Two-Dimensional Protein Patterns of Cereal Cyst Nematodes

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Scientific paper 11,998, Purdue Agricultural Experiment Station. Research supported in part by NSF grant BSR 8214460 and USDA grant 87-CRCR-1-2289.

Technical assistance of Phyllis Lockard is gratefully acknowledged.

Accepted for publication 1 May 1989 (submitted for electronic processing).

ABSTRACT

Ferris, V. R., Faghihi, J., Ireholm, A., and Ferris, J. M. 1989. Two-dimensional protein patterns of cereal cyst nematodes. Phytopathology 79:927-933.

Comparisons of protein patterns obtained by two-dimensional polyacrylamide gel electrophoresis of 10 isolates of cereal cyst nematodes from nine locations in Sweden revealed greater differences between the two groups of Swedish *Heterodera avenae*, referred to as strict *H. avenae* and the Gotland strain of *H. avenae*, than were anticipated on the basis of pathogenicity tests and morphological data. Within each of these two major groups, each geographic isolate showed small and unique pattern differences. No pattern similarities or differences could be found to distinguish all members of any given pathotype from isolates of other pathotypes.

Additional keywords: 2-D PAGE.

The development of a diagnostic biochemical assay for races or pathotypes of plant-parasitic nematodes is an attractive possibility (12,30). Previously, we have found protein patterns obtained by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) to be useful in distinguishing unlike taxa that appear similar by morphological criteria, and we have looked for protein constellations that might be useful in distinguishing races of soybean cyst nematode (SCN). We have found many protein pattern differences between geographic isolates that belong to race 3 of SCN on the basis of the reactions of the standard differentials (15,16,20) but no differences that distinguish all of our isolates of race 3 from isolates of other races. Differences in one-dimensional protein patterns have been sought for use in diagnosing pathogenic races of cyst nematodes of several species, but in those research projects only one isolate seems to have been examined for each race studied (8,22,29).

Cereal cyst nematode (*Heterodera avenae* Wollenweber) has a worldwide distribution but has been particularly well studied in several European countries, including Sweden. Multiple geographic isolates are known for many pathogenic groups categorized on the basis of host plant genetics. A collection of isolates of cereal cyst nematode from Sweden, characterized by an assortment of international test differentials, constitutes a good model system in which to determine whether differences in protein patterns obtained by 2-D PAGE are characteristics of pathotypes or only of one particular isolate (or subset of isolates) within a pathotype.

Swedish isolates of *H. avenae* compose two major groups, commonly referred to as strict *H. avenae* and the Gotland strain of *H. avenae* (10) (Table 1). Several terminologies exist for pathotypes within the strict group of *H. avenae*. A recent one that is widely used in Europe is based on the fact that at least three monogenic dominant genes for resistance to *H. avenae* are known in barley (1). These genes are termed *Ha*1 (found in the cultivars Drost and Ortolan), *Ha*2 (found in the cultivars KVL 191 and Siri), and *Ha*3 (found in the cultivar Morocco). All populations avirulent to cultivars containing the gene *Ha*1 are said to belong to the Ha1 pathotype resistance group. When different pathotypes occur within a resistance group, each is given two numbers, with the second number designating the resistance group: e.g., Ha11 and Ha21 are two different pathotypes that are avirulent to cultivars containing the gene *Ha*1. A similar numbering sequence exists for Ha2 pathotypes.

The Gotland strain of *H. avenae* is thought to be similar to a population found in Britain and known there as pathotype 3 (10,34). In Sweden two different pathotypes of the Gotland strain are distinguished, the West Gotland and East Gotland strains (23).

MATERIALS AND METHODS

Isolates examined. We compared 10 isolates from nine locations in Sweden (Table 2 and Fig. 1). The isolates of strict *H. avenae* included two of pathotype Ha11 (one from Ask and one from Naum) and two of pathotype Ha12 (one each from Nässja and Alnarp, site 1). We also included a unique population (from Våxtorp) considered to be a pathotype of strict *H. avenae* belonging to resistance group Ha3. Since no other Ha3 pathotypes of strict *H. avenae* occur in Sweden, some Swedish workers replace the first number in the pathotype designation for this isolate by a question mark, i.e., Ha?3 (24). Two isolates of the West Gotland pathotype (from Hjelmsäter and North Härene) and three of the East Gotland pathotype (from Etelhem, Stacketorp, and Alnarp, site 2) were included. Voucher specimens are retained in the Department of Plant and Forest Protection, Swedish University of Agricultural Sciences, Alnarp.

Obtaining test samples. Following a 6-wk period during which cysts were held at 4 C, all isolates were increased in plant growth chambers on the susceptible barley cultivar Varde (*Hordeum vulgare* L.) grown in 9- × 16-cm plastic pouches (24). The temperature of the seedling cultures was maintained at 10 C for the first 2 wk and then 18 C for the remainder of the growth period. After 8–9 wk, when young female nematodes (in the white cyst stage) were visible through the clear plastic, 30 nematodes per sample were picked from the roots and cleaned by rinsing in tap water.

