. Notwithstanding these differences, these three isolates had higher pairwise Jaccard coefficients with each other than with the isolates from northern Indiana. The Vanderburgh isolate also had a high similarity coefficient of 0.830 with the Mississippi isolate (Table 1). This degree of similarity resulted at least partly from shared features in the protein constellation of sector 9 (Figs. 1C, 3, 4). The Mississippi pattern also shared with the three southern Indiana isolates a nearly identical arrangement of the small paired protein spots in the upper half of sector 3 (Figs. 1A, arrow; 3). For each of the three isolates from southern Indiana, the lowest pairwise similarity coefficient was with the isolate from Japan (Table 1).

The two isolates from Benton and White counties in northern Indiana (Fig. 2) showed special similarities as well as differences from each other and from the isolates from southern Indiana. The Benton and White isolates shared a third protein spot at the top of sector 4 (the left spot in the chain of three large spots in sector 4, Fig. 2) that we have not observed in any other SCN pattern. Below this spot the Benton pattern had an additional large unique protein spot (Fig. 2A). At the lower part of sector 4 (Fig. 2B), the pattern for the isolate from White County had a spot that seemed to be identical to one observed (5) in a pattern for an isolate from Pulaski County. The Benton pattern had a dis-

tinctive dark spot in the upper part of sector 10 (Fig. 2A) that we also observed in the Pulaski pattern (5). The Benton pattern was most similar to the patterns of the other northern Indiana isolates, Pulaski (SC = 0.840) and White (SC = 0.805), and least similar to the Japan pattern (SC = 0.659). Although the White pattern was most similar to that of Benton, the White pattern had relatively low pairwise similarity coefficients with all the patterns under comparison, including that of the Pulaski isolate (SC = 0.766). The White, Benton, and Pulaski isolates, however, had a similar pattern of the small paired protein spots in the upper half of sector 3 (Fig. 2) that differed from the pattern of the southern Indiana isolates and the Mississippi isolate (Figs. 1, 3). Elsewhere we have seen a pattern of these particular spots similar to the northern isolates only in a Race 2 isolate collected near Holland Station, Suffolk, Virginia (unpubl.).

DISCUSSION

We found it relatively easy to identify a given SCN isolate by examination of its protein pattern, particularly those isolates with conspicuous protein differences like those from Pulaski County and Japan (5). The real value of studying 2-D PAGE protein patterns is not to provide a means of ready identification of nematode taxa or isolates, however. It is likely that one-dimensional isozyme tests will prove to be effective for this purpose and simpler to

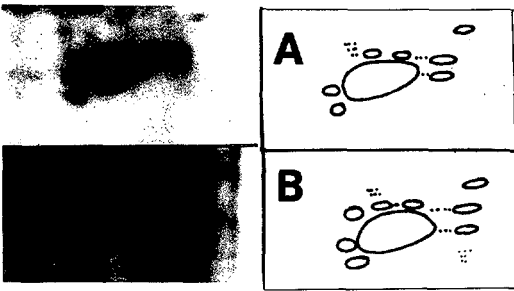


FIG. 4. Enlargement of 2-D PAGE pattern of *Heterodera glycines* protein constellation of sector 9 showing similarity between the Mississippi isolate (A) and the Vanderburgh County, Indiana, isolate (B), as discussed in text. Photographs of autoradiographs (left) and sketches of typical patterns (right).

devise and perform on a routine basis (2). The value of 2-D PAGE protein analysis, as we have used it here, is that it provides a way to visualize and measure relative evolutionary divergence among isolates across a broad spectrum of different kinds of proteins, including structural and regulatory proteins as well as monomorphic and polymorphic enzyme proteins, with the patterns probably reflecting degree of divergence over the entire genome. Our data showed that two isolates may be different in some sectors of the pattern and similar in others, and any type of protein analysis that is restricted to one type of protein could be misleading with respect to overall divergence.

Although the protein pattern for each of our geographical SCN isolates was unique, patterns for the three from southern Indiana showed more similarity to each other than to those from northern Indiana. The northern isolates showed some special similarity to each other, but as a group they were less similar to each other than were the southern Indiana isolates. Such relationships probably result from some facet of their biogeographical history that we do not yet understand, perhaps related to Pleistocene glaciations of the area. Between the regional groupings (northern vs. southern) the protein patterns appeared to coordinate more closely with behavior of the isolates on breeding line differentials (3) than with morphological similarities and differences (4).

Comparisons of these patterns plus others from Indiana and from other U.S. isolates of SCN (including the Mississippi pat-

tern discussed here) and from Japan (5) suggest that many of the protein spots now present in only some isolates may have been present in a widespread ancestral population (i.e., they are plesiomorphic attributes) and were subsequently lost in a mosaic pattern as divided subpopulations continued to evolve in isolation. One such spot (the large dark spot in the upper part of sector 10, Fig. 2A) shared by the Pulaski and Benton isolates seems to be present also in the pattern of an Indiana isolate of *H. trifolii* (data not shown) and may be a remnant protein of an early ancestor of what we now call *H. glycines*. A few of the unique protein spots, and some shared by geographically close populations (e.g., the large protein spot of sector 4 [Fig. 2] shared only by the White and Benton isolates), may be derived (i.e., apomorphic) proteins. Examination of additional isolates and species should provide tests for these suggestions and new data for evaluating our current concepts of species limits across taxa.

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