COMMONWEALTH INSTITUTE OF HELMINTHOLOGY

HELMINTHOLOGICAL ABSTRACTS

Series B, Plant Nematology

Vol. 48 December, 1979 No. 4

Biochemical Systematics of Nematodes—A Review

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Nematode systematics is in a new era where classification and phylogenetic studies do not have to be based solely on the classical approach to taxonomy. New approaches to nematode taxonomy, such as systems analyses (Maggenti, 1970), numerical taxonomy (Moss and Webster, 1970), cytogenetics (Triantaphyllou, 1970), and biochemical systematics (Hansen and Buecher, 1970), are providing new and valuable information about these organisms and their phylogenetic relationships. These new approaches to characterizing and studying relationships among nematodes will be valuable in complementing and enhancing the information provided by classical nematode systematics.

According to Allen and Sher (1967) the problems associated with taxonomy of phytoparasitic nematodes are not necessarily unique to this group of organisms. These problems, viz. variation in many morphological characters and the occurrence of numerous physiological races (pathotypes) recognizable only by their reproduction on certain plants, have resulted in investigators searching for means other than morphological and anatomical characters to assist in the identification and characterization of species and races of nematodes.

In the past decade, because of the worldwide importance of members of Heteroderidae and Meloidogynidae as plant pathogens and the difficulty often encountered in identifying species in these two families, attention has been focused on applying new approaches to studying the systematics of these complex cyst and root-knot nematodes. The genus *Meloidogyne* contains species with various physiological races (Taylor and Sasser, 1978), and a major character used for identification, the female posterior cuticular pattern, is often variable (Whitehead, 1968). Cytogenetics and biochemical analyses, however, have provided significant information and have been shown to be useful in taxonomic and phylogenetic investigations of species of this genus (Triantaphyllou and Hussey, 1973). From these studies, it is apparent that problems encountered in nematode systematics and in elucidating phylogenetic relationships will be best resolved by combining information obtained from morphological, cytological, and biochemical investigations, thereby characterizing nematodes more completely than hitherto. This paper reviews and evaluates biochemical approaches being used in studying the systematics and phylogeny of nematodes, with emphasis on the work that has been done with phytoparasitic nematodes.

BIOCHEMICAL ANALYSES

Biochemical systematics depends on elucidation of the subtle molecular differences which underlie taxonomic variation (Hansen and Buecher, 1970). The primary problem is determining which chemical characters will be most valuable in providing information for taxonomy. In order for a chemical character to be useful in taxonomy it must have properties of a good taxonomic character. The chemical character (i) should not vary within samples being studied, (ii) should not be susceptible to environmental influence, and (iii) should correlate with existing classifications constructed using other characters (Boulter and Thurman, 1968).

The ultimate goal in systematics should be to classify the genotypes of organisms. The principal flow of genetic information within an organism is from the deoxyribonucleic acid (DNA) to ribonucleic acid (transcription) to protein molecules (translation). Zuckerkandl and Pauling (1965) have designated these molecules that carry the information of the gene as semantides—DNA molecules are called primary semantides, messenger-RNA molecules are designated secondary semantides, and proteins are referred to as tertiary semantides. Semantides are the most relevant molecules when investigating phylogenetic relationships. Practical methods for analyses of the nucleotide sequence of a gene are unavailable. Comparison of DNA molecules, however, can be made through DNA hybridization studies (Leone, 1964), although this has not been used to any extent with nematodes. Proteins, on the other hand, are a manifestation of the sequence of nucleotides in a gene and analyses of these macromolecules provides a reliable approach for comparing genotypes of organisms. Analyses of the amino acid sequence of proteins would provide the most valuable information as this is a direct manifestation of the genome. This approach, however, is very laborious and not presently feasible with nematodes; therefore, biochemical systematics of nematodes has relied primarily on comparing properties of proteins via gel electrophoresis and serology.

Before further considering the application of biochemical systematics to nematology, the problems of availability of nematodes and the separation of different stages to eliminate age-dependent variation need to be considered.