Preparation of protein samples. The nematodes were homogenized in 0.2 M sodium borate buffer at pH 9 (with or without the reducing agent 100 mM thiodiglycol) in an ice bath, with a ground-glass homogenizer with a motor-driven pestle. The homogenate was centrifuged (at 12,800*g* for 5 min), and the supernatant dialyzed against 0.2 M sodium borate buffer at pH 9 and then stored over liquid nitrogen. The proteins were labeled

in vitro by reductive methylation with formaldehyde and sodium (^{3}H) borohydride (26). This labeling procedure is mild and specific for α-amino groups of amino-terminal residues and ϵ-amino groups of lysyl residues. It is well suited for labeling small quantities of protein (25–50 μg per sample) for electrophoresis under denaturing conditions. Urea sample buffer, which contained 9.5 M urea, 2% (v/v) Nonidet P-40, and 5% (v/v) β-mercaptoethanol, was added to each labeled, precipitated, and washed protein sample (washed in acetone/ether, 1:3), and the samples were stored at −80 C.

Preparation of gels. Electrophoresis was carried out essentially as described by O'Farrell (28). Proteins were first separated by isoelectric focusing in a tube gel (125 × 3.4 mm, inside diameter) with a Bio-Rad model 155 gel electrophoresis cell. The protein sample (25–50 μl per tube) was loaded at the top and overlaid with sample overlay solution (28). The anode electrode solution was 0.01 M H_3PO_4, and the cathode electrode solution was 0.02 M NaOH (degassed just prior to use). Electrophoresis was carried out at 400 V for 18 hr. The power was then increased to 800 V for 1–2 hr to focus the proteins.

Following the first-dimension electrophoresis, each tube gel was equilibrated in buffer (28) for 20 min and then placed on top of a sodium dodecyl sulfate 12% (w/v) polyacrylamide slab gel (1.2 mm thick). Electrophoresis was carried out in a Bio-Rad Protean dual vertical slab gel electrophoresis cell at 20 mA per gel for approximately 5 hr; it was stopped when the indicator dye was 1 cm from the bottom.

At the outset, protein determinations were made by the method of Lowry et al (27), labeling efficiencies were calculated for complex protein mixtures from several nematode taxa, and a number of test gels run with different quantities of protein. Subsequently, on the basis of the amount of radioactivity incorporated into a given sample (determined by counting a 5-μl subsample in a liquid scintillation counter), sufficient protein was loaded on the first-dimension gel to produce a satisfactory autoradiograph after an exposure of the film for 1–2 days at −80 C. The quantity of protein loaded varied between 10 and 25 μg, and within this range the quantity loaded did not affect the protein pattern.

In our study proteins from each isolate were run in both dimensions in the same electrophoresis cell with proteins from other isolates to permit the tracing of small variations in protein positions to peculiarities of individual gel runs. During the several years of the study some changes in reagents and methods were necessary, including a mid-course shift to a new batch of ampholytes (owing to a depletion of our supply of the original batch). This affected the precise positioning of the proteins in the first dimension (28). In addition, we changed our method of preparation of the second-dimension gel mixture from that of O'Farrell (28) to that of Bravo (9), because we found the latter method to give more consistent results. Although the autoradiographs shown in the photographs below were made from gels prepared at various times throughout the period, all comparisons discussed were based on patterns obtained with the same procedures and reagents for the isolates being compared. All autoradiographs paired together in the figures were made from gels run under the same conditions.

Molecular weight standards (Bethesda Research Laboratories, Gaithersburg, MD) were run in the second dimension with the nematode proteins. The standards included ^{14}C-labeled

TABLE 1. Reactions of host differentials to pathotypes of *Heterodera avenae* and the Gotland strain of *H. avenae* found in Sweden[a]

	H. avenae pathotype[b]			Gotland strain pathotype	
Host	Ha11	Ha12	Ha?3	West	East
Barley					
Emir	S	S	S	R	S
Ortolan	R	S	S	R	S
Siri	R	R	S	R	S
Morocco CI 3902	R	R	R-(R)	R-S	(R)-S
Varde	S	S	S	S	S
KVL 191	R	R	S	(R)-S	S
Bajo Aragon 1-1	R	R	R	R	R
Herta	S	S	ND	ND	ND
Martin 403-2	R	R	(R)	(R)-S	S
La Estanzuela 750-1-15	(R)	R	R-(R)	R	R
Harlan 43	R	R	S	(R)-S	(R)-S
Oats					
Sun II	S	S	S	(R)-S	(R)-S
640318-40-2-1 (CI 3444)	R	R	R	(R)-S	(R)-S
Silva	(R)	(R)	(R)	(R)-S	(R)-S
Avena sterilis Cc.4658 (I.376)	R	R	R	R-(R)	R-(R)
Nidar II	S	S	S	S	S
Selma	S	S	S	R	R
Hedvig	R	R	R	R	R
Wheat					
Capa	S	S	S	S	S
63/1-7-15-12 (Loros)	R	R	R	S	S
AUS 10894	R	R	R	S	S
Psathias	S	S	(R)-S	R	R

[a]Based on Andersen and Andersen (1) and adapted from Ireholm (24).
[b]S = susceptible; R = resistant; (R) = moderately resistant; ND = not determined.

