8 Reproduction, Physiology and Biochemistry

David J. Chitwood[1] and Roland N. Perry[2]
[1]*USDA, ARS, BARC-West, Beltsville, Maryland, USA;* [2]*Rothamsted Research, Harpenden, Hertfordshire, UK and Ghent University, Ghent, Belgium*

8.1 Introduction

The extensive amount of information on the reproduction and cytogenetics of species of *Meloidogyne* contrasts with the limited information on physiology, biochemistry and biochemical pathways. In common with other species of plant-parasitic nematodes, the obligate parasitism and small size of *Meloidogyne* make research on physiology and traditional biochemistry challenging. This chapter aims to discuss the reproductive strategies of species of *Meloidogyne* and to discuss the data available on aspects of their physiology, biochemistry and sensory biology.

8.2 Reproduction and Moulting

The structure of the reproductive system of *Meloidogyne* and the basic features of the development of the gonads are given in Eisenback and Hunt, Chapter 2, this volume. The various reproductive mechanisms of free-living and plant-parasitic nematodes have been reviewed in detail by Evans (1998). Although the plethora of information about the development of *Caenorhabditis elegans* is not matched by data on other species of nematodes, the information available on the cytogenetics of *Meloidogyne* is more extensive than that on any other genus of plant-parasitic nematodes. In the following sections, it is possible only to give a summary of available information.

8.2.1 Reproduction mechanisms and cytogenetics

Among all areas of root-knot nematode biology, none induces admiration from many researchers more than the complex cytogenetics of *Meloidogyne*. A genus with less complicated reproductive cytogenetics would not have attracted the historical or contemporary interest devoted to the root-knot nematodes.

  Root-knot Nematodes (eds R.N. Perry, M. Moens and J.L. Starr)

8.2.1.1 Mode of reproduction

Three types of reproduction exist within the genus: (i) amphimixis, in which sperm from males fertilize oocytes in females and meiosis subsequently occurs; (ii) facultative meiotic parthenogenesis, in which amphimixis occurs in the presence of males but, in their absence, meiosis occurs within oocytes, but two of the nuclei with reduced chromosomal complement (the egg pronucleus and the second polar body) subsequently fuse (automixis); and (iii) obligate mitotic parthenogenesis, where males are not involved (apomixis or amixis), in which one of the two nuclei produced during an initial mitotic division within the oocyte deteriorates and the other becomes the predecessor of the subsequent embryo. Only seven of 37 species of *Meloidogyne* studied to date are amphimictic. Like many soil nematodes, most *Meloidogyne* spp. are parthenogenetic. Some are facultative meiotic parthenogens, and several of the most widespread and economically important species are obligate mitotic parthenogens. Populations of the same *Meloidogyne* species may differ in mode of reproduction; for example, 29 of 32 populations of *M. hapla* reproduced by facultative meiotic parthenogenesis, the others were mitotic parthenogens (Triantaphyllou, 1966). A slightly different form of meiotic parthenogenesis in *M. floridensis* was reported by Handoo *et al.* (2004), where there was a suppression of the second maturation division, indicating that this species has a type of parthenogenesis intermediate between the meiotic form with two maturation divisions and mitotic parthenogenesis.

8.2.1.2 Sex ratios

In *Meloidogyne*, as with *Globodera* and *Heterodera*, sex chromosomes are absent and the sex ratio may be influenced by environmental factors. In the species reproducing by meiotic parthenogenesis, overcrowding, food shortage, temperature extremes or other adverse environmental stresses may lead to the formation of males. These males rarely inseminate females; even when they do, a mitotic division in the oocyte initiates embryogenesis without any fusion with the spermatozoon nucleus. The opportunity for engineering gender reversal in some species is an attractive, although unrealized, crop protection target. In the mitotically parthenogenetic species, the production of males is also induced by unfavourable environmental conditions. As these males were genetically destined to become females, they are produced by a process of sex reversal. Indeed, the timing of the reversal influences the morphology of the males produced: an early reversal results in males with only one testis very similar to normal males; a later reversal induces the production of males with two testes of unequal size; a still later reversal yields males with two testes akin to the two ovaries of females (Papadopoulou and Triantaphyllou, 1982).

8.2.1.3 Chromosome complement

The fascinating cytogenetics of *M. arenaria*, *M. hapla*, *M. incognita* and *Meloidogyne javanica* were elucidated by Triantaphyllou (1962, 1963, 1966, 1981, 1985), whose monumental studies involved the painstaking preparation and microscopic examination of tens of thousands of stained gonads. More recently, the area has been reviewed in detail by Castagnone-Sereno (2006).