Axenic culturing is the ideal method of propagating nematodes for biochemical analyses because the influence of a host on the nematode's metabolism is removed, culture conditions can be standardized and large quantities of worms can be obtained easily. Phytoparasitic nematodes, being obligate parasites, cannot be cultured axenically and usually are propagated xenically on greenhouse-grown plants or less frequently, monoxenically on callus tissue. Thus standardization of culture conditions is often impossible, and the host plant may influence quantitatively and/or qualitatively the chemical compounds selected as taxonomic characters. This potential

influence of a host plant on a phytoparasitic nematode's metabolism will be discussed later. Several microbivorous nematodes, however, can be cultured axenically, which makes them more suitable for biochemical studies. Obtaining large quantities of nematodes, particularly phytoparasitic ones, is not easy and, consequently, biochemical techniques often need to be adapted to analyze small amounts of material. Many micromethods (Neuhoff, 1973) are now available which can be used in biochemical studies of nematodes. For example, micro-gel electrophoretic techniques are available which permit analysis of enzymatic and nonenzymatic proteins from a single nematode specimen (Dalmasso and Berge, 1978). Techniques such as these offer tremendous possibilities for studying nematode biochemistry and applying this information to systematics, population genetics, and elucidation of phylogenetic relationships.

An important problem to overcome once nematodes are in culture is separating the different developmental stages. It is now well established that certain chemical characters are age-dependent. Chow and Pasternak (1969) clearly demonstrated the occurrence of stage-specific electrophoretic enzyme patterns in the microbivorous nematode, *Panagrellus silusiae*. Five discrete bands of malate dehydrogenase were detected for the L3 larvae whereas only three, two, and one band were found for L4, adult, and L2 stages, respectively. Other differences in lactate dehydrogenase and esterase profiles also occurred among these stages. Dickson *et al.* (1971) also found differences in several enzyme patterns when comparing homogenates of eggs, second-stage larvae and adult females of *Meloidogyne incognita* by electrophoresis. These studies illustrate the importance of working with a single developmental stage when conducting biochemical systematic investigations. Procedures are available for separating the different vermiform stages of nematodes (Chow and Pasternak, 1969; Myers *et al.*, 1971). Phytoparasitic nematodes which exhibit sexual dimorphism, as do members of the Meloidogynidae and Heteroderiadae, are particularly suited for biochemical analyses since living, globose adult females can be readily separated from nematodes in other developmental stages (Hussey, 1972). This permits analyses to be carried out on nematodes all in a single developmental stage, thereby eliminating most age-dependent variation. Eggs and second-stage larvae of these nematodes can also be easily collected for biochemical analyses. In fact, Greet and Firth (1978) found that pre-parasitic second-stage larvae of round cyst nematodes provided a more reliable source of proteins for biochemical comparisons than did adult females because these larvae were free of plant host proteins which can contribute to variation in electrophoretic protein profiles of adult females. They felt that pre-parasitic larvae are the best source of uniform and consistent proteins for biochemical studies because (i) host proteins may occur in the guts of nematodes, which will interfere with the protein analysis, and (ii) the uncertainty as to when different nematodes feeding on different host plants reach comparable physiological development. Many phytoparasites, however, can be propagated on a common host, reducing the importance of the influence of host proteins on protein profiles from these nematodes. Caution, nevertheless, must still be exercised when explaining certain data because even nematodes in the same stage but at different points of development within that stage, might still have slightly different chemical characteristics.

GEL ELECTROPHORESIS

Electrophoresis on acrylamide gels provides a high resolution technique for separating protein molecules on the basis of size and net charge (Davis, 1964; Ornstein, 1964). While other support media have been used for electrophoresis, acrylamide gel has become a favourite because of its molecular sieving capabilities. The technique is relatively simple to use proficiently, and several books have been written which describe in detail the procedure and possible modifications (Gordon, 1970; Maurer, 1971). Gel electrophoresis is capable of separating proteins from crude homogenates which makes it very applicable to taxonomic studies where large numbers of samples have to be examined.

Since proteins are the immediate manifestation of an organism's genetic endowment, comparisons of nonenzymatic proteins and enzymes via electrophoresis should reflect direct relationships among the genotypes of the nematode species being studied. Before discussing specific studies, however, I would like to briefly outline the general procedures that have been used in electrophoretic studies of nematode proteins. Homogenates containing proteins are usually prepared by extraction of nematodes with a dilute (0.01 to 0.05 M) aqueous salt solution at an appropriate pH (between 7.4 to 8.0). Seventeen percent sucrose has been included in the buffer in some studies. Reducing agents, such as cysteine or ascorbic acid, need to be added to the extractant when the nematodes are known to contain high concentrations of phenol oxidases. Quinones, the product of phenol oxidase activity on its substrates, are very reactive and can denature proteins. Homogenates of various cyst nematode species of the genera *Heterodera* and *Globodera*, will turn black after standing for a short period of time without these reducing agents, indicating high phenol oxidase activity. Following maceration of the nematodes in a tissue homogenizer, the homogenate is clarified by low speed centrifugation, usually around 20,000 g, and the supernatant fluid serves as the source of proteins.