TABLE 2. Number designations, source locations in Sweden, and pathotypes or strains of isolates used in this study

Number	Source	Pathotype or strain	Symbol
1	Ask	Pathotype Ha11	○
2	Naum	Pathotype Ha11	○
3	Nässja	Pathotype Ha12	●
4	Alnarp, site 1	Pathotype Ha12	●
5	Våxtorp	Pathotype Ha?3	▼
6	Hjelmsäter	West Gotland strain	■
7	North Härene	West Gotland strain	■
8	Etelhem	East Gotland strain	□
9	Stacketorp	East Gotland strain	□
10	Alnarp, site 2	East Gotland strain	□

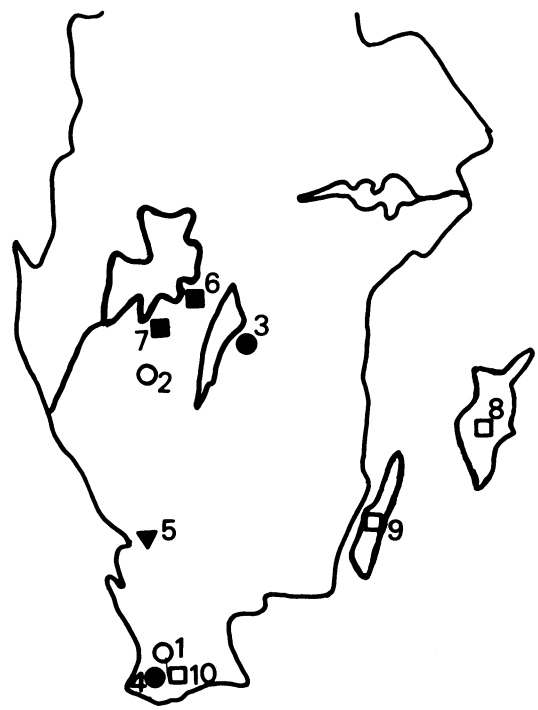

Fig. 1. Partial map of Sweden showing source locations of 10 isolates of *Heterodera avenae*, with numbers and symbols as in Table 2.

phosphorylase B (mol wt 97,400), bovine serum albumin (mol wt 68,000), ovalbumin (mol wt 43,000), α-chymotrypsinogen (mol wt 25,700), β-lactoglobulin (mol wt 18,400), and lysozyme (mol wt 14,300). The pH gradient was measured with a pH meter, using 1-cm segments of the isoelectric focusing gels (28).

Preparation and analysis of autoradiographs. Following separation in two dimensions, labeled proteins were located on the gels by fluorography with the EN^{3}HANCE procedure (New England Nuclear Research Products, Boston, MA). Four to 10 gel patterns were obtained for each isolate, depending on the quantity of protein available. From each of these, several autoradiographs were made at a range of exposure times. Abundant dark proteins were best studied on autoradiographs with short exposures, whereas small or pale protein spots were best studied on autoradiographs with longer exposures (see below, Figs. 2 and 4–7). The transparent autoradiographs were overlaid and compared directly. As is customary for 2-D gels, we used internal "landmark" spots to align gels for comparison (9,32,36). Proteins and polypeptides with identical electrophoretic properties were assumed to be identical (5,6,9,28). For convenience of analysis, the patterns were divided into three sectors (see below, Fig. 3). Each sector contained constellations of spots that seemed to retain precisely the same electrophoretic relationship to each other, despite minor shifts among constellations from gel to gel (a consequence of unavoidable differences between gel runs). Each reproducible spot in each sector was numbered, and its presence or absence was determined for all autoradiographs of the isolates being compared. About 130 spots for each isolate consistently showed good enough resolution to be easily recognized on multiple autoradiographs and scored. A spot consistently pale on patterns of some isolates and dark on others was scored as present in all isolates, with errors probably biased toward higher estimates of similarity rather than difference.

RESULTS

It is clear by inspection of typical autoradiographs (Fig. 2) that the patterns of two of the isolates of strict *H. avenae*, one a pathotype Ha12 isolate from Nässja (Fig. 2C) and the other a pathotype Ha11 isolate from Ask (Fig. 2D), were nearly identical. Likewise, the patterns of two different isolates of the Gotland strain, one a West Gotland pathotype from Hjelmsäter (Fig. 2A) and the other an East Gotland pathotype from Etelhem (Fig. 2B), were very similar to each other. Many differences were evident, however, between the strict *H. avenae*-type pattern and that of the Gotland strain. Obvious differences included the vertical row of ladderlike spots in the patterns of the two isolates of strict *H. avenae* (Fig. 2C and D, arrow d), which was largely missing in the patterns of the two Gotland strain isolates (Fig. 2A and B). Additional differences in protein spots between the two sets of patterns were numerous (Fig. 2). Sketches of all of the spots present in the pattern of the Nässja isolate of strict *H. avenae* (Fig. 3, right) but absent from that of the Hjelmsäter isolate of the West Gotland strain and of the spots present in the pattern of the Hjelmsäter isolate (Fig. 3, left) but absent from that of the Nässja isolate illustrate that each pattern had many spots not shared by the other. In both cases, the protein spots not shared (i.e., the spots that are sketched in Fig. 3) constituted about 75% of the total spots present and scored on the autoradiographs.

