Manual of Agricultural Nematology

edited by
William R. Nickle

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Foreword

I. N. Filipjev's seminal *Manual of Agricultural Helminthology* has served nematologists and agriculturalists for many years; however, an updating of the data has been badly needed. For example, this 1941 text cited 19 genera and 185 species of plant parasitic nematodes which has now grown to 207 genera and over 4832 species of plant parasites as of 1990. The present *Manual of Agricultural Nematology* is an attempt to provide a contemporary exposition of the 1941 *Manual of Agricultural Helminthology*.

To achieve this task, W. R. Nickle has assembled an international group of specialists in the field of nematology, each with particular expertise in one or more groups of nematodes or in a pertinent area of nematology such as anatomy, morphology, or systematics. As one would expect, each author has introduced into the text not only expert commentary but also style and a little personality.

This book will neither solve nor amend all the terrible problems incumbent on modern nematology. It will, however, come as close to achieving that task as one can hope for, and it should certainly prove an indispensable tool in any nematological workplace. It has an undeniable international, individualistic flavor, and at the same time is instilled with a logical, well-coordinated sequence of data. Reviewing this book has been both an education and a pleasure.

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Preface

This book is an attempt to update the *Manual of Agricultural Helminthology* written by the Russian I. N. Filipjev in 1934 and translated and revised by Schuurmans Stekhoven, Jr. in 1941. The format is the same; however, because of the current complexity of the science of nematology, 29 carefully selected, internationally eminent contributors have been called upon to prepare this volume. The contributors were asked to present biology and a conservative taxonomic treatment of the main groups of plant and insect parasitic nematodes.

This book has been designed as a reference text and guide for higher level students and workers in nematology, plant pathology, zoology, horticulture, agronomy, and insect pathology. The most comprehensive treatment of nematode races available, it stands as a companion volume to *Plant and Insect Nematodes* (Marcel Dekker, Inc., 1984).

Plant parasitic nematodes cause over $6 billion in losses to agricultural production in the United States each year. Insect parasitic nematodes are beginning to take a more prominent place in biological control of pest insects as we try to reduce the amount of pesticides in our groundwater. Separate chapters are devoted to nematode morphology, biology and ecology, collection and preparation, and the newest methods of nematode identification including DNA fingerprinting and other techniques. Next is a very learned evolutionary treatise on higher level classification. The major groups of nematodes then follow, with the most important groups taken up first. The root-knot nematodes and cyst nematodes, which are the most serious pests, are presented in detail. Other important groups of plant parasitic nematodes follow with aids to their identification and biology. The last three chapters deal with insect parasitic nematodes.

I am indebted to the Agricultural Research Service, United States Department of Agriculture, for the opportunity to work in the science of nematology since 1965 and to the contributors to this book and colleagues around the world who offered their support and encouragement. Several journals and publishers have generously approved the reproduction of some of the illustrations and descriptions presented in the book, and this cooperation is appreciated. I wish to thank my wife, Cathy, for helping with some of the typing.

This book is dedicated to the 29 contributors who took the time to give us their best efforts in the pages that follow.

William R. Nickle

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Manual of Agricultural Nematology

I

GENERAL MORPHOLOGY AND BIOLOGY OF NEMATODES

1

General Nematode Morphology

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I. INTRODUCTION

Understanding of organismal biology advances only where there is substantial knowledge of the form, structure, and function of the embodied taxa. A knowledge of morphology is essential if we are intent on not only entering but contributing to modern biology in aspects seemingly unrelated to nematode morphology. Without morphology there would be no taxonomy and no systematic organization of nematodes in a classification. Such a vacuum would negate studies in population ecology, pathology, physiology, and, to a large extent, genetics where processes could be studied but application would be limited. Molecular diagnostics might show relationships among individuals or populations but we could not comprehend the broader significance of such relationships.

The more that is known about the organisms one is working with the more significant will be any findings, for knowledge leads to confidence, insight, and to the ultimate objective of almost any research—predictability. Predictions have enhanced value when they are consistent with observable facts. Predictions not founded on fact are conjectures that through repetition become obstacles to the advancement of knowledge.

One of the most important contributions morphologists can make to their respective science is the determination and testing of homologous and analogous structures. Comparative morphology reveals the true nature of structures and thus can lead to significant modifications in our understanding of nemic relationships. Elucidating the analogous nature of the postcorporal valves of Plectidae and Rhabditidae led to a revision of the higher classification of Nemata (Maggenti, 1963). Inglis's (1964) comparative study of the head structures of Enoplida resulted in a clearer understanding of the origin and homologies of stomatal structures throughout Nemata. This knowledge along with the work of Baldwin and Hirschmann (1976) resulted in a new interpretation of the stomatal structures in Tylenchina (Maggenti, 1981). Discussions over whether or not a structure is analogous or homologous will be inevitable wherever morphologists gather in conversation; however, comparative morphology remains the bastion which when consistent with observed facts sustains all biological studies.

II. GAMETOGENESIS AND REPRODUCTION

Genetics elucidates the laws by which some aspects of life may be understood. This is the discipline that very often is able to offer explanations for observed biology. Through genetics the mechanisms of inherent patterns in living things are revealed; disclosure of the mechanism established laws such as the laws of inheritance. Biological observation precedes our understanding of the nature and properties of things. Observation forms the basis of biological theories that are, without knowledge of the mechanisms, logical abstractions of reality.

Mendel (1865) formulated the first laws of inheritance and established the basis on which the later developments of genetics have taken place. The work of Mendel lay dormant and unappreciated for more than 30 years; however, the biological observations on the mechanisms underlying Mendel's work were known. Van Benden's work (1883) with the eggs and spermatozoa of *Parascaris equorum* was basic to the doctrine of the genetic continuity of chromosomes. In this same report he described the process of meiosis and the fact that gametes contained only one-half the chromosome complement of somatic cells.

A. Spermatogenesis

The formation of spermatozoa is accomplished by two consecutive reduction divisions (meiosis) (Fig. 1). The process of spermatogenesis begins when the nuclear reticulum of the primary spermatocyte is resolved into the diploid number of chromosomes. During this process the chromatin material is threadlike and may be in the process of doubling. The chromatin threads then come to lie side by side, forming a monoploid set of conjugating homologous pairs of male- and female-derived chromosomes. The paired chromosome threads at this stage are clearly doubled and held together by a centromere. Each original chromosome now becomes four strands associated in a tetrad of four chromatids. In each tetrad two chromatids separate from the other two and thus each chromatid has a pairing partner. At this time, when the chromosomes are shortened and thickened, they can easily be counted and characterized. At the next step in the process the tetrads align at the spermatocytes' equatorial plate. The paired chromatids separate from the other pair of the tetrad and as dyads move to opposite poles of the cell. The cell now divides and the cell so formed has its own complement of dyads. Therefore, each cell has $1n$ chromosome but $2n$ chromatids. The cell is now a secondary spermatocyte.

The process of spermatogenesis proceeds through the second meiotic division. The dyads of the preceeding program align along the equatorial plate. Upon the separation of the chromatids of each dyad the integrity of the complete chromosome is returned. The now distinct chromosomes move to opposite poles of the cell which then divides and the resulting haploid progeny are called spermatids. Thus four spermatids have been derived from the primary spermatocyte.

The formation of spermatozoa involves mitosis, maturation, and morphogenesis. Among Nemata spermatozoa are variable in shape and motility. The mechanism of spermatozoan motility in *Caenorhabditis* has been studied by Roberts and Ward (1982a,b). Their findings indicate that ameboid spermatozoa propel themselves by tip to base flow of membrane over the pseudopod surface i.e., a new membrane component is inserted at the tip of the pseudopod and these components flow backward to be taken up at the base of the pseudopod. Because the pseudopod membrane is continuously being rebuilt at the tip, new attachment sites are created at the leading edge of the cell which is thus propelled forward.

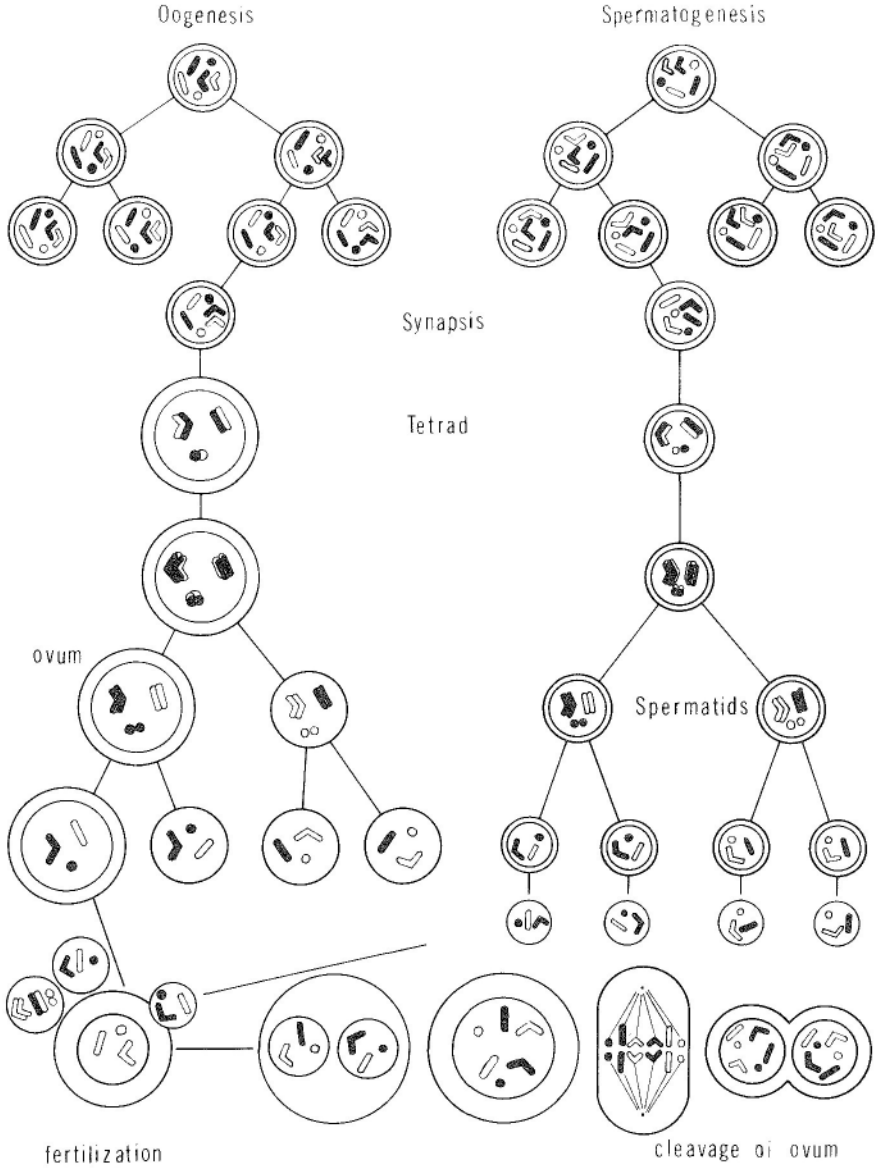

FIGURE 1 Schematic of oogenesis, spermatogensis, fertilization, and cleavage. (Maggenti/Springer-Verlag, 1981; adapted from Huettner.)

B. Oogenesis

A sequence of events similar to that outlined above for spermatogensis occurs in oogenesis (Fig. 1). The chromatin of the primary oocyte forms tetrads which are separated into dyads in the same manner as occurred in the primary spermatocyte. However, one of the cells that is constricted off is called the first polar body. The second maturation division follows the pattern of the first, i.e., the dyads separate into monads. The next division results in the formation of the second polar body and the ovum (egg pronucleus), both of which are haploid.

In nematodes the sperm generally enters the egg prior to or at the time of the second reduction division of the oocyte. At the time of the egg and sperm pronuclei fusion, the eggshell is generally present and the two polar bodies may be seen outside the vitelline membrane.

C. Reproduction

Reproduction in Nemata is sexual. The term asexual should never be used in reference to nematodes or as a synonym for parthenogenesis because the term asexual refers to reproduction by fission, budding, or fragmentation which usually occurs among plants or animals considered evolutionarily lower than nematodes.

Most commonly nematodes are amphigonous (males and females separate) and oviparous (exotokia), meaning that the eggs develop outside the body. Ova are fertilized by sperm stored in a sperm receptacle at the end of the ovary, from sperm in a spermatheca located at the junction of the oviduct and uterus, or, in some monovarial forms, from sperm stored in the vestigial posterior uterus. Though exotoky is common among nematodes, endotoky does occur. When a well-defined eggshell is not seen during embryogenesis and larvae are retained within the uterus until deposited, the condition is called viviparity. When the eggs (normal shell) hatch within the female, the condition is known as ovoviviparity. Larvae may be released in the normal manner; however, larvae may (when females are senile) remain in the body and feed on the decaying female. This phenomenon is often incorrectly designated as *endotokia matricida.* As coined by Seurat (1920) for Heteroderidae and Oxyuridae, endotokia matricida referred to the retention of eggs within the dead female and was not intended to connote matricide.

The most common form of reproduction among nematodes is amphimixis, i.e., the union of two gametes. Parthenogenesis is the second most common form of reproduction and is most often seen as a reproductive strategy among nematode parasites of plants and animals. Three types of parthenogenesis are known among nematodes: meiotic automixis, meiotic parthenogenesis, and mitotic parthenogenesis. Meiotic automixis (facultative meiotic parthenogenesis) occurs when the diploid chromosomal complement is restored in the reduced oocytes by fusion of the second polar body with the egg pronucleus. Meiotic parthenogenesis refers to a reproductive process whereby synapsis takes place and the reduced chromosome number appears at first maturation prophase. The somatic chromosome number (diploid, tetraploid, etc.) is restored by chromosome duplication at anaphase 1; there is no second maturation division. Mitotic parthenogenesis occurs when there is no pairing of homologous chromosomes during prophase and therefore the somatic chromosome number is maintained. The result is the formation of the first polar body and diploid egg pronucleus; from this point embryology proceeds normally.

What may be a precursor step in the development of parthenogenesis is a modified form of parthenogenetic reproduction called pseudogamy or pseudofertilization. In this type of reproduction the sperm enters the oocyte but does not fuse with the pronucleus; it

merely activates further development. The sperm nucleus degenerates and development proceeds by parthenogenesis.

The least common form of reproduction among nematodes is hermaphroditism. It occurs in *Caenorhabditis* (bacterial feeders in nature) (Wood, 1988) and may occur in *Heterogonema* (mermithid parasite of nitidulid beetles) (Van Waerbeke and Remillet, 1973). In both of these genera normal amphimictic reproduction occurs, although less commonly in the former than the latter.

The hermaphrodites of these genera differ: *Caenorhabditis* has two tubular ovotestes, whereas the hermaphrodite of *Heterogonema* has an anterior testis, a posterior ovary, and the secondary sexual characteristics of males (spicules) and normal females occur in the breeding population. *Caenorhabditis* hermaphrodites are self-fertilizing except on those rare occasions when normal males are present and cross-fertilization occurs. On the other hand, in *Heterogonema* cross-fertilization is the rule; self-fertilization, if it occurs, happens only at the end of the hermaphrodite's life when eggs are seen in the posterior female gonad (this may happen by parthenogenesis). Only cross-fertilized females are infective to new coleopteran hosts.

In both genera the form of hermaphroditism seems to serve a senseless purpose. Among other invertebrates, including those considered evolutionarily primitive as compared to nematodes, such as Gastrotrichs, hermaphrodites cross-fertilize each other. Cross-fertilization enhances an organism's reproductive potential in a discontinuous environment and furthermore it assures gene flow as opposed to clonal stagnation. It is interesting that among hermaphrodites gene flow is assured by periodic production of normal males (*Caenorhabditis*) or by cross-fertilization of normal females by hermaphroditic males (*Heterogonema*). Other reports of hermaphroditism among nematodes need to be investigated; the presence of sperm in the spermatheca and the rarity of males does not indicate hermaphroditism. It may only indicate that males are seasonal and sperm can be stored for prolonged periods.

III. EMBRYOGENESIS

Studies of nemic embryology and cell lineage began more than 100 years ago; the most notable effort was that of Boveri in 1892. His study of *Parascaris equorum* is the foundation of modern nematode embryology. Following Boveri were several rather detailed studies and among these the most memorable are the studies of Martini (1903, 1909) and Pai (1927, 1928). Interest in comprehensive studies of embryonic cell lineage waned over the next 50 years and a revitalization did not occur until scientific inquisitiveness was stimulated by the manipulability of *Caenorhabditis elegans* and the availability of modern techniques and equipment. In 1983 Sulston et al. published the embryonic cell lineage of *Caenorhabditis elegans* from zygote to newly hatched larva.

Throughout the years there have been differences of opinion as to what particular cells give rise to in the completed larva. These differences were conceptual rather than based on actual conditions in the given nematode species. This should not be taken as a criticism of early workers. It merely points out that misinterpretations are to be expected when one must follow the development of hundreds of cells.

Caenorhabditis elegans has proven to be an ideal animal for these and other studies because of certain inherent features: it has a short life cycle, it can be easily cultured, it is small enough that large numbers are not a hindrance to laboratory procedures, and it has relatively few cells in the juvenile and mature stages. Embyrogenesis takes about 14 hrs. at 22°C (Wood, 1988), as opposed to 14 days for *Plectus parietinus* (Maggenti, 1961).

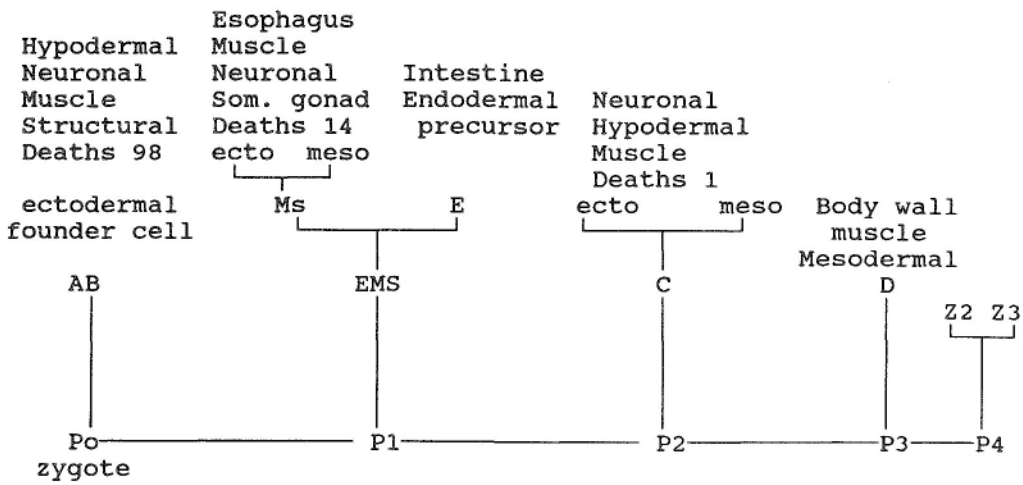

FIGURE 2 Lineage pattern of cleavages in the *Caenorhabditis elegans* embryo. (Modified from Wood, 1988.)

The following summarization of the embryonic development of a *C. elegans* hermaphrodite is based on Wood (1988) and Fig. 2 is a generalized outline of blastomere lineage.

The first cleavage of the ovum (P_0) results in two cells of unequal size. The larger anterior ectodermal founder cell is designated AB (S_1 in traditional nemic embrylogy); the smaller posterior cell (P_1)is the posterior germline cell whose potential is to form the remainder of the embryo. From this point on the cleavages are nonsynchronous. The derivative lineages of cell AB are 254 neuronal cells, 72 hypodermal cells, 23 muscle cells, and 40 other structural cells. In the production of the forgoing there are 98 cell deaths and 389 survivors.

The second cleavage (about 10 min. after the first) results in the second somatic daughter cell designated EMS (ethylmethanesulfonate) and P_2. The EMS cell divides unequally and produces the MS founder cell and the slightly small E founder cell of the mesenteron (20 cells). The MS cell produces 48 muscle cells, 13 neuronal cells, 9 gland cells, 2 somatic gonad cells, and 8 other cells; there are 14 cell deaths and 80 survivors.

Shortly after the formation of EMS, P_2 divides to produce somatic founder cell C and germline cell P_3. C produces 32 muscle cells, 13 hypodermal cells, and 2 neuronal cells; there is 1 death and 47 survivors. P_3 produces founder cell D that gives rise to 20 muscle cells and P_4 that produces the two germinal cells Z2 and Z3.

At eclosion the hermaphroditic larva consists of 558 cells. During embryogenesis 671 cells were formed and of these, 113 underwent programmed death. These cell deaths are specific, autonomous, and invariant from one embryo to the next.

As of now there is no one satisfactory explanation for programmed cell death. The responsible factors could be intrinsic or extrinsic, i.e., cell death could be suicide or murder. The advocates of murder point to the phagocytic activity of surrounding cells. However, phagocytosis can be blocked by mutation and cell death still occurs. There is some evidence that cell death is sex-linked because certain cells die in males that do not die in females and vice versa. Another view is that it is because cell lineage is dichotymous that unnecessary cells or excess cells are produced. Sulston et al. (1983) put forth the idea that cell–cell inter-

actions that were originally essential for developmental decisions have been replaced by autonomous programs that are fast and reliable. In other words, the loss of flexibility was outweighed by efficiency. With this in mind, cell death is a feature that could seemingly be eliminated by a more efficient design—cell death represents a developmental fossil. There may be some circumstantial evidence to support the latter view. Most cell deaths are recorded in the development of the nervous system, and from ancestral to derived forms we see a reduction in the system i.e., the noticeable reduction occurs with tactile sensory hairs from marine to terrestrial nematodes. There is also a simplification of male supplementary organs and a reduction in somatic glands both of which are often associated with simple sensory cells. This circumstantial observation has some support in that most deaths occur in the AB founder line and in the MS founder line, the greatest contributors to the nervous system, somatic musculature, and glands (Fig. 2).

Postembryonically nematodes undergo four molts and pass through five stages, the fifth stage being the adult. Among Adenophorea it is typically the first-stage larva that emerges from the egg. Among Secernentea it is often the second-stage larva that emerges, there being many exceptions among free-living and parasitic Rhabditia; an exception is noted in *Caenorhabditis*, which emerges as a first-stage larva.

IV. INTEGUMENT

The exoskeleton of the nematode consists of the cuticle and its underlying producer, the hypodermis. Together they form a complex organ that protects the animal from detrimental external conditions and functions, in part to maintain the delicate chemical balance internally.

The advent of the electron microscope revived interest in nematode cuticle because it was more easily fixed and less subject to distortion than other tissues of the body. However, it was soon evident that the universal model of nematode cuticle, *Ascaris*, was misleading. As attempts were made to incorporate the two prevalent nomenclatures to the strata of the cuticle, it became apparent that the most studied feature of nematodes was also the least understood. A basic problem is the attempt to apply the same nomenclature to cuticular layers, ignoring the variation occurring throughout Nemata. The number and nature of the layering in *Enoplus* and *Pratylenchus* are not the same; yet attempts are made to apply the same nomenclature and to seek the same number of layers.

The simplistic nomenclature applied to ascarid cuticle simply labels the layers: cortical, matrix (median), and basal. The most comprehensive divides the cuticle into nine layers (Chitwood and Chitwood, 1974): (1) and external cortical layer; (2) an internal cortical layer; (3) a fibrillar layer; (4) a matrix layer (homogeneous layer); (5) a boundary layer; (6–8) external middle and internal fiber layers; and (9) a basal lamella.

Shepherd (1972) proposed a separate nomenclature for Heteroderidae and this scheme was followed by Baldwin (1983). In the Shepherd system the layering is labeled A–D. What needs to be recognized is that this layering has no relationship with the cuticle of *Ascaris* other than the fact that the entire cuticle of Heteroderidae, indeed all Tylenchina, is cortical. There are no fibrillar, matrix, or fiber layers in Tylenchina.

After reviewing the available literature and studying electron micrographs of the cuticle, Maggenti (1979) proposed nomenclature that avoided the traditional designations that had become confused in application. The components of the system were designed after the system applied to other invertebrates. Only those components (strata) that are present are used in a discussion of cuticle. One need not try to apply all nine layers or four strata to every nematode and the system is applicable throughout Nemata. The ancestral cuticle as

seen in *Deontostoma* (Fig. 3) is complete in its strata: epicuticle, exocuticle, mesocuticle, and endocuticle. The crossed-fiber mesocuticlular layer is sometimes replaced by structural struts. Note also that *Ascaris* has no endocuticle and that Tylenchina has neither meso- nor endocuticle. However, there is evidence of sublayering in the tylench exocuticle and this is where the Shepherd–Baldwin system should be employed.

There are few studies on the cuticle that lines the esophagus, vagina, cloacal pouch, and other minor intrusions into the body such as glands or sensory structures. In gross features it appears similar to epi- and exocuticle; however, differential staining shows that there are different isoelectric points between external and internal cuticle. Through differential staining it can also be demonstrated that the cuticle of the cheilostome is more closely related to that of the external body than the esophageal lining (including the esophastome). Other evidence of the differences in external and internal cuticle can be inferred from the virus vectors where the receptor sites vary internally and, as far as is known, are never external.

A. Cuticular Structures

Cuticular structures can be divided into two categories: ornamental and sensory. Those that are classified as ornamentation are not to be interpreted as nonfunctional. Many of these are important taxonomically because they are conservative and biologically because they contribute to the animals' lifestyle, e.g., anterior retrorse spines on animal parasites to give purchase (Fig. 4). Sensory structures are also important taxonomically and they offer a great deal of information about a given taxa's evolutionary state.

B. Ornamentation

There are numerous forms of external cuticular ornamentation and a few that are internal. A common ornamentation seen among Chromadoria and Secernentea is somatic transverse striae; the interstices between striae are called annuli (s. annulus). (The use of annule and annules is incorrect and should be avoided; the word annule does not exist in the English language and is not used in zoology outside of nematology.) In addition to transverse striae there may be longitudinal striae or ridges. These longitudinal ornamentations may be present along with the transverse striae in which case the body facia looks like a corn cob. Striae, either longitudinal or transverse, are rarely seen among Enoplia; however, electron micrographs show that sub-light microscopic striae do occur.

Laterally there may be special longitudinal body striae called the lateral field; these are found among Chromadoria and Secernentea. The number and nature of these striae are commonly used in generic and species characterizations. These lateral longitudinal striae may be broken, diagonal, or joined asymmetrically by other striae (a condition known as areolation). Lateral fields often take on dramatic forms such as extended alae that may be as wide as the body diameter. Lateral alae occur as special structures cervically and caudally. The latter are found only on males.

For the most part male caudal alae are restricted to Secernentea; however, they do occur rarely in Enoplia. Among the animal parasites, especially those in Rhabditia, the caudal ala and its supporting musculosensory rays have great taxonomic significance. In these groups and especially among the strongyles the caudal ala because of the sensory rays and musculature is called bursa copulatrix (Fig. 5B,C). This term should not be used to describe caudal alae outside these specialized groups of nematodes. Though commonly associated with the tylenchid plant parasites, the significance of the form and shape of the caudal alae are only occasionally important in taxonomy. When the caudal alae are restricted to the two

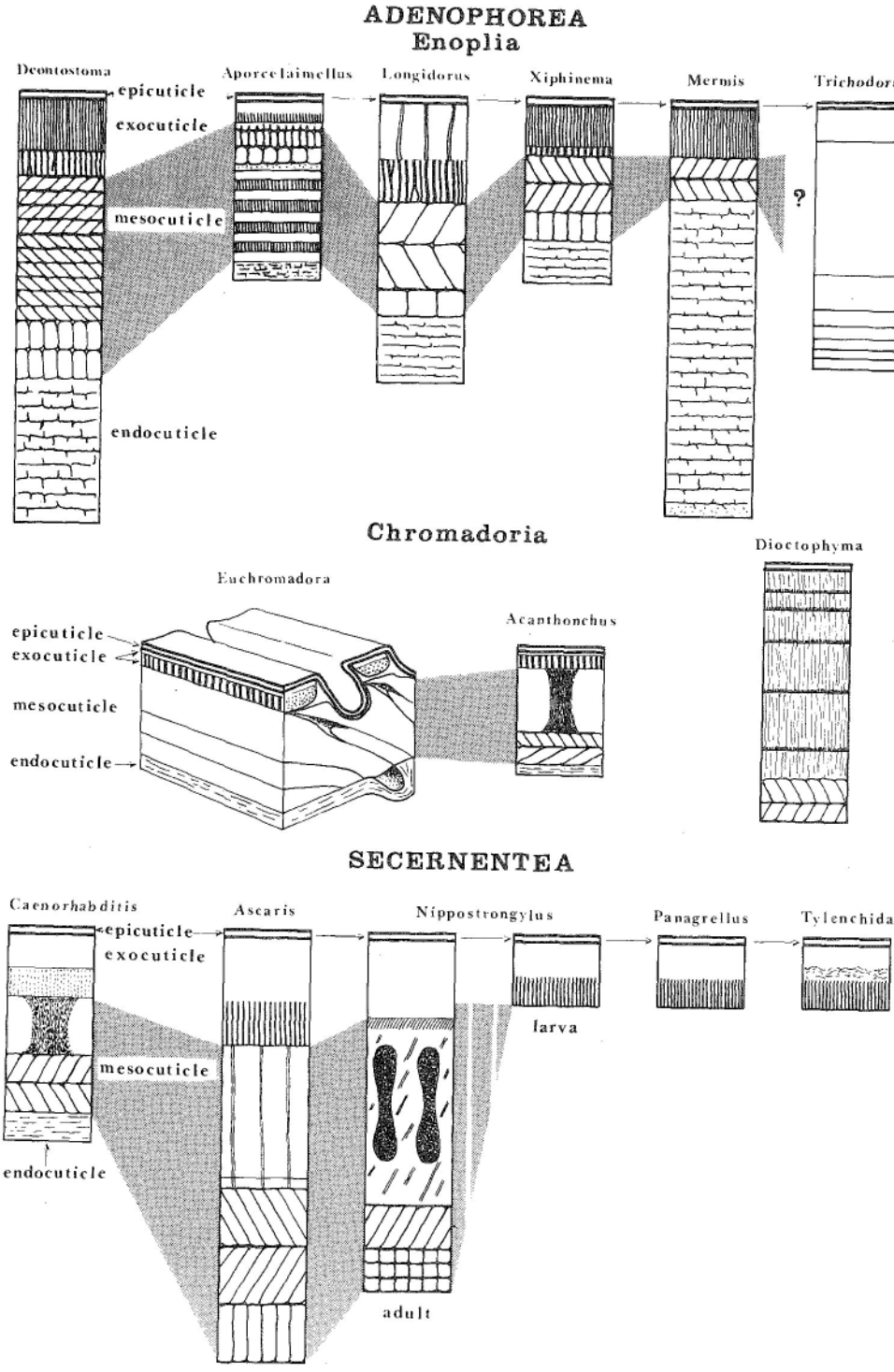

FIGURE 3 Schematic comparison of nematode cuticles and strata relationships. Mesocuticle comparisons are shown by shaded connections. (Maggenti/Springer-Verlag,1981.)

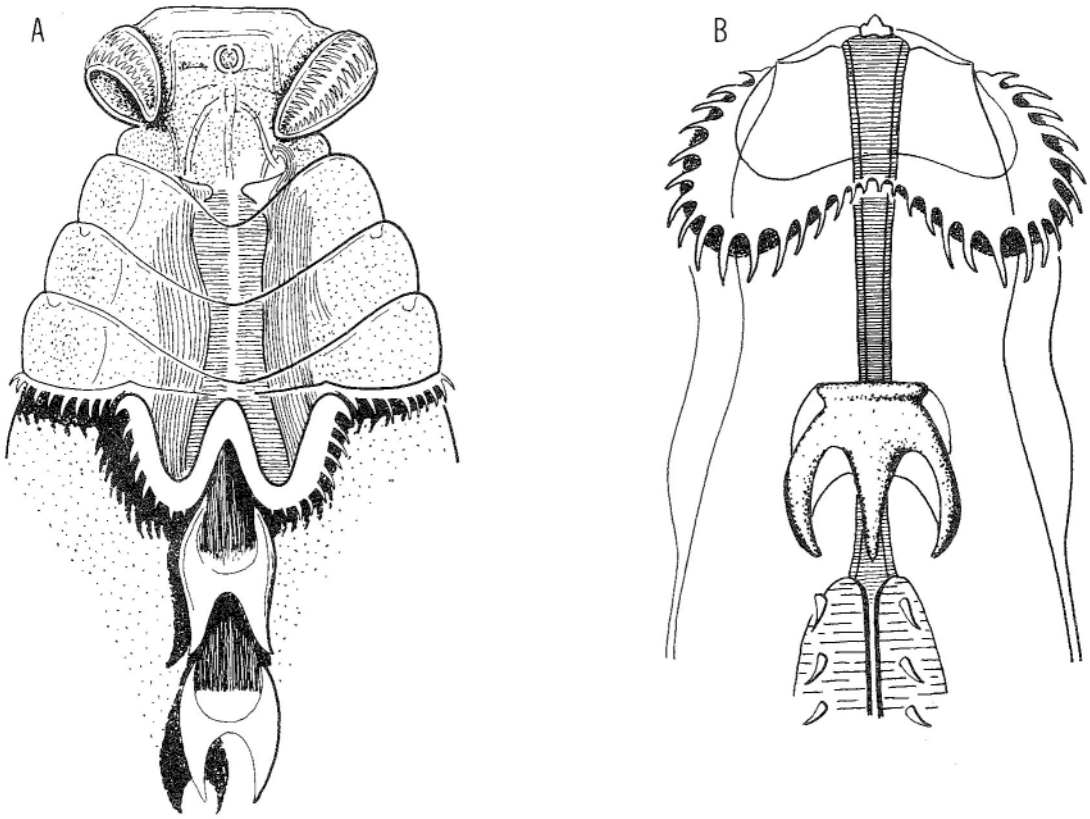

FIGURE 4 Cuticular spines. (A) Oxyuroidea: *Heth* female with lobed collar edged with spines followed by two massive body spines. (Maggenti/Springer-Verlag, 1981; adapted from Steiner.) (B) *Seuratia*, spiny cordons. (Maggenti/Springer-Verlag, 1981; adapted from Seurat.)

sides of the body and do not extend to the tail tip, they are called leptoderan (Fig. 5E). When the alae surround or meet at the tail tip they are called peloderan (Fig. 5A–C). If the caudal alae join both anteriorly and posteriorly (forming a bowl around the cloacal opening), then they are designated as arakoderan (Fig. 5D,F).