After separation of the nematode proteins by electrophoresis, the position of nonenzymatic proteins are located in the gels by using a non-specific protein stain such as Coomassie blue or Buffalo Black NBR. After destaining the gels, proteins appear as discrete coloured bands. The resulting protein profile can then be examined visually or analyzed by scanning the gel in a microdensitometer.

Interpretation of nonenzymatic protein profiles is not always easy. The position of a protein in a gel is determined by its amino acid composition and the size of the molecule. Different protein molecules from two species may carry the same net charge and therefore, have the same electrophoretic mobility, even though their amino acid sequences may be quite different. Identical protein profiles may be obtained from organisms in different taxa although the proteins giving the patterns may not be identical (Boulter and Thurman, 1968).

The sensitivity of gel electrophoresis is greatly increased when specific enzymatic proteins are identified in the gels using standard histochemical techniques. Following electrophoresis, sites of specific enzymatic activity are located in the gels by incubating them in a staining solution that contains the substrate for the enzyme plus co-factors necessary for the reaction to take place. Products of the reaction then combine with a dye in the staining solution to give a coloured product at the site of enzyme activity (Brewer, 1970). Enzymes are known to occur in multiple molecular forms which are called isoenzymes when they have the same catalytic activity. When different forms of an enzyme are known to be produced by different alleles at the same locus, they are designated allozymes (Gottlieb, 1971). Books are available which list staining recipes for identifying 38 different enzymes in gels (Brewer, 1970; Shaw and Koen, 1968; Wilkinson, 1970).

Variability in results of electrophoretic analyses of nematode proteins can occur for many different reasons: e.g., (i) methods of culturing nematodes, (ii) stage of nematode development or the physiological state of nematodes in a particular stage, (iii) protein extraction procedure and conditions of storage of protein extract, and, (iv) methods of protein and enzyme analysis. All of these may influence either the number of protein bands or isoenzymes that are detected, or their electrophoretic mobility, or both. Protein analysis obtained by different investigators will be most useful for nematode systematic and phylogenetic studies if standardization of gel electrophoresis procedures is adopted; otherwise, meaningful comparisons cannot be made. Even so, discrepancies may still occur among studies. Franco (1979 obtained fewer protein

Table 1. Gel electrophoretic studies of nematodes. The numbers shown in the table refer to the references at the end of this review.

Nematode	Stages Examined	References
Aphelenchoides fragariae	Mixed	25
Aphelenchus avenae	Mixed	14, 15, 17
Caenorhabditis dolichura	Mixed	25
Caenorhabditis briggsae	Mixed	19
Caenorhabditis elegans	Mixed	19
Ditylenchus dipsaci	Mixed	3, 14, 15, 31
Ditylenchus destructor	Mixed	17
Ditylenchus triformis	Mixed	14, 15, 31
Ditylenchus myceliophagus	Mixed	17
Heterodera avenae	Cyst	50, 54
Heterodera carotae	Cyst	54
Heterodera glycines	Cyst	14, 15, 34
Heterodera schachtii	Cyst	54
Heterodera trifolii	Cyst	54
Heterodera oryzae	Cyst	34
Globodera rostochiensis	L_2, Cyst	18, 24, 54, 55
Globodera pallida	L_2, Cyst	18, 24, 54, 55
Globodera solanacearum	L_2, Cyst	24
Globodera tabacum	L_2, Cyst	24
Globodera virginiae	L_2, Cyst	24, 54
Meloidogyne incognita	Egg, L_2, Adult female	4, 11, 14, 15, 32, 33, 34, 47, 53
Meloidogyne arenaria	Adult female	4, 11, 14, 15, 33, 47, 53
Meloidogyne hapla	Adult female	4, 11, 14, 15, 34, 47
Meloidogyne javanica	Adult female	4, 11, 14, 15, 47
Meloidogyne mali	Adult female	34
Meloidogyne naasi	Adult female	11
Panagrellus redivivus	Mixed	3
Panagrellus silusiae	Mixed, L_2, L_3, L_4 Adult	8, 25
Pelodera teres	Mixed	25
Rhabditis terricola	Mixed	25

bands for *Globodera rostochiensis* than previously reported by Trudgill and Carpenter (1971), even though procedures were duplicated. The versatility of polyacrylamide-gel electrophoresis and the conditions necessary for pattern reproducibility have been discussed by Chrambach and Rodbard (1971).