Despite the pronounced differences between the isolates of strict *H. avenae* and those of the Gotland strain, some spots and

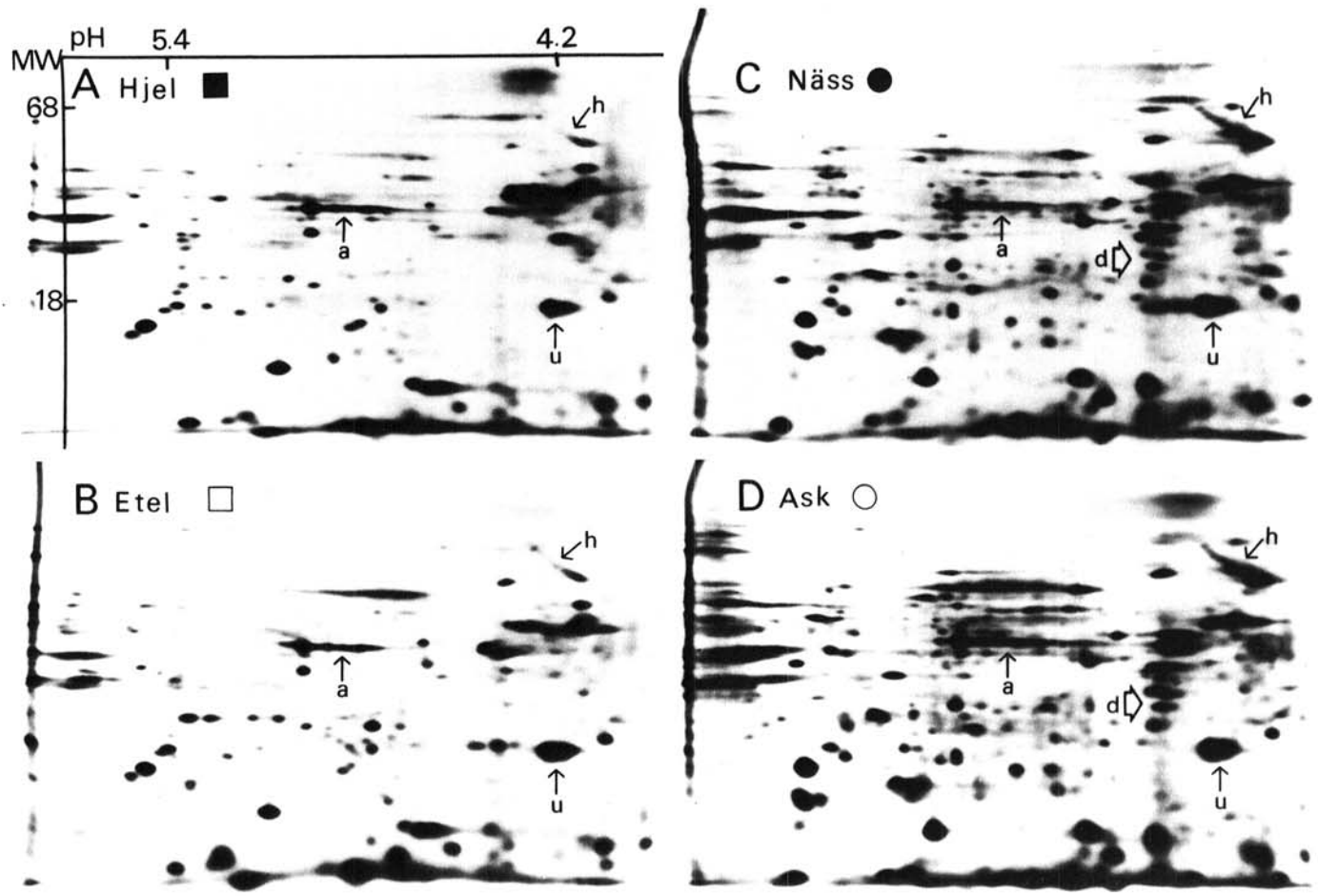

Fig. 2. Typical protein patterns from two-dimensional polyacrylamide gel electrophoresis of the Gotland strain of *Heterodera avenae* (**A** and **B**) and strict *H. avenae* (**C** and **D**). **A,** West Gotland strain isolate from Hjelmsäter. **B,** East Gotland strain isolate from Etelhem. **C,** Pathotype Ha12 from Nässja. **D,** Pathotype Ha11 from Ask. Protein spots a, d, h, and u are referred to in the text. Molecular weights are given in thousands.

constellations of spots were present in all four patterns (Fig. 2). These included the intense spot at the right of each pattern (Fig. 2, arrow u). We have found this "universal" spot to be present in the patterns of all taxa of nematodes we have examined thus far, including rhabditids, tylenchids, and dorylaimids (14,16; V. R. Ferris and J. M. Ferris, *unpublished*). A slanted constellation at the upper right of the patterns (Fig. 2, arrow h) has been present in the patterns of all cyst nematode species that we have examined, including three genera and many species and isolates (Ferris and Ferris, *unpublished*). A string of spots at the upper center of the patterns (Fig. 2, arrow a) was characteristic of all isolates of *H. avenae*.

Some differences existed between the patterns of four isolates (representing two pathotypes) of strict *H. avenae* (Fig. 4) and, therefore, suggested candidates for diagnostic spots to delimit pathotypes. A possibly diagnostic spot for the Ha12 pathotype was the conspicuous spot (Fig. 4A, arrow n) in the pattern of the Nässja isolate. This was missing from the patterns of both the Ask and the Naum isolates of pathotype Ha11 (the symbol ○ in Fig. 4C and D marks the place where the spot would have been if it were present). The spot was, however, also missing from the pattern of the Ha12 isolate from Alnarp, site 1 (Fig. 4B). That isolate, in turn, had a distinct spot (Fig. 4B, arrow x) that was not present in either of the Ha11 isolates (Fig. 4C and

