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), cytotaxonomy (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). Zuckermand 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
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 of the homologous protein sequences 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 staining solution, 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 staining 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 to form a visible band usually 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, 1968).

Variability by low-speed centrifugation, usual analyses of nematode proteins can occur for many different reasons: (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.

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Stages Examined</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Aphelenchoides fragariae</td>
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</tr>
<tr>
<td>Heterodera glycines</td>
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<td>Heterodera schachtii</td>
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</tr>
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<td>Heterodera trifolii</td>
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<td>Globodera pallida</td>
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<td>Globodera tabacum</td>
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<td>Globodera virginiæ</td>
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<td>Meloidogyne incognita</td>
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<td>Panagrellus silusiae</td>
<td>Mixed, L2, L3, L4, Adult</td>
<td>8, 25</td>
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<td>Pelodera teres</td>
<td>Mixed</td>
<td>25</td>
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<td>Rhabditis terricola</td>
<td>Mixed</td>
<td>25</td>
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</table>

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 Chrumbach 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 non-enzymatic proteins and enzymes of phytoparasitic and micro-bivorous nematode species. In their study six sites of non-specific 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 quantitatively 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 differences 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 Meloidogyne 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 Meloidogyne 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 profiles from IF (electrophoretic mobility value) 0.20-0.60 contained the interspecific differences whereas that segment from IF 0.60-0.76 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 be consistently 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 two species. The three enzymes were considered to have the most taxonomic value for distinguishing between 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 being cultivated. The patterns of enzyme oxidase 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. Isibashi (1970) also reported variation in protein and enzyme patterns of root-knot nematodes as affected by the 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 microelectrophoresis to separate proteins from single nematode specimen. They developed a microtechnique where acrylamide gels are cast as slabs (0.4 mm thick) or as cylinders in microhemocytor 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 where 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). Polycrylamide-gel electrophoresis recently has been utilized to compare the proteins among species of Heteroderidae 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 showed differences but not as extreme. 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.
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carrying the H as well as the H₂ gene. Protein profiles of
pathotype A (now G. rostochiensis) and pathotypes B and E
(now G. pallida) consistently differed by three bands. This was
comororated 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 com-
pared 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 avail-
able 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. dipaci using serological techniques. Con-
siderable 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.

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Stages Examined</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Apherlenchoides ritzemabosi</td>
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<tr>
<td>Apherlenchus avenae</td>
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<td>16</td>
</tr>
<tr>
<td>Diplogaster sp.</td>
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<tr>
<td>Ditylenchus dipsaci</td>
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<td>21, 56</td>
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<td>Ditylenchus destructor</td>
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<td>56</td>
</tr>
<tr>
<td>Ditylenchus myceliophagus</td>
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<td>Heterodera trifolii</td>
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<td>Meloidogyne incognita</td>
<td>Adult females, larvae, eggs</td>
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<td>Panagrellus redivivus</td>
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consisting of mixed stages (adults, larva, and eggs) for
analyses. This variation precluded differentiation of the bio-
logical races. Another study showed that A. avenae was not
serologically related to P. redivivus 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 im-
munoprecipitate was unique for M. incognita, whereas another
tight, 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
dose 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 un-
balanced 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 \textit{M. incognita} and \textit{M. arenaria} were obtained when nematode antigen preparations were used undiluted (Hussey, 1972). Two bands near the antisera well, however, were sharper 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 \textit{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 \textit{M. incognita} and \textit{M. arenaria} were compared by immunodiffusion 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). Cytotoxic proteins may be a good source of antigens for such serological studies.

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

Misaghi and McClure (1974) examined the serological relationships of three \textit{Meloidogyne} species using antigens from larvae and eggs. Although a number of precipitin bands was common to all species, larvae and eggs of \textit{M. incognita} possessed one specific precipitin band not present in \textit{M. javanica} or \textit{M. arenaria}. Two precipitin bands were also unique to \textit{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 \textit{Heterodera} and two pathotypes of \textit{Globodera rostochiensis}. The two pathotypes of \textit{G. rostochiensis} were serologically similar and closely related to \textit{H. schachtii} and \textit{H. trifolii}. These three species, however, did not show any serological relationship with \textit{H. cruciferae}, \textit{H. carota} or \textit{H. goettingiana} which all showed a certain degree of relatedness to each other. In the same study three \textit{Ditylenchus} species were serologically distinct.

The usefulness of serology in distinguishing populations of races of nematodes within a given species is still uncertain. In tests comparing populations of \textit{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 \textit{H. glycines} but no differences were detected. Four races of \textit{D. dipsaci} 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 \textit{M. incognita} and \textit{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.

Pyrolytic gas chromatography has been shown to be a reliable method for characterization of certain microorganisms (Derenbush 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 there 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 it is easily understood 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 \textit{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 geneticist, 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 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 amphi-micetic 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.

REFERENCES


20. Gell, P. G. H., 1968. Serotaxonomy of vertebrate soluble proteins. In: Chemotaxonomy and sero-
toxonomy. Hawkes, J. G. (Editor). N.Y.; Aca-
demic Press. pp. 67-76.
tiation of biological races of Ditylenchus dipsaci. J. Nematol. 14, 184-188.
N.Y.; Am. Elsevier Publishing Co., Inc., pp. 7-149.
25. Gysels, H., 1968. Electrophoretic observations on the protein composition of free-living and plant-
parasitic nematodes, with a special reference to some components showing a digestive activity. Nematologica 14, 489-496.
29. Hussey, R. S., 1972. Serological relationships of Melo-
doidyge inognita and M. arenaria. J. Nematol. 4, 101-104.
31. Hussey, R. S. and Krusberg, L. R., 1971. Disc-
electrophoretic patterns of enzymes and soluble proteins of Ditylenchus dipsaci and D. trifurcos.
J. Nematol. 3, 79-84.
ationship of Meloidogyne inognita, M. javanica, and M. arenaria. Phytopathology 64, 698-701.
44. Scott, H. A. and Riggis, R. D., 1971. Immuno-electro-
phoretic comparisons of three plant-parasitic nematodes. Phytopathology 61, 751-752.
47. Stark, J. L., 1979. Peroxidase isozymes from Melo-
doidyne spp. and their origin. J. Nematol. 11, 1-5.
nematode. Nematologica 18, 591-606.
50. Stone, A. R. and Williams, T. D., 1974. The morph-
52. Triantaphyllou, A. C., 1970. Cytogenetic aspects of
 evolution of the family Heteroderae. J. Nematol. 2, 26-32.
trrophoresis and larval dimensions of British, Dutch and other populations of Heterodera rostochiensis, as evidence of the existence of two species, each with pathotypes. Nematologica 18, 141-148.
56. Webster, J. M. and Hooper, D. J., 1968. Serological and morphological studies on the inter- and intra-
specific differences of plant-parasitic nematodes Heterodera and Ditylenchus. Parasitology 58, 879-891.
59. Wright, C. A., (Editor) 1974. Biochemical and immuno-
60. Zuckermandl, E. and Pauling, L., 1965. Molecules as