Comparative studies of nematode proteins by gel electrophoresis have provided information that has been helpful in nematode identification and in elucidating relationships among various nematode species (Table 1). Benton and Myers (1966) were the first to utilize electrophoresis to study nonenzymatic proteins and enzymes of phytoparasitic and microbivorous nematode species. In their study six sites of nonspecific esterase activity were detected in gels following separation of proteins from *Ditylenchus dipsaci* in an anionic electrophoresis system whereas only four were detected for *Panagrellus redivivus*. Esterases from the two species also had different mobilities. Differences also were detected with nematode acid phosphatases in a cationic electrophoretic system.

Gysels (1968), using agar gel electrophoresis, compared proteins of *P. silusiae*, *Aphelenchoides fragariae* and three rhabditid species, *Pelodera teres*, *Rhabditis terricola* and *Caenorhabditis dolichura*. Sufficient differences were detected in the protein profiles to distinguish each species. Influence of sample storage on pattern variability was also investigated and the least variation in mobility occurred when fresh samples or samples refrigerated for less than 18 hours were used. Longer refrigeration, freezing, or lyophilizing samples altered the electrophoretic mobility of several proteins. Protease patterns differed among the species whereas amylase profiles were similar.

Evans (1971) evaluated the ability of gel electrophoresis (primarily starch) to distinguish among populations of *Aphelenchus avenae* and between *Ditylenchus destructor* and *D. myceliophagus*. Esterase, acid phosphatase, amylase, and nonenzymatic protein patterns differed slightly among seven isolates of *A. avenae*. The two closely related species of *Ditylenchus* were distinguishable on the basis of their esterase patterns. He also studied the influence of culture age, temperature, host fungus and host nutrition on the composition of the protein and enzyme profiles for *A. avenae*. The protein patterns were not qualitatively influenced by the parameters studied, but esterase patterns varied inconsistently.

Two races of *D. dipsaci*, separated by the difference in their ability to reproduce on Wando garden pea, did not differ in regard to esterase or catalase profiles but did differ by a single band in the protein profiles (Hussey and Krusberg, 1971). *D. dipsaci* could be distinguished from *D. triformis* by the enzyme patterns. Dickson *et al.* (1971) employing a different extractant, also detected differences between *D. dipsaci* and *D. triformis*.

Although differences were detected in protein and enzyme profiles between different nematode species in the above

studies, analyses were made on mixtures of nematodes in different developmental stages in these investigations. The mixed stages (larval and adult) were probably responsible for most of the variability in the results often noticed by the investigators. Since work has shown considerable differences in enzyme patterns among individual stages (Chow and Pasternak, 1969), biochemical systematic studies should be carried out on nematodes in a single developmental stage, preferably the adult female, since the female is considered the most important stage in the taxonomy of nematodes, and their larger size often permits individual specimens to be analyzed.

As mentioned above, members of the families Heteroderidae and Meloidogynidae have often been used in biochemical systematic studies. One advantage of working with nematodes in this group is that specimens in different developmental stages are easily isolated, enabling analyses to be made on nematodes all in a specific stage. The adult female has been the developmental stage most often investigated.

The first biochemical systematic study with *Meliodogyne* species was conducted by Dickson *et al.* (1970, 1971), who compared protein extracts of adult females by polyacrylamide-gel electrophoresis. Their first paper demonstrated that females of four *Meloidogyne* species (*M. incognita*, *M. arenaria*, *M. hapla*, and *M. javanica*) each possessed characteristic nonenzymatic protein profiles. Two regions of the electrophoretograms (microdensitometer tracings of the protein profiles) were considered to have taxonomic value. One area of the profile was genus specific and another contained interspecific differences. The portion of the profile from Ef (electrophoretic mobility value) 0.20-0.60 contained the interspecific differences whereas that segment from Ef 0.66-0.76 was characteristic for the genus *Meloidogyne* when compared against profiles of female nematodes of three other genera. Four populations of *M. javanica* originating in widely separated geographic regions of the world had very similar protein profiles.

In a companion paper stains were used to identify specific enzymes in the acrylamide gels (Dickson *et al.*, 1971). Although lactate dehydrogenase and acid phosphatase were similar for the four *Meloidogyne* species, differences were detected with α-glycerophosphate dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase and esterases. Some bands were unique for certain species but rarely were they characteristic for populations within a species. Culturing nematodes on four different hosts (tomato, tobacco, cucumber and wheat) did not affect the patterns of any of the above enzymes for the adult female nematodes. Interestingly, no differences were detected in the enzyme patterns from two cytological races of *M. hapla*, one having a reduced chromosome number of 15 to 17 and the other with a somatic chromosome number of 45.