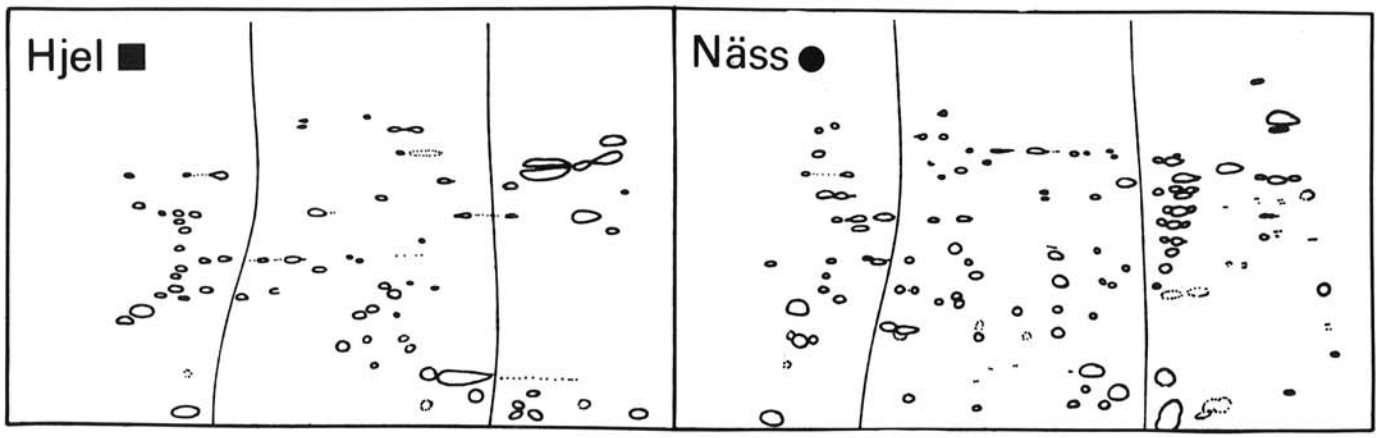

Fig. 3. Sketches of proteins present in the Hjelmsäter isolate (left) but absent from the Nässja isolate, and those present in the Nässja isolate (right) but absent from the Hjelmsäter isolate. The vertical lines divide each pattern into three sectors to facilitate analysis.