C. Spines and Setae

Spines are noncellular cuticular protrusions without muscle or nervous connections. As with most cuticular ornamentations spines are commonly seen among Secernentea, sometimes among Chromadoria, and very rarely in Enoplia. Scales are similar to spines and are distinguished by their bluntness.

Setae and their derivatives are sensory structures. These sensilla are associated with three cells: the tormogen cell that forms the socket, the trichogen cell that forms the seta, and a sensory neuron (Fig. 6A). Sometimes glands are associated with hollow setae (Fig. 6B) and in some instances (*Draconema*) these hollow tubes are long and used in locomotion; these are then called ambulatory setae (Allen and Noffsinger, 1978).

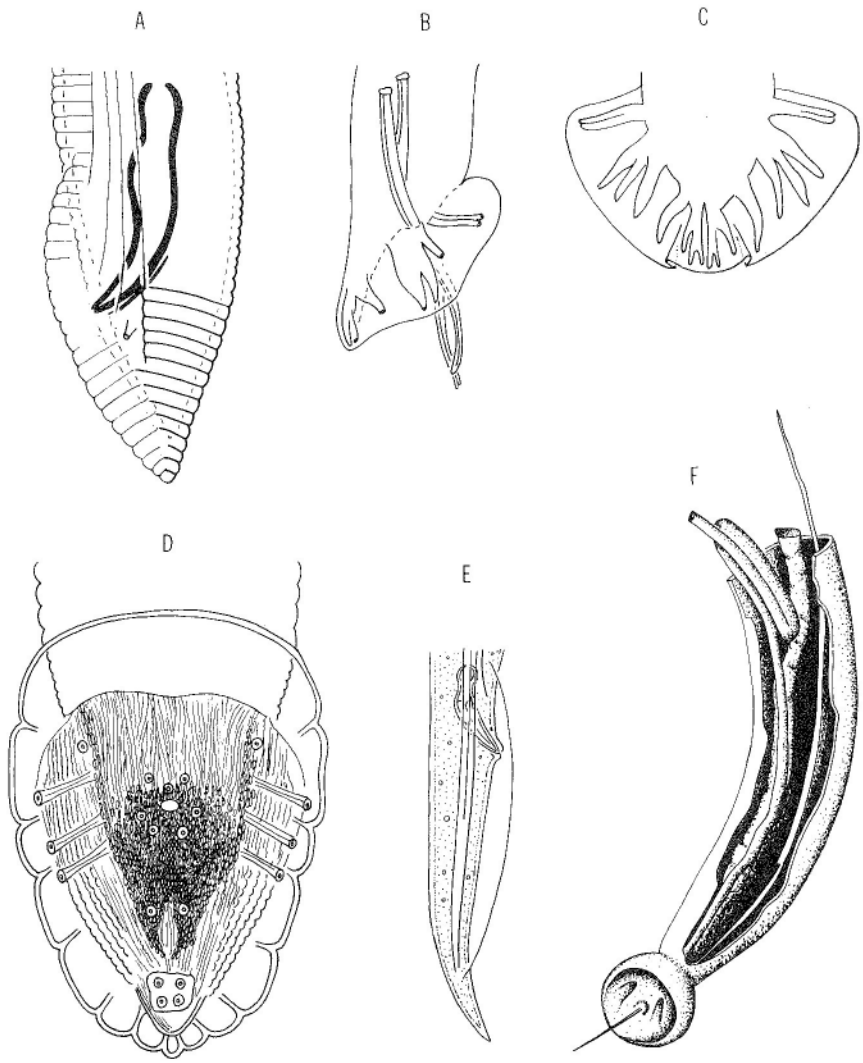

FIGURE 5 Types of caudal alae; Secernentea. (A) Peloderan, Tylenchida: *Rotylenchus*. (Maggenti/Springer-Verlag, 1981; adapted from Thorne.) (B) Peloderan, lateral view with well developed (bursa/caudal alae) bursal rays; Strongylida: *Chabertia*. (Maggenti/Springer-Verlag, 1981; adapted from Yorke and Maplestone.) (C) Peloderan, ventral view with bursal rays; Strongylida: *Delafondia*. (Maggenti/Springer-Verlag, 1981; adapted from Loos.) (D) Arakoderan, ventral view; Strongylida: *Physaloptera turgida*. (Maggenti/Springer-Verlag, 1981; adapted from Chitwood.) (E) Leptoderan, lateral view; Tylenchida: *Ditylenchus*. (Maggenti/Springer-Verlag, 1981; adapted from Thorne.) (F) Arakoderan (bowlshaped); Ascaridida: Dioctophymatoidea. (Maggenti/Springer-Verlag, 1981; adapted from Goeze.)

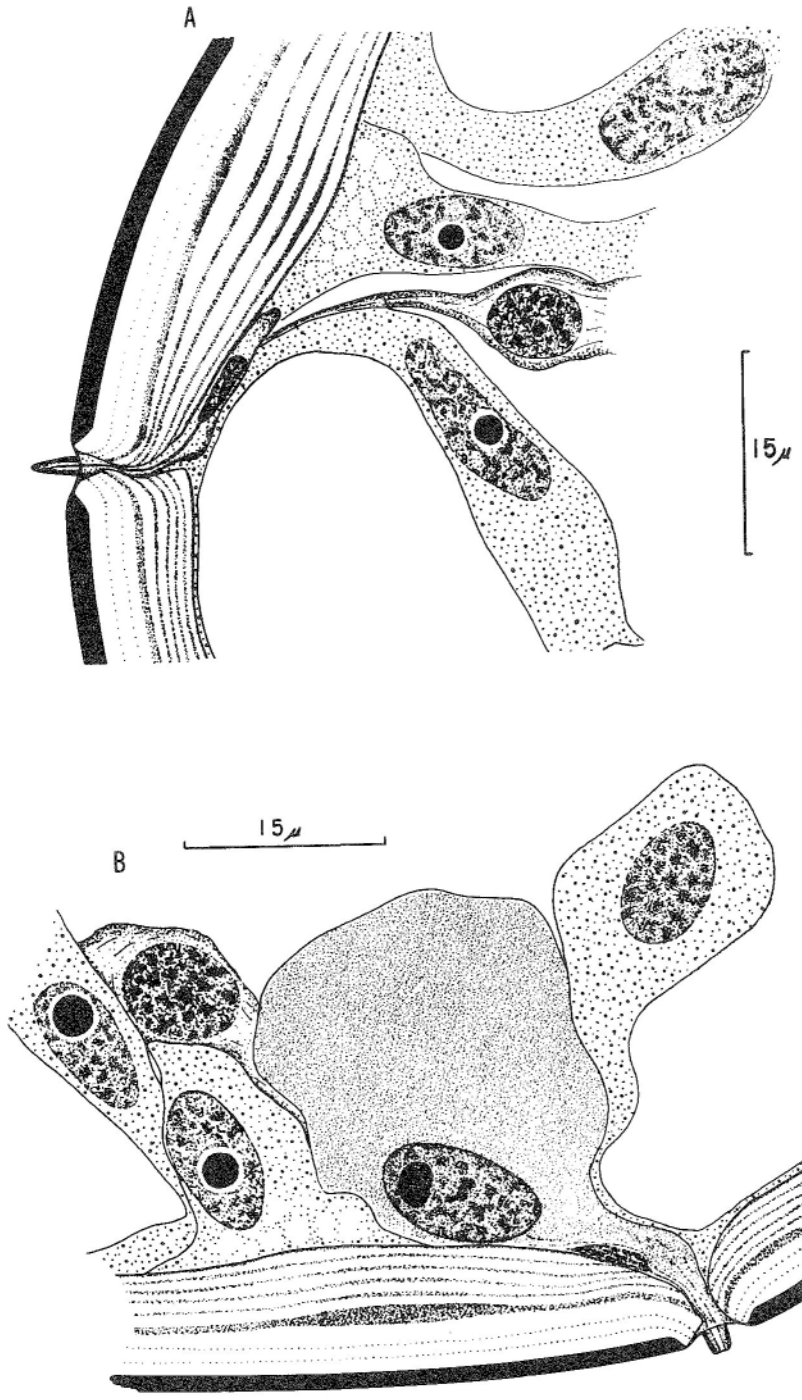

FIGURE 6 Enoplida: *Deontostoma californicum*. (A) Transverse section through sublateral somatic seta showing relationship of hypodermal cells and sensory neurons. (B) Tranverse section through a sublateral hypodermal gland. (Maggenti/Springer-Verlag, 1981.)

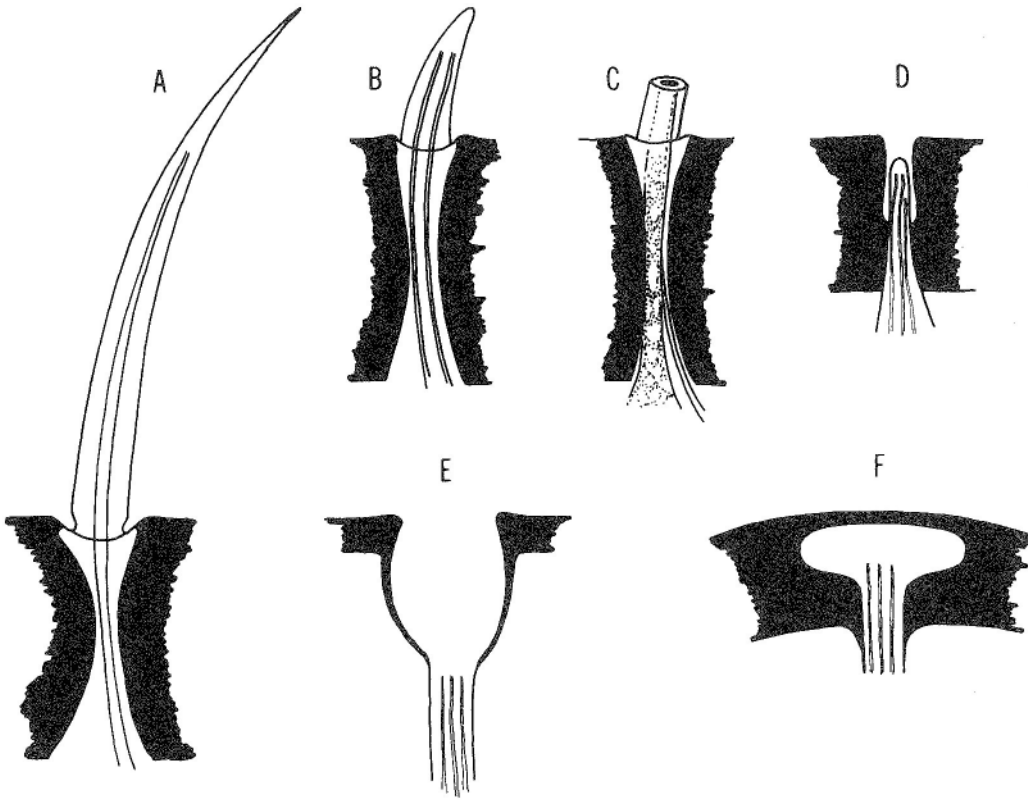

FIGURE 7 Some forms of sensilla found among Nemata. (A) Sensillum trichodeum. (B) Sensillum basiconicum. (C) Sensillum basiconicum. (D) Sensillum coeloconicum. (E) Sensillum ampullastomum. (F) Sensillum insiticii. (Maggenti/Springer-Verlag, 1981.)

Other cuticular ornamentations are cephalic helmets; these are characteristic and prominent in some chromadorids. Similar but only occasionally very prominent is the double cephalic cuticle of enoplids. However, in some groups, such as *Deontostoma*, this cephalic capsule and its fenestrae are highly significant in leptosomatid taxonomy. Tail shape is often an expression of cuticular ornamentation, e.g., long and filamentous, adorned with digits, mucros, etc. Preanal suckers are another feature classed under ornamentation; they are known only among animal parasites and their function is not understood. Suckers are only verified among the Seuratoidea. In this group the sucker is a combination of muscle and glandular tissue.

D. Sensory Structures

There are a variety of morphological manifestations of the sensilla of nematodes (Fig. 7). For the most part the sensilla of nematodes are primary sense cells (Fig. 6A). A primary sense cell receives stimuli either directly or through a distal process. A secondary sense cell is an ectodermal cell that receives stimuli indirectly by way of distal branches of a sensory neuron.

Nematodes exhibit six basic forms of primary sense organs (Maggenti, 1981): sensilla trichodea, with the distal processes elongate setae (Fig. 7A); sensilla basiconica, the distal processes are reduced to pegs or cones (Figs. 7B,C); sensilla coeloconica, the distal processes are peglike and recessed in pores (papillae) (Fig. 7D); sensilla ampullacea are large, or relatively so, deep flask-shaped pouches, such as amphids, that lead to the sensory cell endings (cilia) (Figs. 7D, 8B); sensilla insitica, there is no evidence of external distal processes, the ciliary processes or modified cilia are embedded in the cuticle (Figs. 7F, 8A); sensilla colei, similar to sensilla insitica but the ciliary endings are beneath the cuticle, not embedded in it (Fig. 8B).

These sensory structures of nematodes are important to our understanding of nemic phylogeny. Most notable are the so-called cephalic sensilla that differ by form, number, and placement. There are 16 cephalic sensilla that follow the circlet formula of 6–6–4. The combination and reduction of these sensilla signals derived states in nematode phylogeny (Maggenti, 1981). The use of contemporary cephalic sensilla patterns for interpreting nemic phylogeny utilizes the concept of cephalization. (Cephalization refers to the movement of somatic structures or organs toward the anterior extremity.) Among contemporary Nemata a logical sequence of the cephalization of cephalic sensilla, including the paired amphids, can be developed. Currently, the most ancestral condition of three separate whorls of sensilla, one on the lips and two postlabial, exists among the Oxystominoidea (superorder Marenoplica; order Enoplida). In some taxa of the family Oxystominidae the sensilla are in three separate whorls, are setiform, and are of two forms: sensilla trichodea and sensilla basiconica. Among the Leptosomatidae the first whorl is reduced to six labial sensilla coeloconica and the second and third whorls have combined into 10 postlabial setiform sense organs. The amphids are coincidentally moving anteriorly as well. In the combined state, generally, the organs of the first external whorl are sensilla trichodea and the four of the second external whorl are sensilla basiconica. However, in some taxa (Prismatolaimus) the condition is reversed. The genus *Plectus* shows a further step in that all but the four sensilla of the second external whorl (sensilla basiconica) are on the lip region as sensilla coeloconica. Among Adenophorea the final step is for all sensilla to be on the lips as sensilla coelonconica; however, the amphids always remain postlabial. In Secernentea the sensilla are labial and sensilla coeloconica; the amphids are, with rare exceptions (some larval diplogasterids), also labial. Cephalization among Secernentea is moving toward reduction in sensory organs on the labia to either sensilla insitica or sensilla colei. Among animal parasitic nematodes, especially in Spiruria, it is not uncommon to find only the four sensilla of the second external whorl and the amphids visible. Plant parasitic nematodes vary from all present to 10 or six or only four visible; the amphids remain labial.

E. Amphids

The paired amphids are presumed to be chemoreceptors. Their function has never been verified; nor is it known if they have the same function in all taxa.

Amphids are sensilla ampullacea (Figs. 7E, 8B). The aperture regardless of its external manifestation is followed by a pouch or in some instances a tube in which the ciliary sensors lie. These ciliary sensors are contiguous with the dendritic ending of the amphidial nerve whose neurocytes are clustered posterior to the nerve ring in the laterally located amphidial ganglia. This forms the basic morphology of all amphids.

Phylogenies have been proposed on the basis of amphid aperture morphology (Schuurmans Stekhoven and DeConinck, 1933). These have proven less than satisfactory because the morphological countenance of the external aperture reveals no logical sequential development. Among marine enoplids the amphids are often large and vesiculate but in

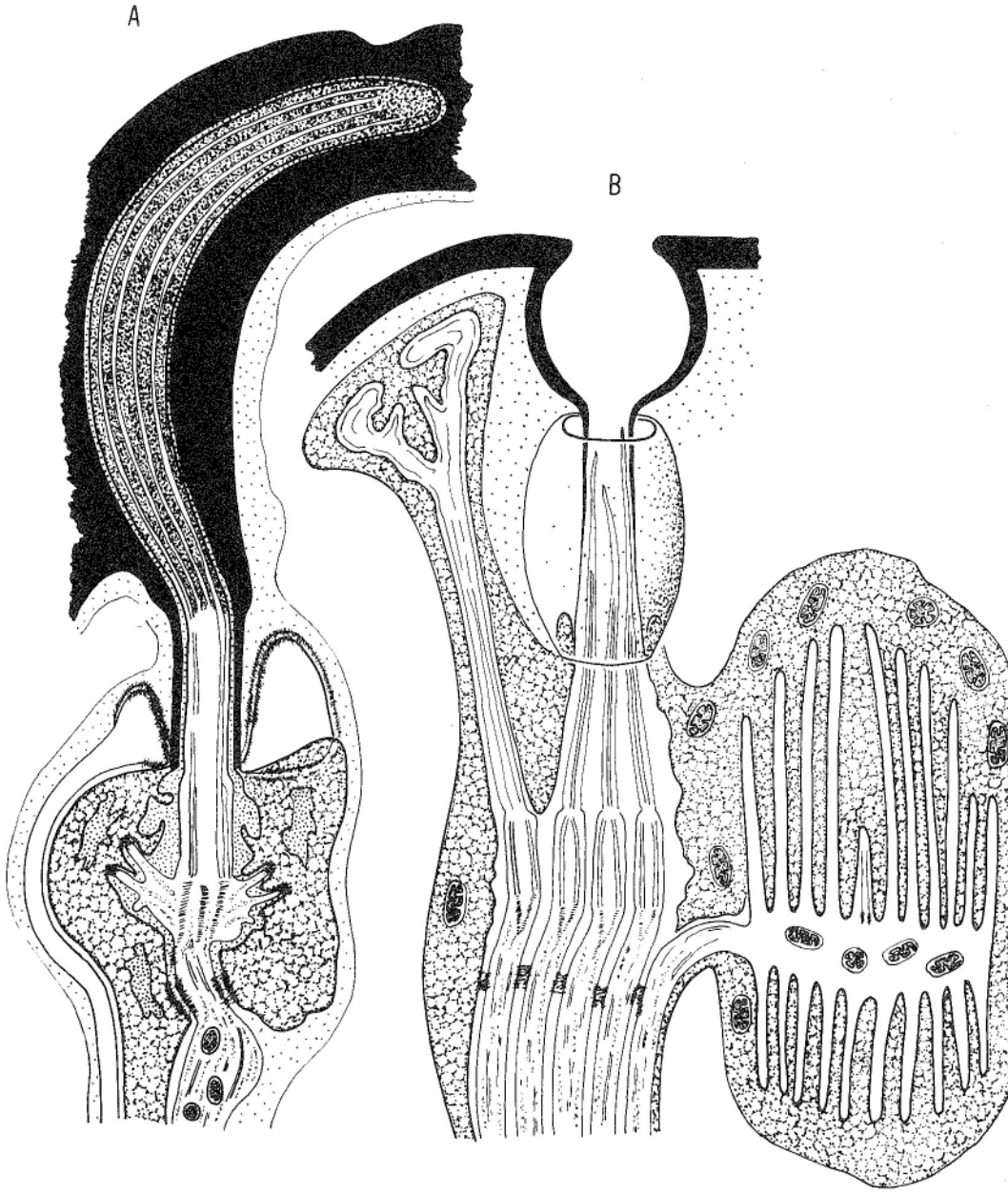

FIGURE 8 Cephalic sensilla of Tylenchida. (A) Sensillum insiticii. (Maggenti/Springer-Verlag, 1981; adapted from DeGrisse.) (B) Sensillum ampullastomum (amphid) with an associated sheath sensillum: sensillum colei. (Maggenti/Springer-Verlag, 1981; adapted from Coomans.)

many instances they are seen as small, hardly discernible oval slits. Terrestrial Enoplia may have slit apertures with large internal pouches so that they appear as inverted stirrups. Still other taxa have simple oval openings with small pouches. The greatest variations are seen among Chromadoria where the apertures range from large and disklike circles through unispirals to multispiral forms. Among Secernentea they vary from porelike, to elongate longitudinal clefts, to relatively large oval pores. It would seem that some extrinsic factor or factors have influenced aperture shape rather than an intrinsic factor that is reflected phylogenetically.

F. Somatic Sensilla

In Adenophorea somatic sensilla are common. This is especially true of marine Enoplia that they have sensory setae scattered along the body sublaterally. In some instances they are combined with glands that open through hollow setae (Fig. 6B). Though somatic sensilla do occur in terrestrial Enoplia, they are generally most obvious on the tail region of females (Fig. 14C). When present along the body they occur most often as papillae or pores. Chromadoria are noted for possessing somatic sensilla, most commonly on the tail of males and females. However, there are some notable exceptions such as *Epsilonema* whose entire body is covered with sensory hairs. It is also among Chromadoria that deirids occur (they are not known from any other group of Adenophorea). These cervical sensory organs are generally found at the level of the nerve ring.

Secernentea are notable for their paucity of somatic sensory organs. The two most commonly observed are deirids and phasmids. Phasmids are similar to amphids or some deirids but are generally confined to the tail region. Phasmids are generally porelike but they may be large and disklike. In addition to their form and size, their location is also variable; in some taxa they are found anterior to the vulva and at different positions on either side of the body. Unusually large phasmids are also found on some vertebrate parasites in Rhabditia and on some earthworm parasites in Spiruiria (Drilonematoidea).

G. Hypodermis

Most hypodermal studies have dealt with Secernentea. As a result a syncytial hypodermis has been misinterpreted as the norm among Nemata. This is not true, as there are no verified findings of multinucleate cells in any taxon of Adenophorea. It may occur to some extent in mermithids but this has not been confirmed (Chitwood, 1974).

In Adenophorea increased body size is accommodated by an increase in the number of individual hypodermal cells. Secernentea accommodate increased body size by syncytial growth of the hypodermis, i.e., nuclear increase without an increase in cell number.

The hypodermis underlies and secretes the cuticle whether it be external or internal. In general, the cell bodies of the hypodermis (where the nuclei are located) protrude into the body cavity dorsally, ventrally, and laterally. The protruding cells are referred to as chords. This nomenclature (and spelling) comes from early morphological studies in which the chords marked the segment of the secant between the muscle intersections with the body curvature. Dorsally the single hypodermal chord normally extends posteriorly only for the length of the esophagus. The ventral chords, in cross-section, may consist of one or two rows of cells. The lateral chords are generally the most prominent and generally they consist of three rows of cells.

From the dorsal, dorsolateral, ventrolateral, and ventral hypodermal cell bodies thin sheets of tissue extend between the cuticle and somatic musculature. The cell bodies that are directly lateral (between the lateral subdorsal/subventral cell bodies) have no sheet like ex-

tension and are called seam cells. The number of cells that make up an individual hypodermal chord longitudinally varies according to the size and species of nematode.

What has here been described as a generalized hypodermis is typical of Adenophorea except for parasitic forms. Among the animal parasitic forms the trend is for an increase in the number of chords, especially in the anterior body. These variations are most commonly seen among mermithids. One variant is to have uninucleate chords as is normal but in addition there are four submedian chords, also uninucleate. In another example there are three rows in each lateral chord, three rows in the dorsal chord, two rows in the ventral chord with two additional chords subventrally; once again all chordal cells are uninucleate. Some chromadorids do show pseudochords in the submedian sectors. This results from the meeting of the subcuticular hypodermal cellular sheets that are then pushed or protruded into the body cavity. Another modification seen among the parasitic adenophores is the bacillary band, which in reality is no more than an "excess" of hypodermal glands mixed with the regular hypodermal cells (Chitwood and Chitwood, 1974). Bands are found on either side or both sides of the body in some Stichosomida.

Among Secernentea multinucleate (syncytial) hypodermal cells are very common. How the syncythium (multinucleate condition) arises has become a matter of disagreement. The majority of observations report that the multinucleate condition results from coenocytic processes, i.e., nuclear division without cell membrane formation. This pheonomenon is commonly seen among animals and occurs in the production of cartilage and connective tissue. The opposing view holds that the multinucleate condition is formed by the amalgamation of separate cells by cell membrane dissolution. This is reportedly the mechanism that occurs in *Caenorhabditis* (Sulston and Horvitz, 1977). Amalgamated cells are not common among plants or animals; among plants certain latex cells are of this type of syncytial development; and some pathological animal tissues are formed in this manner. The most commonly observed syncytial development results from nuclear division without cell membrane formation.

It is disquieting that in the literature on *Caenorhabditis* cell lineage, the initial reports are of nuclei destined to become a part of the lateral syncytia. At this stage there is no reference to cells or cell membranes. It is only after the multinucleate condition is seen that there is reference to formation by cell membrane fusion (Wood, 1988). No mechanistic explanation is given that explains directional cell membrane fusion with immediate cell membrane dissolution! Also there is no explanation given for the fact that the number of nuclei in the multinucleate cell are more reflective of a geometric progression than an arithmetic one: 110 nuclei are reported. Since all divisions are not synchronous, this is still on the order of the expected nuclei number as produced by synchronous geometric progression, i.e., 128. If multinucleate cells are derived from the amalgamation of uninucleate cells, why are the nuclei smaller than those found in nonamalgamated cells? This is not to say dogmatically that the "syncytium" is not formed by cell fusion but that uniqueness among any animal group deserves a very critical viewing. In either event multinucleate hypodermal cells are common among Secernentea. This is one reason put forth for the placement of dioctophymatids in Secernentea: all chords and submedial chords are multinucleate.

H. Molting

Postembryonically nematodes undergo four molts prior to achieving adulthood. The adults do not molt unless one considers *Deladenus siricidicola* as an exception. However, it is not a true molt but a very specialized adaptation to their parasitism of woodwasps. The adult female sheds the final cuticle and the exposed hypodermis develops microvilli whose func-

tion is direct absorption of nutrients from the host hemocele (Riding, 1970). The lack of cuticle also allows the females to undergo inordinate growth.

All known or verified observations of Adenophorea indicate that the first-stage larva emerges from the egg. Secernenteans, on the other hand, may emerge as either a first- or second-stage larva from the egg. In Diplogasteria the emergence of the second-stage larva at hatching is common and apparently it is universal in Tylenchida.

Though frequently observed, molting is among the most poorly understood phenomena in Nemata. Two differing processes of molting are reported for nematodes (Bird and Rogers, 1965; Lee, 1970). In one form of molting the entire cuticle with all layers intact is shed; in the other process only the epicuticle is shed while other "layers" are dissolved. In addition to the above we also know that as the molting process begins there are significant changes in the hypodermis. Increased cellular activity is manifested by a thickening of the hypodermis underlying the cuticle with a concomitant increase in mitochondria and enlargement of the hypodermal cell nuclei and nucleoli.

Prior to the time of the loosening or dissolution of specific cuticular layers, the new epicuticle is layed down completely. This could mean that the molting fluid does not activate until the underlying new cuticle is protected by the epicuticle.

V. SOMATIC MUSCULATURE

The longitudinal obliquely striated somatic muscles are located peripherally and attached to the hypodermis as a single layer of spindle-shaped cells. Most often the muscle cells are separated into four muscle fields by the hypodermal chords: two dorso-and two ventrosubmedian. When there are submedian protrusions of the hypodermis into the body cavity there may be six or eight fields.

Unusual, though not unique to nematodes, is the phenomenon of the muscle sending an elongate "innervation" process from the noncontractile portion of the cell to the central nervous system rather than having nerves extend from the central nervous system to the muscle cell (Fig. 9A). This unusual situation has also been recognized among Echinodermata and Cephalochordata. These extensions are seen throughout the body of the nematode from the nerve ring posteriorly. Anteriorly, because there are no longitudinal nerve cords, the muscle innervation processes enter directly into the nerve ring.

Transmission electron microscopy is antiquating many terms that in the past were applied to the somatic musculature. Some of the terms remain useful as points of reference but they have lost value in the development of phylogenies. Among these are the descriptive morphological terms for the position of the contractile elements in muscle cells: platymyarian, coelomyarian, and, in some restricted instances, circomyarian. The configurations seen in the light microscope are actually formed by the intrusion of the sarcolemma, sarcoplasmic reticulum, or sarcoplasma among the contractile elements of the muscle fiber. *Xiphinema* (Dorylaimid) is described as having coelomyarian musculature, as are *Ascaris* (Ascaridida) and *Deontostoma* (Enoplida), yet each is different. The appearance of coelomyarian muscle "bundles" is created in *Xiphinema* because the contractile elements are separated by fingers of sarcoplasmic reticulum (Fig. 10). *Ascaris*, on the other hand, has discrete elements apically and basally. The same differences occur in so-called platymyarian muscles. In *Desmoscolex* (Desmoscolecida) the contractile fibers are separated by sarcoplastmic reticulum and sarcoplasma. Tylenchida are also described as having platymyarian musculature. However, the contractile elements are not clearly separated but have sarcoplasmic reticulum penetrating the contractile elements irregularly. Two other terms of limited phylogenetic value are meromyarian and polymyarian. These terms refer to

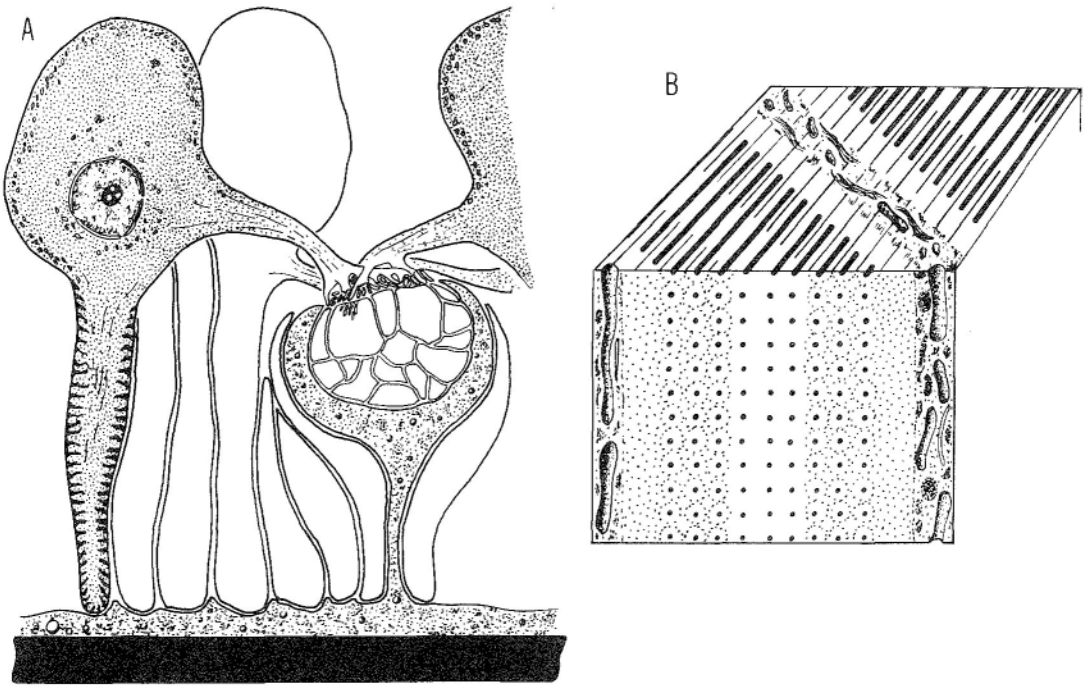

FIGURE 9 (A) Schematic transverse section of muscle cells and their armlike myoneural extensions; the ventral nerve cord is embedded in troughlike hypodermis. (B) Schematic of a muscle fiber showing striation patterns in two planes. The obliqueness is greatly exaggerated in this diagram; in reality, the angle of the striations is less than 6°. (Maggenti/Springer-Verlag, 1981; adapted from Rosenbluth.)

the number of muscle cells in a quadrant; mero- refers generally to less than six and poly- to six or more. These are arbitrary terms. Some authors have made attempts to utilize these conditions as markers for the ancestral and derived states (Chitwood and Chitwood, 1974). The premise was that the ancestral nema was an areolaim, which is meromyarian. However, the nemas now considered as ancestral representatives, oxystominids, are polymyarian-coelomyarian as adults. This proposal breaks down because all known nemas are platymyarian-meromyarian in at least the first juvenile stage. This was pointed out by Filipjev (1934) where he referred to this pheonomenon among derived forms as pedogenesis.

Throughout the body there are specialized somatic muscles that serve special functions or are associated with the secondary sexual organs. Among the better known are the somatointestinal, somatoesophageal, copulatory, bursal, spicular, gubernacular, and vulval muscles. These are generally converted somatic muscles that are recognized as such because the noncontractile portion containing the nucleus is located on the body wall.