The chromosomal complement of *Meloidogyne* spp. reflects the complexity of their reproduction. Perhaps as would be expected in a largely parthenogenic group of often polyploid species, chromosome numbers are not exact multiples of a simpler haploid chromosomal complement. The generally accepted haploid number is $n = 18$ (Triantaphyllou, 1985; Castagnone-Sereno, 2006), although that number might have originated from a chromosomal complement doubling (see next section for discussion). Fertile tetraploid populations of *M. hapla* and *M. microcephalus* have been discovered (Triantaphyllou, 1984; Triantaphyllou and Hirschmann, 1997). The chromosome number is quite variable (Table 8.1) and populations of the same parthenogenetic species may differ greatly in number. For example, various isolates of *M. arenaria* contained 36, 51, 53 or 54 chromosomes (Triantaphyllou, 1966); 220 populations of *M. incognita* had primarily 40–46, although some isolates had only 32–36 chromosomes (Triantaphyllou, 1981), and one female had 88, the sole obviously polyploid specimen in this mammoth study involving the visual counting of approximately 100,000 tiny chromosomes. In 29 facultatively meiotic parthenogenic

Table 8.1. Chromosome number and mode of reproduction of some species of *Meloidogyne*.

Species	Mode of reproduction	Chromosome number	Reference
M. arenaria	obligatory mitotic parthenogenesis	$2n$ = 30–38, 40–48, 51–56	18, 22
M. carolinensis	amphimixis	n = 18	8
M. chitwoodi	facultative meiotic parthenogenesis	n = 14–18	22, 25
M. cruciani	obligatory mitotic parthenogenesis	$2n$ = 42–44	22
M. enterolobii	obligatory mitotic parthenogenesis	$2n$ = 42–44	16, 22
M. ethiopica	obligatory mitotic parthenogenesis	$2n$ = 36–44	2
M. exigua	facultative meiotic parthenogenesis	n = 18	15, 22
M. fallax	facultative meiotic parthenogenesis	n = 18	25
M. floridensis	facultative meiotic parthenogenesis	n = 18	9
M. graminicola	facultative meiotic parthenogenesis	n = 18	22
M. graminis	facultative meiotic parthenogenesis	n = 18	22
M. hapla cytological race A	facultative meiotic parthenogenesis	n = 13–17 (polyploids n = 28 & 34)	13, 19, 22
M. hapla cytological race B	obligatory mitotic parthenogenesis	$2n$ = 30–32, 43–48	19, 22
M. hispanica	obligatory mitotic parthenogenesis	$2n$ = 33–36	19, 22
M. incognita	obligatory mitotic parthenogenesis	$2n$ = 32–38, 41–46	21, 22
M. inornata	obligatory mitotic parthenogenesis	$2n$ = 54–58	4
M. izalcoensis	obligatory mitotic parthenogenesis	$2n$ = 44–48	3
M. javanica	obligatory mitotic parthenogenesis	$2n$ = 42–48	22
M. kikuyensis	amphimixis	n = 7	23
M. konaensis	obligatory mitotic parthenogenesis	$2n$ = 44	Triantaphyllou, pers. comm. in 7
M. megatyla	amphimixis	n = 18	8
M. microcephalus	obligatory mitotic parthenogenesis	$2n$ = 36–38	22, 24
M. microtyla	amphimixis	n = 18	8, 22
M. minor	facultative meiotic parthenogenesis	n = 17	van der Beek, pers. comm. in 12
M. morocciensis	obligatory mitotic parthenogenesis	$2n$ = 47–49	17
M. naasi	facultative meiotic parthenogenesis	n = 18	22

M. oryzae	obligatory mitotic parthenogenesis	2*n* = 51–55	22
M. ottersoni	facultative meiotic parthenogenesis	*n* = 18	22
M. paranaensis	obligatory mitotic parthenogenesis	3*n* = 50–52	1
M. partityla	obligatory mitotic parthenogenesis	2*n* = 40–42	14
M. petuniae	obligatory mitotic parthenogenesis	2*n* = 47	Triantaphyllou, pers. comm. in 5
M. pini	amphimixis	*n* =18	11, Triantaphyllou, pers. comm. in 6
M. platani	obligatory mitotic parthenogenesis	2*n* = 42–44	22
M. querciana	obligatory mitotic parthenogenesis	2*n* = 30–32	22
M. salasi	obligatory mitotic parthenogenesis	2*n* = 36	22 (as '*Meloidogyne* sp. from rice')
M. spartinae	amphimixis	*n* = 7	20
M. subarctica	amphimixis	*n* = 18	20
M. trifoliophila	facultative meiotic parthenogenesis		10, based on similarity to *M. graminicola*