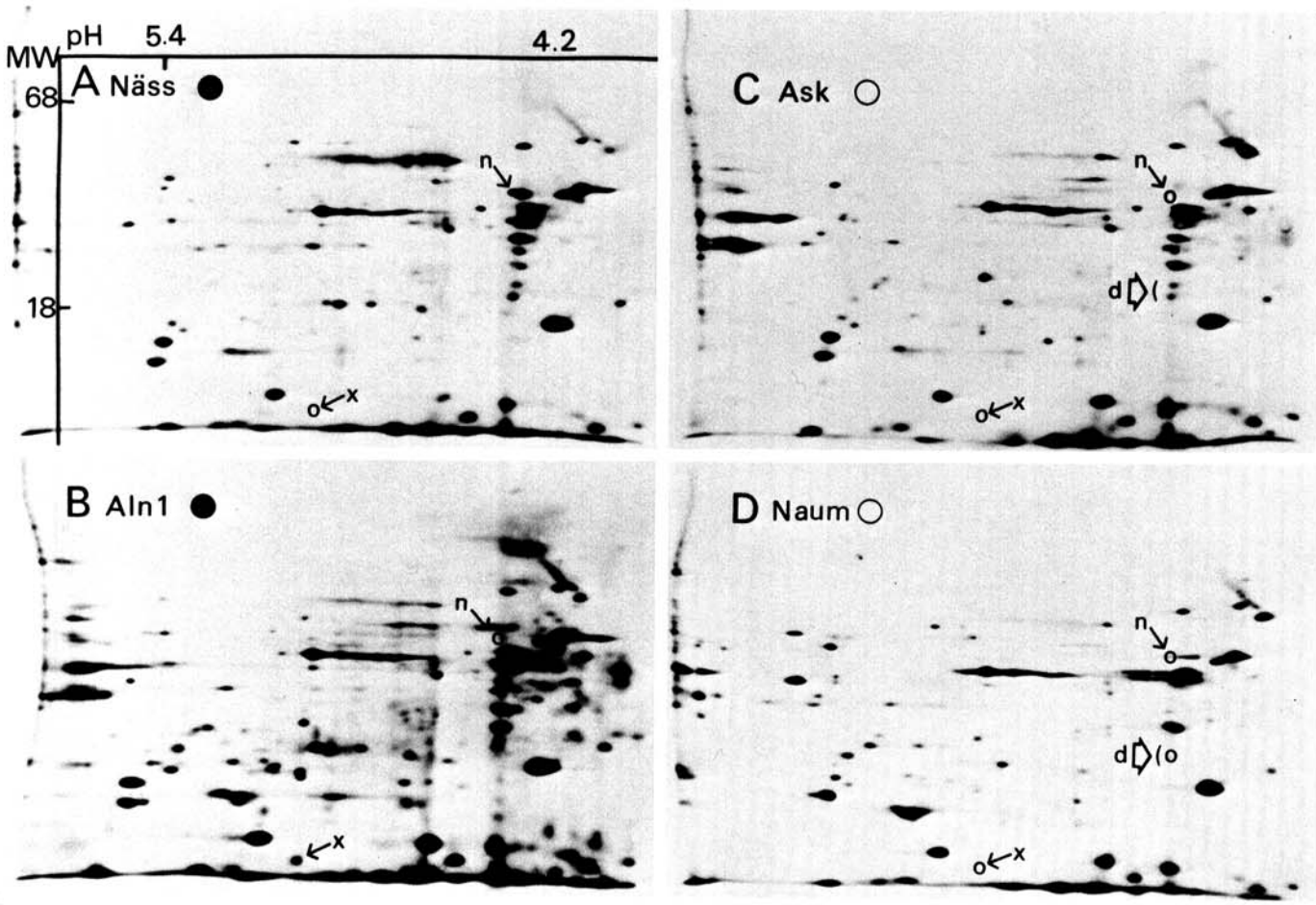

Fig. 4. Protein patterns from two-dimensional polyacrylamide gel electrophoresis of four isolates of strict *Heterodera avenae*—two of pathotype Ha12, from Nässja (**A**) and Alnarp, site 1 (**B**), and two of pathotype Ha11, from Ask (**C**) and Naum (**D**). Protein spots d, n, and x are referred to in the text. The symbol ○ indicates the absence of a protein spot. Molecular weights are given in thousands.

D), but this spot was also not present in the Nässja isolate of pathotype Ha12 (Fig. 4A). The Ha11 isolate from Naum (Fig. 4D) was missing two of the ladder-type spots that were characteristic of the two Ha12 isolates, but these spots were clearly present in the Ask isolate of Ha11 (Fig. 4C, arrow d), and so they could not be used to separate pathotypes Ha11 and Ha12.

The pattern of the Våxtorp isolate (Fig. 5), considered to be a pathotype of strict *H. avenae*, resembled those of the other isolates of strict *H. avenae* (Figs. 2C and D and 4), but there were conspicuous differences. Many of the ladder-type spots at the right of the pattern that are typical of isolates of strict *H. avenae* were missing, as they were for the Naum isolate (Fig. 4D), but those remaining differed from those of the Naum isolate. The "diagnostic" spot n in the Nässja isolate (Fig. 4A) was also present in the Våxtorp isolate (Fig. 5, arrow n). In addition to differences from each of the other isolates of strict *H. avenae* in small spots throughout the pattern, the Våxtorp isolate had a unique spot (Fig. 5, arrow v) that was not present in the patterns of any of the other isolates of strict *H. avenae*.

An intense protein spot was present in the upper right of the pattern of both isolates of the West Gotland pathotype (Fig. 6A and B, arrow w). This spot was missing in two of the isolates of the East Gotland pathotype, from Etelhem and Alnarp, site 2 (Fig. 7A and B), and was a candidate for diagnosing isolates

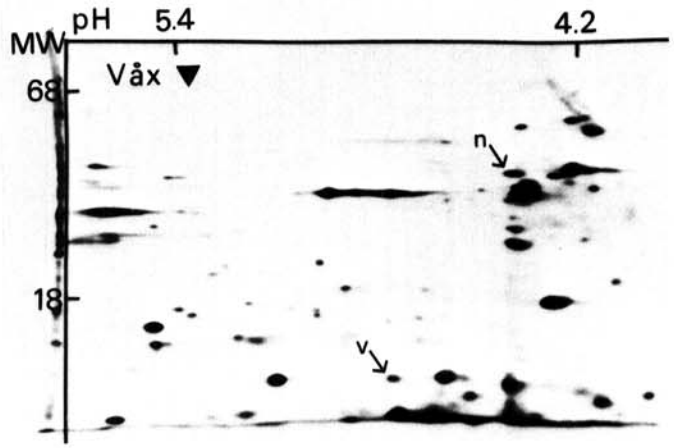

Fig. 5. Protein pattern from two-dimensional polyacrylamide gel electrophoresis of the Våxtorp isolate. Protein spots n and v are referred to in the text. Molecular weights are given in thousands.

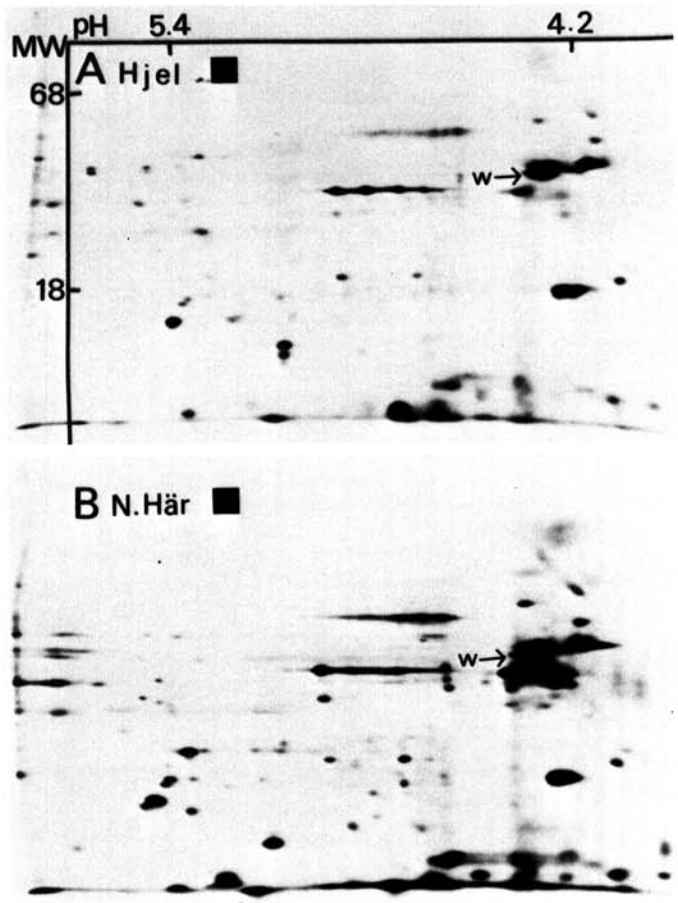

Fig. 6. Protein patterns from two-dimensional polyacrylamide gel electrophoresis of two West Gotland strain isolates, from Hjelmsäter (**A**) and North Härene (**B**). Protein spot w is referred to in the text. Molecular weights are given in thousands.