The somatic muscles of nematodes are obliquely striated at about 6°. It should be noted that the obliqueness as illustrated by Rosenbluth (1967) is 60° in the figure for convenience of illustration (Fig. 9B). Oblique musculature is known to occur in other invertebrate groups and is typical of the longitudinal tentaclular muscles found in cephalopods. In squids the obliquely striated muscles are capable of a much greater range of lengthening and shortening than the cross-striated perpendicular muscles and they have a faster reaction time. The versatility of movement attributed to tentacles, tongues, and elephant trunks is

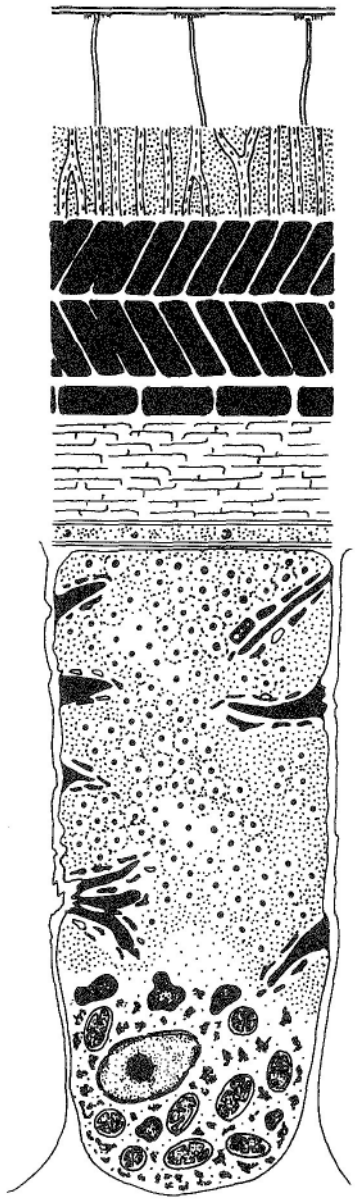

FIGURE 10 Portion of a transverse section through *Longidorus* showing the cuticle, underlying hypodermis, and a transverse section through a muscle cell showing fingerlike extensions of the sarcolemma. (Maggenti/Springer-Verlag, 1981; adapted from Aboul-eid.)

credited to the peripherally arranged longitudinal muscles, and internally perpendicular and transverse muscles that form a muscular hydrostatic skeleton (Smith and Kier, 1989).

Much emphasis has been placed on the role of the hydrostatic skeleton (fluid-filled pseudocoelom) in nematode movement since the introduction of the terminology into nematological literature by Harris and Crofton (1957). In nematodes it is true that a part of the skeleton support system is provided by the liquid-filled pseudocoelom as well as the enteron but such a hydrostatic skeleton alone does not explain the diversity of movement exhibited by nematodes. Perhaps the latter can only be understood when nematode hydrostatics are viewed as intermediate between hydrostat movement as exemplified by polyps along with other wormlike invertebrates with large, fluid-filled cavities/"coeloms," and muscular hydrostats as seen in the organs of cephalods and many mammals. In a muscular hydrostat (Kier and Smith, 1985) the musculature itself effects movement and provides skeletal support for that movement. Skeletal support is provided because the muscle is composed primarily of an incompressible liquid and is thus constant in volume. This mechanism offers advantages over the hydrostatic skeleton, which provides support through large, liquid-filled cavities and thus allows only unlocalized movement.

In muscular hydrostats where structures are capable of complex bending, there are peripherally arranged muscle cells parallel to the long axis (longitudinal muscles). These are arranged helically around the long axis by a slightly offset orientation or by a combination of obliquely striated muscle cells and staggered arrangement that creates a helix. (The somatic musculature of nematodes is peripherally located, longitudinally arranged, obliquely striated, and helically arranged by alternation of muscle cells and by not being oriented on the direct longitudinal axis.)

The most important biomechanical feature of a muscular hydrostat is its constant volume (incompressibility at physiological pressures). Without some means of resisting longitudinal compression, unilateral shortening will not produce bending. Nematodes lack antagonistic muscles and hence bring into play the hydrostatic skeleton and the cuticle to prevent shortening by maintaining a constant diameter. If a constant diameter is not maintained, then the muscular hydrostat organ will shorten but not bend (Smith and Kier, 1989). The helical arrangement allows, through directed contraction along the helix, the animal to twist. The muscle cell arrangement diagrams (Fig. 11) of Ohmori and Ohbayashi (1975) indicate that the nematode could contract a helical in either a left-handed or right-handed direction that would result in a left or right twist.

The foregoing is still speculative for nematodes but does offer an alternate explanation for the diverse movements of nematodes not allowed by a high-pressure hydrostatic skeleton that functions as an antagonist to the longitudinal somatic musculature.

VI. SYSTEMS OF EXCRETION

The cells of the excretory system in nematodes are hypodermal (ectoderm) in origin. The system consists of a sinus cell (= excretory cell, rennete, ventral gland), a pore or socket cell (tormogen); and, depending on the class, collecting tubules may be present. However, within Nemata there are alternate systems of excretion that may or may not involve tissue of hypodermal origin. Therefore, an excretory system is not universal among nematodes but a system of excretion is.

In an orthodox discrete excretory system there are never less than two cells contributing to the form of the organ: the excretory cell and the pore-forming cell. This is the basic system as seen among those Adenophorea that exhibit an excretory system. The system is not universally present among Adenophorea but appears to be constantly present in Secer-

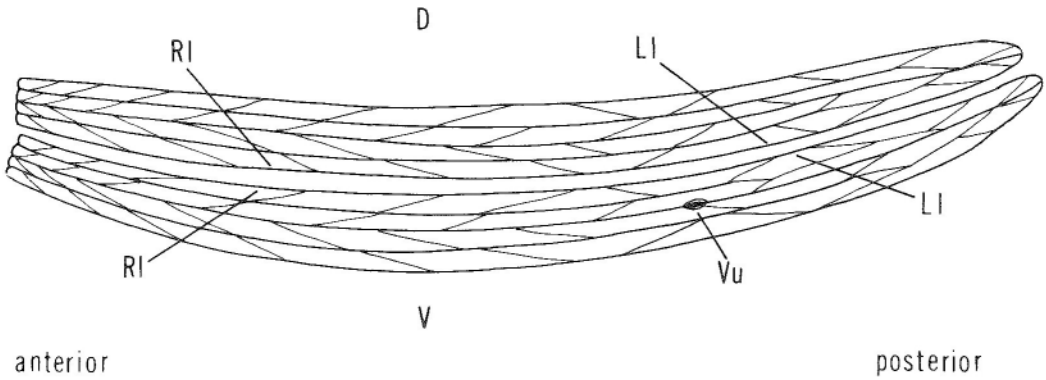

FIGURE 11 Diagram of the muscle cell arrangement in *Decrusia additicta*. The nematode is split through the lateral line. The lower sector represents the ventral portion of the body and the upper section the dorsal sector of the body. Anterior is to the left, posterior to the right. Abbreviations: V, ventromedial line; D, dorsolateral line; Ll, left lateral line; Rl, right lateral line; Vu, vulva. (Maggenti/Springer-Verlag, 1981; adapted from Ohmori and Ohbayashi.)

nentrea where the organ is more complex and may consist of as many as six cells: the pore cell, the duct cell, the excretory cell, tubule cell(s), and fused coelomocytes in the preadult and adult.

Collecting tubules and elongate cuticularized ducts are unknown among Enoplia and in this subclass more taxa possess an excretory system in Marenoplica than among Terrenoplica. In Chromadoria some taxa possess an elongate cuticularized duct (*Plectus*) and some exhibit pseudo-collecting tubules (nonhollow) as are found in *Anonchus*.

It is axiomatic that when an excretory system is absent, as it is in many taxa of Adenophorea, the vital function of excretion must be assumed by some other organ or tissue. There are several candidates among the Adenophorea: hypodermal glands, caudal glands, tubular gland setae, coelomocytes, and the prerectum. It may be coincidental but as the excretory system becomes smaller relative to body size the number of hypodermal glands increases. Numerous hypodermal cells would seem more efficient that a single anteriorly restricted excretory cell.

As previously mentioned, the system in Secernentea appears to be universal but its form is variable. The basic system, possibly the ancestral secernentean system, is found in Rhabditia and is referred to as the "H" system, i.e., consisting of two anteriorly directed and two posteriorly directed collection tubules. All the systems seen in Secernentea are modifications of this condition. All taxa have one or two posteriorly directed collection tubules and these then may or may not be associated with anterior tubules.

In Rhabditia an unusual and unexplained condition is seen; it was first described in depth by Waddell (1968) in the kidney worm *Stephanurus dentatus*. Juveniles, stages 1–3, have two separate subventral coelomocytes that are located near the excretory cell. In the preadult and adult these coelomcytes join the excretory system and are the basis for the observation that the system in rhabdits has three cells. The function of these cells in the early juveniles is unknown; perhaps they have some juvenile hormone role that ceases to function in the pre- and adult stage. Cellular changes in function are known to occur with age in Arthropoda.

Taxa in the order Tylenchida most often have a single collecting tubule that has both an anteriorly and posteriorly directed branch. This tubule may be located on the right or left side of the body. Among the taxa in Tylenchulinae that produce a gelatinous matrix, it is the excretory cell that is the source of the gelatin (Maggenti, 1962). It is not unusual for the excretory cell in these nematodes to occupy one-half or more of the body volume.

VII. ALIMENTARY CANAL

The alimentary canal or enteron of a nematode is divisible into three major sections: the stomodeum, mesenteron, and proctodeum. The stomodeum and the proctodeum are lined with cuticle which is absorbed and/or shed at each molt.

The stomodeum can be subdivided into the stoma, esophagus, and esophagointestinal valve. The lips, even though they are not strictly a part of the stomodeum, will be discussed here. "Lips" is somewhat of a misnomer inasmuch as they are seldom movable except in some marine taxa and animal parasites.

A. Lip Region

Among Adenophorea the labial region is most often distinctly or indistinctly hexaradiate and, as discussed earlier, the lip region may bear from one to all three whorls of cephalic sensilla but never the amphids. The lip region may be smoothly rounded or each sector may be prominent and pyramidal or conical. The shape appears to have more to do with the biology of the taxa than its phylogenetic placement.

Amphid apertures among Adenophorea are highly variable. They are laterally placed and they may be simple ovals, laterally elongate slits, huge dorsoventral ellipses that occupy much of the width of the cervical region, large circles, uni- or multiple spirals, or simple pores. Sometimes the amphid aperture is guarded by a tonguelike or flaplike cuticular accessory piece (Maggenti et al., 1983; Hope, 1988).

There is far more variability in the lip regions of Secernentrea; the basic plan is hexaradiate but in many taxa this is not discernible. Though the lips may not be discernible the cephalic sensilla are, and they do occupy that region designated as labial and in almost all cases the amphidial apertures are also components of the region. Ascarids have three lips, spirurids two, and Diplogasteria exhibit almost all available combinations from hexaradiate to little more than an oral plate.

The plant parasites in Tylenchida display a wide range of en face views. Rarely is a full complement of sensilla visible; most often the second whorl of six sensilla (surrounding the oral opening) and the outer whorl of four sensilla are visible. In many taxa only the four sensilla of the third whorl are seen. DeGrisse et al. (1974) demonstrated that the first and second whorls may subside into the anterior stoma.

Lips as such do not exist in Tylenchida. The lip region is often reduced to an unlobed labial plate or there may be two or six lobes with an undivided oral plate (*Meloidodera*). Terms that have been applied to the various structures associated with the labial region are lobes, pseudolips, liplets, etc. What needs to be done is a systematic morphological comparison of the "labial region" so that homologies and analogies can be determined.

The amphidial apertures are highly variable among the Tylenchida. In the family Tylenchidae the apertures may be elongated sinuous slits extending posterolaterally, or arc-shaped to rounded pits on the labial plate; rarely are they seen as oblique slits on the labial plate. In Anguinidae the apertures are elliptical and directed toward the oral opening. In the remainder of Tylenchina they are round, oval, elliptical, (the latter two are dorsoventrally

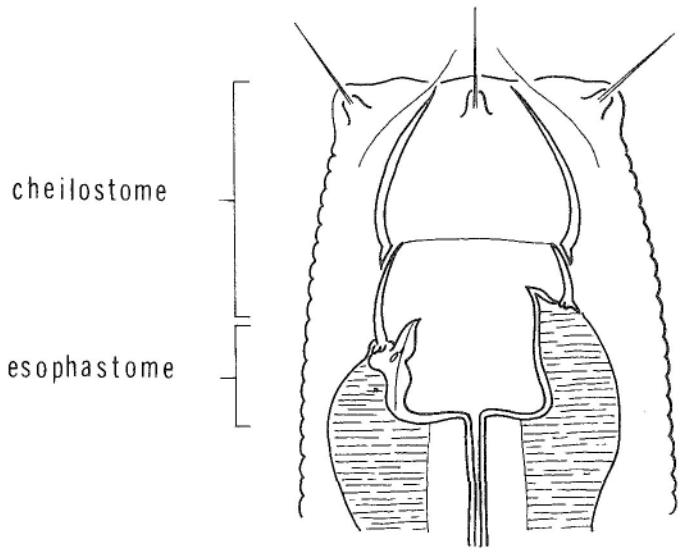

FIGURE 12 Stomatal division in Nemata. (Maggenti/Springer-Verlag, 1981; *Butlerius* head adapted from Goodey.)

directed), and located laterally. Often the amphids are abutting the labial disk and in some instances they are on the disk. The obvious difference among these apertures is their relative dorsoventral length.

B. Stoma

The nematode stoma, though highly variable, has limited phylogenetic value when overviewing the phylum. However, because the form, shape, and armature of the stoma is an indication of the animal's biological habits, its form is often valuable for the separation of lower taxa.

In ancestral nematodes the stoma is part of the primary invagination that forms the esophagus (= pharynx) and is called the esophastome. Generally, these stomas are described as collapsed or undeveloped. However, in some more derived nematodes the esophastome may be highly developed, bulbous, and armed or guarded by teeth (*Bullbodacnitis*; Seuratoidea; Spirurida).

In most nematodes the stoma is constructed of two parts that originate from the primary invagination (esophastome) and a secondary invagination (cheilstome) (Fig. 12). One or both of these invaginations form the stoma throughout Nemata and they do allow comparative homologies to be made. Among derived taxa the cheilostome is generally well developed and contributes to stomatal armature such as teeth, denticles, plates, and stylets (Fig. 13). The foundation of movable armature (though the armature is cheilostomal) is part of the esophastome and the operating musculature is most commonly esophageal; retractor muscles are generally converted somatic muscles. It is for this reason that all movable armature is dorsal or subventral.

The adjudication of which portion of the stoma is cheilostome or esophastome is determined by the anterior-most extent of esophageal tissue. The rhabdion designations of the stoma by Steiner (1933) are not useful in determining homologies because they are ap-

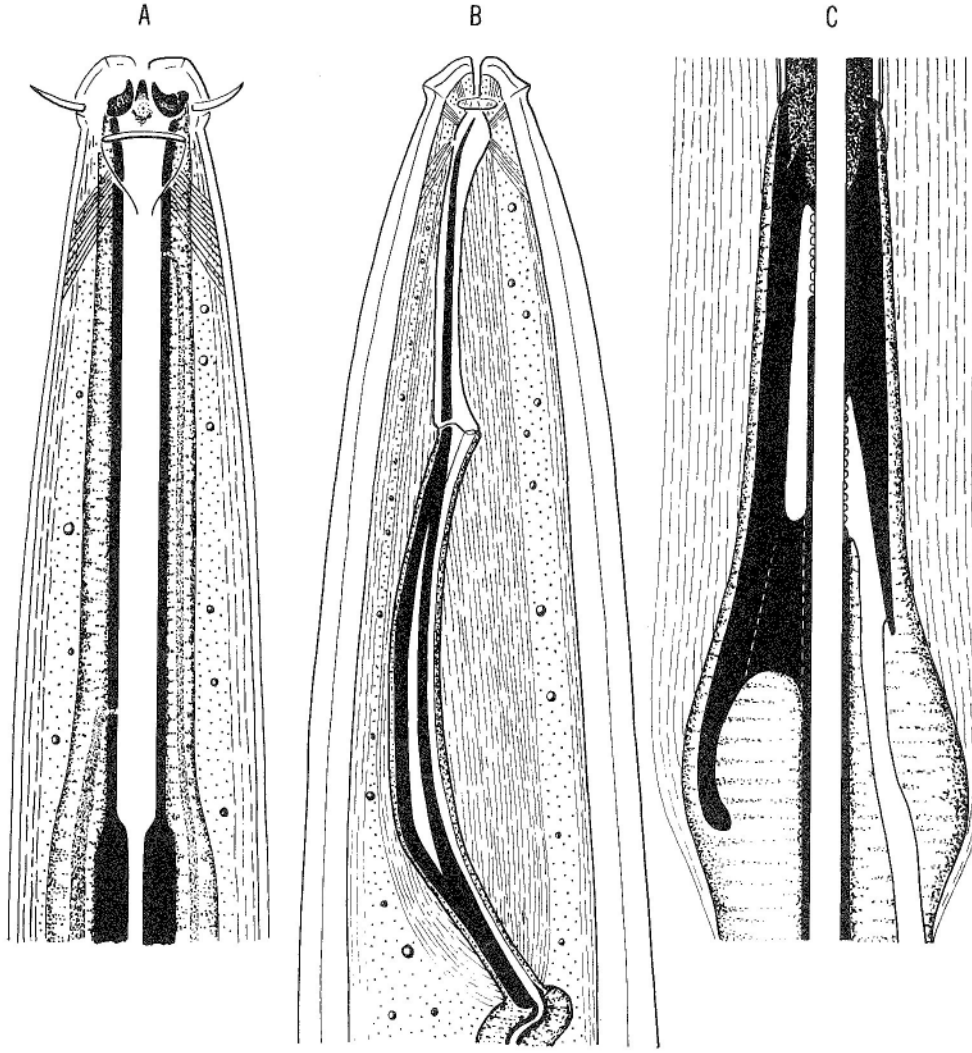

FIGURE 13 (A) Ironidae: *Ironus*; anterior movable teeth, cheilostomal; elongated cylindrical portion of stoma, esophastome. (Maggenti/Springer-Verlag, 1981, adapted from Van der Heiden.) (B) Diphtherophoroidea: *Trichodorus*; the anterior slender portion of the tooth (extending from where the esophageal lumen attaches to the cheilostome) is cheilostomal in origin; the basal portion of the tooth is esophastomal. (Maggenti/Springer-Verlag, 1981, adapted from Allen and Noffsinger.) (C) Esophastomal flanges (odontophore) of *Xiphinema* showing the internal sensory "gustatory" organs. (Maggenti/Springer-Verlag, 1981, adapted from Robertson.)

plied without regard for the rhabdion origin, i.e., whether they are of cheilostomal or esophastomal origin.

Stomatal structure alone cannot determine the feeding habits of a nematode. For example, the variety of stomas found among vertebrate parasites encompasses the array of types seen in predators and bacterial feeders whereas plant parasitic nematodes share stomal characteristics with the cellular (algae and fungi) feeders and predators whose commonality is a hollow (or derivative therefrom) axial spear capable of piercing cells of both lower and higher plants. Such armature occurs independently in both the subclass Enoplia and Diplogasteria.

In Adenophorea plant parasitic nematodes are known only from the order Dorylaimida in the suborders Dorylaimina and Diphtherophorina. The suborder Diptherophorina contains plant parasites that utilize a modified solid "tooth" derived from a hollow spear for feeding on plant roots (Fig. 13B). Features common to all Adenophorean plant parasites are: all are below ground root parasites, all are ectoparasitic, and they all are capable of transmitting specific plant viruses. The hollow axial spear of dorylaims is the basic model for those seen in the plant parasitic Longidoridae (Fig. 14A). The spear is derived from the two stomal sections: the anterior odontostyle is cheilostomal and the posterior odontophore is esophastomal. The specialized cell in the anterior esophagus is hypodermal and belongs to the somatic cheilostomal hypodermis, not to that of the esophagus. The cell is embedded in the anterior esophagus but its opening is at the junction of the cheilostome and esophastome. The ancestral cheilostome remains as the "guiding rings." In reality this structure is the membranous walls of the stoma that extend from the oral opening (may include heavily sclerotized structures as in *Actinolaimus* or *Carcharolaimus*) posteriorly to where it is attached at the base of the odontostyle at the ferrule junction. The illusion of rings is created by the folding of the membrane to accommodate the protrusion of the retractable stylet. Longidoridae differ from this model by great elongation of the odontostyle and odontophore. The latter in some taxa is ornamented by flanges (apodemes for muscle attachment). Within the odontophore of Longidoridae are three sinuses (Fig. 13C) that contain sensory organs that have been designated as gustatory organs (Robertson, 1976).

The "stylet" of the trichodorids is derived from a hollow axial spear that can be seen in the ancestral taxa of Diphtherophorina. Much of the original spear is atrophied back to the membranous cheilstome. However, the dorsal segment (originating from the dorsal odontophore) and the extended cheilostomal "mural tooth" remain to form the feeding apparatus. The ancestral membranous stoma projects posteriorly from the oral opening to the junction of the anterior "tooth" and the dorsal, mural odontophore. From this juncture the lumen of the esophagus is visible running down the ventral surface of the odontophore (Fig. 13B). As in all Dorylaimida the odontostyle (in this instance, the anterior mural tooth) is formed by a specialized cheilostomal hypodermal cell embedded in the anterior esophagus.

The hollow axial spear of Tylenchida does not differ significantly in architecture from that found in Dorylaimida. The tylench spear is thought to be derived from the diplogasterid fossoria. A much closer link is seen in *Neodiplogaster* and *Tylopharynx*; both have stomatal armature reminiscent of the tylench spear (Maggenti, 1963). The cheilostomal structure is somewhat more complex than that described in Longidoridae; in tylenchs it includes the cephalic framework, the stomatal cavity (vestibule), and the spear cone (Fig. 15). The cephalic framework is the anterior modification of the cheilostome that extends like an umbrella over the modified anterior esophagus. The framework has two supposed functions: it supports the labial region as an internal skeleton, and it provides apodemal structure to the protractor muscles of the spear. The so-called guiding apparatus is the ancestral stoma and it extends from the oral opening to the ferrule junction of the ante-

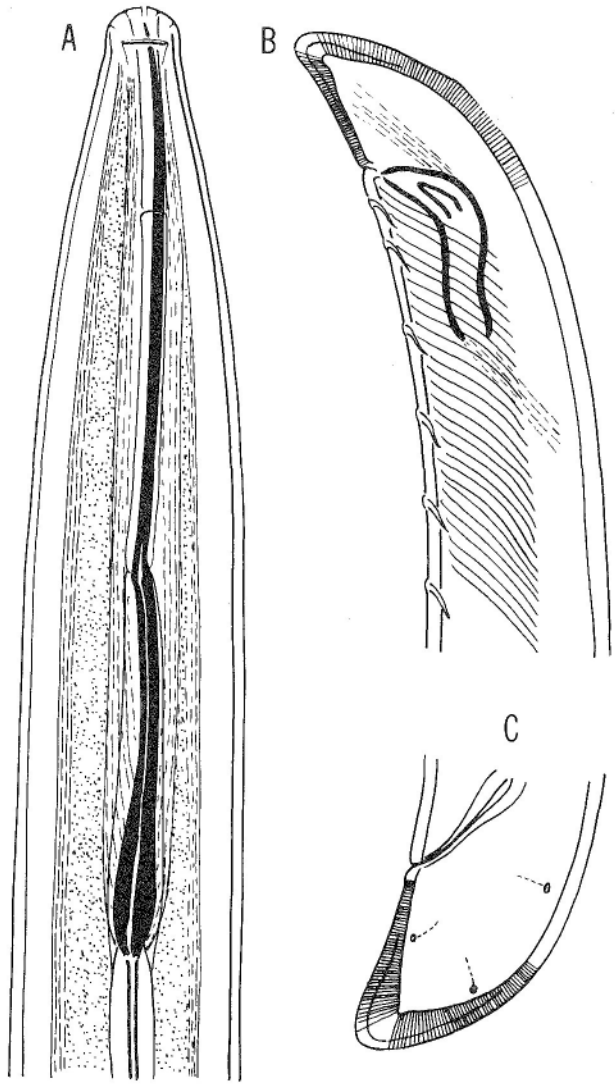

FIGURE 14 *Longidorus* spp. (A) Female anterior. (B) Male tail. (C) Female tail. (Maggenti/ Springer-Verlag, 1981.)

rior cone and the esophastomal shaft. The opening of the spear cone is subterminal and ventral. The esophastome consists of the spear shaft and accompanying apodemal knobs (one dorsal and two subventral) (Fig. 15); these are not well developed or seen at the base of the spear in all taxa. The protractor muscles are modified from the three anterior esophageal muscles. The contractile portion of the muscles have their origin on the knobs and their insertion on cephalic framework and body wall. The noncontractile cell bodies are located in the anterior esophagus. There are no retractor muscles as described for dorylaims.

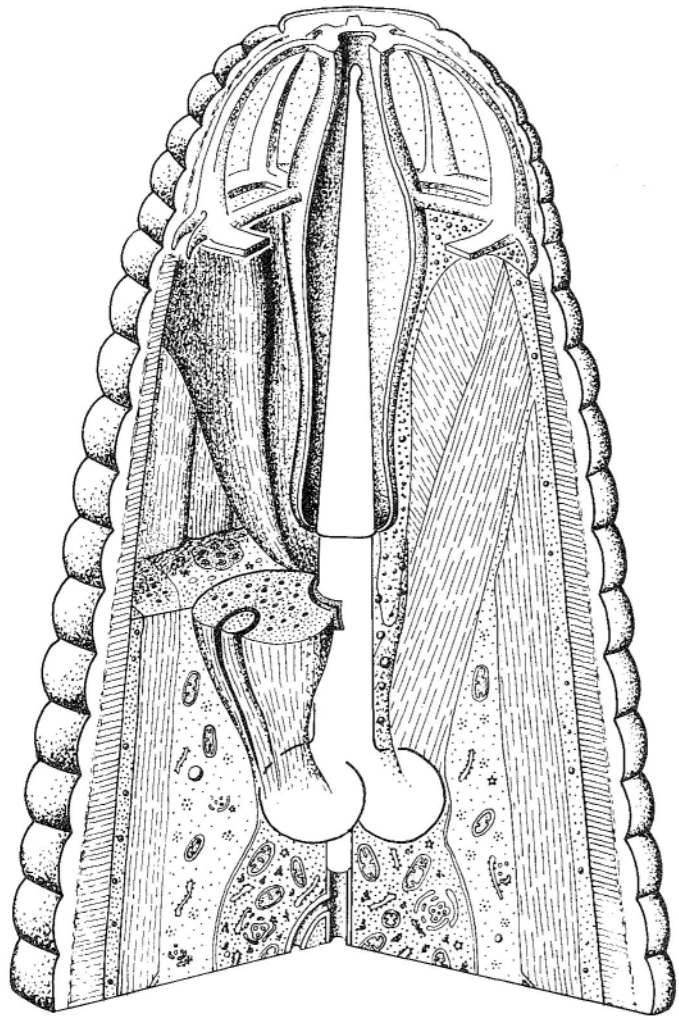

FIGURE 15 A three-dimensional reconstruction of the anterior extremity of Tylenchina illustrating the cheilostome as consisting of the basketlike cephalic framework and the tubelike extension attached to the base of the spear cone (also cheilostomal). These parts are contributed to by the external hypodermis seen lining the right side of the central tube. The shaft and knobs composing the base of the spear are esophageal in origin as are the protractor muscles that are conversions of the three anterior esophageal muscles. (Maggenti/Springer-Verlag, 1981; adapted from Baldwin and Hirschmann.)

C. Esophagus

The second region of the stomodeum is the esophagus or pharnyx. The latter term has had ambiguous use in nematology. Among helminthologists it is used correctly and refers to the esophastome. It also seems misleading to equate the esophagus of nematodes with the mastax (pharnyx, trophi) of rotifers that is designated posteriorly as an esophagus. There is no proof of any homology of the structures other than functionally, i.e., the esophagus transports food from the stoma to the intestine. [The original hypothesis of homology was based on a *Rhabditis/Plectus* ancestor as compared to rotifers. This view of nemic phylogeny was discarded a quarter of a century ago (Maggenti, 1963).] The pharnyx in other invertebrates is a recognizable division of the esophagus that links the stoma and esophagus proper. As such helminthologists are correct when they refer to the esophastome as the pharynx. Confusion was engendered by Hyman's (1951) unsubstantiated claim that the pharynx (mastax) of rotifers is homologous to the esophagus of nematodes and this interpretation was perpetuated by Roggen (1973). Terms that are used to support unfounded evolutionary relationships, such as "pharynx" sensu Hyman–Roggen, purporting homology with rotifers and other so-called pseudocoelomates are misleading and detrimental to the advancement of our knowledge of nematode evolution. Esophagus refers to the primary embryological invagination of the stomodeum and does not imply a single- or multiple-tissue organ. By definition pharynx is, when recognizable, a subdivision of an esophagus. In the absence of any proof of homology with rotifers the term as a substitute for esophagus should be avoided.

The esophagus is the most complex organ in the nematode body. This one organ has nerve, muscle, gland, and hypodermal constituents. Nemic esophagi are highly variable in shape and functional parts. Esophageal form is indicative of the animal's trophic behavior and an important structure in the reconstruction of nemic phylogeny.

In ancestral adenophorean taxa the constituent cells and their nuclei are evenly distributed throughout the esophagus; there is no congregation noted anteriorly or posteriorly (Chitwood and Chitwood, 1974). Externally the esophagus is either cylindrical or long and tapering to a broad posterior that houses the five glands, all of which open posterior to the nerve ring. Derived forms may have the same external shape; however, internally the cell nuclei are aggregated anteriorly and posteriorly. The area that is devoid of nuclei is, in derived taxa, called the isthmus. When the nuclei are so aggregated the anterior region is called the corpus and the posterior region the postcorpus. These two regions are, by rough estimation, marked by the nerve ring. Figure 16 compares these regions in Adenophorea and Secernentea.

Esophageal musculature has been primarily reported on the number of radial nuclei observed. As has been shown with the detailed studies of *Caenorhabditis* (Wood, 1988), nuclear studies can be misleading as to the number of individual cells, since some cells have multiple nuclei. In the ancestral state (Enoplia) there appears to be a total of 36 muscle nuclei in the esophagus. This is a recurring number throughout Adenophorea though the distribution varies. Dorylaims have 24 radial nuclei in the corpus and 12 in the postcorpus. Among chromadorids the distribution of radial nuclei is commonly 12 in the procorpus, 12 in the metacorpus, and 12 in the postcorporal bulb. In Secernentea the number varies with the subclass: Spiruria in general have 36 radial nuclei (18 corpus, 18 postcorpus); Rhabditia 24 (6 procorpus, 6 metacorpus, and 12 in the postcorporal bulb) (20 radial nuclei are reported for *Caenorhabditis*); and Diplogasteria/Tylenchida 12, divided between the procorpus and the metacorpus, and rarely are even remnants of the radial nuclei seen in the glandular postcorpus.

The esophageal nervous system as described in *Caenorhabditis* is fundamentally bilateral in symmetry in contrast to the triradiate symmetry of the esophagus. The neuropile

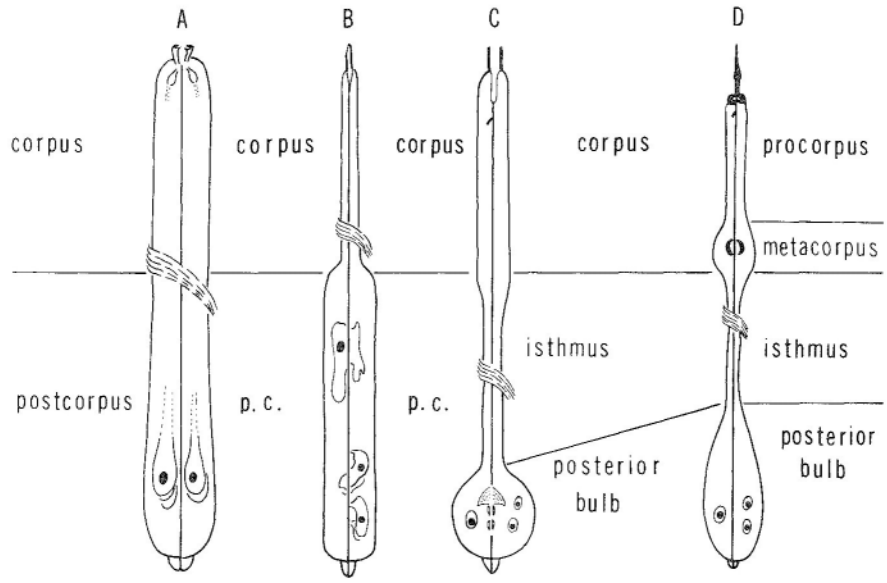

FIGURE 16 Diagramatic comparison of homologous regions of nemic esophagi. (A) Enoplida. (B) Dorylamida. (C) Rhabditida. (D) Tylenchina. (Maggenti/Springer-Verlag, 1981.)

is for the most part organized into two nerve rings, one in the metacorpus and the other in the postcorporal bulb. Connection with the central nervous system is by way of two interneurons that penetrate the basement membrane of the esophagus in the procorpus. White (1988) suggests that the postsynaptic interactions with the nonesophageal nervous system indicates that information flows unidirectionally from the central nervous system to the esophagus. White further reports that with the exception of one motor neuron, "M4," the esophageal nervous system is not required for pumping. Therefore, the system may be for sensory-mediated modulation and inhibition of pumping.