Hussey *et al.* (1972), also studied enzyme profiles of *Meloidogyne* species but changed the extractant solution previously used by Dickson *et al.* (1971). All other procedures were essentially similar. This change resulted in an increase in the number of esterase bands obtained for *M. arenaria* and *M. incognita* over the number previously reported for these species by Dickson *et al.* (1971). Seven more sites of esterase activity were detected for both species. Two additional sites of α-glycerophosphate dehydrogenase activity were also identified for *M. arenaria*. *M. arenaria* and *M. incognita* could consistently be differentiated on the basis of the number of sites of activity for malate dehydrogenase and α-glycerophosphate dehydrogenase. Although the same number of sites of esterase activity was detected for both species, characteristic differences did occur with respect to the relative activity at certain sites and the migration rates of other esterases in the gels. These three enzymes were considered to have the most taxonomic value in distinguishing these two *Meloidogyne* species with the greatest differences occurring among the esterases. Patterns of six other enzymes were identical for these two species. Although characteristic nonenzymatic protein profiles were obtained for the two *Meloidogyne* species, the differences were not as striking as the differences in the enzyme patterns.

Contrary to a report by Dickson *et al.* (see above), enzyme patterns associated with nematodes were shown by other investigators to be influenced by the host plant on which the parasite is propagated. Differences have been detected in peroxidase and α-glycerophosphate dehydrogenase patterns of extracts from *M. incognita* propagated on different hosts (Hussey and Sasser, 1973; Hussey *et al.*, 1972; Starr, 1979). Patterns of several other enzymes, including esterases, were not influenced by varying the host, suggesting that the host may only influence certain enzymes. Ishibashi (1970) also reported variation in protein and enzyme patterns of root-knot nematodes as affected by host plants and growing conditions of the host. He found that there could be variation in esterase profiles as did Berge and Dalmasso (1975).

Dalmasso and Berge (1978) were the first to use micro-electrophoresis to separate proteins from single nematode specimens. They developed a microtechnique where acrylamide gels are cast as slabs (0.4 mm thick) or as cylinders in microhematocrit tubes (1.1 mm internal diameter). These exceedingly thin gels were used to separate proteins from one adult female specimen of *Meloidogyne* species. The micro-slab technique enables extracts of 8 to 10 individual nematodes to be separated on a single gel.

Dalmasso and Berge (1978) used the microtechniques to study enzymes and nonenzymatic proteins from several populations of the four common *Meloidogyne* species and one population each of *M. naasi* and an unknown species. To assess the variability at each locus studied, 80 individuals from wild populations and 20 specimens from single egg mass populations were analyzed, making a total of 22,000 individual specimens examined. Although some differences were apparent in the nonenzymatic protein profiles from individual female nematodes, as reported in previous studies with *Meloidogyne* species, the greatest differentiation was obtained with the enzyme analyses while non-specific esterase patterns proved to be the most valuable. As one would expect, however, fewer enzyme bands were usually obtained (esterases in particular) from individual specimens than from homogenates of hundreds of nematodes. The migration rates of the enzymes also differed from those previously reported (Hussey *et al.* 1972). Nevertheless, this study demonstrates the feasibility of using individual nematodes for enzyme analyses. Although the designation of allelic variants for certain enzyme loci could not be confirmed by breeding experiments, grouping of *Meloidogyne* species by their allelic differences corresponded to previous groupings based on chromosome numbers and host range.

Taxonomic problems also occur with cyst nematodes and particularly with the *Globodera* genus where few morphological differences exist among the species (Stone, 1977). Polyacrylamide-gel electrophoresis recently has been utilized to compare the proteins among species of *Heterodera* and *Globodera* and pathotypes within certain species. Unfortunately, only nonenzymatic proteins have been compared among cyst nematodes with no effort having been made to study enzymes. I feel that enzyme analyses eventually will prove to have more taxonomic value than other proteins for species identification of cyst nematodes as has proven to be the case with *Meloidogyne* species. As stated earlier, more than one nonenzymatic protein may migrate to the same site. Detection of enzymes, however, has the distinct advantage of enabling specific proteins to be identified from among the many present in an extract.