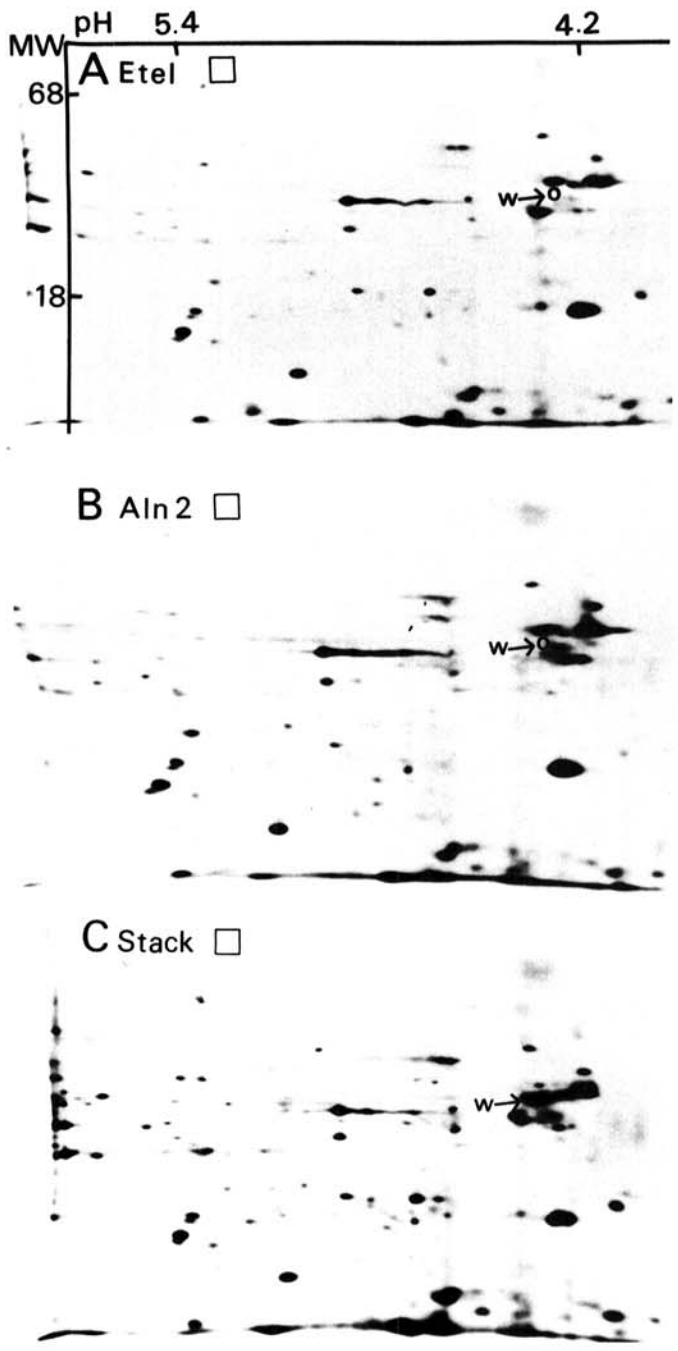

Fig. 7. Protein patterns from two-dimensional polyacrylamide gel electrophoresis of three East Gotland strain isolates, from Etelhem (**A**); Alnarp, site 2 (**B**); and Stacketorp (**C**). Protein spot w is referred to in the text. The symbol O indicates the absence of a protein spot. Molecular weights are given in thousands.

of the two pathotypes of the Gotland strain. The spot was, however, present in a third isolate of the East Gotland pathotype, from Stacketorp (Fig. 7C, arrow w) and therefore cannot be considered diagnostic for isolates of the West Gotland pathotype.

DISCUSSION

The degree of dissimilarity between the protein patterns of the isolates of strict *H. avenae* as a group and those of the Gotland strain isolates was unexpected, even though the Gotland strain isolates are distinguished from strict *H. avenae* by the fact that they do not attack the oat cultivar Selma but do attack wheat cultivars that are resistant to the isolates of strict *H. avenae* (Table 1). In addition, Gotland strain nematodes differ slightly from strict *H. avenae* in several morphological characteristics, including the fenestrae, bullae, underbridge, and cyst color (3,10,34). Nematologists, however, have traditionally been divided regarding the significance in cyst nematodes of differences in morphological characters such as these (34,38).