The vertebrate parasites show a similar degree of variation in the external morphological diversity of esophageal form as the so-called free-living taxa. However, plant parasitic nematodes have two basic types of esophagi according to class: a two-part esophagus (Adenophorea) or a three-part esophagus (Secernentea).

The two-part adenophorean esophagus consists of a cylindrical corpus followed by either an elongated cylindrical muscular/glandular region (Longidoridae) or a short pyriform muscular/glandular region (Trichodoridae). (The postcorpus is here designated as muscular/glandular rather than simply glandular as is seen commonly throughout the literature. The distinction is being made here because of the nature of the postcorpus in secernentean plant parasites where the postcorpus is truly glandular.) The generalized esophagus of Dorylaimida contains 24 muscles in the corpus and 12 muscles in the glandular postcorpus. Six of the muscles of the corpus are the protractor muscles of the stomatal armature. The number of glands in the postcorpus varies from three to five; indications are that the plant parasitic Dorylaimida may have five glands: one dorsal and four subventral. In all known instances the orifices for these glands are located near the gland body posterior to the nerve ring.

The three-part esophagus of secernentean plant parasites finds it ancestral form in diplogasteroids. In these forms the esophagus can be subdivided into the corpus (procorpus

and metacorpus), isthmus, and glandular postcorpus (= posterior bulb). The postcorporal glands are variable from three to five, rarely more. Among the derived Tylenchida the metacorporal valve may be present or absent, and the absence does not necessarily denote taxonomic divergence beyond the species or generic level with the exception of Sphaerulariina. Musculature is limited to the 12 muscles of the corpus; there are no muscles in the postcorpus. A recurring phylogenetic development is seen in the enlargement and overlap of the glands constituting the postcorpus. The glands may overlap the anterior intestine slightly or they may extend posteriorly for a considerable distance. This developmental trend is seen in many families of Tylenchina. The assumption is that overlapping glands are indicative of the more derived forms (Luc et al., 1987). In Tylenchina when the stylet is elongated there may be an amalgamation of the procorpus and the metacorpus with the lumen of the procorpus becoming tortuous (*Dolichodorus* in Tylenchoidea and in many taxa of Criconematoidea).

Tylenchoidea and Aphelenchoidea are divergent in some features of their esophagi; these differences along with others are used in separation of the suborders Tylenchina and Aphelenchina. In aphelenchs the dorsal esophageal gland orifice is located in the metacorpus anterior to the valve, whereas in tylenchs the orifice is in the procorpus and generally near the base of the stylet. A "typical" three-part esophagus is seen among aphelenchs only in Paraphelenchidae. The other families in Aphelenchina are distinguished not only by having the postcorpus glands overlapping the intestine but by the glands taking the form of an appendage coming directly from the metacorporal bulb (Aphelenchoidea) or as a distinct cecalike appendage of the columnar isthmus.

D. Mesenteron (Intestine)

The single-layered mesenteron is derived from embryonic endoderm and is the first tissue invaginated during gastrulation. The two parent intestinal cells lie ventrally and posteriorly on the blastula just prior to gastrulation. At the start of gastrulation (Wood, 1988) the two parent intestinal cells sink inward. Later, after the somatic musculature and sex cell have invaginated, the esophageal precursors sink into the interior. Through further divisions of the endoderm and esophageal precursor cells, a central cylinder is formed that in the completed embryo becomes the esophagus and intestine.

Some taxa show subdivisions of the mesenteron and these are designated as the ventricular region, midgut, and prerectum. In totomounts the three regions are seldom recognized. However, the prerectum in Dorylaimida is easily recognized by the conspicuous change in the nature of the constituent cells and their long microvilli. The ventricular region is somewhat arbitrary and is distinguished by packed-cell inclusions and insoluble spherocrystals. Among the plant parasites in the order Tylenchida subdivisions of the mesenteron are not recognized. Animal parasitic nematodes may have the mesenteron separated from the stomodeum and proctodeum, in which case it is a food storage organ and is called a "trophosome."

The ventricular region is in some parasitic forms described as having diverticula (cecae) projecting anteriorly, posteriorly, or in both directions. In all known instances anteriorly directed cecae are ventricular in origin and posteriorly directed cecae may be either ventricular or esophageal.

In all regions of the intestine the internal border of the cells are covered by microvilli which in older literature is referred to as the bacillary layer or brush border. Below the microvilli there may be an area of dense fibrils known as the terminal web which is perforated by cytoplasmic connections to the remainder of the cell cytoplasm. The terminal web

may extend into the base of the microvilli. This condition, though unexplained, was observed with light microscopy and was called the subbacillary layer (Fig. 17A).

The cells throughout the intestine may have the same or differing characteristics, and such conditions are designated homocytous and heterocytous, respectively. If all the cells in cross section are of equal height, then the intestine is isocytous; if different in height the condition is called anisocyty. Nomenclature is also applied to the total number of cells in the intestine: oligocytous, up to 128 cells; polycytous, 256 to 8192 cells;and myriocytous, 16,384 cells or more. These figures are based on theoretical divisions of the endoderm and the actual numbers are seldom achieved. Cell shape is also affected by number (Fig. 18): Oligocytous intestinal cells are longitudinally elongate and rectangular (64) (Fig. 18B, C, E, F) to hexagonal (128); polycytous intestines have cuboidal cells (Fig. 18A) and myrioytous intestines have tall columnar cells (Fig. 18D). The shape of the intestinal lumen is also dictated by the number of intestinal cells: oligocytous, cylindrical/rounded lumen; polycytous, subpolygonal lumen; myriocytous, the lumen is a multifolded or flattened tube. Intestinal cell descriptions are further complicated by being uninucleate to polynucleate. The latter condition is only known among mermithids in Adenophorea but is common among Secernentea. Syncytial intestines are reported for the highly derived secernentean plant parasites such as *Meloidogyne*.

E. Proctodeum

The proctodeum is the posterior ectodermal invagination counterpart to the anterior ectodermal invagination of the stomodeum (Fig. 17B). In cross-section the rectum (proctodeum) is flattened subtriangular, or an irregular tube that anteriorly is surrounded by a sphincter muscle and posteriorly ends at the surface orifice, the anus. In addition to the sphincter muscle, internally there may be an intestinorectal valve (= pylorus), formed from intestinal epithelium. Two muscles may be associated with the rectum, the uncommon dilator ani and the universal depressor ani (the H-shaped muscle). In association there may be rectal glands, six in the male and three in the female. An exception to this occurs in mature females of *Meloidogyne* (Tylenchina) there are six rectal glands that produce the gelatinous matrix. The proctodeum in males is complicated by the secondary sexual organs and will be discussed with the reproductive system. Suffice it to say here that the proctodeum of males joins the reproductive system to form a cloaca.

VIII. REPRODUCTIVE SYSTEM

Nematodes are mostly dioeceous i.e., ordinarily only one sex is represented in any one individual. A few rare instances are known of hermaphroditic nematodes in which both sexes are represented. The best known example is *Caenorhabditis elegans*. The reproductive system of nematodes is quite similar in both sexes (Figs. 19 and 20) and is not unlike a single ovariole or testicular tubule of arthropods. Generally the reproductive system is composed of one or two (rarely multiple) tubular gonads.

The complete reproductive system consists of the primary sex organs and the secondary sex organs. The primary sex organs are mesodermal in embryonic origin. The mesodermal parts of the genitalia not only house the germ cells but also provide for their development and nutrition. The secondary sex organs are ectodermal in origin and are produced by invaginations of the body wall.

Sexual dimorphism, other than the secondary sex structures, is not a common feature of nematodes. When it does occur it is most often evident among parasitic groups and

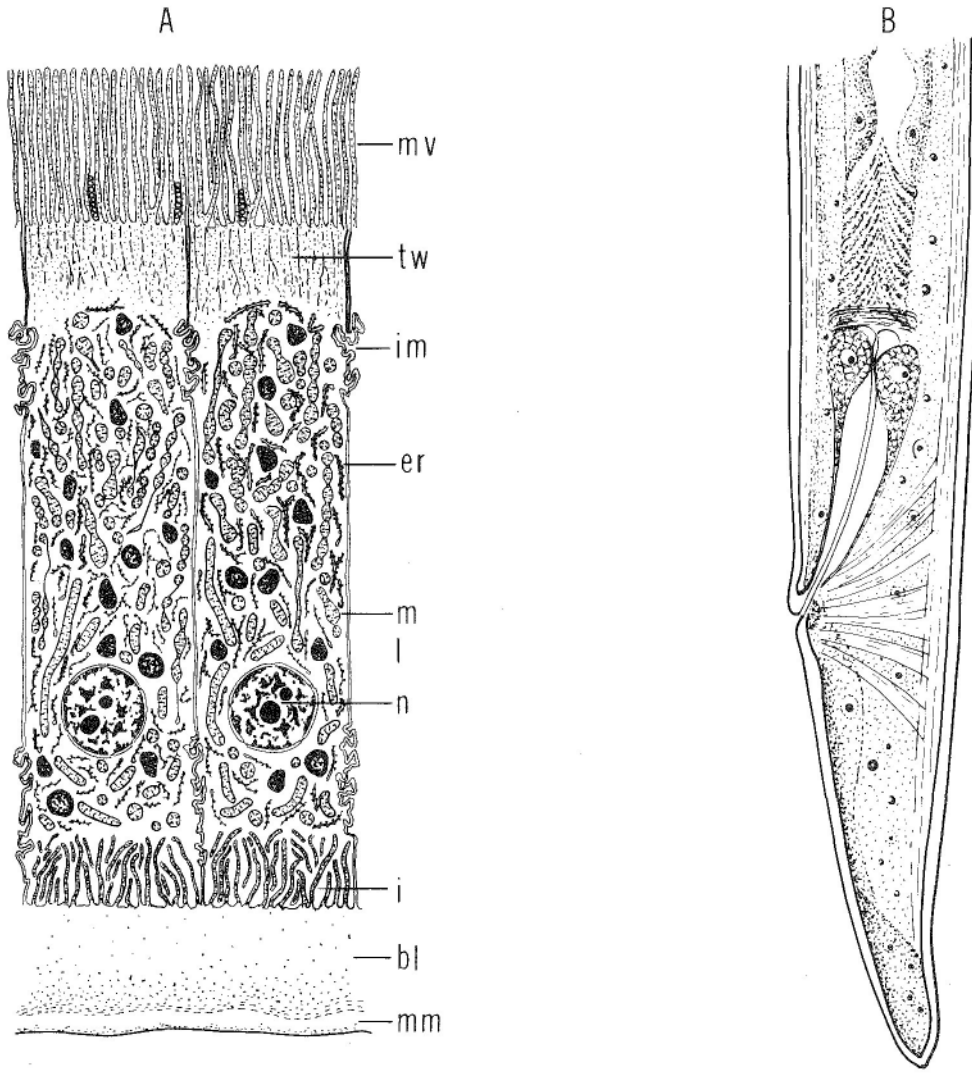

FIGURE 17 (A) Intestinal cells of *Ascaris*. Abbreviations: mv, microvilli; tw, terminal web; im, infolding of cell membrane; er, endoplasmic reticulum; m, mitochrondria; l, lipid inclusions; n, nucleus; i, infoldings of plasma membrane; bl, basal lamella; mm, mesenterial membrane. (Maggenti/Springer-Verlag 1981; adapted from Kessel et al.) (B) Female tail of *Bulbodacntis* showing intestinorectal valve, sphincter muscle, rectal glands, and depressor ani muscle. (Maggenti/Springer-Verlag, 1981.)

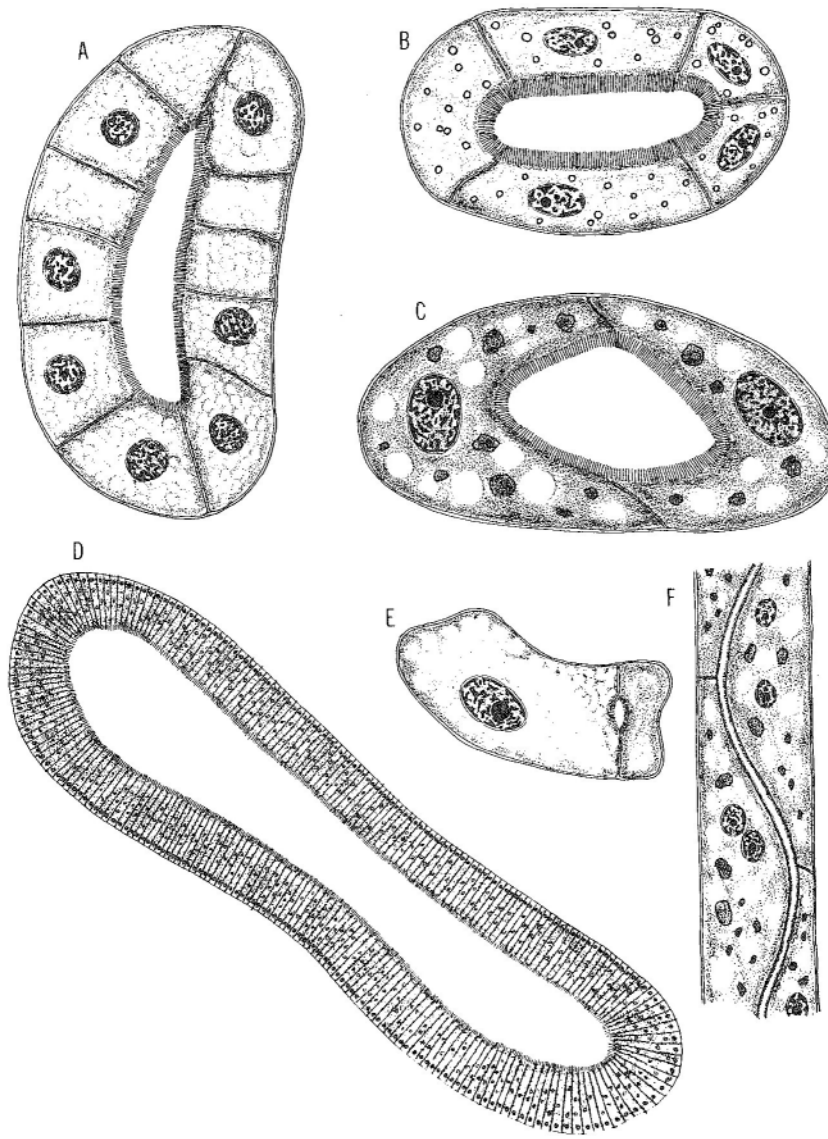

FIGURE 18 Histological sections of nemic intestines. (A) Enoplia: *Deontostoma.* (B) Chromadoria: *Axonolaimus.* (C) Rhabditia: *Rhabditis.* (D) Spiruria: *Ascaris.* (E) Diplogasteria: *Ditylenchus.* (F) Diplogasteria: *Ditylenchus* (longitudinal section). (Maggenti/Springer-Verlag, 1981; adapted from Chitwood and Chitwood.)

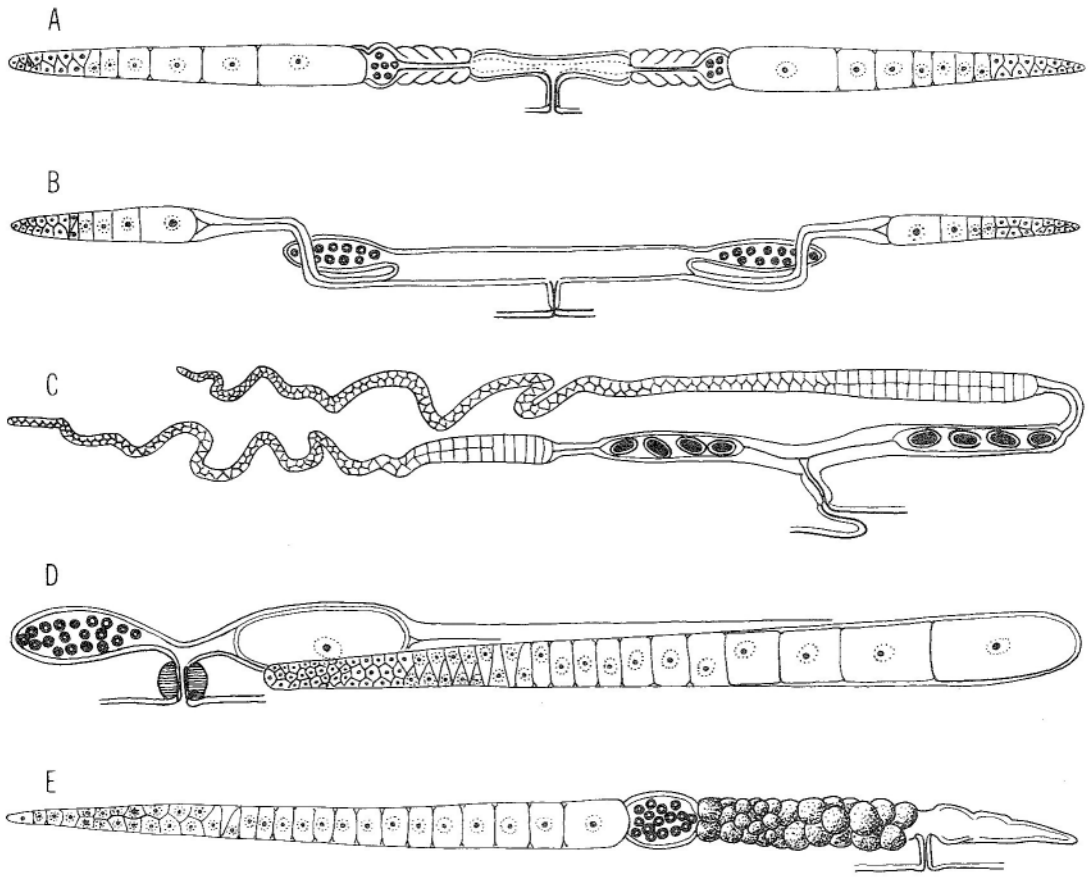

FIGURE 19 Amphidelphic female reproductive systems. (A) Ovaries outstretched. (B) Specialized spermatheca between oviduct and uterus. (C) Anterior ovary outstretched with flexures; posterior ovary reflexed and anteriorly directed with flexures. (D) Amphidelphic postvulval reproductive system (postpudenal). (E) Amphidelphic prevulval reproductive system (antepudendal). (Maggenti/Springer-Verlag, 1981; adapted from Chitwood and Chitwood.)

rarely in freeliving, most often marine, taxa. The commonest example is the swollen saccate female in contrast to the vermiform male. Other illustrations of sexual dimorphism among nematodes include atrophy of the male feeding apparatus, notable differentiation of cuticular ornamentation or sense organs (amphids), and the degeneration of the female into a reproductive sac or the prolapse and growth of the reproductive system independent of the female body (*Sphaerularia bombi*).

Aberrant individuals that are gynandromorphs (individuals in which male and female characteristics or structures occur) are not uncommon. Generally, the female gonad is complete and only portions of the male secondary sexual characteristics are present. These individuals are not hermaphrodites.

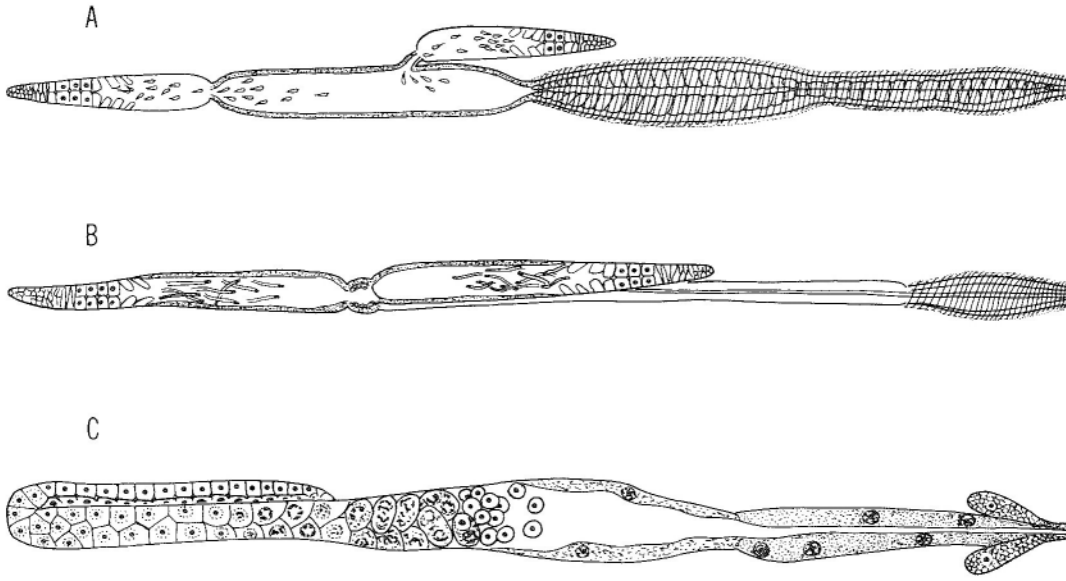

FIGURE 20 Male reproductive systems. (A) Enoplia: *Enoplus*; seminal vesicle and vas deferens heavily muscled. (B) Chromadoria: *Tobrilus*; musculature limited to vas deferens. (C) Secernentea: *Rhabditis*; musculature lacking but glands present. (Maggenti/Springer-Verlag, 1981; adapted from Chitwood and Chitwood.)

A. Female Reproductive System

The typical system consists of two ovaries, one anterior and one posterior (Fig. 19A–C). The ovaries are proximally connected to their respective oviduct and uterus, which in turn are connected proximally to the cuticularly lined vagina that terminates at the vulva. The vulva is generally located at or near the midbody. There are many variants from this typical plan; the ovaries may be outstretched or reflexed; there may be one, two, or more ovaries (32 in *Placentonema gigantissima*). A spermatheca may be present at the junction of the ovary and oviduct or at the junction of oviduct and uterus, the former being the most common. The uterus may be divided into columned uterus (= tricolumella, quadricollumella) and uterus vera; the vagina may also be divided, generally in parasitic taxa, into a vagina uterina (mesodermal) and vagina vera (ectodermal). When only one complete gonad is present, then the vestigial uterus of the second gonad may act as a seminal receptacle for sperm storage (*Ditylenchus, Anguina*) even though a functional spermatheca may be present. The ovaries of nematodes are of three types: panoistic, teleotrophic, and hologonic. The most common ovarial type is the panoistic ovary in which new germ cells originate at the blind distal end from one cell. *Caenorhabditis* and *Ascaris* are examples of teleotropic ovaries. New germ cells are produced in the same manner as in the panoistic ovary; however, the developing oocytes receive nutrients from the central rachis rather than through the single-layered epithelial covering that absorbs nutrients from the body cavity. The attachment of the rachis persists until the oocytes are ready for fertilization. In a hologonic ovary (*Trichuris*) the germinal cells reportedly are proliferated from a series of germinal areas extending the length of the ovary either on one or on both sides.

Seurat (1920) ascribed explicit phylogenetic designations to the types of morphological variability seen among nematode reproductive systems. The essence of Seurat's nomenclature is the position and direction of the uteri (not the ovaries) and how this can then be applied to studies of nematode phylogeny. Many nematologists have lost sight of the original purpose of the terms, their contributory significance to understanding nematode phylogeny, and the inherent genetic significance imparted by the correct application of this terminology. Seurat's nomenclature and definitions are as follows:

Amphidelphic: uteri opposed
Opistodelphic: uteri parallel and posteriorly directed
Prodelphic: uteri parallel and anteriorly directed

All plant parasitic nematodes are amphidelphic whether they have one or two ovaries anteriorly or posteriorly directed. It is interesting to note that when adenophorean plant parasites have a single ovary it is posteriorly directed, and if there is a shift in vulval position from midbody, it is toward the anterior. When secernentean plant parasites have one ovary it is anteriorly directed and any shift of vulva position is toward the posterior.

To apply Seurat's terminology incorrectly is to ignore the inherent knowledge of the system and to miss the entire phylogenetic message contained in the reproductive system under consideration. For the examples *Pratylenchus vulnus* and *Xiphinema bakeri*, some nematologists would teach that *Pratylenchus* is prodelphic and that *Xiphinema bakeri* is opisthodelphic; this is erroneous, misleading, and devoid of factual information. Both examples are amphidelphic. By recognizing this we know the ancestral condition, that there has been a reduction from the diovarial condition to monovarial, that this was followed by a reduction of one uterus (the extent of reduction is variable), and that the gonad reduction occurred anteriorly or posteriorly.

The value of the information gained by the correct application of terminology was recently demonstrated in the genus *Helicotylenchus* (two ovaries, amphidelphic) and its synonym *Rotylenchoides* with a well-developed anterior ovary and an atrophied posterior ovary. The cline of ovarial reduction throughout the genus *Helicotylenchus* and the recognition of the amphidelphic condition strongly supports the synonomy of these once separate genera (Fortuner, 1984). Designation of *Rotylenchoides* as prodelphic would by definition mean that the genetic histories and compositions of the two genera are different and therefore they could not be synonymized.

Many have questioned the condition in *Meloidogyne* which is not an example of prodelphy but the victim of secondary prodelphy induced by the swelling of the female with the coincident posterior shift of the vulval position. In the Heteroderidae, the swelling of the body is genetically controlled, whereas the condition of the reproductive system is an architectural accommodation. This is proven by such ancestral heteroderid genera as *Meloinema*, *Nacobbodera*, and *Bursadera*, where the young adult female is vermiform and amphidelphic but later becomes swollen, sedentary, and secondarily prodelphic. Hopefully, these examples clarify how biased misinterpretations of established terminology obstructs enhanced communication and the advancement of factual knowledge.

B. Male Reproductive System

In general organization the male reproductive system is similar to that of the female in that it consists of one or two testes, associated ducts, and sperm reservoirs and outlets to the outside of the body (Fig. 20). The typical system consists of three parts: the testis, the seminal receptical, and the vas deferens. Rarely, there is a vas efferens between the testis and the

seminal receptical. The testes are of two types: panoistic or hologonic. The hologonic testes are known only from the same parasitic taxa (*Trichuris*) that had females with hologonic ovaries.

There are differences between the male reproductive systems of Adenophorea and Secernentea. As a rule adenophorean males have two testes (diorchic) (exception: *Trichodorus* and some taxa in Chromadoria). Secernentean males have a single testis (monorchic) (exception: sex-reversed males in *Meloidogyne*). Other differences are evident in the musculature associated with the proximal end of the system (Fig. 20). In males of Enoplia the ejaculatory duct is heavily muscled and this is easily detected (Fig. 20A). Chromodorids also have a muscle layer surrounding the ejaculatory duct but it is weak and difficult to detect (Fig. 20B). In Secernentea the males lack ejaculatory muscles but there are well-developed ejaculatory glands that are often mistaken for muscle (Fig. 20C).

The secondary sexual organs of the male are more prominent than those associated with the female reproductive system. The secondary sex organs referred to are the cloaca, spicular pouch, spicules, gubernaculum, copulatory muscles, supplements, and caudal alae. Caudal alae have already been discussed.

The presence of a cloaca in male nematodes and the absence of it in females is one of the characteristics that separates nematodes from other pseudocoelomates. A cloaca is known in females of two genera, *Lauratonema* and *Rondonia*, neither of which is considered ancestral. There is a difference in the cloaca between adenophoreans and secernenteans. The difference adds evidence to the assumption that Adenophorea is ancestral and Secernentea derived. In the adenophorean cloaca the confluence of the rectum and vas deferens is posterior as a result a distinguishable rectum persists. (This could indicate that the progenitors of Nemata as males and females had separate gonopore openings.) In males of Secernentea the cloaca is formed by the entrance of the vas deferens into the hindgut either at or just posterior to the intestinorectal valve: thus no distinguishable rectum exists.

C. Spicules

On the dorsal wall of the cloaca there are specialized cells called the spicula primordia that by a quasi-evagination form the spicular pouch and by invagination form the spicules. As such the spicules do not lie directly within the cloacal pouch but in an offset pouch. The spicules are extruded to the exterior by way of the cloacaspicular orifice.

Spicules are not, as often supposed, flat bladelike structures (Fig. 21A). Each spicule is in cross-section crescentic or tubelike, and in all instances with a cytoplasmic core in which sensory nerves may be embedded (Fig. 21C). Like most nematode structures, terminology of parts is not consistent because proposals were made independently in the various fields of helminthology, plant nematology, marine nematology, etc. The proximal end of the spicule modified for muscle attachment and contiguous with the spicular pouch is called the manubium (= capitulum, head). Beyond the head the spicule may narrow to a section known as the calomus (= shaft). The main portion of the spicule is the lamina (= blade). The blade may have a longitudinal, winglike, membranous extension called the velum.

The basic number of spicules is two but there may be only one or none. In addition to variability in number, the paired spicules may be very unequal in length. For example, males of *Viguiera hawaiiensis* del Prado Vera, Maggenti, and Van Riper, 1985 have extremely unequal spicules. The short right spicule is 0.14–0.17 mm and the long left spicule 3.35–4.10 mm long. The long spicule in this genus is nearly 60% of the total body length (average length of male is, 6.1 mm). Each spicule has both a protractor and a retractor muscle.

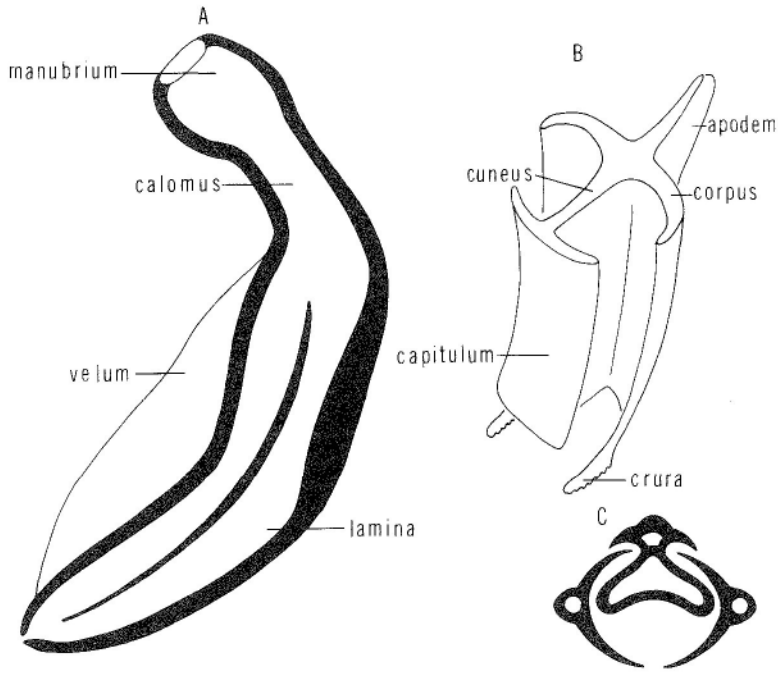

FIGURE 21 (A) Spicule. (B) Gubernaculum. (C) Transverse section through spicules and gubernaculum showing relationship to each other. (Maggenti/Springer-Verlag, 1981.)

D. Gubernaculum

The gubernaculum is a cuticular thickening of the dorsal wall of the spicular pouch and acts as a guide during spicular protrusion. The simplest gubernaculum is merely a thickened plate or trough called the corpus. In its most complex form protruding from the corpus and at right angles to it, is an additional longitudinal plate that keeps the spicules separate; this plate is termed the cuneus (Fig. 21B). In turn the cuneus may be tipped by a transverse plate called the capitulum. Toothed lateral extensions at the bottom of the corpus are called crura. Extending posteriorly from the corpus there may be apodemes for muscle attachment.

Most commonly there are two pairs of muscles that operate the gubernaculum: the protractor gubernaculi that extend from the ventral body wall anteriorly to the gubernaculum and the retractor gubernaculi that extend from the gubernaculum to the dorsal body wall. In addition, there may be paired seductor gubernaculi muscles that extend from the lateral body wall to the gubernaculum.

E. Telamon

The telamon is a specialized structure known only from strongyles. In strongyles it is an immovable thickening of the ventral cloacal wall; it is independent of the spicular pouch. This structure is essential in strongyles because the spicular pouch orifice is not directly opposite the cloacal (anal) orifice as in other Nemata. Therefore, the telamon turns the spicules to the exterior as they are being protruded.

The term should not be applied to the spicular accessories associated with males of tylenchid plant parasites. The so-called "telamon" of hoplolaims is merely the capitulum of the gubernaculum.

Additional secondary sexual characters often useful in identifying taxa are the preanal and postanal male supplements. In Adenophorea preanal supplements are in a single medioventral row; in Secernentea they are subventral and paired. When the supplements are large and extensible, as they are in some marine Adenophorea, they are called appendicules.

IX. NERVOUS SYSTEM

One of the responsibilities of the nervous system is to transmit stimuli received externally by way of somatic sensory organs to the central nervous system and then to the internal tissues where they are translated into a "proper" response. The nervous system acts to mediate all of the animal's activity through stimulation, coordination, and responsive actions.

The basic operative unit of the nervous system is the neuron. The neuron has a large cell body (neurocyte) with a conspicuous nucleus and two or more protoplasmic processes. The process transmitting stimuli to the neurocyte is called the dendrite and the protoplasmic process carrying impulses away from the neurocyte is the axon. There are two basic types of neurons, bipolar and multipolar. Bipolar neurons have one dendrite and one axon. Multipolar neurons have multiple dentrites and one axon. The axon which is generally unbranched may have a collateral branch near the neurocyte. A group of neurocytes constitute a ganglion.

Neurons fall into three categories: sensory neurons (afferent), motor neurons (efferent), and adjustor neurons (internuncial or associative). Sensory neurons transmit impulses to the central nervous system; motor neurons conduct impulses to the effectors and internuncial neurons interconnect with sensory and motor neurons so that more than one effector may be activated.