References: [1]Carneiro *et al.* (1996); [2]Carneiro *et al.* (2004); [3]Carneiro *et al.* (2005); [4]Carneiro *et al.* (2008); [5]Charchar *et al.* (1999); [6]Eisenback *et al.* (1985); [7]Eisenback *et al.* (1994); [8]Goldstein and Triantaphyllou (1982); [9]Handoo *et al.* (2004); [10]Hugall *et al.* (1999); [11]Karssen and Moens (2006); [12]Karssen *et al.* (2004); [13]Liu and Williamson (2006); [14]Marais and Kruger (1991); [15]Muniz *et al.* (2009); [16]Rammah and Hirschmann (1988); [17]Rammah and Hirschmann (1990); [18]Triantaphyllou (1963); [19]Triantaphyllou (1966); [20]Triantaphyllou (1971); [21]Triantaphyllou (1981); [22]Triantaphyllou (1985); [23]Triantaphyllou (1990); [24]Triantaphyllou and Hirschmann (1997); [25]van der Beek and Karssen (1997).

populations of *M. hapla*, the haploid chromosome number was 15–17 (with three presumably polyploid mitotic pathenogens possessing 45 chromosomes) (Triantaphyllou, 1966). Four facultative meotic parthenogenic strains of *M. hapla* had $n = 16$ (Liu and Williamson, 2006).

8.2.1.4 *Evolution of* Meloidogyne *species*

The relationships among species of *Meloidogyne* are examined by Adams *et al.* (Chapter 5, this volume), but the evolution of the genus, especially in relation to the mode of reproduction, has also attracted interest. Triantaphyllou (1985) summarized the speculations about the evolution of *Meloidogyne* spp. based upon cytogenetics. He regarded the obligate amphimictic species (e.g. *M. megatyla*, *M. microtyla* and *M. carolinensis*) with $n = 18$ or 19 as the current species most closely related to the ancestral predecessors of *Meloidogyne* spp. He also speculated that the low chromosomal numbers in most other nematodes (generally $n = 4–12$; see Coghlan, 2005) offered support for a polyploid origin of nearly all of the species of *Meloidogyne*. At that time *M. spartinae* was regarded as being in a now-defunct closely related genus, *Hypsoperine*, but its low chromosomal complement ($n = 7$) was regarded as additional evidence for a condition of tetraploidy in the many species of *Meloidogyne* with $n = 14–18$. Plantard *et al.* (2007) consider that the $n = 7$ chromosome number found in only a few species of *Meloidogyne* is a derived character from species with $n = 13–19$. Triantaphyllou (1985) regarded parthenogenetic species with 30–38 chromosomes as diploids, having arisen from diploid amphmictic species with $n \sim 18$, and species with *c.* 54 chromosomes as being triploids produced by the fusion of the chromosomal complements of diploid and haploid forms. The previously discussed existence of naturally occurring polyploid individuals in diploid populations provides additional support for polyploidy as a force in evolution, with aneuploidy or chromosomal fragmentation further modifying the chromosomal complement.

Triantaphyllou (1985) pointed out that as most species of *Meloidogyne* reproduce by mitotic parthenogenesis and have variable chromosome numbers, their status as distinct species may be unclear. The exceptions are *M. javanica* and *M. incognita*, where their distinct biological features represent defined species. Even with the obligate amphimictic species, reproductive isolation tests are extremely difficult because of host specialization of these species.

8.2.1.5 *Origin and evolution of parthenogenesis*

Evans (1998) pointed out that, although conventional understanding indicated that amphimictic reproduction, with full genetic reassortment, would be the only long-term method enabling species to adapt to environmental change, the most sophisticated and successful genus of plant-parasitic nematodes, *Meloidogyne*, has flourished using, primarily, mitotic parthenogenesis. Such success seems counter to neo-Darwinian wisdom! However, there are advantages to parthenogenesis and, as detailed below, genetic mixing can exist with this mode of reproduction. Parthenogenesis is a reproductive strategy speculated to have advantages in colonizing new ecological niches or in improving the odds of reproductive success in an environment in which the few potential mates may have difficulty in finding females (Ritz and Trudgill, 1999).