Many data now suggest that 2-D gel protein patterns are useful taxonomic tools for sorting out taxa for which morphological data are equivocal (2,5–7,13–17,33). Species, subspecies, and strains of medically important trypanosomes have recently been distinguished by 2-D protein patterns (2). Similarly, the 2-D protein patterns of two species of *Leishmania* showed a degree of disparity not previously revealed by isozyme analyses or DNA cross-hybridization (33). Proteins are believed to change stochastically over long periods of time, whether or not observable changes occur in the gross morphology of the organisms during those same periods. This lack of synchrony between morphological change and biochemical change has been established for taxa of many kinds of organisms (6,18,21,37). In a comparison of published data for overall measures of electrophoretic protein similarity (one-dimensional patterns) between taxa in a wide assortment of organisms, Thorpe (35) concluded that the published data indicate no great differences in protein evolution in mammals, reptiles, amphibians, fishes, or invertebrates and suggested that the amount of protein divergence between conspecific populations and congeneric species may be roughly similar across a wide range of taxa. Aquadro and Avise (4) used 2-D protein patterns to quantify evolutionary divergence in rodents and found 92–95% similarity in conspecific field mice, 81–85% similarity in congeneric species, and only 50–60% similarity between species of different genera. Gabriel and Ellingboe (19) found the 2-D PAGE protein pattern of a new field isolate of the fungus *Erysiphe graminis* DC. to be nearly identical to that of a culture maintained in the laboratory for 19 yr. Such data suggest that changes that do occur over time in the 2-D protein patterns of organisms do so at a slow rate.

Our data and those of others (5–7) indicate that each species of nematode has a distinctive and highly conserved 2-D PAGE protein pattern. Each of our 2-D gels showed a spectrum of different kinds of proteins revealed by our particular experimental protocols, and comparisons of 2-D patterns probably reflected a degree of similarity and difference across whole genomes. For nematodes we have not observed the 2-D pattern to change with the host plant or with experimental perturbations of environmental conditions. In geographic conspecific isolates of free-living dorylaimids collected on different continents or widely separated Pacific islands, we found pattern similarities of about 90% or higher (14). In cyst nematodes we found nearly identical patterns for *Globodera virginiae* Miller & Gray collected in Virginia and in Mexico (13). Although 2-D protein patterns for widely separated isolates of SCN from the United States and Japan were more dissimilar than we expected (suggesting long isolation from each other), they were far more similar than were the two groups of isolates of *H. avenae* from Sweden (15,16).

Bakker and Bouwman-Smits (5,6) and Bakker and Gommers (7) found conspecific geographic isolates (all from Europe) of *Globodera rostochiensis* Wollenweber and *G. pallida* Stone to be more than 95% similar in 2-D protein patterns, whereas the congeners, *G. rostochiensis* and *G. pallida*, shared only about 30% of the proteins scored. The latter observation is particularly relevant to our study because *G. rostochiensis* and *G. pallida* have only small morphological differences, the significance of which for taxonomy was debated prior to the description of *G. pallida* in 1973 (38). Our observation that the Gotland strain isolates shared with the isolates of strict *H. avenae* only about 25% of the proteins and polypeptides that we scored supports the view that the Gotland strain is a distinct species (3). Differences such as these exceed those we would expect in conspecific geographic isolates, even if they were separated by great distances.

An explanation of our inability to find protein differences diagnostic for pathotypes must be speculative. Diagnostic protein markers for cereal cyst nematode pathotypes may not exist, or they may exist but are not revealed by our particular experimental protocols. It is also possible that diagnostic protein spots are present in our patterns but are too small or too similar electrophoretically to be discernible by us. In addition to the kinds of comparisons we made, Bakker and Bouwman-Smits (5) and Bakker and Gommers (7) also used a technique in which proteins of two samples under comparison are mixed together prior to electrophoresis in the two dimensions, and the resulting pattern is one of superimposed spots. In this manner very small suspected differences in isoelectric points can be verified. Although this additional test is useful in special circumstances when a close comparison of only a few isolates is desired, the cost and effort required are not practical for cross-comparisons of many isolates and many pathotypes. Further, protein spots with electrophoretic differences so small as to be revealed only by such protocols would not be very useful for a diagnostic assay.

Although it seems to be a common assumption that so-called races or pathotypes of nematodes constitute coherent biological groups diagnosable by criteria other than their behavior on a designated set of host differentials, this may not be the case (11). Bakker and Bouwman-Smits (5) found protein differences within *G. pallida* pathotypes to be often larger than those between pathotypes, a finding similar to ours for races of SCN (16; Ferris and Ferris, *unpublished*). Dropkin (11) has suggested that the different geographic isolates of cereal cyst nematode represent ecotypes, which may or may not be manifest as distinct pathotypes separated by their reproduction on different sets of hosts. Multiple geographic isolates should be examined prior to reporting diagnostic biochemical assays (protein or DNA) for races or pathotypes of any plant-parasitic nematode species. This has not been emphasized in past research (22,25,29,31), but if we had not seen the protein pattern of the Stacketorp isolate, we might have been tempted to report the discovery of a protein spot diagnostic for the West Gotland pathotype.

As discussed above, in the pathotype scheme favored at the present time (Table 1), the Våxtorp isolate belongs to resistance group Ha3. When this scheme is extended to include the Gotland strain isolates, those isolates also become members of pathotype resistance group Ha3 (1). The data reported here, together with other observations (3,23,24,34), indicate that strict *H. avenae* and the Gotland strain of *H. avenae* constitute distinct species. It is difficult to see how a single pathotype group can contain variants of two different species, inasmuch as the function of the term *pathotype* is to designate intraspecific variants (11). This dilemma underscores the desirability of new biochemical tools for discriminating between geographically separated populations of nematodes that are morphologically similar. Information from such investigations needs to be used together with other data, including information on resistance and susceptibility, to devise stable systems of nomenclature useful to nematologists, plant pathologists, and breeders.

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