Trudgill and Carpenter (1971) first characterized cyst nematodes by nonenzymatic protein profiles. Large differences were observed in the protein profiles comparing adult females of lemon vs. round, cyst nematodes. Genera within each group also differed in this respect, but to a lesser extent. At the time of this study *Globodera pallida* and *G. rostochiensis* were known as pathotypes of *H. rostochiensis* which differed in their ability to reproduce on resistant potato varieties

carrying the H as well as the H_2 gene. Protein profiles of pathotype A (now *G. rostochiensis*) and pathotypes B and E (now *G. pallida*) consistently differed by three bands. This was corroborated by later studies (Trudgill and Parrott, 1972) and helped support the decision to separate the pathotypes into two species. These pathotypes also differed in measurements of males and larvae, female cyst colour and poor ability to interbreed (Stone, 1973). Proteins from different aged cysts, separated by colour (white, yellow, and brown) were compared. White and yellow cysts yielded similar protein profiles while those obtained for the older brown cysts were considerably different.

Stone and Williams (1974) were unable to distinguish between two pathotypes of *H. avenae* using gel electrophoresis of proteins. Nor were differences noted in the morphology or measurements of second-stage larvae, suggesting that the pathotypes belonged in a subspecific rank.

The above studies clearly demonstrate the usefulness of gel electrophoresis in identifying nematode enzymes and provide evidence that this technique should prove to be a valuable tool in taxonomic and phylogenetic studies of nematodes.

SEROLOGY

The protein constitution of two organisms can also be compared by serology. Because of the relative specificity of the antigen-antibody reaction, serological techniques are regarded as very reliable in determining homologies between proteins of different species (Gell, 1968). No attempt will be made here to go into the specifics of serological techniques that have been applied in systematic studies. Excellent books are available for this information (Crowle, 1973; Smith, 1976; Leone, 1964).

Serological comparisons are most useful for determining phylogenetic relatedness of different species. The antigen-antibody reactions are primarily studied using gel diffusion or immunoelectrophoretic techniques. These techniques have been employed in determining differences or similarities among antigens (soluble proteins) extracted from nematodes (Table 2).

Gibbins and Grandison (1968) attempted to differentiate among races of *D. dipsaci* using serological techniques. Considerable variation occurred in their analyses and it was speculated that this variation was the result of using samples

Table 2. Serological studies of nematodes. The numbers in the table refer to the references at the end of this review.

Nematode	Stages Examined	References
Aphelenchoides ritzemabosi	Mixed	21
Aphelenchus avenae	Mixed	16
Diplogaster sp.	Mixed	16
Ditylenchus dipsaci	Mixed	21, 56
Ditylenchus destructor	Mixed	56
Ditylenchus myceliophagus	Mixed	56
Globodera rostochiensis	Cysts	56
Globodera pallida	Cysts	56
Heterodera betulae	Cysts	44
Heterodera carotae	Cysts	56
Heterodera cruciferae	Cysts	56
Heterodera glycines	Cysts	44
Heterodera goettingiana	Cysts	56
Heterodera schachtii	Cysts	56
Heterodera trifolii	Cysts	56
Meloidogyne incognita	Adult females, larvae, eggs	29, 33, 39
Meloidogyne arenaria	Adult females, larvae, eggs	29, 33, 39
Meloidogyne javanica	Larvae, eggs	39
Panagrellus redivivus	Mixed	16

consisting of mixed stages (adults, larva, and eggs) for analyses. This variation precluded differentiation of the biological races. Another study showed that *A. avenae* was not serologically related to *P. redivinus* or a *Diplogaster* species (El-Sherif and Mai, 1968).

Serology failed to demonstrate great differences in antigens between *M. incognita* and *M. arenaria* (Hussey, 1972). Double-diffusion (Ouchterlony) tests, however, showed that one immunoprecipitate was unique for *M. incognita*, whereas another eight, based on band position and coalescence, were common to both species. The large number of common precipitin bands that formed in the double diffusion tests indicated a close serological relationship between the two *Meloidogyne* species. Preliminary results comparing antigens of *M. hapla* and *M. javanica* with *M. incognita* and *M. arenaria* suggest that *M. javanica* is more closely related to *M. incognita* and *M. arenaria* than is *M. hapla* (Hussey, unpublished results). Fewer precipitin bands were obtained with extracts of *M. hapla* than with those of the other three species, and spurs formed on several of the *M. hapla* precipitin bands that did develop.