The central nervous system of nematodes (Figs. 22 and 23) consists of a large aggregate of ganglia that are situated dorsally (two subdorsal ganglia), laterally (six ganglia), and ventrally (a bilobed ganglion) around the esophagus and are anteriorly connected to the major nerve bundle surrounding the esophagus, i.e., the circumesophageal commissure or nerve ring (Chitwood and Chitwood, 1974; Wood, 1988). Anterior to the "brain" (nerve ring and associated ganglia) are six small ganglia that receive nerves (dendritic fibrils) from the cephalic sensory organs; axonic nerves transmit the impulses through the nerve ring to internuncial neurons in the lateral ganglia. The amphids and deirids are innervated from separate ganglia that are located laterally and posteriorly to the nerve ring.

The area under the medial ventral body wall where the two arms of the nerve ring amalgamate before proceeding posteriorly as the ventral nerve cord is called the hemizonid. In lateral view the hemizonid stands out as a refractive body. All the refractive structures called "cephalids" are really subcuticular commissures. Just posterior to the hemizonid is the anterior-most and largest ganglion of the ventral nerve cord: the retrovesicular ganglion. The ventral nerve trunk proceeds posteriorly passing to the right of the excretory pore and to the right of the vagina. Two loose aggregates of neurocytes anterior and posterior to the vagina are designated by some authors as the pre- and postvulvar ganglia. At the rectum (Fig. 23) the ventral nerve trunk sends off two commissures. Each one is directed dorsally around the rectum or cloaca and laterally enters the laterorectal ganglion. The commissure then proceeds dorsally where the two arms now merge in the dorsorectal ganglion. The nerve originating from this ganglion innervates the tail.

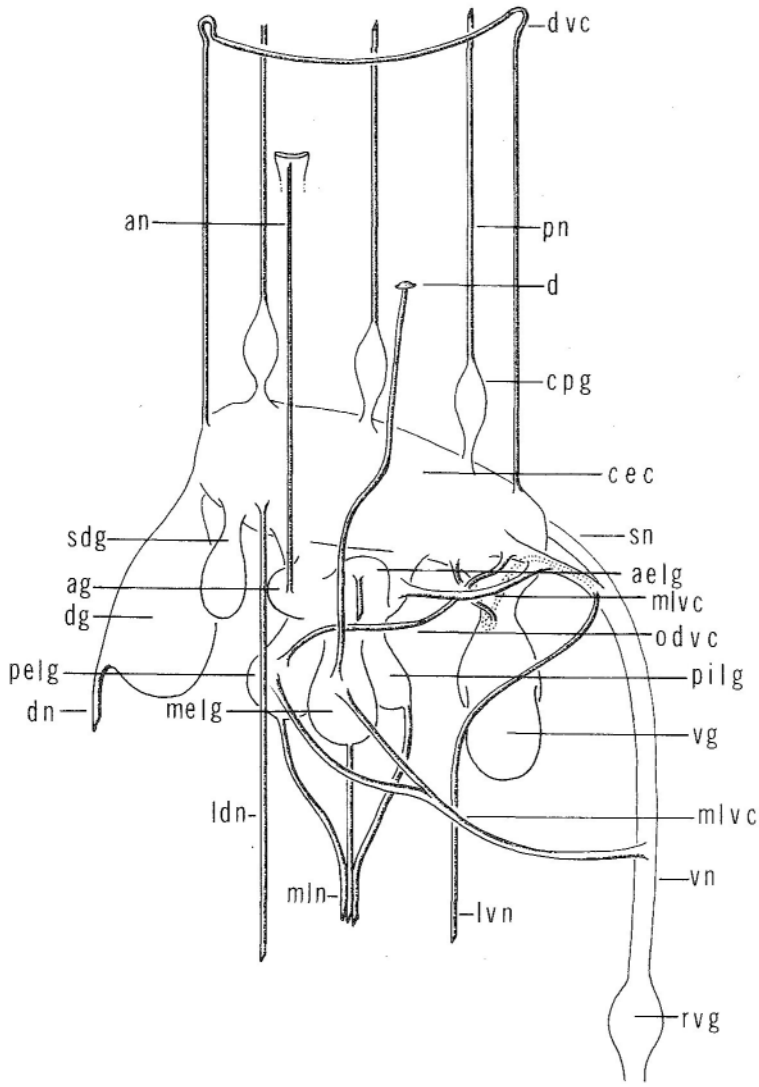

FIGURE 22 Generalized anterior central nervous system (right side) illustrating nerve ring, associated ganglia, nerves, and commissures. Abbreviations: dvc, dorsoventral commissure: an, amphidial nerve; pn, papillary nerve; d, deirid; cpg, cephalic papillary ganglia, cec, circumesophageal commissure; sn, subventral nerve; sdg, subdorsal ganglion; ag, amphidial ganglion; aelg, anterior externolateral ganglion; mlvc, major lateroventral commissure; dg, dorsal ganglion; odvc, oblique dorsoventral commissure; pelg, posterior externolateral ganglion; dn, dorsal nerve; melg, median externolateral ganglion; pilg, posterior internolateral ganglion; vg, ventral ganglia; mlvc, minor lateroventral commissure; ldn, laterodorsal nerve; mln, mediolateral nerve; lvn, lateroventral nerve; vn, ventral nerve; rvg, retrovesicular ganglion. (Maggenti/Springer-Verlag, 1981.)

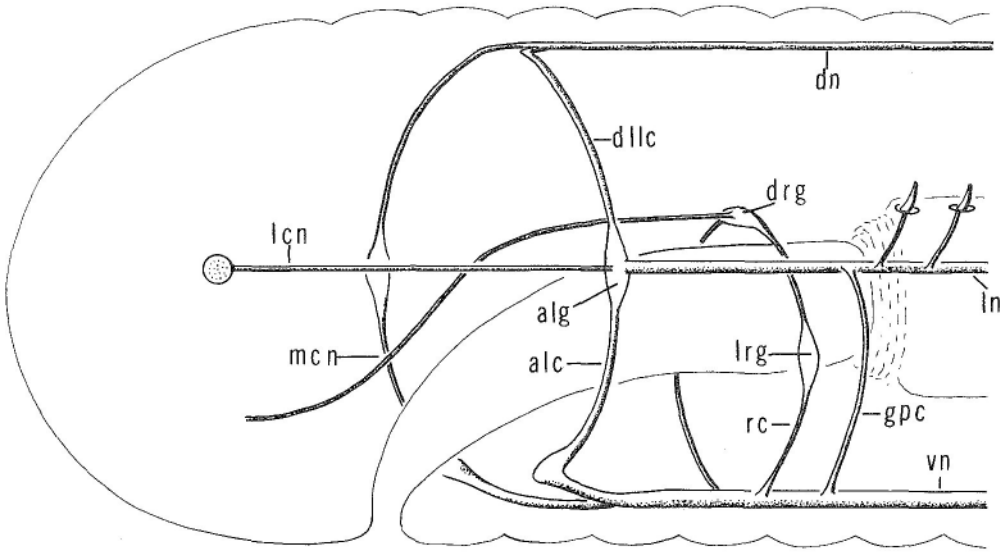

FIGURE 23 Generalized central nervous system, caudal region. Abbreviations: dn, dorsal nerve; dllc, dorsolateral lumbar commissure; drg, dorsorectal ganglion; lrg, laterorectal ganglion; mcn, median caudal nerve; ln, lateral nerve; rc, rectal commissure; alg, anolumbar ganglion; lcn, laterocaudal nerve; alc, anolumbar commissure; rc, rectal commissure; gpc, genitopapillary commissure; vn, ventral nerve. (Maggenti/Springer-Verlag, 1981.)

Caudal sensory organs (including the phasmids) are innervated from the nerve that emanates from the anolumber ganglion which terminates the lateral nerves and receives commissures that connect the ventral nerve with the dorsal nerve.

The subsidiary somatic nerves are the dorsal nerve cord and the lateral nerve cord. Just as the muscles of the subventral sectors send processes to synapse with the ventral nerve cord, so also do the muscles of the subdorsal sectors send processes to the dorsal nerve, which is primarily motor. Muscles anterior to the nerve ring connect directly into it. The lateral and ventral nerves are composed of both motor and sensory nerves. The entire system is connected by commissures that connect the dorsal nerve with the lateral and ventral nerves and vice versa.

Among Adenophorea there appears to be a peripheral nerve net that extends from the anterior extremity to the posterior extremity forming plexuses at the lip region, vulva, and in males along the entire caudal region. A notable feature is the linking of the somatic setae and papillae, which in turn are individually connected to sensory neurons of the peripheral nervous system.

The system is controversial but has been seen several times and in several genera of Enoplia and Chromadoria when silver-stained as described by Croll and Maggenti (1968). They postulated that the network coordinates impulses from seta to seta and thence to the central nervous system, thereby compensating for the paucity of sensory organs over the body. A network has not been demonstrated in Secernentea; however, free nerve endings under and in the cuticle (sensilla insitica and sensilla colei) have been and these could act as proprioceptors.

REFERENCES

Allen, M. W. and Noffsinger, E. M. 1978. A revision of the marine nematodes of the superfamily *Draconematoidea* Filipjev, 1918 (Nematoda: Draconematina). University Calif. Pub. Zool. Vol. 109 Univ. Calif. Press, Berkeley.

Baldwin, J. G. 1983. Fine structure of bodywall cuticle of females of *Meloidodera charis, Atalodera lonicerae* and *Sarisodera hydrophila* (Heteroderidae). *J. Nematol.* 15: 370–381.

Baldwin, J. G., and Hirschmann, H. 1976. Comparative fine structure of the stomatal region of males of *Meloidogyne incognita* and *Heterodera glycines*. *J. Nematol.* 8:1–17.

Bird, A. F., and Rogers, G. E. 1965. Ultrastructure of the cuticle and its formation in *Meloidogyne javanica*. *Nematologica* 11:224–230.

Boveri, T. 1892. Ueber die Entstehung des Gegensatzes zwischen den Geschlechtszellen und der somatischen Zellen bei *Ascaris megalocephala*, nebst Bermekungen zur Entwickelungsgeschichte der Nematoden. Sitsungsb. *Gellsch. Morphol. Physiol.* 8:114–125.

Chitwood, B. G., and Chitwood, M. B. 1974. *Introduction to Nematology.* University Park Press, Baltimore.

Croll, N. A., and Maggenti, A. R. 1968. A peripheral nervous system in Nematoda with a discussion of its functional and phylogenetic significance. *Proc. Helminthol. Soc. Wash.* 35:108–115.

DeGrisse, A. T., Lippens, P. L., and Coomans, A. 1974. The cephalic sensory system of *Rotylenchus robustus* and a comparison with some other tylenchids. *Nematologica* 20:88–95.

Filipjev, I. N. 1934. The classification of the free-living nematodes and their relation to the parasitic nematodes. *Smithsonian Misc. Coll.* 89:1–63.

Harris, J. E., and Crofton, H. D. 1957. Structure and function in the nematodes: Internal pressure and the cuticular structure in *Ascaris*. *J. Exp. Biol.* 34:116–130.

Hope, W. D. 1988. *Syringonomus dactyltus,* a new species of bathyal marine nematode (Enoplida: Leptosomatidae) and a supplementary description of *Syringonomus typicus* Hope and Murphy, 1969. *Proc. Biol. Soc. Wash.* 101: 717–729.

Hyman, L. H. 1951. The Invertebrates: Acanthocephala, Aschelminthes and Entoprocta. The Pseudoscoelomate Bilateria, *Vol. 3. McGraw-Hill, New York.*

Inglis, W. G. 1964. The marine Enoplida (Nematoda): A comparative study of the head. *Bull. Br. Mus. (Nat. Hist.) Zool.* 2:263–376.

Kier, W. M., and Smith, K. K. 1985. Tongues, tentacles and trunks: The biomechanics of movement in muscular hydrostats. *Zool. J. Linn. Soc.* 83:307–324.

Lee, D. L. 1970. Moulting in nematodes: The formation of the adult cuticle during the final moult of *Nippostrongylus brasiliensis*. *Tissue and Cell* 2:139–153.

Luc, M., Maggenti, A. R., Fortuner, R., Raski, D. J., and Geraert, E. 1987. A reappraisal of Tylenchina (Nemata). 1. For a new approach to the taxonomy of Tylenchina. *Revue Nématol.* 10:127– 134.

Maggenti, A. R. 1961. Morphology and biology of the genus *Plectus* (Nematoda: Plectidae). *Proc. Helminthol. Soc. Wash.* 28:118–130.

Maggenti, A. R. 1962. The production of the gelatinous matrix and its taxonomic significance in *Tylenchulus* (Nematoda: Tylenchulinae). *Proc. Helminthol. Soc. Wash.* 29:139–144.

Maggenti, A. R. 1963. Comparative morphology in nemic phylogeny. In *The Lower Metazoa Comparative Biology and Phylogeny*, E. C. Doughtery, eds. Univ. Calif. Press, Berkeley, pp. 273–282.

Maggenti, A. R. 1979. The role of the cuticular strata nomenclature in the systemaics of Nemata. *J. Nematol.* 11:94–98.

Maggenti, A. R. 1981. *General Nematology.* Springer-Verlag, New York.

Maggenti, A. R., Raski, D. J., Koshy, P. K., and Sosamma, V. K. 1983. A new species of *Chronogaster* Cobb, 1913 (Nemata: Plectidae) with an amended diagnosis of the genus and discussion of cuticular ornamentation. *Revue Nématol.* 6:257–263

Martini, E. 1903. Ueber Furchung und Gastrulation bei *Cucullanus elegans* Zed., *Ztschr. Wissensch. Zool.* 74:501–556.

Martini, E. 1909. Ueber Subcuticula und Seitenfelder einiger Nematoden. *Ztschr. Wisssensch. Zool.* 93:535–624.

Mendel, G. 1865. Versuche uber Pflanzen Hybriden. *Verh. naturf. Ver. in Brunn, Abhandlungen, IV*: 3–47. [English Trans.] 1956. Harvard Univ. Press, Cambridge, MA.

Ohmori, Y., and Ohbayashi, M. 1975. Arrangement of the somatic muscle cells of meromyarian nematodes. *Jap. J. Nematol.* 24:294– 299.

Pai, S. 1927. Lebenzyklus der *Anguillula aceti* Ehrbg., *Zool. Anz., Leipzig* 74:257–270.

Pai, S. 1928. Die Phasen des lebenscyclus der *Anguillula aceti* Ehrbg. und ihre experimentell-morphologische Beeingflussung. *Ztschr. Wissensch. Zool.* 131:293–344.

Riding, I. L. 1970. Microvilli on the outside of a nematode. *Nature* 226:179–180.

Roberts, T. M., and Ward, S. 1982a. Centripetal flow of pseudopodial surface components could propel the amoeboid movement of *Caenorhabditis elegans* spermatozoa. *J. Cell Biol.* 92: 132–138.

Roberts, T. M., and Ward, S. 1982b. Directed membrane flow on the pseudopods of *Caenorhabditis elegans* Spermatozoa. *Cold Spring Harbor Symp. Quant. Biol.* 46:695–702.

Robertson, W. M. 1976. A possible gustatory organ associated with the odontophore in *Longidorus leptocephalus* and *Xiphinema diversicaudatum. Nematologica* 21:443–448.

Roggen, D. R. 1973. Functional morphology of the nematode pharynx. 1. Theory of the soft-walled cylindrical pharynx. *Nematologica* 19:349–365.

Rosenbluth, J. 1967. Ultrastructural organization of obliquely striated muscle fibers in *Ascaris lumbricoides. J. Cell Biol.* 25:495–510.

Schuurmans Stekhoven, J. H., and DeConinck, L. A. 1933. Morphologische Fragen zur Systematik der freilebenden Nematoden. *Verhandl. Deutsch. Zool. Gesellsch.* 35:138–143.

Seurat, L. G. 1920. Histoire Naturelle des Nematodes de la Berbérie, Alger.

Shepherd, A. M., Clark, S. A., and Dart, P. J. 1972. Cuticule structure in the genus *Heterodera. Nematologica* 18:1–17.

Smith, K. K., and Kier, W. M. 1989. Trunks, tongues, and tentacles: Moving with skeletons of muscle. *Am. Scientist* 77:28–35.

Steiner, G. 1933. The nematode *Cylindrogaster longistoma* (Stefanski) Goodey, and its relationship. *J. Parasitol.* 20:66–68.

Sulston, J. E., and Horvitz, H. R. 1977. Postembryonic cell lineages of the nematode *Caenorhabditis elegans. Dev. Biol.* 56:110–156.

Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J. N. 1983. The embryonic cell lineages of the nematode *Caenorhabditis elegans. Dev. Biol.* 100:64–119.

Van Beneden, E. 1883. *Recherches sur la maturation de l'oeuf, la fecondation et la division cellulaire.* Gand and Leipzig, Paris.

Van Waerbeke, D., and Remillet, M. 1973. Morphologie et biologie de *Heterogonema ovomasculis* n. sp. (Nematoda: Tetradonematidae) parasite de nitidulidae (Coleoptera). *Nematologica,* 19:80–92.

Waddell, A. H. 1968. The excretory system of the kidney worm *Stephanurus dentatus* (Nematoda). *Parasitology* 58:907–919.

Wood, W. B. (ed.) 1988. The nematode *Caenorhabditis elegans.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

2

Biology and Ecology of Nematodes

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I. INTRODUCTION

Nematodes are the most numerous multicellular organisms present in agroecosystems and can be found at densities up to 30 million/m^2. They are found exploiting every niche provided by the vegetation, soil, and other biota as resources. Filipjev and Schuurmans Stekhoven (1941) limited their view of nematodes that are important for agriculture to plant and insect parasitic species; hence the emphasis in the current tome. It is clear, however, that taxa belonging to other trophic groups or guilds have an impact on agricultural production that exceeds the direct effects of parasitism (Anderson, 1987; Yeates and Coleman, 1982; Yeates, 1987). Thus, there are historical perceptual limits on what taxa or guilds of nematodes should be studied or included in a manual of agricultural nematology. Nematology has suffered because of these perceptions (Stone et al., 1983). Nonetheless, our emphasis here will be on plant parasitic nematodes; it is the acute interaction between plant pathogenic nematodes and their economically important hosts that directs our attention to the plant parasites.

Taxonomic limits are also imposed on the study of nematode ecology: May and Seger (1986) deplored the unsatisfactory state of systematics, especially with invertebrates, and they recognized the importance of knowing what is where as a basis for understanding and managing the ecosystem. Problems exist even with extant nematode identifications let alone the myriad of species yet to be described. The limitations on studies of nematode biology and ecology due to sampling and extraction procedures are well documented but not inimical to valuable research (Barker and Noe, 1988; Ferris and Noling, 1987; McSorley, 1987).

All the limitations notwithstanding, research into nematode ecology has progressed increasingly rapidly in the past decade. Movement in mathematical and molecular ecology should enhance rather than replace studies in the classical and traditional areas. In this treatment, we do not wish merely to present a collation of facts but to give examples as a basis for understanding these organisms, an inadequate as our understanding may be. Nor are these examples presented to elucidate a "principle." Although one cannot ignore the human factor

in discussions of agriculture, nematodes are not inclined to behave according to human-contrived principles. We do search for common patterns, but the student, matriculating or lifelong, should not be conditioned to think or search in learning from principles to cases. Thus, our examples, but we hope that they are pertinent.

Our knowledge and ability to manage nematode populations when desired is still much too meager. This chapter can only be a preliminary statement concerning a phase of nematology in which most questions are yet to be answered. Even so, the literature is too vast to be exhaustively treated here, so the reader is referred to review articles and books wherever possible.

II. GENERAL BIOLOGY

A. Life Cycles

Life cycles of most plant nematodes are not complex. There are six stages, including the undifferentiated egg, four juvenile stages (J1–J4), and the adult. In the Secernentea, the "tylenchid" plant parasites, the J1 forms in the egg where it molts into the J2, which is the stage that hatches. In the Adenophorea, the "dorylaimids," the hatching stage is the J1.

Hatching, along with various stimulants and depressants of the process, has received attention as a possible target of control measures aimed at preventing it or inducing it in the absence of a host (Perry, 1987). Preceded by induced changes in eggshell permeability, hatching may be either a physical or an enzymatic process. Plant nematodes, such as *Rotylenchulus reniformis* and *Meloidogyne incognita*, may hatch freely in water at appropriate temperatures with some increase in percentage hatch in the presence of a host. Others, such as *Globodera rostochiensis*, may require a specific hatch-inducing signal from a host. Some may undergo diapause in the egg (e.g., *Heterodera avenae, Meloidogyne naasi*; Evans, 1987); other forms of arrested development will be discussed in Section III. D. A naturally occurring chemical isolated from red kidney bean induced hatch of *Heterodera gylcines* (Masamune et al., 1982), but specific stimuli operative in soils have not been identified.

Molting separates each of the J1–adult stages in all nematodes, involving a sequence of events similar to that of the insects: (a) apolysis, separation of the cuticle from the hypodermis; (b) cuticle formation; and (c) ecdysis (Wharton, 1986). Molting may represent an evolutionary relict, a mechanism in which the cuticle is modified between stages, or in which the cuticle is modified between stages, or in which excess nitrogen is excreted. It may simply be an accommodation to growth proceeds; for example, Bird (1983) found that the volume growth of adult *Rotylenchulus reniformis* was 17–19% lower than that of the J2 stage. *Paratylenchus projectus* is dependent on a host stimulus for molting (Ishibashi et al., 1975).

Dorylaimid plant parasites remain vermiform throughout their life cycles, but among the tylenchids there is some variation. Most species remain vermiform, but the females of some important plant parasitic species become sedentary in the J2 stage and increasingly saccate to globose as they mature, e.g., *Meloidogyne* and *Heterodera* spp. Sexual primordia are visible in the J3 stages of the most plant nematodes.

B. Reproduction

Plant nematodes reproduce amphimictically or autokonously, never asexually. In species that have males, unbalanced sex ratios are common and show a general trend toward an

increased proportion of males when a population is subjected to environmental stress, even when sex is genetically determined (Yeates, 1987). Life cycles vary from less than 5 days, as with some neotylenchids, to a year or more, as with some longidorids, and within the limits imposed by biology are influenced primarily by temperature and substrate quality. Population cycles of most soil- and root-inhabiting nematodes have overlapping generations. Some nematodes, such as the anguinids *Anguina tritici* and *Subanguina calamagrostis*, begin their life cycles as a distinct cohort and progress separately through each of their developmental stages with little overlapping of generations.

Egg production is high (300+) for species whose eggs are confined, as in plant tissue (*Ditylenchus dipsaci*), an egg sac (*Meloidogyne* spp.), or the body of the female (*Heterodera* spp.). It is more difficult to obtain information with nematodes where eggs are deposited in the soil because of difficulty in recovery and identification. Because of the rapidity with which some migratory nematodes increase in a season (e.g., *Pratylenchus* spp.), egg production must occur rapidly. Fecundity is usually seasonal in temperature zones. A question remaining is whether females keep reproducing as long as the environment is favorable or there is a period in the migratory species in which the female can live after fecundity ceases. Female cyst nematodes, *Heterodera* spp., and species of related genera die with the maturation of the adult. See Wharton (1986) for a discussion of reproductive energetics.

C. Feeding

The feeding processes of dorylaimid and tylenchid plant parasites have been documented on videotape and described in detail by Wyss (1981, 1987). Feeding is by means of a stylet or spear, usually containing a hollow tube through which secretions from pharyngeal glands are injected into plant cells, and cell contents are ingested. The secretions may partly digest cell cytoplasm before ingestion, induce the formation of specialized feeding sites, or have other activity (Hussey, 1989).

Most plant nematodes parasitize underground parts, but some feed on above-ground parts. Nematode feeding habits are often described as ecto- or endoparasitic, with each category subdivided into migratory and sedentary, or sessile, habits. Sometimes an additional category, semiendoparasitic, is included in the scheme. Galling may be induced by certain species in each group. True ectoparasites can feed on epidermal or deeper tissues, depending on stylet length and other factors, their bodies remaining outside the plant tissue. Endoparasites penetrate tissues completely. Feeding habits are not always easily delimited because some species fit into different categories at different life stages. *Heterodera glycines* females are sedentary, endoparasitic during development until sexual maturity when their swollen bodies erupt through the root cortex; they continue feeding semiendoparasitically. *Hoplolaimus galeatus* individuals can be endoparasitic or semiendoparasitic during the same development stage. *Pratylenchus agilis*, a member of a genus of endoparasites, can feed ectoparasitically in vitro, but it is unknown whether *P. agilis* exhibits this behavior in nature (Rebois and Huettel, 1986).

In the root, most migratory endoparasites feed in the cortical parenchyma, but a few species, such as *Pratylenchus vulnus*, penetrate beyond the endodermis and even into the more lignified tissues. Migratory endoparasites may cause extensive necrotic lesions in the tissues in which they feed, leaf-feeding nematodes usually feed on mesophyll tissue, and may feed either ecto- or endoparasitically (*Aphelenchoides* spp.). Sedentary root endoparasites and some deep-feeding ectoparasites induce development of specialized feeding sites that act as metabolic sinks in or adjacent to stelar tissue (e.g., giant cells, syncytia, nurse cells). The pinewood nematode *Bursaphelenchus xylophilus* invades the resin canals

of many coniferous trees and feeds on the epithelial lining of the canals. Knowledge of feeding habits is essential in choosing appropriate extraction methods.

D. Host Reaction

The overall effects of nematode parasitism on plants range from stimulatory to lethal, even within single plant–nematode interactions (Nickle, 1984; Oostenbrink, 1966). Host reactions are characterized along a continuum from susceptible to resistant (even "immune"), which designations may or may not include reference to the nematode's ability to reproduce on the host (Cook and Evans, 1987). Yield responses of susceptible annual hosts are often a function of initial, or preplant, densities of the parasite and all of the conditions affecting the interaction. In deleterious (to the host) associations, above-ground parasites may cause characteristic malformations of various plant organs. Root-knot (*Meloidogyne* spp.), stubby root (*Paratrichodorus* and *Trichodorus* spp.), and some other root parasites cause characteristic root symptoms, but most below-ground parasites cause nonspecific shoot symptoms.

For many economically important plant nematodes and their hosts, genetic variability in the interaction results in a situation in which there is some degree of host cultivar–nematode isolate specificity. The nomenclature applied to the nematode varies, including race, strain, ecotype, and pathotype, but there are many examples, including the interactions between *Ditylenchus dipsaci* and various hosts, *Globodera rostochiensis* and potato, *Heterodera glycines* and soybean, and *Bursaphelenchus xylophilus* and pine (Dropkin, 1988). This variability causes a number of problems, not the least of which are the difficulties presented to plant breeders attempting to provide growers with nematode-resistant cultivars, but it is characteristic of nematode–host relations.

E. Locomotion and Dissemination

All nematodes have at least one motile stage. In agroecosystems, motile stages most often occur in the soil. Locomotion is by out-of-phase waves of muscle contraction in the dorsoventral plane, resulting in draconic (rather than snakelike) serpentine undulations. In contrast, in the Criconematidae the waves are in phase, so that movement is earthwormlike. The repertoire of body movements exhibited by nematodes is somewhat limited (Crofton, 1971), and the plant parasites in soil probably move actively only a few to several centimeters per year. Plant nematodes tend to be more sluggish than microbivorous nematodes. Soil nematodes in general are capable of their most rapid movement when body length is about three times the average diameter of soil particles (Nicholas, 1984). Because nematodes are essentially aquatic creatures, their movement in soil is in the water phase and is affected by soil characteristics affecting moisture, e.g., texture, structure, slope position, rainfall, compaction, and so on.

Plant nematodes are probably attracted by metabolic products or other factors emanating from roots, but few specific attractants have been identified. Root feeders may be attracted to root tips as invasion sites (*Meloidogyne* spp.), to young tissues farther back (*Pratylenchus* spp.), or to older tissue (*Helicotylenchus dihystera*). Temperature and CO_2 have been shown to attract plant parasitic nematodes in increasing gradients (Dusenberry, 1987). Often roots contain or produce feeding deterrents or toxins (Anderson, 1987).

Long-distance movement can be by any means that transports soil or infected plant parts, such as farm machinery, animals (Fig. 1), wind, water, root crops, seed, soil peds in seed lots, or nursery stock. There may be a striking similarity in the species comprising plant nematode communities on crops growing in a region exhibiting similar macroclimatic char-

FIGURE 1 Two common ways of spreading nematodes: farm machinery and animals. (Iowa Agricultural Experiment Station.)

acteristics (Ferris et al., 1971a,b), but this region/crop similarity is exceeded by the efficiency of humans in distributing plant nematodes (Niblack, 1989; Norton, 1978). Nematodes can be carried in guts of rodents, birds, and probably other animals. Dissemination is a natural phenomenon and is difficult to stop, although it can be inhibited is some situations by quarantines and sanitation practices.

III. POPULATION ECOLOGY

We are fortunate in nematology to work with organisms of unitary structure. We are not confronted with modular organisms such as plants with continuous growth and clonal reproduction. Although nematodes can be counted as unitary organisms, there are problems involved in obtaining reliable estimates of populations, and this should be kept in mind when reading generalizations about nematode ecology.

"Ecological" studies of plant parasitic nematodes most often involve single nematode species or populations. This is a natural consequence of the impact of some plant nematodes on their very economically important hosts. Population ecology in this chapter is meant to include species populations and their interaction with biotic and abiotic influences, and subjects independent of a population–community (synecological–autecological) dichotomy. Nematode community studies range from the purely qualitative, i.e., lists of species found in association with a given host, to more quantitative analyses. The community ecology section in this chapter will deal with subjects involving more than one nematode species population at a time.

TABLE 1 Some Factors that Affect Populations of Plant Parasitic Nematodes

I. Abiotic	II. Biotic
A. Topographic	A. Host
1. Elevation	1. Suitability
2. Slope	2. Availability of feeding site
3. Exposure	
4. Surface	
	B. Parasite
B. Soil environment	1. Life cycle
1. Moisture	2. Reproductive rate
Rainfall	3. Survival mechanisms
Snowfall	4. Sex ratios
Runoff	5. Infectivity
Internal drainage	
Frost	C. Human
2. Temperature	1. Cultural practices
Mean	Plowing
Extremes	Rotation
Duration of extremes	Resistant varieties
Cumulative heat units	Pesticides
3. Aeration	2. Conservation practices
4. Texture	
5. Structure	
6. pH and fertility	D. Other biota
7. Organic matter	1. Fungi
8. Gas exchange	2. Bacteria
	3. Nematodes
	4. Viruses and related forms
	5. Insects and mites
	6. Other fauna

A. Biotic and Abiotic Factors Affecting Populations

Some factors that affect distributions and dynamics of populations of plant parasitic nematodes are outlined in Table 1 and many were discussed in reviews (Norton, 1978; Wallace, 1971; Yeates, 1981, 1987). A little thought should illustrate possible ways in which these factors, alone or in combination, can or could act on nematode populations. Naturally, some factors are more important than others and must be determined for individual species under different circumstances. Any combination of factors can change the carrying capacity of a habitat for a species or act as screens to limit the number of species present in a community of parasites.

The host plant, of course, has primacy in the biotic factors affecting nematodes. Its myriad effects on every facet of nematode biology and ecology were reviewed by Yeates (1987). Other biotic factors, including pathogens, parasites, and predators, represent a field of study that has and will provide wide scope for study (Poinar and Jansson, 1988) and perhaps application.

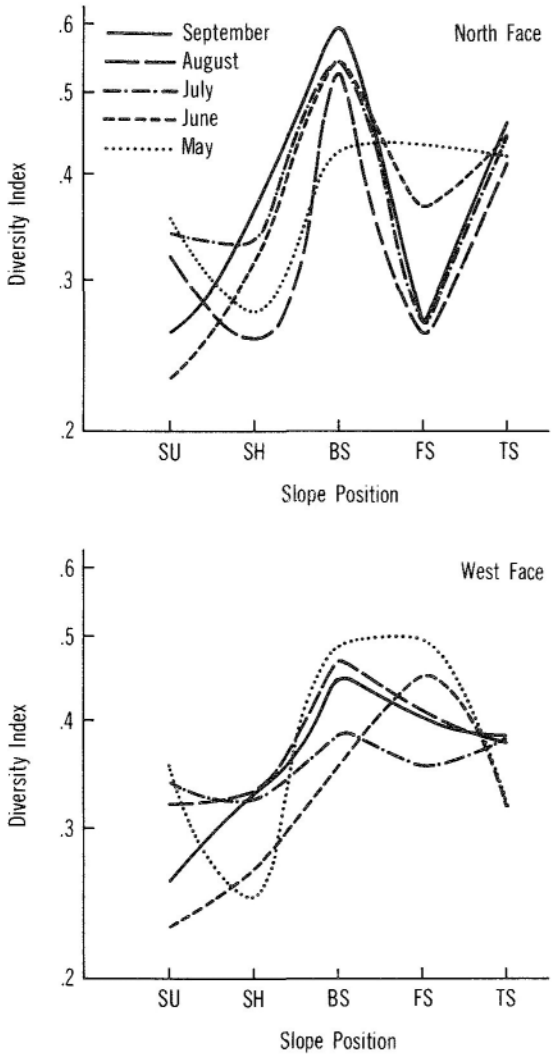

FIGURE 2 Diversity (H') indices of nematodes along a maize toposequence. SU = summit; SH = shoulder; BS = backslope; FS = footslope; TS = toeslope. (Reproduced by permission from Norton and Oard, 1981.)

Among the abiotic factors that govern nematode populations, moisture and temperature are generally considered to be the most important. Nematode densities vary considerably with time, edaphic conditions, and slope aspect (Fig. 2). The effects of soil properties on nematode populations may be direct or indirect.