The origin of parthenogenesis in *Meloidogyne* spp. and its evolution, have been the subjects of frequent comment, a situation undoubtedly enlivened because of the polyploidy and aneuploidy in the mitotic parthenogenetic species. Parthenogenesis in *Meloidogyne* has been speculated to evolve from two, not necessarily mutually exclusive, pathways: hybridization and mutation (Triantaphyllou, 1985; Trudgill and Blok, 2001; Castagnone-Sereno, 2006; Lunt, 2008), probably with a reticulate evolutionary pattern (Hugall *et al.*, 1999; Trudgill and Blok, 2001). Although van der Beek and Karssen (1997) produced hybrid females from crosses between *M. fallax* and *M. chitwoodi*, these females failed to produce viable second-stage juveniles (J2s). None the less, the exchange of genetic information, as evidenced by hybrid enzyme patterns in the females, would be an important component of speciation.

In a study of AFLP (amplified fragment length polymorphism)-quantified variation in *M. incognita* and facultatively meiotic *M. hapla*, van der Beek and Pijnacker (2008) expectedly discovered almost no variation among *M. incognita*

females derived from single juvenile inoculations of tomato roots. By contrast, the variation between sixth-generation descendents of a single egg mass of *M. hapla* was quite large and exceeded the variation between representatives of two different egg masses from the parental population. This variation was regarded as indicative of inverted meiosis, i.e. a process in which chromosomal reduction occurs during the second division instead of the first. The results apparently conflicted with those of Liu *et al.* (2007), who reported a strong tendency to homozygosity in meiotically parthenogenic *M. hapla.*

Although parthenogenetic reproduction would tend to minimize the genetic variation of offspring descending from a single individual, the lack of genetic recombination among different individuals of a parthenogenetic species can result in enhanced accumulation of mutation-initiated divergence of allelic sequences within that species, in comparison with sexually reproducing species (Lunt, 2008). Particularly if only one or a very few parental females were the source of an apomictic species, and if this parental material had originated as a hybrid between two divergent species, the variation between two alleles in the species could be striking. Such individual females might have been the progenitors of parthenogenetic *Meloidogyne* spp. (Hugall *et al.*, 1999; Castagnone-Sereno, 2006; Lunt, 2008). Additional sources of genetic variation in parthenogenetic *Meloidogyne* spp. could result from the abundant transposable elements occurring in these species (Castagnone-Sereno, 2006; Abad *et al.*, 2008; Opperman *et al.*, 2008).

In a nuclear gene sequence study which supported the hybridization ontogeny, Lunt (2008) discovered enhanced sequence divergence within two of three studied genes from mitotic parthenogenetic species (*M. arenaria*, *M. incognita*, *M. javanica* and *M. enterolobii* (=*M. mayaguensis*)), compared with species in which sexual reproduction can occur (*M. chitwoodi*, *M. hapla* and *M. fallax*). Interestingly, Davies *et al.* (2008) reported that cuticular variation in a line derived from a single J2 of mitotically parthenogenetic *M. incognita* was surprisingly high, as reflected by the ability of *Pasteuria penetrans* endospores to attach, and was equal to that of a similar line derived from a single J2 of the facultatively meiotic parthenogen, *M. hapla*. The explanation for the high degree of variation could be that the homologous chromosomes within the polyploid *M. incognita* possess substantial heterozygosity, or that unknown epigenetic mechanisms are responsible for the variation in *M. incognita*.

8.2.2 Moulting

Like most nematodes, *Meloidogyne* moults four times during development to adult. The first moult occurs in the egg, when the small, vermiform, first-stage juvenile (J1) moults to become the infective J2, which subsequently hatches. Details of hatching are given by Curtis *et al.* (Chapter 6, this volume). The infective J2 invade a suitable host plant, initiate a permanent feeding site and feed and grow. The resulting swollen J2 moult into third- and fourth-stage juveniles, which do not feed. Fourth-stage juveniles destined to become males revert to a vermiform shape after the third moult, whereas juveniles destined to become females remain swollen (Fig. 1.1). Both types of fourth-stage juveniles moult once more to become either a mature male or a female.