Immunoprecipitin patterns comparing multiple antigen systems in Ouchterlony tests often are difficult to interpret. Differences in precipitin bands can be obscured due to the complex nature of a precipitin pattern or to diffuse precipitin bands. Diffuse precipitin bands primarily arise from unbalanced antigen-antibody systems. When multiple antigen systems are used, each reactant cannot be adjusted to its optimum concentration. The greatest number and the best overall sharpness of precipitin bands in Ouchterlony tests

comparing *M. incognita* and *M. arenaria* were obtained when nematode antigen preparations were used undiluted (Hussey, 1972). Two bands near the antiserum well, however, were sharpest when the antigen preparations were diluted 1:1 (v/v), whereas the other bands in the pattern were very weak. The strong precipitin bands coalesced and were not helpful in distinguishing between the two *Meloidogyne* species. More striking serological differences probably would be obtained by making comparisons with specific purified nematode proteins. Multiple antigen systems, therefore, are best analyzed by immunoelectrophoresis where only the presence or absence of antigens between species is noted (Gell, 1968). A larger number of precipitin bands developed when antigens of *M. incognita* and *M. arenaria* were compared by immunodisc electrophoresis rather than by Ouchterlony tests (Hussey *et al*, 1972). Gel double diffusion tests, on the other hand, provide the most reliable information when single kinds of molecules are compared to show their structural similarity (Gell, 1968). Cuticular proteins may be a good source of antigens for such serological studies.

Absorption of antisera to remove antibodies which would react with similar antigens from two *Meloidogyne* species produced a species-specific antiserum (Hussey, 1972). When *M. incognita* antiserum previously absorbed with *M. arenaria* antigens was reacted with homologous and heterologous antigens, a precipitin band formed only between the homologous antigens and antiserum. Absorbed antisera may be useful for identification of *Meloidogyne* species.

Misaghi and McClure (1974) examined the serological relationships of three *Meloidogyne* species using antigens from larvae and eggs. Although a number of precipitin bands was common to all species, larvae and eggs of *M. incognita* possessed one specific precipitin band not present in *M. javanica* or *M. arenaria*. Two precipitin bands were also unique to *M. javanica*. The species-specific antigens were confirmed by cross-absorption tests.

A few serological studies have been done with cyst nematodes. The most notable being that by Webster and Hooper (1968) who compared four species of *Heterodera* and two pathotypes of *Globodera rostochiensis*. The two pathotypes of *G. rostochiensis* were serologically similar and closely related to *H. schachtii* and *H. trifolii*. These three species, however, did not show any serological relationship with *H. cruciferae*, *H. carotae* or *H. goettingiana* which all showed a certain degree of relatedness to each other. In the same study three *Ditylenchus* species were serologically distinct.

The usefulness of serology in distinguishing populations or races of nematodes within a given species is still uncertain. In tests comparing populations of *M. incognita* from Taiwan and Peru, nine distinct precipitin bands formed. All precipitin bands, however, coalesced, indicating that these two populations were serologically similar (Hussey, 1972). Scott and Riggs (1971) tried to use serology to distinguish between two races of *H. glycines* but no differences were detected. Four races of *D. dipasci* also were serologically indistinguishable (Webster and Hooper, 1968).

Serological techniques provide reliable procedures for determining similarities in nematode antigens; hence, these techniques have great potential for elucidating the phylogenetic relationships of nematodes and may facilitate the identification of certain species.

OTHER BIOCHEMICAL ANALYSES

Compounds other than nucleic acids and proteins may have taxonomic value for classification of nematodes, although little attention has been given to investigating other chemical characters for this purpose. In plants, however, a wide variety of chemical substances including phenolics, lipids, waxes, carbohydrates, alkaloids, terpenoids and steroids have provided significant taxonomic evidence (Smith, 1976).

Nematode lipids may have potential in species identification (Hansen and Buecher, 1970), although the fatty acids in adult females and eggs of *M. incognita* and *M. arenaria* were qualitatively identical (Krusberg *et al.*, 1973). Relative quantities of certain fatty acids did differ in these two stages between the two species.

Pyrolysis gas chromatography has been shown to be a reliable method for characterization of certain microorganisms (Derenbach and Ehrhardt, 1975) but this technique has not yet been tested with nematodes.