B. Distribution of Populations

1. Geographic Distribution

Many nematodes have a wide host range, occur in a wide range of environments, and are cosmopolitan. These are apt to be rather primitive types within a taxon in that they are not

especially specialized morphologically or in host–parasite reactions; however, some highly specialized parasites, such as *Meloidogyne* spp., can also be characterized by a wide range and wide distribution. Some species are restricted by environment, but not necessarily exclusively. For example, *Meloidogyne incognita*, the "southern root-knot nematode," and *Meloidogyne hapla*, the "northern root-knot nematode," tend to be most common in North America in the ranges to which their common names refer (Anonymous, 1984). *Belonolaimus longicaudatus* has a widespread distribution in the United States but is restricted to soils containing 80–90% sand (Robbins and Barker, 1974). Other species may be distributed in other patterns and for reasons that are not entirely clear. For example, *Hoplolaimus galeatus* is more common and better known in the middle and eastern parts of the United States than in the western part (Anonymous, 1984).

2. Local Distribution

Nematode populations can vary in three spatial dimensions and over time, and changes affecting variation in one dimension may or may not be reflected in others. For example, *Belonolaimus longicaudatus* populations sampled at a depth of 5–15 cm exhibited large fluctuations in densities over time, while densities at 25–50 cm were fairly constant (Barker et al., 1969). In row crops, plant nematodes are often distributed "lengthwise," in the direction of tillage. Horizontal distribution refers to distribution within and between rows.

a. Horizontal distribution

It is generally agreed that of the basic distribution patterns,—uniform, random, and clustered,—the last is by far the most common for plant and soil nematodes. Among the causes of clustering are (a) occurrence of qualitative differences within hosts, resulting in some parts attracting and nourishing some nematodes more than others; (b) production of eggs in clumps by sedentary females; (c) production of several generations by nematodes with short life spans that flourish in some habitats and not in others; (d) competition among various fauna and microflora for nutrients or space; (e) inhibition by local environmental factors such as toxic substances; and (f) crop management practices in agroecosystems that may reduce numbers of some species and increase others.

Horizontal spatial patterns will vary with temporal ones. Within a field species and numbers of nematodes will vary widely among plants or even from one side of a plant to another (Alby et al., 1983; Barker and Nusbaum, 1971). Frequency distributions are often positively skewed (Fig. 3) in that large populations occur in relatively few samples while most samples contain few nematodes. It is this high degree of variability that makes measuring of nematode populations so difficult and imprecise. This skewing has serious implications for damage forecasting when the imprecision is associated with overestimates of crop loss (Noe and Barker, 1985; Seinhorst, 1973). A number of distribution functions have been used to describe horizontal distribution of nematode populations and can help reduce the risk involved in basing crop damage estimates on nematode population estimates (Barker and Noe, 1988; McSorley, 1987).

b. Temporal distribution

Populations of nematodes rarely remain constant for long. Some populations peak early in the season and then decline, abruptly or steadily, for the remainder of the season, while others increase throughout the season only to be limited by a reduction in resource, by the physical-chemical environment, or competition, or predation by other biota. Some plant nematodes, such as *Xiphinema* and *Longidorus* spp., are long-lived, and their densities may vary little within a year (Flegg, 1968). Differences in temporal distributions may reflect

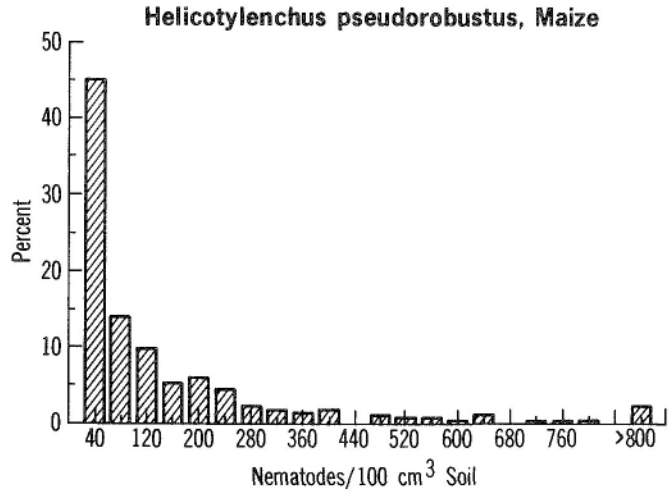

FIGURE 3 Typical nematode frequency distributions when many samples are collected. (Iowa Agriculture Experiment Station.)

inherent differences among nematode species, or be associated with seasonal changes in the quantity or quality of plant material, or both. This allows cohabitation of roots by pathogenic species and complicates studies of competition. Yeates et al. (1985) found sequential, complementary distributions of species of *Meloidogyne, Heterodera*, and *Pratylenchus* on white clover sampled over several months at 3-week intervals. Kraus-Schmidt and Lewis (1979) found similar relationships among *Hoplolaimus, Meloidogyne*, and *Scutellonema* on cotton.

c. *Vertical distribution*

In agroecosystems, the largest numbers of plant nematodes are found in the top 15–20 cm of soil, but some may be found at depths of 240 cm (Raski et al., 1965). Nematodes of many species are stratified in soil. *Criconemella xenoplax*, for example, is found to a depth of 1 m on peach roots, whereas *C. ornata* is found mostly in the upper 15 cm on peanut (Barker, 1982). Root distribution may control distribution of plant nematodes but is not the only factor. Shaping of citrus trees will change soil temperatures, compared with nonshaded ones, with the result that *Radopholus similus* will be closer to the surface in shaded areas than in nonshaded areas, even though abundant roots are present in both levels (Reynolds and O'Bannon, 1963).

Vertical migration probably is largely controlled by temperature, moisture tensions, and root distribution (O'Bannon et al., 1972; Schmitt, 1973), but often evidence of migration is circumstantial. The possibility of preferential colonization at various levels as a response to environmental changes at various levels over time must be considered. Prot (1980) reviewed the literature on migration. In any study of migration, however, techniques must be examined carefully to ensure that conclusions are not based on artifacts of methodology. Doubtless short distance migration occurs, but documentation of the distances migrated in soils are difficult to obtain.

C. Biogeography

As with all organisms, nematodes undergo speciation, radiation, and extinction, and as such these processes are pertinent in biogeographic studies. Although a part of ecology biogeographic studies usually examine widespread distribution as patterned over time, whereas traditional ecology studies include habitat relationships, population increase, survival, age structure, and similar local phenomena. These local facets are important in evaluating more extensive distributions.

It probably is premature to do much more than speculate on regional or global geographic distributions of nematodes and their causes, but a start has been made. Boag and Topham (1985) and Topham et al. (1985) found that certain nematode species were associated with and could be used to detect small populations of virus vector species, demonstrating the potential usefulness of such information. Nematologists in only a few states of the United States have published general accounts of the occurrences of plant nematodes in their respective states, even in what is probably the most surveyed country in the world. Mere listing with minimal annotated remarks and infrequent updating are of limited value. The publication *Distribution of Plant Parasitic Nematode Species in North America* (Anonymous, 1984) was an attempt to collate the known occurrences up to 1984. Although it accomplished much, periodic revisions are necessary.

In studies of nematode distributions and their causes, caution should be made to avoid introductions, i.e., agricultural settings. Patterns of distribution can best be shown where natural forces, barriers such as oceans or climatic forces, limit long-range spread of species. Ferris et al. (1986) related the distributions of some dorylaimids to plate tectonics. As might be expected, there is a tropical fauna in which circumstantial evidence indicates that certain species would not become established in higher latitudes even if introduced. Some examples are *Rotylenchulus reniformis* and many species of *Xiphinema*, among others.

Taxonomic problems further confuse the problems in studying wide distributions of plant parasitic nematodes. For example, *Helicotylenchus dihystera* collected from Iowa prairies are not the same as those collected from maize in South America (Norton, unpublished). Geographic isolation, eliminating gene flow among related populations, is one of the most important causes of speciation. Local abiotic conditions exert selection pressures and, combined with the well-documented effects of host on morphometric variation (Yeates, 1987), result in morphological changes through evolutionary processes such as natural selection and genetic drift. Small morphological changes result in ecotypes or geographic variants. Eventually, some of these variants may evolve into new species.

Although in science we often state that the best experiments are the simplest ones, we must keep in mind that in a holistic view of ecology, as well as other phases of biology, simplistic explanations of phenomena are usually to be avoided.

D. Survival

Doubtless many species of nematodes have become extinct, and doubtless many are now endangered as habitats are being destroyed or chemicals applied. Purposeful local eradication has evidently been successful in a few instances, as with *Globodera rostochiensis* in Delaware and upstate New York in the United States. Small local populations of nematodes are in greater danger of extinction than large populations unless survival capabilities of the former permit persistence. Planting of resistant or nonhost crops may so reduce a population that severe climatic and edaphic conditions may eliminate populations locally. Some plants

nematodes have greater capabilities for survival than others. Nematodes with long life cycles frequently survive, although numbers may be few.

Plant parasitic nematodes are able to survive unfavorable conditions by entering dormant states or states of arrested development classified as quiescent or diapause (Evans, 1987; Antoniou, 1989). Quiescent states are induced by unfavorable environmental conditions; facultative quiescence is a readily reversible response to sudden environmental changes, and obligate quiescence is life stage-specific, requiring specific environmental signals to end. Either form may be induced by lack of water (anhydrobiosis), high salt concentration (osmobiosis), lack of oxygen (anoxybiosis), low temperature (cryobiosis), and high temperature (thermobiosis) (Antoniou, 1989). Anhydrobiosis is the best characterized and documented (Antoniou, 1989; Demeure and Freckman, 1981), and may allow nematode survival for a few months up to 39 years.

Quiescence is not confined to one stage of development. In an Iowa native prairie, evidence indicated that *Helicotylenchus pseudorobustus, Merlinius joctus*, and *Xiphinema americanum* overwintered mainly as eggs, while many vermiform individuals of *H. leiocephalus, Tylenchorhynchus maximus, T. nudus*, and *T. silvaticus* survived the winter (Schmitt, 1973). The host may also affect survival. Koenning et al. (1985) found that survival of *Pratylenchus brachyurus* was less in a winter cover of wheat than in fallow soil, and that winter survival was generally density-independent.

Diapause, like quiescence, may be either a facultative or an obligate state (Evans, 1987), but differs from quiescence in that endogenous factors are responsible for the arrest in development. Most of the plant nematodes known to exhibit diapause do so in the egg (*Meloidogyne, Heterodera*, and related species).

E. Population Dynamics

1. Parameters of Populations

Population dynamics, according to Ferris and Wilson (1987), is a term "used to convey changes in the numbers, age class distribution, sex ratio, and behavior of a population through time and space, determined by inherent characteristics of the individuals and mediated by environmental condition, food resources, and interacting biotic agents." Plant nematode populations have often been characterized as r or K strategists (Ferris and Wilson, 1987; Nicholas, 1984; Wharton, 1986), but these categories are not mutually exclusive and tend to most useful in comparisons within rather than between taxonomic groups (Yeates, 1987).

Densities of nematodes, totals or broken down into stages, are the usual data collected in population studies. Feedbacks that decrease density by decreasing births, survivorship, growth rates, or immigration are negative feedbacks; the opposite are positive feedbacks. Density-vague populations are characterized by high variances that can only be weakly explained by density (Strong, 1984). Often variances are so high that any density effects on population regulation are either absent or not discernable. Except in extreme pathological situations that may occur with plant pathogenic nematodes (e.g., *Meloidogyne*), plant parasitic nematodes do not deplete their resources. Their populations are density-vague or density-independent, governed by other factors such as abiotic ones, host compatibilities, or combinations of such.

Although numbers are used most commonly in analyzing nematode abundance in populations and communities, the use of biomass is often intuitively more satisfying. Biomass is generally defined as the amount of living matter in a given volume or area of habitat. (It seems counterintuitive when abundance and biomass data on nematodes are

given per square meter, despite their existence in three dimensions; however, there are historical arguments in favor of retaining the two-dimensional mindset.) Nematode biomass is frequently calculated by the Andrássy equation:

$$\text{Biomass } (\mu g) = W^2 \times L \div (16 \times 100{,}000)$$

where W = greatest body width (μm) and L = body length (μm) (Yeates, 1988). The biomasses of adults of several plant parasitic species are listed by Waliullah (1983).

One measurement that probably would give the best information on the biology of an organism is body weight (Brown and Gibson, 1983). Body weight, however, must be used in conjunction with feeding habits and many ecological parameters. Different expressions of population change can result depending on whether actual nematode densities (counts) or biomass is used (Fig. 4). Similarly, Yeates (1988) showed how a related parameter, biovolume, does not vary linearly with abundance. Duncan and Freckman (in Freckman, 1982) found alarming disparity between biomass calculated by the Andrássy formula given above and by a more laborious method, itself based on several assumptions. Because other estimates are based on the calculation of biomass (e.g., respiration, production), they suggested that this area needs research attention.

2. Modeling

Studies on the seasonal fluctuations of populations of plant nematodes and the influences thereon by soil characteristics, management practices, host suitability, community structure, and other factors are legion. They have demonstrated a wonderful variety of interactions among nematode reproduction, host response, and environmental influences. There are several comprehensive reviews of the theories and various implementations of population modeling as an end in itself or as a basis for nematode management (Barker and Noe, 1988; Duncan and McSorley, 1987; Ferris and Noling, 1987; Ferris and Wilson, 1987). For economically important plant parasites, empirical models can easily be constructed and applied for predictive purposes using field data and regression analyses. Recently, work has increased on more complex and biologically descriptive simulation models whose parameters do not have to be redefined for changes in environmental conditions, for example.

The most important factor affecting populations of plant nematodes is the presence of a suitable host. For annual crops, the critical point for measuring nematode population density is at planting. This reflects the biological as well as economic reality that crop yields are related to initial nematode population densities (P_i) and that currently available nematode management strategies must be applied at planting. A general, well-known model for relating nematode P_i to crop yield was proposed by Seinhorst (1965) (Fig. 5). The model describes several characteristics of a given nematode–host interaction: the sigmoid relationship between P_i due to intraspecific competition. Duncan and Ferris (1982) expanded the model to describe the effects of multispecies infestations. Economic threshold concepts can easily be applied to this and similar models to be used as a basis for optimizing management decisions. While useful for modeling interactions in annual crops, a different approach must be taken for perennials (Duncan and McSorley, 1987; Ferris and Noling, 1987).

Evolving technology has and will have a profound effect on the development and implementation of predictive modeling for nematode management (Bird and Thomason, 1980), e.g., in the development of expert systems. The sensitivity of the annual crop–pathogenic nematode interaction to initial conditions would seem to make their long-term interactions a suitable system for description using chaos theory.

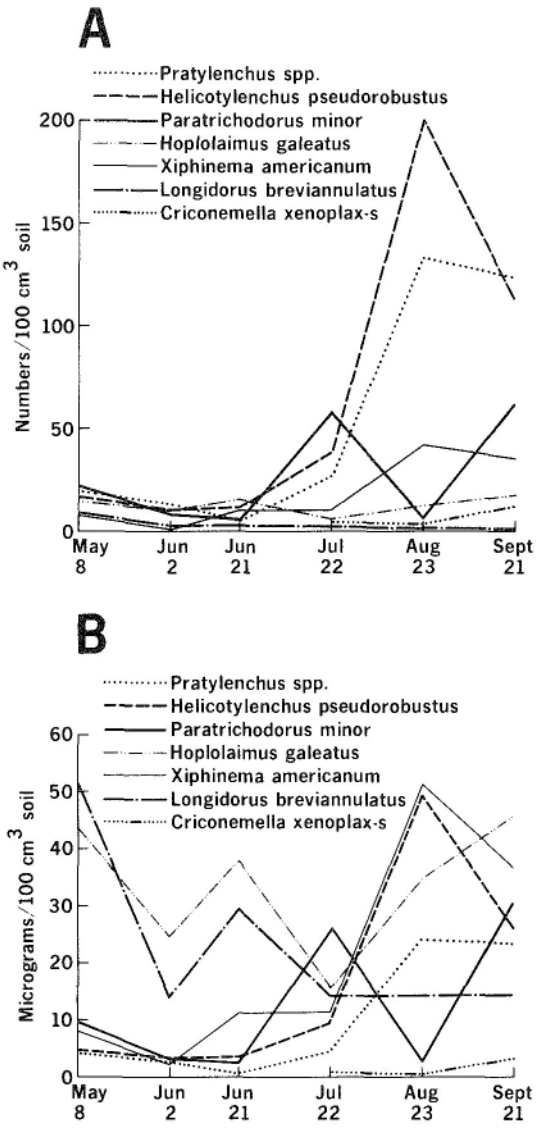

FIGURE 4 Comparison of the same nematode populations in a maize field on the basis of densities (A) and biomass (B). (Iowa Agriculture Experiment Station.)

F. Interactions with Other Microorganisms

Although known for some time but largely ignored until the 1950s, that nematodes influence other organisms and their effects on plants has now been widely accepted. The comparative ease of working with single species may have obscured more complex interactions. While often true that if one species of nematode is controlled, a severe disease situation may be mitigated so that it is no longer economically important, other complexes may not be solved so readily. Atkinson (1892) was perhaps the first to find that a disease, fusarium wilt

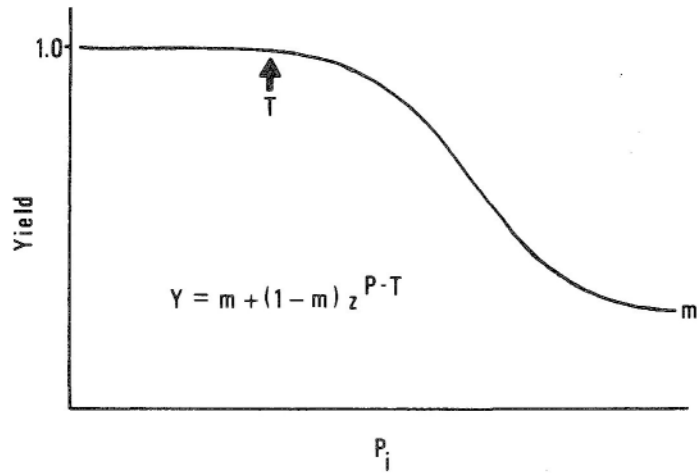

FIGURE 5 Generalized relationship between plant parasitic nematode densities at planting (P_i) and relative yield of a susceptible host. T = tolerance limit; m = minimum yield; z = a constant representing the amount of root not attacked by the nematodes. (From Seinhorst, 1965.)

of cotton, was more serious in the presence of a nematode (a *Meloidogyne* sp.) than in its absence. The range of interactions among plant parasitic nematodes and other microorganisms as they affect plant growth have been extensively reviewed (e.g., Huang, 1987; Hussey and McGuire, 1987; Lamberti and Roca, 1987; Powell, 1979; Sikora and Carter, 1987; Smith, 1987). The "other microorganisms" involved include fungi, bacteria, and viruses. Nematodes also interact with larger creatures (e.g., earthworms, mites, insects, and rodents), but these interactions are not well characterized except for nematode–nematode interactions, discussed below. Powell (1971) and his students pioneered this aspect of nematology. Any change caused by an organism in one part of the plant affects the physiology of other parts of the plant and thus may act some distance from the source; such studies now provide a basis for investigations at the cellular and molecular level.

Nematodes may be involved in disease caused by fungi (*Cylindrocladium crotalariae, Fusarium* spp., *Rhizoctonia solani, Verticillium* spp., and others) that invade the roots, as well as foliage pathogens (Nicholson et al., 1985). Much of the early and most current work was with *Meloidogyne* spp., but fungal–nematode disease interactions are now known with *Belonolaimus, Pratylenchus*, and others. A range of interactions among hosts, nematode species, and mycorrhizal fungi have been reported (Smith, 1987). As with fungi, a range of interactions with bacterial plant pathogens exists. Nematodes may be involved in or required for development of certain plant diseases caused by bacteria (see below), interact positively or negatively with rhizobial symbionts of legumes (Huang, 1987), or feed on plant pathogenic bacteria (Nicholas, 1984).

As with other areas of nematode ecology, there are a number of problems that complicate interaction studies (Sikora and Carter, 1987; Wallace, 1983) because of the number of variables involved, especially in soil systems. The task is easier, but not without complications, with interactions of nematodes with other organisms in above-ground plant parts. Examples are the association of *Anguina tritici* and *Corynebacterium tritici* that results in a different symptom alone from that in combination (Gupta and Swarup, 1972); the association of *Subanguina calamagrostis* and the fungus *Dilosphora alopecuri* (Norton et al., 1987); and *Anguina agrostis* and *Corynebacterium rathayi* (Bird, 1981). In the latter, the

nematode is a vector for the bacterium as a surface contaminant, the galls induced by the nematode become toxic to animals only when the galls are colonized by the bacteria.

Since the classic work of Hewitt et al., (1958), the transmission of plant viruses by nematodes has received much attention (Lamberti and Roca, 1987). About 20 nepoviruses are known to be transmitted by species of *Longidorus* or *Xiphinema*. At least two tobraviruses, tobacco rattle and pea early browning, are transmitted by species of *Paratrichodorus* or *Trichodorus*. Transmission of viruses by members of the Tylenchida has not been established. Nematodes ingest viruses when they feed on virus-infested plants, and evidently act mainly as a mechanical transfer for viruses, as the viruses do not multiply in the vector. However, viruses are retained in specific sites in the nematode, due partly at least to the nature of the protein surface of the virus particles, and perhaps the surface charge (Raski et al., 1973; Taylor and Robertson, 1977). It appears that many viruses are transmitted by a few nematodes, but because many viruses have wide plant hosts ranges, and some of the vectors are common and widespread, it is easy to comprehend that virus transmission is common.

G. Bioenergetics

Bioenergetics is the study of energy transformations and energy exchanges within and between living things and their environments. The scope of ecological energetics (versus biochemical-molecular energetics) and a derivation of the generalized formula used to describe energy flow through a population or community was given by Phillipson (1975) as:

$$C = P + R + F + U$$

where C (consumption) = total intake of food energy during a specified time interval; P (production) = energy content of the biomass of food digested less that respired or rejected; R (respiration) = energy converted to heat and loss in life processes; F (egesta) = energy content of food not digested; and U (excreta) = energy content of digested material passed from the body. The preferred energy unit used is the kilojoule (1 calorie = 4.816 J).

Each of these components requires a number of estimates (total densities, growth rates, reproduction, etc.) and laboratory determinations (oxygen consumption, biomass, energy content), and though the generalized formula appears quite simple, doing the actual calculations is complex. Yet some have attempted it, to allow comparisons to be made among populations, trophic groups, or habitats. Sohlenius (1980) and Yeates (1979) reviewed the literature on the contribution of the total nematode component to energy flow in terrestrial ecosystems. Sohlenius (1980) estimated that nematodes contribute only about 1% to total soil respiration, perhaps 10–15% of animal respiration. Yet bacterial-feeding nematodes can consume as much as 50% of the annual production of microfloral biomass (Paul and Clark, 1988); thus, as regulators of the primary decomposers combined with plant parasites as primary consumers, the contribution nematodes make to energy flow of a system is substantial, as their numbers would indicate. With respect to plant parasitic nematodes, analysis of energetics can also provide insight into their pathogenic effects on plants, and the interrelatedness of the biology of a parasite and its host (Atkinson, 1985).

IV. COMMUNITY ECOLOGY

A. Populations and Communities

A community, in the sense of bioenergetics, must be composed of primary producers, herbivores, and carnivores. However, the term "animal community" is used to describe assemblages of animals in a given habitat, assumed to have food web dependencies as well as mutualistic interactions (Boughey, 1973). A nematode community is an assemblage of nematodes, including primary consumers to predators. It can be studied from several aspects: by numbers of species, numbers of individuals, biomass, physiological or ecological activity, trophic groups, and so on. Techniques applied in population ecology can be applied to communities; community changes can result from normal cyclic patterns, competition or other density-dependent factors, environmental constraints, and many other events. Their interactions may be weak or tightly knit. If species become established, they fill a niche, but niches tend to complement each other and do not work in direct competition (Whittaker, 1975). Usually an increase in niches means an increase in productivity (Boughey, 1973).

A question often asked about communities is whether additional parasitic species could be introduced so that they persist and reproduce without causing extinction of other species. Most species capable of attacking a host species are not locally available and therefore we do not know how much species packing can occur. If an annual monocropping system could be perpetuated for hundreds of years, and if enough different nematode species could eventually be available, would there be greater species packing than there is now? There is evidence of increased species packing in the short term in that there was a trend toward an increase in numbers of obligatory parasites and total nematodes in separate alfalfa plots monitored during 1–3 and 3–5 years (Wasilewska, 1967, 1979). Longer term experiments are needed to allow small populations low and undetectable species to increase (Sohlenius et al., 1987).

The makeup of a community is not entirely a fortuitous one, although chance is important. The following factors in community formation, modified from Mueller-Dombois and Ellenberg (1974), seem to be pertinent for nematodes.

1. The species of an area provide the basic materials for a community.
2. Because of limited short-term dispersal and barriers of various kinds, most plant parasitic nematodes do not come in contact with a given host (see also Price, 1986; Holmes, 1986).
3. Properties of the nematodes themselves, especially their life forms, life cycles, and physiological requirements, allow different species to coexist, persist, and reproduce.
4. The niche is the total of factors operative in a given habitat.
5. Communities change over time; the time elapsed from the initial occupancy to any desired point will affect the community.
6. In addition is the biology, including the ecology, of the host. Although the host is part of a nematode's habitat, it is so important that a separate category is justified.

B. Habitats

The number of nematode species in a community varies with habitat. Prairies and woodlands generally are richer in all nematode species than are cultivated fields; and prairies are richer than woodlands (Burkhalter, 1928; Egunjobi, 1971; Wasilewska, 1979; Weaver and Smolik, 1987; Yeates, 1979), but the number of species in a cultivated field can be as high as

74 (Baird and Bernard, 1984). Plant parasites generally compose a higher percentage of the total nematode population in agricultural settings than in natural or lightly managed areas (Ferris and Ferris, 1974; Niblack and Bernard, 1985), with up to 16 species in a site.

In agroecosystems, many nematode species are an integral part of the community through parasitism of the host or interactions with other nematodes. Others are residual from a preceding crop or associated weeds. Probably most species are rare most of the time (Caughley and Lawton, 1981). Around a given crop, only a limited number of nematode species occur regularly. Other will be erratic, but most species will be absent. For example, over 170 plant parasitic nematode species have been associated with maize, but usually only three to eight species are found around a plant at any time. Similar number of species per site have been shown for other crops (Niblack, 1989; Yeates, 1987); Yeates (1987) suggested that around seven species per guild or trophic group per habitat is "normal" for nematodes.

Humankind is a great dispenser of nematodes, noted above, but most species in cultivated fields are probably residuals from natural areas before cultivated agriculture appeared. As is true with insects (Brown and Gibson, 1983; MacArthur, 1972), most introduced contemporary nematode species became established in disturbed areas. Agricultural systems generally develop toward monoculture, and associated management practices restrict herbage diversity. Cultivation, or compaction in no-till regimes, results in soil structural changes which in turn causes more moisture and temperature fluctuations than are found in noncultivated areas. These changes result in conditions often exceeding the ecological amplitude of many nematodes; the resulting unstable habitats inhibit or prevent many nematodes from becoming established or persisting. Agriculture favors some nematodes whose genetic makeup allows survival and reproduction under frequent environmental changes, including nutritional resources. Thus, nematodes in agricultural systems generally have lower richness and diversities than in natural areas.

C. Trophic Groups

During the long course of evolution, morphological and biochemical modifications result in a diversity of nematode feeding types and habits; thus trophic groups make a useful classification for community studies. Trophic groups have been variously categorized (e.g., Freckman and Caswell, 1985; Niblack, 1989; Overgaard-Nielsen, 1960; Wasilewska, 1971a,b; Yeates, 1971, 1979), and usually the categories are based on known feeding habits or pharyngeal morphology. They usually include those that feed primarily on bacteria (bacterivores, microbivores), fungi (mycophages, fungivores), higher plants (phytophages, plant parasites), small animals (carnivores, predators), and those that feed on a variety of substrates (omnivores). Freckman and Caswell (1985) provided descriptions of each category and presented a model of how interactions among them might occur (Fig. 6). Demarcations are not always clear and may overlap. For example, some of the primitive tylenchids feed on fungi as well as on higher plants. Several species of rhabditid genera (e.g., *Acrobeloides, Cephalobus, Panagrolaimus*) may be able to obtain nourishment from plant tissue (Poinar, 1983). A system of trophic classification that could be applied to nematodes and would reflect the importance of size among soil biota was proposed by Heal and Dighton (1985) to include microtrophic, mesotrophic, and macrotrophic groups.

Our main interest is with plant parasites, but they are only one component of a community, and thus may be influenced directly or indirectly by other biota, including other nematodes. Whether the nematode component of the soil biota represents a community of interacting members, or interactions are minimal among groups exploiting different resources, is a matter of question (Niblack, 1989; Yeates, 1984). Yeates (1987) reviewed the studies showing positive correlations between total nematode abundance and primary pro-

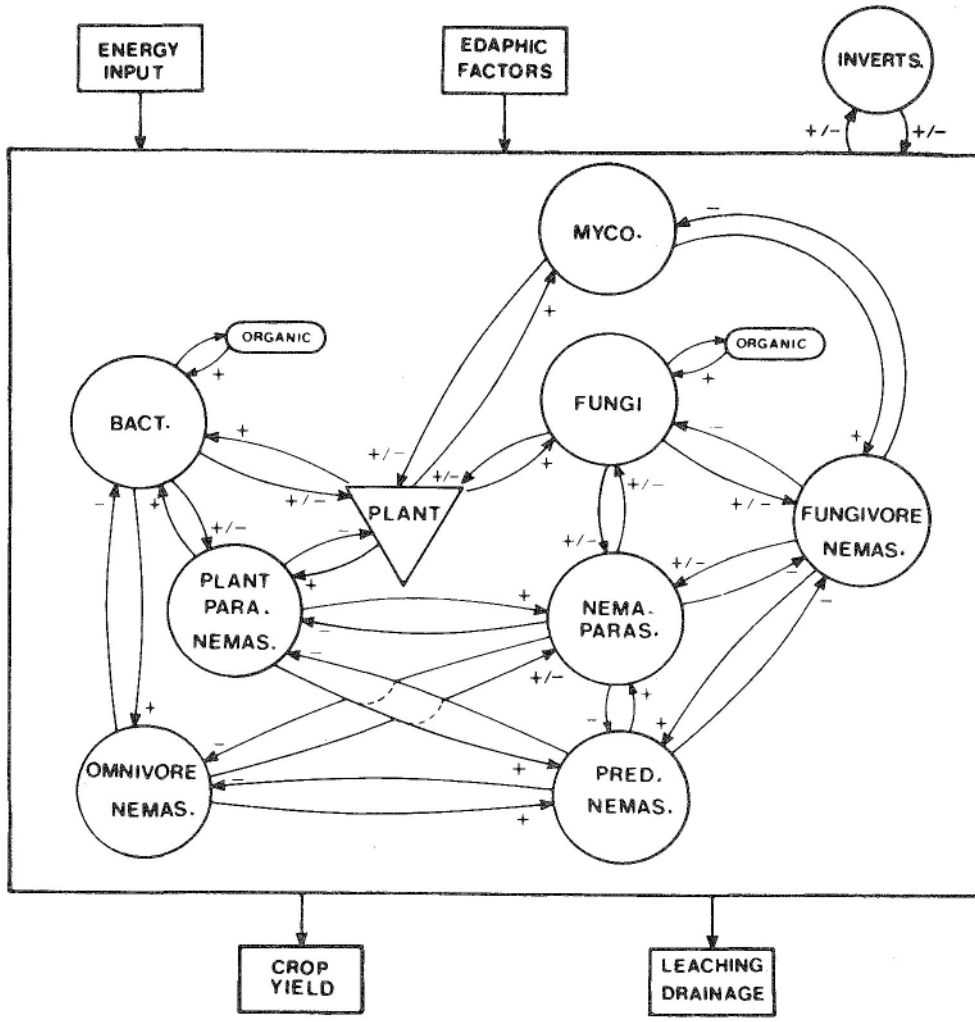

FIGURE 6 Interactions among some components of an agricultural food web. Arrows indicate the direction of the influence; + and – indicate a positive or negative effect. Bact. = bacteria; Fungivore Nemas. = fungivorous nematodes; Inverts. = invertebrates; Myco. = mycorrhizae; Nema. Paras. = parasites and predators of nematodes; Omnivore Nemas. = omnivorous nematodes; Organic = organic matter; Plant Para. Nemas. = plant parasitic nematodes; Pred. Nemas. = predatory nematodes. (Reproduced with permission from Freckman and Caswell, 1985.)

duction in natural or lightly managed ecosystems. Obviously, the nematode community has an impact on the vegetation that is not limited to the deleterious effects of parasitism, but few researchers have looked into this question in agroecosystems. Investigations of nematode community structure in agroecosystems can help explain whether the correlation is coincidental or, if not, determine the basis for the relationship.

D. Structure

Community structure means different things to different people. Most agree that community structure involves patterns of species occurrence, their relative abundance, and resource use (Holmes, 1986). Pielou (1972) takes the position that there has to be interdependence among the species of a community to have structure and that a haphazard assemblage of species with no interaction is devoid of structure. In studies of a complete nematode fauna there is more likely to be evidence of structure if the community is analyzed in terms of its trophic group (or some group other than taxonomic) composition, but this is a matter of controversy (Niblack, 1989).