Moulting of the J1 depends on food reserves stored in the egg. All of the energy required for the three additional moults is contained within the J2. The cuticle of *Meloidogyne* comprises three layers: cortical, medial and basal. The cortical layer is also divided into three layers. At the start of moulting in *M. javanica* the hypodermis becomes thickened and filled with ribosome-like granules and the old cuticle separates from the hypodermis (Bird and Rogers, 1965). The hypodermis first starts to secrete the external cortical layer and then the rest of the new cuticle. The space between the cuticles becomes filled with particles that may be associated with the enzymatic breakdown and reabsorption of the innermost layers of the old cuticle, so that finally only the external cortical layer of the old cuticle is left. After moulting, the new cuticle retains its close cytoplasmic relationship with the hypodermis and increases in thickness. Resorption of the cuticle and recycling its proteins may be an adaptation to endoparasitism, because a sedentary nematode, such as *Meloidogyne*, may have difficulty escaping from a cuticle if it was not absorbed (Lee and Atkinson, 1976).

Having complete sequences of the genomes of *M. hapla* and *M. incognita* (see Abad and Opperman, Chapter 16, this volume) will provide information about the genes involved in moulting in *Meloidogyne*, and this information may aid in the identification of novel control targets. The process of moulting has already attracted attention as a putative target. Soriano *et al.* (2004) examined the effects of the ecdysteroid 20-hydroxyecdysone (20E), a major moulting hormone of insects, on *M. javanica*. Exogenous application of 20E resulted in immobility and death of J2. Furthermore, invasion was partially inhibited and development was halted in spinach with induced high levels of endogenous 20E; however, in the few J2 that invaded, no abnormal moulting was observed. The biosynthesis of ecdysteroids by any nematode has yet to be demonstrated, and specific efforts to detect 20E and its precursor, ecdysone, in *M. arenaria* and *M. incognita* were unsuccessful (Chitwood *et al.*, 1987).

8.3 Physiology

The small size of *Meloidogyne* has limited experimentation on aspects of its physiology. Thus, information on respiration, metabolism and excretion, for example, is limited, and data on the associated biochemical pathways are at best fragmentary and at worst completely lacking.

8.3.1 Respiration

In common with other plant-parasitic nematodes, *Meloidogyne* is sufficiently small for diffusion across the cuticle to provide enough oxygen for aerobic respiration. The limit is likely to be a partial pressure of oxygen of 15 mmHg; below this, nematode activity is adversely affected (Wright and Perry, 2006). Reduced oxygen availability in soils retarded development of *M. javanica* (Van Gundy and Stolzy, 1961), and hatch of J2 from single eggs and from egg masses was reduced at low oxygen concentrations (Baxter and Blake, 1969). Nematodes are more likely to be exposed to low oxygen conditions in soils, especially in waterlogged soil, than in plant tissue, although the root tissues of mangroves and paddy rice may have low oxygen tensions. Robinson and Carter (1986) demonstrated that respiration in J2 of *M. incognita* was essential to survive changes in water potential; when aerobic respiration was prevented, J2 were unable to regulate their volume.

Fumigant nematicides, such as 1,3-D and methyl bromide, are likely to affect biochemical pathways of respiration; those that release methyl isothiocyanate also act on respiration, because once inside the nematode cyanide prevents the utilization of oxygen. The respiration of J2 of *Meloidogyne* spp. treated with 0.5 g methylene bisthiocyanate/ml for 5 min increased significantly but declined when treatment times longer than 5 min were used (Qi *et al.*, 2008). Nordmeyer and Dickson (1989) found that J2 of *M. arenaria* consumed more oxygen than J2 of *M. incognita*, which consumed more than J2 of *M. javanica*. The sensitivity of these three species to nematicides varied in the *in vitro* tests and may relate to differential sensitivity in the field.

8.3.2 Effects of osmotic and ionic stress

Several studies have examined the behaviour of J2 in response to treatment with various solutions (see Curtis *et al.*, Chapter 6, this volume, and Evans and Perry, Chapter 9, this volume), but it is unclear how differences in responses relate to the behaviour of nematodes in soils of varying ionic content. There have been no direct studies on osmotic and ionic regulation by species of *Meloidogyne*. It is probable that they are able to tolerate marked fluctuations in water potentials within plants, particularly at times of drought or nematode-induced stress.

Two complementary osmoregulatory mechanisms are found in animals: isosmotic intracellular regulation, where the osmolarity is adjusted to conform with the extracellular osmotic pressure, and anisosmotic extracellular regulation, where the extracellular fluid is maintained hypo- or hyperosmotic to the external environment (Wright and Perry, 2006). The pseudocoelom is the principal extracellular fluid compartment in nematodes and may act as a primitive circulatory system. In actively moving nematodes, sinusoidal waves of contraction and accompanying internal pressure changes will result in some mixing of the

pseudocoelomic fluid. The body wall, the intestine and the secretory–excretory system have been suggested as sites of urine production in nematodes capable of volume regulation in hypoosmotic environments, but there is no direct evidence for *Meloidogyne*. Regular removal of material by defaecation in actively feeding nematodes suggests that the intestine has an important role in fluid excretion.