PROSPECTS

The future for biochemical systematics in nematology appears bright and as more studies are undertaken the full significance of contributions from using this approach to assist in the classification of nematodes will become more obvious. In assessing the current status of biochemical systematics in nematology, a few observations and comments seem appropriate. The studies reviewed herein clearly show that a biochemical approach to nematode taxonomy has considerable potential for assisting in the identification and characterization of these organisms as well as establishing phylogenetic relationships. Nevertheless, nematologists are still in an experimental stage with biochemical systematics. The techniques necessary to work with these small organisms are still evolving and exactly which compounds will have the most taxonomic value with nematodes is not completely known. Several enzymes have already proven beneficial in separating nematode genera and species, but these are only a few of an arsenal of enzymes that are available (Shaw and Koen, 1968). Also Stone (1977) suggests that caution should be exercised in utilizing these new approaches in nematology because to fully understand the significance of a particular character, a new approach should be evaluated by applying it to well established taxa instead of problem areas of nematode taxonomy where it is often applied first. Instead of a cursory examination of different nematode genera where large differences are to be expected, a large number of species within a single genus needs to be carefully studied to critically evaluate the potential and usefulness of biochemical systematics. Thus far only a few species of any particular nematode genus have been studied and, therefore, the techniques have not been thoroughly appraised.

The progress of biochemical approaches, especially gel electrophoresis, in systematics and population genetics has developed rapidly with other organisms (Avise, 1974). The use of gel electrophoresis in multi-loci studies began with *Drosophila* in 1966 (Hubby and Lewontin, 1966), which was the same year electrophoresis was first used to compare proteins of phytoparasitic and microbivorous nematodes (Benton and Myers, 1966). Look at what has been achieved using this tool with other organisms (Avise, 1974) compared to the progress made with nematodes! In nematology, progress has been slow to develop and almost seems destined to continue at this pace. There are too few investigators interested in biochemical systematics of nematodes and those with this interest only seem to be able to spend a small portion of their research time on such studies as no author has published more than two or rarely three papers dealing with this topic. Therefore, I believe the greatest advances in the future will have to come from the effort of a team of scientists—perhaps a taxonomist, a genetist, and a biochemist—cooperatively working on the biochemical systematics and genetics of nematodes. The vast amount of knowledge that is being generated today in each of these fields makes it difficult for a single scientist to have the necessary expertise in all subject areas. An alternative approach would be to entice scientists outside of nematology to include nematodes in their programmes. Some of the difficulties often encountered when working with nematodes, e.g. their small size, the problems in propagating phytoparasites, many species reproducing parthenogenetically, and relatively long life cycles, may, however, make them unattractive organisms for similar studies by other scientists. Nonetheless, opportunities are available for making numerous significant contributions to nematology.

Several important contributions can develop from a bio-

chemical approach to nematode systematics. Nematode species will be more completely characterized and this approach should facilitate their identification. The potential is there for the development of a relatively quick and reliable procedure for identifying nematode species accurately. This is well illustrated by a recent study with strains of *C. briggsae* (Friedman *et al.*, 1977). Four out of five strains received as putative *C. briggsae* were identified as *C. elegans* based primarily on malate dehydrogenase and nonenzymatic protein profiles obtained by gel electrophoresis. Since these two species are separated primarily by bursal ray arrangement in males which are uncommon in cultures, electrophoretic analysis of proteins provided a reliable and accurate means of species identification. The usefulness of identification based on biochemical techniques is augmented by the development of micro gel techniques and would also be aided by the development of species-specific antisera. Knowledge of species and even race distribution is often important with phytoparasitic nematodes. Since nonchemical control strategies (crop rotation and resistant varieties) for phytoparasitic nematodes are becoming more important in agriculture, it is beneficial to determine the distribution of specific nematode species and resistance breaking races. Also, with *Meloidogyne* species, only second-stage larvae are extracted from soil samples and species identification by morphology cannot be made on this stage alone. Since different *Meloidogyne* species are important pathogens on different agronomic crops, the species present in a field needs to be accurately and often rapidly identified. Perhaps in the future, second-stage *Meloidogyne* larvae will be easily identified to species by a simple biochemical procedure. The same is hoped for the identification of physiological races, although separating them biochemically may be more difficult. Avise (1974) has pointed out that multi-loci studies of conspecific populations of amphimictic organisms have shown that they usually have a high percentage of biochemical similarity making it very difficult to identify subspecies biochemically.

In summary, the capabilities of these biochemical techniques to differentiate and characterize nematode species makes them useful; through their application, taxonomic categories can be better defined and ultimately they may expedite nematode identification. The value of these techniques in the taxonomy of other microorganisms, animals, and plants has been examined, and they are considered to be an important adjunct to the classical approach of classifying these organisms (Avise, 1974; Hall, 1969; Leone, 1964; Smith, 1976; Wright, 1974).

The objectives of nematode taxonomy are to obtain a complete understanding of the kinds and diversities of nematodes and their phylogenetic relationships and to present this information in the simplest manner possible (Bird, 1971). Biochemical systematics will undoubtedly play an important role in fulfilling these objectives.

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