The appearance of structure will depend on the level of resolution of the study. In agroecosystems, studies often focus on the plant parasitic nematodes only. Take, for example, two papers mentioned earlier. First, the work of Boag and Topham (1985) on species associations demonstrated a useful structure that might allow prediction of the occurrence of virus vector nematodes. Second, Yeates et al. (1985) investigated a temporal structure allowing coexistence of three potentially pathogenic nematode populations. In the first instance, the structure involves particular species, and in the second, the particular species are not important to the conclusion of temporal structure.

The most important first step in studying a nematode community is to make a faunistic list or to classify the community in some way (e.g., Baird and Bernard, 1985; Bostrom and Sohlenius, 1986; Sohlenius et al., 1987). Except in rare or unusual circumstances, nematode communities are not fully censused but rather are estimated as we sample the total population, recovering only those species that are at a detectable level based on the extraction technique used. Thus, estimates are nearly always conservative. Different lists can be obtained within the same area depending on the thoroughness of sampling. The rare species that might be missed are usually not important unless they have a large biomass, such as *Longidorus* spp., or carry a virus.

Once the classification and enumeration of the community is complete, the numbers can be investigated in a variety of ways. We use whatever expression is appropriate to the question, such as diversity or concentration of dominance, prominence values, dispersion, and so on, combined with proper analytical techniques (Niblack, 1989).

E. Diversity

It is a common belief that natural ecosystems are more diverse and thus more stable and resistant to perturbations than habitats disturbed by humans (Mindermann, 1956; Wasilewska, 1979). Although it is often stated that community diversity leads to stability, modern thought and evidence support the contention that environmental stability leads to community stability, which permits high diversity (Pielou, 1975). Diversity can be expressed in several ways (Pielou, 1975), most simply by counting up the number of species and comparing their proportions in a community. However, a single expression is useful, allowing for statistical analyses and comparisons among communities. The expression most commonly used in some modification of the Shannon function, usually the Shannon–Wiener index:

$$H' = -\sum_{i=1}^{s} \log_2 p_i$$

where s = number of species, p_i = proportion belonging to the i_{th} species, and H' estimates the probability of correctly predicting the species of an individual randomly drawn from the

population. H' confounds the number of species and their evenness, and it is desirable to keep them distinct (Pielou, 1975). Therefore, a measurement of evenness (often called J or J') can be used:

$$J' = \frac{H'}{H'_{max}}$$

where $H'_{max} = \log_2 s$.

What have diversity studies shown in plant nematology? We know that there are differences in community composition when β diversity is measured along a toposequence or over time (Fig 2; Norton and Oard, 1981). Yeates (1984) found in his study of nematode populations in seven soils with grazed pastures that diversity was not related to pasture productivity, and evenness was negatively correlated with the abundance of the abundance of the dominant species. Richness of species and diversity can vary greatly in a generalized biome. For example, the diversity of plant parasitic nematodes in forests of the Adirondack Mountains of New York State decreases as elevation increases due greatly to the more rigorous climate at the higher elevations (Norton and Oard, 1982). H' was lower in a maize field when biomass rather than numbers was used to calculate the statistic (Fig.4; Norton and Edwards, 1988). This is because a few large nematodes, including *Longidorus brevianulatus*, dominated the community biomass whereas they constituted a small numerical part of the community. Similarly, H' averaged over 15 sites in an Iowa prairie was lower when nematode biomass was used rather than numbers (Norton and Schmitt, 1978). That H' can vary with crops and soils can be demonstrated by calculating it from data given by Ferris and Bernard (1971b, Table 1) from rotation crops in Illinois; H' tended to be higher around corn than with soybeans. Niblack and Bernard (1985) reported that H' was higher around maple than peach or dogwood in Tennessee nurseries. H' was positively correlated with age of tree in dogwood, but not with maple sites, and only weakly correlated with degree of weed cover or number of weed species present; thus increasing diversity of herbage did not increase herbivorous nematode diversity. Species richness and diversity were not affected by soil cultivation in an annual compared with a perennial cropping system in Sweden (Bostrom and Sohlenius, 1986; Sohlenius et al., 1987).

F. Interaction Among Plant Parasitic Nematodes

It is common for three or more species of plant parasitic nematodes to occupy the same general area and feed on the same host at the same time, and these nematodes may each have measurable effects on their hosts. Thus, it is reasonable to expect that interactions between or among the nematodes will affect either the nematodes or plant growth, or both. Indeed, the interactions documented range from neutral (no measurable interaction) to stimulatory to deleterious to one or more of the participants. Most research on interactions has been under confined conditions such as in pots in the greenhouse and often with numbers much larger than those found in nature. Nonetheless, nematodes do interact in reality, with consequences for agriculture. The literature has been reviewed by Eisenback and Griffin (1987).

Oostenbrink (1966) attributed the polyspecific nature of plant parasitic nematode communities to four factors, three of which have been mentioned in previous sections of this chapter: (a) the effect of humans in distributing nematodes in soil and plant parts; (b) the wide host ranges of many plant nematodes; (c) their ability to survive; and (d) the low incidence of interspecific competition among them. Opinions on the existence and importance of competition, difficult to study in any event, have varied from almost complete disregard

to those of the "competitionists," who consider competition to be a major characteristic of interactions among species (Lawton, 1984; Schoener, 1982). In studies of population changes, it is sometimes tempting to ascribe inverse peaks between them. This pattern has lead to the assumption that one nematode can outcompete another, but population changes may merely be a matter of differences in niche dimensions and have nothing to do with interactions among species. For instance: (a) nematodes are highly aggregated, and their distribution patterns may not overlap; (b) life cycles vary so that some nematodes are common or rare at different times; (c) several kinds of substrates occur in a root system, such as root hairs and other epidermal cells, cortical parenchyma, stelar tissue, and so on, that may be preferred by different nematodes; (d) even when parasitized by large numbers of nematodes, usually there is a lot of tissue that is not colonized. A clear distinction should be made between true competition and population decline of a species due to natural cyclic patterns or indirect interactions.

Competition is not likely to occur when many niches occur, when there are vacant niches, when the substrate is rapidly growing, when there is slow colonization, or when the substrate is ephemeral (Price, 1986). Competition is most likely to occur when reproduction of the parasite is rapid and the host tissue damage is great. At the beginning of the season, at least with annual crops in temperate regions, the resources are growing faster than most plant parasitic nematodes can multiply. Thus, there is apt to be no competition unless the initial population is so large that root growth is markedly reduced or there are physiology changes in the host that may favor the nematode.

Competition theory is difficult to test with nematodes for at least three reasons: (a) suitable controls are difficult to establish; (b) growing seasons are often too short for long-term interactions to be evident; and (c) the change in substrate by nematode destruction is too rapid to allow continuous "healthy" nutritional resource. Theoretically, competition should increase diversity by forcing some nematodes to feed on a less favorable substrate. Because of a diversity of substrates and physical and chemical factors, and thus many niches, competition is more apt to occur intraspecifically than interspecifically. The fact remains that, in general, plants are not being eliminated by nematodes, and a plant is capable of supporting a far greater nematode population than it usually does.

V. CONCLUSIONS

In this chapter, we have often emphasized how many problems there are with studies of nematode ecology, even at the most prosaic level. We cannot begin to suggest that all questions have been addressed, much less answered. Our discussions have also frequently included such statements as, "A range of interactions exist, from one extreme to another." One cannot assume that the "average" nematode falls somewhere in the middle, and overgeneralizations are a constant hazard. Every level of resolution gives a new perspective on the complexity of the nematodes' milieu. Because of space constraints, we have dealt with a number of subjects in a cursory way. Even so, it should be obvious that the major theme in the biology and ecology of nematodes is interrelatedness. Changing even the smallest component of a community will have far-reaching effects, whether or not they are immediately obvious or even measurable. Yet communities retain remarkable stability in the absence of catastrophe. There is increasing emphasis on developing sustainable agricultural production systems (i.e., with less harsh environmental effects) given the increasing demands of human populations on productivity. This is the best reason for plant nematologists to take a broad view of the role nematodes play in agroecosystems and still satisfy what Bird (1981)

called our "understandable tendency to study organisms only when they impinge on [our] economy."

REFERENCES

Alby, T., Ferris, J. M., and Ferris, V. R. 1983. Dispersion and distribution of *Pratylenchus scribneri* and *Hoplolaimus galeatus* in soybean fields. *J. Nematol.* 15: 418–426.

Anderson, D. C. 1987. Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *Quart. Rev. Biol.* 62: 261–286.

Anonymous. 1984. *Distribution of Plant-Parasitic Nematode Species in North America.* Society of Nematologists, Hyattsville, MD.

Antoniou, M. 1989. Arrested development in plant parasitic nematodes. *Helminthol. Abstr. Ser. B.* 58: 1–19.

Atkinson, H. J. 1985. The energetics of plant parasitic nematodes: A review. *Nematologica* 31: 62–71.

Atkinson, G. F. 1892. Some diseases of cotton. *Alabama Agr. Expt. Sta. Bull.* 41: 1–65.

Baird, S. M., and Bernard, E. C. 1984. Nematode population and community dynamics in soybean-wheat cropping and tillage regimes. *J. Nematol.* 16: 379–386.

Barker, K. R. 1982. *Criconemella* in the Southeastern United States. In *Nematology in the Southern United States*, R. D. Riggs, ed. Southern Cooperative Series Bulletin 276, pp. 150–156.

Barker, K. R., and Noe, J. P. 1988. Techniques in quantitative nematology. In *Experimental Techniques in Plant Diseases Epidemiology*, J. Kranz and J. Rotem, eds. Springer-Verlag, New York, pp. 223–236.

Barker, K. R., and Nusbaum, C. J. 1971. Diagnostic and advisory programs. In *Plant Parasitic Nematodes*, Vol. 1, B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Academic Press, New York, pp. 281–302.

Barker, K. R., Nusbaum, C. J., and Nelson, L. A. 1969. Seasonal population dynamics of selected plant-parasitic nematodes as measured by three extraction procedures. *J. Nematol.* 1: 232–239.

Bird, A. F. 1981 The *Anguina-Corynebacterium* association. In *Plant Parasitic Nematodes*, Vol. 3, B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Academic Press, New York, pp. 303–323.

Bird, A. F. 1983. Growth and moulting in nematodes: Changes in the dimensions and morphology of *Rotylenchulus reniformis* from start to finish of moulting. *Int. J. Parasitol.* 13: 201–206.

Bird, G. W., and Thompson, I. J. 1980. Integrated pest management: The role of nematology. *Bioscience* 30: 670–674.

Boag, B., and Topham, P. B. 1985. The use of association of nematode species to aid the detection of small numbers of virus-vector nematodes. *Plant Pathol.* 34: 20–24.

Bostrom, S., and Sohlenius, B. 1986. Short-term dynamics of nematode communities in arable soil. Influence of a perennial and an annual cropping system. *Pedobiologia* 29: 345–357.

Boughey, A. S. 1973. *Ecology of Populations.* Macmillan, New York.

Brown, J. H., and Gibson, A. C. 1983. *Biogeography.* Mosby, St. Louis.

Burkhalter, M. 1928. Die Verbreitung der freilebenden Erdnematoden in verschiedenen Gelandarten in Massif der Rochers de Naya (2045 m.) *Rev. Suisse Zool.* 35: 389–437.

Caughley, G., and Lawton, J. H. 1986. Plant-herbivore systems. In *Theoretical Ecology: Principles and Applications*, R. M. May Ed. Sinauer, Sunderland, MA.

Cook, R., and Evans, K. 1987. Resistance and tolerance. In *Principles and Practices of Nematode Control in Crops*, R. H. Brown and B. R. Kerry, eds. Academic Press, New York, pp. 179–222.

Crofton, H. D. 1971. Form, function, and behavior. In *Plant Parasitic Nematodes*, Vol. 1, B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Academic Press, New York, pp. 83–116.

Demeure, Y., and D. W. Freckman. 1981. Recent advances in the study of anydrobiotic nematodes. In *Plant Parasitic Nematodes*, Vol. 3, B. M. Zuckerman and R. A. Rohde, eds. Academic Press, New York, pp. 205–226.

Dropkin, V. H. 1988. The concept of race in phytonematology. *Ann Rev. Phytopathol.* 26: 145–161.

Duncan, L. W., and Ferris, H. 1982. Interactions between phytophagus nematodes. In *Nematodes in Soil Ecosystems*, D. W. Freckman, ed. University of Texas Press, Austin, pp. 29–54.

Duncan, L. W., and McSorley, R. 1987. Modeling nematode populations. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD, pp. 377–389.

Dusenberry, D. B. 1987. Prospects for exploring sensory stimuli in nematode control. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD, pp. 131–135.

Egunjobi, O. A. 1971. Soil and litter nematodes in some New Zealand forests and pastures. *N. Zealand J. Sci.* 14: 568–579.

Eisenback, J. D., and Griffin, G. D. 1987. Interactions with other nematodes. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD, pp. 313–320.

Evans, A. A. F. 1987. Diapause in nematodes as a survival strategy. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD, pp.180–187.

Ferris, H., and J. W. Noling. 1987. Analysis and prediction as a basis for management decisions. In *Principles and Practices of Nematode Control in Crops*, R. H. Brown and B. R. Kerry, eds. Academic Press, New York, pp. 49–85.

Ferris, H., and Wilson, L. T. 1987. Concepts and principles of population dynamics. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD, pp. 373–376.

Ferris, V. R., and Bernard, R. L. 1971a. Crop rotation effects on population densities of ectoparasitic nematodes. *J. Nematol.* 3: 119–122.

Ferris, V. R., and Bernard, R. L. 1971b. Effect of soil type on population densities of nematodes in soybean rotation fields. *J. Nematol.* 3: 123–128.

Ferris, V. R., and Ferris, J. M. 1974. Interrelationships between nematode and plant communities in agricultural ecosystems. *Agro-Ecosystems* 1: 275–299.

Ferris, V. R., Goseco, C. G., and Ferris, J. M. 1976. Biogeography of free-living soil nematodes from the perspective of plate tectonics. *Science* 193: 508–510.

Filipjev, I. N., and Schuurmans Stekhoven, J. H., Jr. 1941. *A Manual of Agricultural Helminthology*. E. J. Brill, Leiden.

Flegg, J. J. M. 1968. The occurrence and distribution of *Xiphinema* and *Longidorus* species in southeastern England. Nematologica 14: 189–196.

Freckman, D. W. 1982. Parameters of the nematode contribution to ecosystems. In *Nematodes in Soil Ecosystems*, D. W. Freckman, ed. University of Texas Press, Austin, pp. 81–97.

Freckman, D. W., and Caswell, E. P. 1985. The ecology of nematodes in agroecosystems. *Ann. Rev. Phytopathol.* 23: 275–296.

Gupta, P., and Swarup, G. 1972. Ear-cockle and yellow ear-rot diseases of wheat. II. Nematode bacterial association. *Nematologica* 18: 320–324.

Heal, O. W., and Dighton, J. 1985. Resource quality and trophic structure in the soil system. In *Ecological Interactions in Soil: Plants, Microbes, and Animals*, A. H. Fitter, D. Atkinson, D. J. Read, and M. B. Usher, eds. Blackwell Scientific, Oxford, pp. 339–354.

Hewitt, W. B., Raski, D. J., and Goheen, A. C. 1958. Nematode vector of soil-borne fanleaf virus of grapevines. *Phytopathology* 48: 586–595.

Holmes, J. C. (1986). The structure of helminth communities. In *Parasitology: Quo Vadit? Proceedings of the Sixth International Congress of Parasitology*, M. J. Howell, ed. Australian Academy of Science, Canberra, pp. 203–208.

Huang, J.-S. 1987. Interactions of nematodes with rhizobia. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD, pp. 301–306.

Hussey, R. S. 1989. Disease-inducing secretions of plant parasitic nematodes. *Ann. Rev. Phytopathol.* 27: 123–141.

Hussey, R. S., and McGuire, J. M. 1987. Interaction with other organisms. In *Principles and Practices of Nematode Control in Crops*, R. H. Brown and B. R. Kerry, eds. Academic Press, New York, pp. 292–328.

Ishibashi, N., Kondo, E., and Kashio, T. 1975. The induced molting of 4th stage larvae of pin nematode, *Paratylenchus aciculus* Brown (Nematoda: Paratylenchidae) by root exudate of host plant. *Appl. Ent. Zool.* 10: 275–283.

Koenning, S. R., Schmitt, D. P., and Barker, K. R. 1985. Influence of selected cultural practices on survival of *Pratylenchus brachyurus* and subsequent effects on soybean yield. *J. Nematol.* 17: 464–469.

Kraus-Schmidt, H., and Lewis, S. A. 1979. Seasonal fluctuations of various nematode populations in cotton fields in South Carolina. *Plant Dis. Reptr.* 63: 859–863.

Lamberti, F., and Roca, F. 1987. Present status of nematodes as vectors of plant viruses. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD, pp. 321–328.

Lawton, J. H. 1984. Herbivore community organization: General models and specific tests with phytophagous insects. In *A New Ecology*, P. W. Price, C. N. Slobodchikoff, and W. S. Gaud, eds. John Wiley and Sons, New York, pp. 329–352.

MacArthur, R. H. 1972. *Geographical Ecology, Patterns in the Distribution of Species*. Harper and Row, New York.

May, R. M., and Seger, J. 1986. Ideas in ecology. *Am. Scientist* 74: 256–267.

Masamune, T., Anetai, M., Takasuge, M., and Katsui, N. 1982. Isolation of a natural hatching stimulus, glycinoeclepin A, for the soybean cyst nematode. *Nature* 297: 495–496.

McSorley, R. 1987. Extraction of nematodes and sampling methods. In *Principles and Practices of Nematode Control in Crops*, R. H. Brown and B. R. Kerry, eds. Academic Press, New York, pp. 13–41.

Mindermann, G. 1956. Aims and methods in population researches on soil-inhabiting nematodes. *Nematologica* 1: 47–50.

Mueller-Dombois, D., and Ellenberg, H. 1974. *Aims and Methods of Vegetation Ecology*. John Wiley and Sons, New York.

Niblack, T. L. 1989. Applications of community structure research to agricultural production and habitat disturbance. *J. Nematol.* 21:437–443 .

Niblack, T. L., and Bernard, E. C. 1985. Plant-parasitic nematode communities in dogwood, maple, and peach nurseries in Tennessee. *J. Nematol.* 17: 132–139.

Nicholas, W. L. 1984. *The Biology of Free-Living Nematodes*, 2nd ed. Clarendon Press, Oxford.

Nicholson, R. L., Bergeson, G. B., DeGennaro, F. P., and Viveiros, D. M. 1985. Single and combined effects of the lesion nematode and *Colletotrichum graminicola* on growth and anthracnose leaf blight of corn. *Phytopathology* 75: 654–661.

Nickle, W. R. (Ed.). 1984. *Plant and Insect Nematodes*. Marcel Dekker, New York.

Noe, J. P., and Barker, K. R. 1985. Overestimation of yield loss of tobacco caused by the aggregated spatial pattern of *Meloidogyne incognita*. *J. Nematol.* 17: 245–251.

Norton, D. C. 1978. *Ecology of Plant-Parasitic Nematodes*. John Wiley and Sons, New York.

Norton, D. C. and Edwards, J. 1988. Age structure and community diversity of nematodes associated with maize in Iowa sandy soils. *J. Nematol.* 20:340–350.

Norton, D. C., and Oard, M. 1981. Plant-parasitic nematodes in loess toposequences planted with corn. *J. Nematol.* 13: 314–321.

Norton, D. C., and Oard, M. 1982. Occurrence and diversity of some Criconematidae and Paratylenchidae in the Adirondack Mountains of New York State. *Proc. Iowa Acad. Sci.* 89: 11–14.

Norton, D. C., and Schmitt, D. P. 1978. Community analyses of plant-parasitic nematodes in the Kalsow Prairie, Iowa. *J. Nematol.* 10: 171–176.

Norton, D. C., Cody, A. M., and Gabel, A. W. 1987. *Subanguina calamagrostis* and its biology in *Calamagrostis* spp. in Iowa, Ohio, and Wisconsin. *J. Nematol.* 19: 260–262.

O'Bannon, J. H., Radewald, J. D., and Tomerlin, A. T. 1972. Population fluctuation of three parasitic nematodes in Florida citrus. *J. Nematol.* 4: 194–199.

Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. *Meded. Landouwhogeschool Wageningen* 66–4.

Overgaard-Nielsen, C. 1949. Studies on the soil microfauna. II. The soil inhabiting nematodes. *Natura Jutland.* 2: 1–131.

Paul, E. A., and Clark, F. E. 1988. *Soil Microbiology and Biochemistry*. Academic Press, New York.

Perry, R. N. 1987. Host–induced hatching of phytoparasitic nematode eggs. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD, pp. 159–164.

Phillipson, J. 1975. Introduction to ecological energetics. In *Methods for Ecological Bioenergetics*, W. Grodzinski, R. Z. Klekowski, and A. Duncan, eds. Blackwell Scientific, Oxford, pp. 3–13.

Pielou, E. C. 1972. Measurement of structure in animal communities. In *Ecosystem Structure and Function*, J. A. Weins, ed. Oregon State University Press, pp. 113–135.

Pielou, E. C. 1975. *Ecological Diversity*. Wiley–Interscience, New York.

Poinar, G. O., Jr. 1983. *The Natural History of Nematodes*. Prentice-Hall, Englewood Cliffs, N.J.

Poinar, G. O., Jr., and Jansson, H.-B. 1988. *Diseases of Nematodes*. CRC Press, Boca Raton.

Powell, N. T. 1971. Interactions between nematodes and fungi disease complexes. *Ann. Rev. Phytopathol.* 9: 253–273.

Powell, N. T. 1979. Internal synergisms among organisms inducing disease. In *Plant Disease, Vol. 4*, J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York, pp. 113–133.

Price, P. W. 1986. Evolution in parasitic communities. In *Parasitology: Quo Vadit? Proc. 6th Inter, Congr. Parasitol.*, M. J. Howell, ed. Australian Acad. Sci., Canberra, pp. 209–214.

Prot, J.-C. 1980. Migration of plant parasitic nematodes towards plant roots. *Rev. Nematol.* 3: 305–318.

Raski, D. J., Hewitt, W. B., Goheen, A. C., Taylor, C. E., and Taylor, R. H. 1965. Survival of *Xiphinema index* and reservoirs of fanleaf virus in fallowed vineyard soil. *Nematologica* 11: 349–352.

Raski, D. J., Maggenti, A. R., and Jones, N. O. 1973. Location of grapevine fanleaf and yellow mosaic virus particles in *Xiphinema index*. *J. Nematol.* 5: 208–211.

Rebois, R. V., and Huettel, R. N. 1986. Population dynamics, root penetration, and feeding behavior of *Pratylenchus agilis* in monoxenic root cultures of corn, tomato, and soybean. *J. Nematol.* 18: 392–397.

Reynolds, H. W., and O'Bannon, J. H. 1963. Factors influencing the citrus nematode and its control on citrus replants in Arizona. *Nematologica* 9: 337–340.

Robbins, R. T., and Barker, K. R. 1974. The effects of soil type, particle size, temperature, and moisture on reproduction of *Belonolaimus longicaudatus*. *J. Nematol.* 6: 1–6.

Schmitt, D. P. 1973. Population fluctuations of some plant-parasitic nematodes in the Kalsow Prairie, Iowa. *Proc. Iowa Acad. Sci.* 80: 69–71.

Schoener, T. W. 1982. The controversy over interspecific competition. *Am. Scientist* 70: 586–595.

Seinhorst, J. W. 1965. The relation between nematode density and damage to plants. *Nematologica* 11: 137–154.

Seinhorst, J. W. 1973. The relation between nematode distribution in a field and loss in yield at different average nematode densities. *Nematologica* 19: 421–427.

Sikora, R. A., and Carter, W. W. 1987. Nematode interactions with fungi and bacterial plant pathogens: Fact or fancy. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD, pp. 307–312.

Smith, G. S. 1987. Interactions of nematodes with mycorrhizal fungi. In *Vistas on Nematology*, J. A. veech and D. W. Dickson, eds. Society of Nematologists, pp. 292–300.

Sohlenius, B. 1980. Abundance, biomass, and contribution to energy flow by soil nematodes in terrestrial ecosystems. *Oikos* 34:186–194.

Sohlenius, B., Bostrom, S., and Sandor, A. 1987. Long-term dynamics of nematode communities in arable soil under four cropping systems. *J. Appl. Ecol.* 24: 131–144.

Stone, A. R., Platt, H. M., and Khalil, L. F., eds. 1983. *Concepts in Nematode Systematics*. Academic Press, New York.

Strong, D. R. 1984. Density-vague ecology. In *A New Ecology*, P. W. Price, C. N. Slobodchikoff, and W. S. Gaud, eds. Wiley-Interscience, New York, pp. 313–327.

Taylor, C. E., and Robertson, W. M. 1977. Virus vector relationships and mechanics of transmission. *Proc. Am. Phytopathol. Soc.* 4: 20–29.

Topham, P. B., Alphey, T. J. W., Boag, B., and De Waele, D. 1985. Comparison between plant-parasitic nematode species association in Great Britain and in Belgium. *Nematologica* 31: 458–467.

Waliullah, M. I. S. 1983. Biomass of some selected plant-parasitic and soil nematodes. *Ind. J. Nematol.* 13: 32–47.

Wallace, H. R. 1971. Abiotic influences in the soil environment. In *Plant Parasitic Nematodes*, Vol. 1, B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Academic Press, New York, pp. 257–280.

Wallace, H. R. 1983. Interactions between nematodes and other factors on plants. *J. Nematol.* 15: 221–227.

Wasilewska, L. 1967. Analysis of the occurrence of nematodes in alfalfa crops. II. Abundance and quantitative relations between species and ecological groups of species. *Ekol. Pol. Ser. A.* 15: 347–371.

Wasilewska, L. 1971a. Nematodes of the dunes of the Kampinos Forest. II. Community structure based on numbers of individuals, state of biomass, and respiratory metabolism. *Ekol. Pol.* 19: 651–688.

Wasilewska, L. 1971b. Trophic classification of soil and plant nematodes. *Wiadm. Ekol.* 17: 379–388.

Wasilewska, L. 1979. The structure and function of soil nematode communities in natural ecosystems and agrocenoses. *Ekol Pol.* 27: 97–146.

Weaver, T., and Smolik, J. 1987. Soil nematodes of northern Rocky Mountain ecosystems: Genera and biomass. *Great Basin Nat.* 47: 473–479.

Wharton, D. A. 1986. *A Funcional Biology of Nematodes*, Johns Hopkins University Press, Baltimore.

Whittaker, R. H. 1975. *Communities and Ecosystems*. Macmillan, New York.

Wyss, U. 1981. Ectoparasitic root nematodes: Feeding behavior and plant cell responses. In *Plant Parasitic Nematodes*, Vol. 3, B. M. Zuckerman and R. A. Rohde, eds. Academic Press, New York, pp. 325–354.

Wyss, U. 1987. Video assessment of root cell responses to dorylaimid and tylenchid nematodes. In *Vistas on Nematology*, J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD., pp. 211–220.

Yeates, G. W. 1971. Feeding types and feeding groups in plant and soil nematodes. *Pedobiologia* 11: 173–179.

Yeates, G. W. 1979. Soil nematodes in terrestrial ecosystems. *J. Nematol.* 11: 213–229.

Yeates, G. W. 1981. Nematode populations in relation to soil environmental factors: A review. *Pedobiologia* 22: 312–338.

Yeates, G. W. 1984. Variation in soil nematode diversity under pasture with soil and year. *Soil Biol. Biochem.* 16: 95–102.

Yeates, G. W. 1987. How plants affect nematodes. *Adv. Ecol. Res.* 17: 61–113.

Yeates, G. W. 1988. Contribution of size classes to biovolume, with special reference to nematodes. *Soil Biol. Biochem.* 20: 771–773.

Yeates, G. W., and Coleman, D. C. 1982. Role of nematodes in decomposition. In *Nematodes in Soil Ecosystems*, D. W. Freckman, ed. University of Texas Press, Austin, pp. 55–80.

Yeates, G. W., Watson, R. N., and Steele, K. W. 1985. Complementary distribution of *Meloidogyne, Heterodera*, and *Pratylenchus* (Nematoda: Tylenchida) in roots of white clover. *Proc. 4th Aust. Conf. Grassland Invert. Ecol.*, pp. 71–79.

II

TECHNICAL METHODS FOR COLLECTION AND PREPARATION OF NEMATODES

3

Methods for Collection and Preparation of Nematodes

Part 1. Field Sampling and Preparation of Nematodes for Optic Microscopy

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I. INTRODUCTION

This chapter reviews the techniques used for studies of nematode systematics and identification: collection, fixation, mounting, and related studies.

The excellent book *Laboratory Methods for Work with Plant and Soil Nematodes* has been in wide use since it was first published by T. Goodey in 1949. It was recently updated for the fifth time (Southey, 1986a) to include detailed descriptions of all published techniques up to 1984. The present chapter will not duplicate information available from Southey's book, but it will review the pros and cons of the various techniques.

II. COLLECTION OF NEMATODE SAMPLES

Southey (1986b) and Barker (1985a) gave accounts of the problems and errors attached to nematode sampling due to the patchy distribution of most nematode species. Southey discusses mostly cyst nematodes, and Barker is interested in *Meloidogyne*, but their comments are true for other plant parasitic nematodes. Nematode distribution is aggregated, and the distribution pattern varies depending on seasonal fluctuation, crop and the species considered. Vertical distribution patterns also vary.

Nematode populations most often are fitted to a negative binomial distribution, described by two parameters: the mean and an aggregated index k, that reflects clumping. Ag-

gregation is also measured by Taylor's power law where the aggregation index also increases with clumping.

Merny and Déjardin (1970) sampled the nematode population levels in two 1-hectare fields in Ivory Coast by taking 100 samples per hectare. The populations followed Taylor's law with an aggregation index of 1.65. This level of sampling allows a reasonable estimate of the population after log transform of the raw data, but only if the mean population is high enough. If only 10 samples per hectare are taken, it is reasonable to estimate the population levels within the following five classes:

Very low: Very high variability; or four samples or more have no nematodes,
Low: Lower end of the confidence interval of the mean < 25 nematodes per liter of soil,
Average: Lower end of confidence interval between 25 and 99 nem/liter soil,
High: Lower end of confidence interval between 100 and 399 nem/liter soil,
Very High: Lower end of confidence interval > 400 nem/liter soil.

The sampling method must be adapted to circumstances and to the purpose of sampling. Vertical distribution should be considered, as most plant parasitic nematodes follow the root distribution. Sampling for nematodes associated with trees should be done on the drip line where the actively growing rootlets can be found. Quénéhervé and Cadet (1986) described a sampling technique for banana roots separated into roots attached to the mother plant, and those attached to first- and second-generation shoots. Barker (1985a) reviews several patterns for sample collection in the field. Southey (1986b) discusses the problem of sampling for regulatory purposes, and he gives a table with the percentage chances of detection and failure to detect various population levels. Sampling should be done across the rows, but a deliberate sampling bias may be introduced. For example, in California strawberry nurseries the strawberries are planted by a machine that does four rows at a time. There is one plant container per row, so the first container will do rows 1, 5, 9, etc. Field sampling at the end of the growing season is biased so that plants that came from all four containers have the same chance to be sampled.

When investigating the eventuality that nematodes are responsible for a patch of poor growth, sampling should be done from the center of the diseased patch toward a place outside the patch where the plants are still in good condition. If a nematode is responsible for the damage, the largest populations will probably be found at the boundary of the patch, where the plants are still able to provide an abundant source of food to the parasites. Wallace (1971) gave the results of such a sampling for damages of *Helicotylenchus dihystera* on turf in Australia.

The biology of the nematodes should be known, particularly if a particular species is targeted. Searching for an infestation by *Xiphinema americanum* by placing carefully scrubbed roots in a mist extractor will surely fail because all *Xiphinema* are ectoparasites.

Knowing the life cycle of the nematodes can help sampling. In South Dakota, gravid females are found only from late April to early June, when new roots are produced by the hosts. In samples collected during August not a single gravid female was found and very few adult were present (Thorne and Malek, 1968). The life span of the species sampled should also be considered (Barker, 1985a).

Obviously, the field samples should be kept cool and moist, and be processed as soon as possible. Some species are very fragile and special precaution must be taken for their recovery. Trichodorids may be killed in a soil sample dropped from a height. The mortality may be very high when the sample is dropped repeatedly (Brown and Boag, 1988). In Africa, the trichodorids often disappear completely when samples are brought back from

the field via dirt roads with washer-board surface. It was found that the best way to recover trichodorids was to force an aluminum can into the soil and carefully remove the can with the soil core inside. The can protected the nematodes until extraction.

Hooper (1986a) gave some general advice: recovery depends on host plant, sampling depth, soil type, and type of nematode. Samples should be kept in plastic bags as survival decreases in dry soil. However, saturated soils also are adverse to survival. Direct sunlight and excessive heat should be avoided. The samples should be stored at 5–20°C and processed as soon as possible. A few days of storage may increase recovery probably due to hatching of eggs. This increases the chances for detecting a species present in low numbers, but newly hatched individuals should not be counted when estimating populations.

Air photography may be useful for evaluating damages of some species. Barker (1985a) reviewed historical attempts. Shesteperov (1986) described trials held in the USSR for evaluation of damages caused by *Globodera rostochiensis* on potato from aerial photographs. The method was fairly successful from the time of bud formation to the flowering of potatoes, but overall success was only 50% against 95% accuracy from examination of soil samples.

III. EXTRACTION

Numerous methods exist that take advantage of various characteristics of the nematodes for separating them from the substrate: difference in size, in density, in motility, etc. Some methods are more suited for soil extractions, others for extractions of nematodes from vegetal tissues. Any method is rarely used alone, but rather in combination with other methods. For example, the residue on the sieve in the sieving method can be cleaned by Baermann funnel, migration, or centrifugation.

A. Direct Examination

Root material can be cleaned from most debris. They can be dissected under the dissecting microscope for direct observation of the nematodes within the tissues. This method is particularly suited when juvenile forms of species with obese mature females have been found in a soil sample. Roots from nearby plants may be dissected for recovery of the adult females. Staining can help by selectively coloring the nematodes within the roots.

B. Sieving

Sieving is mostly used for soil, but also for cleaning nematode suspensions after recovery from soil and roots by other methods.

Sieving discards the particles either smaller or larger than the nematodes. Soil and nematodes are suspended in water. After a short wait to allow heavy particles to sink, the supernatant is poured through a sieve. Cobb recommends using a series of sieves, with smaller and smaller mesh size.

Nematode recovery is improved by briefly (less than 5 min) soaking the soil in water before the extraction. The clay particles can be dispersed using a mixer or automatically shaking the sieves, or by various chemicals, such as sodium oxalate, or detergents containing sodium (Seinhorst, 1956; Wehunt, 1973). Meerzainudeen et al. (1984) put small stones in the sieves to prevent clogging.

Specimens can be lost by passing through sieves with too large mesh size. Sieve openings should not be greater than one-tenth of the nematode length, and even then there

should be repeated sieving for reasonably accurate results. However, repeated sieving has its own faults, when specimens are trapped on the screen and cannot be washed out.

C. Migration

Nematode suspension contaminated by debris that hinders observation can be cleaned by active migration through a filter, generally a tissue paper filter. Inactive nematodes and many nematodes with body cuticular appendages and ornamentations (criconematids) are unable to go through the filter. There is also a risk of toxicity from components of the tissue paper.

Ryss (1987) described a filter composed of a 1-cm layer of coarse sand placed in a 1-mm mesh sieve partially submerged in water. Active nematodes, including large-size nematodes, migrated through the sand in one-half to 12 hr. Results were better than with tissue paper filters, but criconematids were lost.

Sudakova et al. (1986) report a technique for extracting *Aphelenchoides avenae* from thick fungal cultures using the nematodes' ability to migrate toward water-filled chambers. It is conceivable to improve this technique for the selective recovery of plant parasitic nematodes by using a solution of nematode attractant (root extract) instead of pure water.

D. Flotation

1. General Principles

Flotation methods take advantage of the difference of gravity between nematodes and debris. They process either by elutriation in a stream of water (including the Seinhorst two-flask method) or by centrifugation.

2. Seinhorst Two-Flask Technique

This type of extraction from soil can be done in the field with very simple material. It is remarkably efficient for small and medium-sized nematodes. It was found to be slower and less efficient than sieving for large nematodes such as longidorids (Brown and Boag, 1988).

3. Elutriation

In the elutriation technique, the nematode and soil particles are poured at the top of a column where is maintained an upward current of water. The current allows the heavier particles to settle while retaining the nematodes and the smaller particles in the column. Glass elutriators are fragile and easily broken, but there exist metallic models. The upward flow of water must be monitored and adjusted. The size of the soil sample is limited by the size of settling contained at the bottom of the apparatus. An advantage of the method is that it can be automatized (Byrd et al., 1976).

Winfield et al. (1987) describe a new soil elutriator, the Wye Washer, which they claim achieves extractions as good as or better than those from existing techniques, as well as easier operation.

4. Centrifugation

During centrifugation the nematodes float in a solution with a density greater than the average density of the nematode. The method is very good for extracting sluggish forms such as criconematids. It is generally more efficient than the other methods, and it may be used to

clean extracts obtained from sieving or elutriation. The density depends on the species of nematode: *Pratylenchus vulnus* and *Meloidogyne incognita* seem to be recovered at lower densities than *Criconemella xenoplax*, while *Xiphinema index* requires even higher densities (Viglierchio and Yamashita, 1983). Recovery also depends on the solute used. *Pratylenchus vulnus* is recovered at 1.060 with "Percoll" (a colloidal silica with polyvinylpyrrolidone), but at 1.100 with zinc sulfate (Viglierchio and Yamashita, 1983). Sugar is the most used solute because it is cheap. Sulfate of magnesium does not have the stickiness of sucrose. Sulfate of zinc has fewer osmotic effects but is more acid and toxic. Other manufactured solutes (Ludox, Ficoll, Percoll) have advantages over the simple chemicals but are more expensive. It is important to verify the specific gravity of the solution after mixing the solute. In hypertonic solutions, the nematode shrinks longitudinally, and the body assumes an accordion appearance, distorts, and collapses. It may or may not recover after being transferred back to water. In extreme cases, membrane functions collapse, allowing free inflow of the hypertonic solution through the body wall. Dead nematodes precipitate and they are eliminated in the pellet. Use of sucrose is not recommended for extraction of specimens to be studied with SEM (Eisenback, Part 2 of this chapter).

5. Settling Methods

Gravity is also used in "settling" methods (Barker, 1985a), where nematodes are allowed to settle at the bottom of a beaker or a test tube. Settling also occurs as the final step of most extraction processes, when extra water is siphoned out of the test tube containing the nematodes. Enough time should be allowed for all nematodes to settle (see "Mist Extraction," below). The rate of sedimentation of the nematodes is not as critical as the time it takes for the water to come to a complete rest after the initial nematode suspension has been poured into the tube. Differences of temperature may increase this time by allowing convection currents to further disturb the water.

E. Maceration

1. General Principles

Maceration (called root incubation by Hooper, 1986b) is used mostly for nematodes inside vegetal tissues because nematodes tend to leave roots immersed in water. Leaves and stems are generally not suitable for maceration techniques; however, the method was successful for *Aphelenchoides ritzemabosi* in chrysanthemum leaves (Cranston and Newton, 1965). Most nematodes emerge within 4–7 days. After about 2 weeks, a new generation appears that has developed within the plant tissues. The roots or shoots and leaves can be stored moist or immersed in water. The water can be either still (jars, plastic bags, Baermann funnel with closed stem) or flowing (mist extractor with open stem). Enzymes or chemicals can be added for speeding the decomposition of plant material.

2. Baermann Funnel

The Baermann funnel uses little labor and simple equipment: a funnel, a piece of rubber tube, and a clamp. It is used mostly for nematode extraction from plant material, but also from soil finely crumbled, or to clean nematode suspensions extracted using another technique. Active nematodes leave the decaying tissues, go through the cloth, and sink to the bottom of the funnel. Lack of oxygenation can kill or immobilize some nematodes. To limit this risk, it is better to use a polyethylene tube through which oxygen can diffuse, or add H_2O_2 to water, or use an air stream. This method allows recovery of active nematodes only,

and specimens can be trapped by the tissue and the sides of the funnel. The tissue material can be toxic and kill the nematodes. There is also a possibility of contamination of the water by bacteria or fungi that attack nematodes.

3. Mist Extraction

With this method, there is no risk of nematode death by lack of oxygen. A mist sprayer send a mist of water to several funnels, usually arranged in a square below. There may not be enough water in the corners for proper humidification of the plant material, or there may be too much water under the spray cone, creating a strong current that may carry individuals away, particularly good swimmers such as *Aphelenchoides* spp. Viglierchio and Schmitt (1983a) found that the sedimentation rate for various nematode species ranges from about 0.004 cm/sec for small *Meloidogyne* juveniles to 0.1 cm/sec and more for large nematodes such as *Xiphinema index*. Losses due to overflow or adherence to the funnel are negligible except with extremely low (10-min cycle with less than 10% spray time) or high (more than 50% spray time) mist cycles. The water pressure may not remain constant; the pipes and spray nozzles may have deposits that diminish the water flow and/or modify the spatial distribution of the spray. The system should be regularly cleaned and retested. To achieve more uniform spray, the mist system at the ORSTOM lab in Abidjan was changed to a battery of individual mist chambers, each chamber with its own sprayer and a single funnel. The funnel chambers were arranged in a single row to avoid water spillage and contamination that may occur when a funnel is removed from the back of a large chamber with several rows of funnels. Viglierchio and Schmitt (1983a) linked poor recovery to the quality of the paper tissue used. Some tissues may have a low permeability to nematodes. Permeability varies with each brand of paper tissue and with each lot of the same brand. Recovery of *Meloidogyne incognita* varied from 37 to 90% depending on the tissue used.

F. Flocculation

In soil samples, flocculating agents such as Separan or ferric chloride ($FeCl_3$) help with the separation of nematodes from flocculated soil particles. This method cannot really be used alone, but it is a first step to other methods (Byrd et al., 1966). There is a risk that the flocculate traps the smaller nematodes.

G. Shredding

With the shredding method (often called maceration) a blender or mixer is used to shred or lacerate roots or other plant material. The shedding time should be adjusted for the blender used and for the plant material. It should not be too long as to damage the nematodes. About 5 sec at full speed is generally adequate. This technique would not be practical for processing large numbers of samples because of the time wasted between each extraction to thoroughly wash the blender. As for soil flocculation, shredding should be completed by another method, such as migration, centrifugation, flocculation, or sedimentation.

H. Comparison of the Various Techniques

Many authors have compared the methods for nematode extraction as listed by Viglierchio and Schmitt (1983b) and, more recently, McSorley et al. (1984), Shesteperov et al. (1984), and Clayden et al. (1985). Results depend on the nematode, the soil, and the host. Most

methods or combinations of methods do not even achieve a 50% recovery of the inoculum, and results are extremely variable.

Generally speaking, routine is the enemy of accuracy. It is recommended to check the efficiency of the extraction techniques at least once when they are first implemented. Then, the procedure followed by the technical staff should be checked at least once a year. Checking should be done by running known numbers of specimens through the system with at least 10 replicates.

I. Extraction of Heteroderid Cysts

Cyst extraction requires special techniques and procedures, as recently reviewed by Shepherd (1986). The cysts are first extracted from the soil by a gravity method, either flotation (Fenwick can and its various modification) or centrifugation. The cysts must then be separated from the debris, either directly (wet debris) or after drying. The cysts also can be floated away from thoroughly dried debris by ethanol or glycero-ethanol mixtures. An improved machine for the rapid separation of cysts from dried root debris is described by Faulkner and Greet (1984).

The reliability of cyst extraction appears to be higher than that of soil and root nematodes. Miller (1983) studying the variation of results of *Heterodera schachtii* cyst extraction in different labs found that collecting the cysts from debris is the most critical phase. Cyst collection is helped by separation in ethanol. Intensive training of the staff and standardized procedures are necessary to attain uniform results. Cooke et al. (1983) compared several methods (Fenwick and flotation) for cyst recovery. All results were consistent and the methods were equally good. Caswell et al. (1985) described a Fenwick technique and separation of cysts by an ethanol-glycerine mixture. Recovery depended on soil type and on the number of cysts present. It was almost 90% in the best cases. Reilly and Grant (1985) found that recovery depended on cyst density. Centrifugal method is to be preferred at or above 400 cysts/100 cm^3 of soil. Flotation method gave better results when cyst density was under this figure. Rajan and Swarup (1985) compared several techniques for extraction of cysts of *Heterodera cajani*. Sieving and the Fenwick can are best. For separation of cysts from debris, acetone or acetone carbon tetrachloride is the most effective, but all evaluated chemicals affect hatching and root penetration in biological studies.

J. Waste Disposal

Waste is composed of solid material (part of the sample not used for the extraction; bulk of the material left at the end of the extraction procedure) and liquid material (overflow of water used during the extraction; water suspension with the nematodes after examination). It is recommended to dispose of this waste to avoid contamination of the environment with exotic nematodes. This is particularly true for regulatory and quarantine labs. Treatment of residues can be by dry heat, steam, or fumigation, under proper conditions. For example, the California Department of Food and Agriculture recommends various ranges of temperature and time: dry heat from 230–249°F for 16 hr to 430–450°F for 2 min; steam heat, 15 lb pressure for 30 min. The water used to process samples may be boiled, chemically treated, or filtered. The residues left on the filter should be burned. Contaminated shipping containers may also be burned.

IV. IDENTIFICATION AND COUNTING

A. Observation

At the end of the extraction process, the nematodes are typically recovered in a test tube, in about 25 cm^3 of water. Observation and counting of nematodes present is usually done in a smaller container with only 5 cm^3 of water. Identification and eventually counting of the forms present can be to genus only (species to be determined later) or immediately to species after making temporary slides (nematodes heat-killed in a drop of water on a glass slide, coverslip with a temporary seal made with a mixture of eight parts paraffin wax to three parts petroleum jelly). Immediate identification is required for regulatory action.

Nematode populations can be expressed (Hooper, 1986a) as (a) number of nematodes per unit volume of soil (per liter, per 100 cm^3), but this causes large operator errors due to difficulty of packing a volume of moist soil; (b) number of nematodes per volume of soil, determined by displacement of water, but results are affected by compaction and moisture content of soil; (c) number of nematodes per unit weight of soil, preferably dry weight. Hooper recommends that the number N of nematodes recovered from Z g of moist soil with a moisture content of Y g of water/100 g of dry soil be reported to:

$$N \times \frac{100 + Y}{100} \times \frac{200}{Z} \text{ nematodes/200 g dry soil}$$

B. Reporting Results

The absolute density of a species (also called abundance) is the number of specimens of this species per unit of volume or weight of soil independent of other species that may have been present.

For comparing the populations of different species in one sample, the various absolute densities can be compared, or the relative densities can be calculated as the number of individuals of each species divided by total number of individuals in this sample, in percent.

When several samples have been collected, e.g., from several fields during a general survey, the frequency (also called constancy) indicates how widely distributed is a species, regardless of its density. The absolute frequency is the percentage of samples where the species is observed (number of samples containing a species divided by total number of samples collected, in percent). Comparison between several species is facilitated by computing their relative frequency, which is the absolute frequency of a species divided by the sum of the absolute frequencies of all the species present, in percent.

The importance of each species depends on both its absolute density (population level in the fields where the species has been found). This can be shown graphically (e.g., Fortuner, 1976) or by computing a prominence value for this species equal to the absolute density multiplied by the square root of the absolute frequency of this species.

Finally, the importance of a species in a community also depends on its size expressed as its biomass. Biomass is computed by dividing the volume of the species (body length multiplied by square of body width) by a correction factor equal to 1.6×10^6 (Andrássy, 1956). Robinson (1984) proposed a computer program for calculating nematode volume from its dimension obtained with a digitizing tablet. The importance value is equal to the sum of relative frequency, relative density, and relative biomass.

V. CULTIVATION OF NEMATODES

A. Interest of Cultivation for Systematic Studies

It may be necessary to establish a lab culture of the specimens to obtain an accurate identification. For example, *Deladenus siricidicola* cultured on a young fungus is a free-living, mycetophagous species, but old and brown cultures (usually after a month at 22°C) produce insect parasitic forms that are morphologically very different from the mycetophagous ones (Bedding, 1973). Until recently, the mycetophagous form and the insect parasitic form were classified into different genera, even different families (Fortuner and Raski, 1987). Another example is the development of the esophageal glands that varies with age in *Ditylenchus myceliophagus* where the long overlap observed in second-stage juveniles later regresses until it almost disappears in the oldest females (Fortuner, 1982).

Another interest of nematode lab cultures is that field populations are often represented by a small number of specimens, which makes it impossible to give a good account of variability. Also, specimens obtained from a single host do not give a good account of the large environment-induced variability. Lab culture allows the placement of limits on the extent of this variability.

B. Culture Techniques

1. Agnotobiotic or Xenic Cultures

The culture is said to be agnotobiotic or xenic when the nematode is cultivated with an unknown number of associated organisms, e.g., a mixture of fungi and/or bacteria. Greenhouse culture on a whole plant belongs in this category.

2. Gnotobiotic or Monoxenic Cultures

In gnotobiotic cultures, the nematodes are cultivated with known associated organisms. When there is only one such organism, the culture may be called monoxenic. Monoxenic cultures include cultures on callus tissues or excised roots.

3. Axenic Cultures

In axenic cultures, there are no associated organisms and the nematodes are cultivated on a chemical nutritive medium that contains no living organisms, or part of organisms, other than the nematodes themselves. Bolla (1987) gave a comprehensive account of the current problems for axenic culture of plant parasitic nematodes. Success was achieved only for aphelenchids. Mechanical problems (need for solid substrate, feeding tube, stylet size and action) and biochemical problems (host attraction, nutrient concentration) have so far prevented the establishment of truly axenic cultures for tylenchs.

4. Surface Sterilization

In addition to the sterilization techniques reviewed by Hooper (1986e), Krusberg and Sardanelli (1984) report the use of a glass chromatography column filled with small glass beads and wrapped in aluminum foil. The column is sterilized, then filled with a solution of streptomycin sulfate, penicillin G, and potassium salt in sterile distilled water. Xenic nematodes are rinsed several times in the above solution then transferred to the top of the column. As they work their way down the column the nematodes are surface-sterilized while the contaminating microorganisms are left behind on the top of the column. The glass beads pre-

vent convection currents that would carry the microorganisms to the bottom of the sedimentation column.

C. Nematode Banks

As an alternative to lab culture of nematodes in individual labs, Plant Genetics, Inc. in California is offering "Nematest," large populations of several nematode species (*Meloidogyne hapla, M. incognita, M. chitwoodi, Pratylenchus penetrans, Ditylenchus dipsaci*) to nematologists studying these species.

Mai and Riedel (1987) call for the creation of a large germplasm bank for nematodes that would supply large quantities of well-defined plant parasitic species for research and teaching. An alternative to maintaining a large collection at a single location would be a network of universities and research centers maintaining local collections of nematodes species. Bridge and Ham (1985) describe a technique for cryopreservation of living specimens of *Meloidogyne graminicola* that could be used for long-term preservation in a germplasm bank.

D. Staining Nematodes

Hooper (1986b) gave a review of the traditional stains used for nematodes: acetic orcein, nile blue B and toluidine blue, gold chloride, silver nitrate, and, for vital staining, methyl red and neutral red pH indicator dyes. Premachadran et al. (1988) used Coomassie brilliant blue G to stain secretions from amphids, phasmids, excretory system of live nematodes.

Meyer et al. (1988) compared seven stains for distinguishing live and dead eggs of *Heterodera glycines*. With bright-field microscopy, chrysoidin, eosin Y, new blue R, and nile blue A gave the best results, even better with added DMSO (dimethylsulfoxide). No single stain was consistently better with fluorescence microscopy, and the results depended on the combination filter/stain.

VI. PREPARATION OF SLIDES

Recent reviews on methods for killing, fixing, and mounting nematodes were given by Hooper (1986c) and Santos and Almeida (1989). It must be stressed that there exists no technique for eternally preserving lifelike dead nematodes (Maggenti and Viglierchio, 1965), and that fixation and mounting always result in a certain amount of distortion that tends to increase with age of the slide. After studying the effect of various methods, Brown and Topham (1984) concluded that some of the differences in published morphometrics of *Xiphinema diversicaudatum* are due to differences in processing methods. It is important that methods used be recorded and published with each description.

A. Killing

Killing is generally done by heat, with or without simultaneous fixation. If only a few specimens have to be killed, they are placed in a drop of water on a glass slide, and the slide is heated over an alcohol lamp. Heating should stop as soon as the nematodes are dead. As a rule of thumb, the specimens are often dead when condensation droplets that appear when the slide is first placed over the flame have evaporated. The specimens should be checked with the dissecting microscope and if some are still twitching the slide should be put back a few more seconds over the flame.

Mass killing is often done with hot fixative following the methods of Seinhorst (1966), Netscher and Seinhorst (1969), or Netscher (1971). Safe mass killing of nematodes can be done by slowly heating the test tube with the nematode suspension in a beaker with hot or boiling water. The temperature of the suspension should be monitored with a thermometer placed in the test tube. The nematodes die after a few seconds at 60°C.

Other killing methods have been proposed (e,g., vapor phase perfusion, Maggenti and Viglierchio, 1965) but they are not in common use.

B. Fixing

TAF was widely used in the 1950s as a fixative because of the remarkable lifelike appearance of the specimens, but it was soon discovered that long-term storage in TAF prior to mounting resulted in distortion of the specimens. For this reason its use has decreased dramatically. However, specimens fixed in TAF and mounted lactophenol or glycerin within a year remain in good condition (Hooper, 1987).

Mixtures of formalin (40% formaldehyde) and either glacial acetic acid or propionic acid (FA 4:1 of Seinhorst, 1954; FP 4:1 of Netscher and Seinhorst, 1969) currently are the most widely used fixatives. Olowe and Corbett (1983) tested several fixatives for *Pratylenchus brachyurus* and *P. zeae*. None were perfect but F4 and FP 4:1 were the most satisfactory.

It is best to conserve part of the nematodes in mass collection in case the mounted specimens deteriorate. Long-term storage runs the risk of slow evaporation, but MacGowan (1986) solves this problem by storing preserved nematodes in heat-sealed glass ampules.

C. Mounting

Lactophenol mounts allow an excellent preservation of the specimens, even after 30 years, but it is difficult to obtain a good seal and many slides dry out (Hooper, 1987).

Glycerine mounts are the favorite. A number of techniques exist that allow processing the specimens through alcohol to glycerine with minimum time and efforts (Hooper, 1986c, 1987).

Nematodes are often mounted on Cobb's slides that allow observations at high magnifications (oil immersion) from either side of the specimens. The coverslips must always be supported to avoid squashing the specimens. Glass rods or beads are often used, but it is difficult to select supports of the same diameter than the specimens. ORSTOM labs uses tungsten filaments of calibrated diameter, by 5-µm increments. Huang et al. (1984) use polyester base adhesive transparent tapes 45–50 µm thick with a 9-mm-diameter opening where the glycerol and nematodes are placed, then covered with the usual coverslip.

Taxonomy and identification of *Meloidogyne* rest in part on characteristics seen in lip region profile, excretory pore region, and perineal pattern in mature globose females. Gerber and Taylor (1988) describe a method which by the removal of the contents of the posterior half of the body followed by cutting away a quarter of the cuticle close to the perineal pattern allows mounting of whole specimens clearly showing all three regions.

Esser (1988) describes how whole cysts, freshly taken from the root, can be set on water-agar on a microscope slide and the cone area examined directly without having to be cut and trimmed. This method is said to give excellent results, even though the light is passing lengthwise through the cyst.

D. Labeling

It is important to record the origin of the specimens and to maintain proper records of the slides and their contents. The system advocated by Thorne for numbering slides by genus name, with each species in a genus identified by a number, and each slide with a particular species distinguished from the others by a letter, is still used in many labs. The inconvenience of such a system is obvious because of the continuous changes in nomenclature. Also, it is impractical for computerized records.

Several collections have already stored, or are in the process of storing, their records on computer. It would be preferable if all nematode collections would follow the same format. It would then be conceivable to regroup all records in a central computer for easier search and use of the records. A comprehensive format has been proposed by the present author, and it is implemented at UC–Riverside and UC–Davis, while curators of several other collection have expressed interest in this concept.

VII. COLLECTING DATA

A. Microscope

A top-quality research microscope is the most indispensable piece of equipment for systematic studies. It should be properly installed and maintained. In bright-field microscopy, Köhler illumination gives the best resolution and least glare (Winfield and Southey, 1986). Most details are seen only with oil immersion objectives of 100x magnification. Immersion oil can also be placed between the condenser and the bottom of slide to achieve maximum resolution.

Oil-smeared slides must be cleaned after use. Placing some alcohol on the slide makes the oil gather in a small drop that is then easily picked up by a brush. What is left of the oil on the slide can then be wiped by a clean brush, a piece of cotton, or a tissue while avoiding crushing the specimens. Xylene is a good solvent for immersion oil but it releases toxic fumes.

Interference microscopy, and particularly the differential interference contrast of Nomarsky, reveals morphological details invisible with bright-field microscopy. It is necessary to describe the observation methods employed (microscope, magnification, bright-field or interference microscopy) in the "Materials and Methods" paragraph of taxonomical papers because the same feature may appear differently in different setups.

B. Measurements

Some authors take measurements directly from the ocular micrometer, but it may be difficult to exactly position the micrometer in reference to the feature to be measured. Error becomes prohibitive when the feature is longer than the graduated bar of the micrometer.

The outline or the axis of the feature can be traced on a piece of paper using a traditional camera lucida or, better, a drawing tube available with most microscopes. Most standard objectives are subject to field curvature resulting in differences of magnification between the center and the edges of the field.

Measurements can be taken from the drawing with a ruler and a map measurer for curved lines. Actual measurements in millimeters are written on a piece of paper, then converted to micrometers. Cheap hand-held calculators allow easy computation of mean and standard deviation. Any published description should state these basis statistics (Fortuner, 1984).

Semiautomatic measurement systems have been available for some time on microcomputers (Boag, 1981). Current systems allow electronic magnification and enhancement of morphological details by selection of grey levels. Measuring, converting, and computing data is done automatically and accurately by the computer after the operator has marked the beginning and the end of the feature to be measured. The results can be obtained as square matrix, ready to be loaded into a statistical analysis package. Cost is about $4000 for the software, $4000–$5000 for specialized hardware (digitizing pad, video camera, frame grabber, graphic card, high-resolution monitor, etc.), plus the cost of the computer itself.

Statistical packages such as SAS PC are now available on microcomputers and they make using elaborate statistical procedures such as discriminant function analyses a relatively easy task.

C. Descriptions

Accurate illustrations, made from feature outlines taken with drawing tube or camera lucida, should accompany every description. All observed shapes of each feature should be fully illustrated.

Photographs are rarely used for illustrating shapes because the narrow depth of field at high magnifications keeps most of the subject out of focus. Eisenback (1988) described a technique whereby a single exposure of, e.g., 40 sec is replaced by four exposures of 10 sec each, at four different levels of focus.

Part 2. Preparation of Nematodes for Scanning Electron Microscopy

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I. INTRODUCTION

The scanning electron microscope (SEM) is a useful tool to study nematode morphology and taxonomy (Baldwin and Powers, 1987; Eisenback, 1985; Hirschmann 1983). This instrument has been used to detect differences among populations or races of species, to group species within a genus or genera within a family, and has helped in some studies to reconstruct proposed phylogenies (Hirschmann, 1983). Clearly, the SEM has clarified many difficult taxonomic questions in several nematode groups, and descriptions of new species increasingly include scanning electron micrographs. The value of the SEM to nematode morphology and taxonomy will probably increase in the future.

The most useful taxonomic characters revealed by SEM include morphology of the anterior end, in particular lip patterns, and details of the posterior end and general body region (Hirschmann, 1983). In addition, the SEM is useful to visualize surface morphology of nematode eggs and dissected body parts including stylets (Eisenback and Rammah, 1987), spicules (Rammah and Hirschmann, 1987), sperm (Eisenback, 1985), and other tissues (Abrantes and Santos, 1989). Details revealed by SEM often aid in a more precise interpretation of morphology as seen in the light microscope (LM); as a result, the LM is made more useful (Fig. 1).

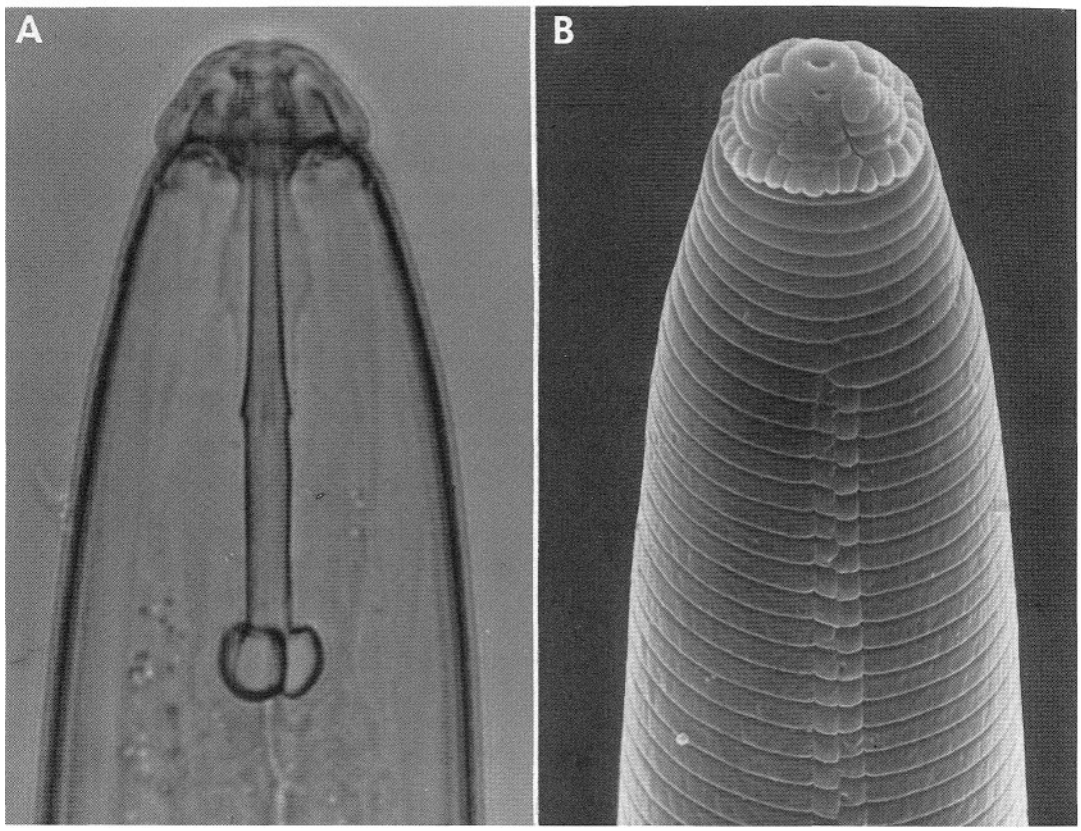

FIGURE 1 Anterior end of a female of *Hoplolaimus galeatus* (Cobb) Filip'ev and Schuurmans Stekhoven. (A) Light micrograph showing that the specimen is transparent to light and revealing that the depth of focus is low. (B) Scanning electron micrograph showing the three-dimensional image and large depth of focus. (From Eisenback, 1985.)

Proper preparation of the specimens is important for SEM observations. Poor preparation may obscure small details and often produces artifacts that interfere with the correct interpretation of the scanned image. At times the utility of information obtained by SEM is limited by adequate specimen preparation (Eisenback, 1986).

Preparing nematodes for SEM usually involves numerous steps. The success of the preparation is dependent on the success of each individual step. Shrinkage, swelling, and surface precipitation that occurs during the initial stages of preparation are likely to worsen in the final stages.

Techniques for preparing nematodes for SEM are now available that produce specimens that are stable in the microscope and relatively free of artifacts (Eisenback, 1986). The description of the technique that follows is adequate for most genera of plant parasitic nematodes, although slight modifications of the procedure may be required for the optimum preservation of detail, depending on the genus or nematode tissues. In cases where these modifications are known, they will be described for each individual genus.

II. SELECTION AND HANDLING NEMATODES

A. Handling

Careful selection and handling of specimens are necessary for proper preparation (Eisenback, 1985). Extraction techniques that are harsh may cause artifacts and give poor results even though the remaining procedures are adequately performed. Harsh extraction techniques include those that use bleach, sucrose, or flocculating agents, and those that allow the increase of contaminating organisms or anaerobic conditions.

Hand-picking individual specimens with a small, fine wire, such as a dental pulp canal file, or other suitable pick ensures that each specimen will be of adequate quality, and of the same species and developmental stage (Eisenback, 1985). Micropipetting is a useful alternative method of handling specimens provided that specimens are in good physiological condition, in a relatively clean monospecific solution, and in good supply. This technique is particularly useful for large, saccate forms.

B. Processing Containers

Processing nematodes for SEM usually involves placing the specimens in many different types of solutions and equipment. A chamber that allows the bulk transfer of many specimens from one liquid to another is necessary to facilitate the preparation procedures. An ideal container allows for rapid exchange of fluids, prevents loss of few specimens, and minimizes additional artifacts (Eisenback, 1985).

Several containers have been described; however, a modified epoxy embedding (BEEM) capsule is perhaps the most widely used and is easily assembled (Fig. 2) (Eisenback, 1985). The container is made by cutting the conical end off the capsule. A perforated cap is fashioned with a small hole punch. A small piece of fine-mesh nylon screen is held in place over the hollow cylinder as the cap is snapped into position (Fig. 2C). Specimens are placed in the modified BEEM container inside a small glass Stendor dish. Fluids are exchanged by withdrawing one solution from the Stendor dish and pipetting in a new one.

III. KILLING AND FIXATION

A. General Precepts

Proper fixation is perhaps the most important step in preparing good specimens for SEM (Fig. 3). In most cases, fixatives and techniques commonly used for preparing nematodes for light microscopy are inadequate for SEM (Fig. 3C) (Eisenback, 1986). Therefore hot formalin-based fixatives are not useful; instead, a sequential fixation with cold glutaraldehyde-based fixative or mixtures of glutaraldehyde and formalin are usually adequate for most species and genera (Fig. 3D).

Fixation is usually a two-part process. Primary fixation of protein molecules occurs by an aldehyde or mixture of aldehydes, and the unsaturated fats are postfixed with osmium tetroxide. Glutaraldehyde is the aldehyde of choice because each molecule of the fixative has two reactive groups that are capable of crosslinking proteins (Hopwood, 1969, 1972).

The fixatives can greatly upset the tonicity of the tissues and must be used with a suitable buffering system (Schiff and Gennaro, 1979). Sodium cacodylate and phosphate buffers are most commonly used for biological material. The selection of the buffer is dependent on the preference of the user. Sodium cacodylate contains arsenic and is thus toxic to contaminating organisms, but may be hazardous to use; whereas the phosphate buffers