The ionic composition of the pseudocoelomic fluid in several animal-parasitic species suggests that ionic regulation must occur. However, there is only limited, largely indirect, physiological and biochemical evidence for the ion channels and pumps that would be required to maintain electrochemical gradients across nematode epidermal and intestinal cells (Thompson and Geary, 2002). There is molecular evidence for K–Cl cotransporter protein in *M. incognita*, which could be involved in ionic and osmotic regulation, and the gene was expressed in both mobile and sedentary stages (Neveu *et al.*, 2002). These authors hypothesized that the gene is involved in the regulation of osmotic pressure of cells in order to maintain nematode body fluids hyperosmotic to the environment.

8.3.3 Secretory–excretory products

In general, nematodes are ammonotelic, with the majority of nitrogenous waste product being ammonia (Wright, 1998). Ammonia is easily soluble in water and poses no problems for nematodes in an aqueous environment. However, ammonia is also toxic, and where water for dilution and diffusion is limited, ammonia is converted to a less toxic end product, such as urea. Information about the excretory products from *Meloidogyne* is lacking, and it is unknown what the excretory end product is once a feeding site has been established. There is no evidence for an excretory function by the secretory–excretory system of *Meloidogyne*, and the role of the system and detailed information of the molecules it secretes remain to be ascertained.

The surface of *Meloidogyne* appears to be covered by glycoprotein and protein, at least some of which appear to be secreted via the nematode secretory–excretory system (Bird *et al.*, 1988) and amphids (Davis *et al.*, 1988; McClure and Stynes, 1988; Davis and Kaplan, 1992). Glycoprotein is also a major component of the gelatinous matrix in the egg mass (Sharon and Spiegel, 1993), and the importance of the gelatinous matrix in the survival of unhatched J2 of *Meloidogyne* is discussed by Evans and Perry, Chapter 9, this volume. Other sources for secretions are the pharyngeal gland cells, and through the cuticle itself. Much of the early work on nematode secretions and their origins has been reviewed by Jones and Robertson (1997). Blaxter and Robertson (1998) reviewed information on the nematode cuticle and pointed out that it plays an important role in nematode physiology, including protection from the environment and excretion, and there may be proteins with potential roles in host recognition.

The secretion of proteins during migration through the host tissue is an essential component of the host–parasite interaction. The source of these proteins and the genes encoding them are being defined, and the functions of secreted proteins coded by the *Meloidogyne* parasitism genes are being elucidated; some are discussed by Atkinson *et al.*, Chapter 15, this volume. The spectrum of proteins associated with species of *Meloidogyne* is discussed in the next section.

8.4 Biochemistry

8.4.1 Enzymes

Investigation of the biochemistry of *Meloidogyne* spp., as in similar studies with other plant-parasitic nematodes, has been hindered by the inability to culture the nematodes independently from their host plants. Much of the early literature on the biochemistry of root-knot nematodes focused on biochemical or histochemical assays for characterizing nematode enzymes or other proteins, or direct analysis of nematode homogenates, extracts or secretions for specific components. Employing electrophoretic or cytochemical techniques, numerous investigators reported discoveries of numerous enzymes in root-knot nematodes, such as acid and alkaline phosphatases, ATPase, catalase, cytochrome oxidase, diaphorase, esterase, β-galactosidase, glucose-6-phosphate

dehydrogenase, glucose phosphate isomerase, β-glucosidase, glutamate oxaloacetate transaminase, α-glycerophosphate dehydrogenase, lactate dehydrogenase, lipase, malate dehydrogenase, peroxidase, 6-phosphogluconate dehydrogenase, succinic dehydrogenase, and superoxide dismutase (Ishibashi, 1970; Dickson *et al.*, 1971; Hussey *et al.*, 1972; Dalmasso and Berge, 1978; Starr, 1981; Marwah and Khera, 1988; Esbenshade and Triantaphyllou, 1990; Navas *et al.*, 2001; Molinari *et al.*, 2005). For nearly two decades, a few of these, especially esterase (EST) and malate dehydrogenase, have proved to be useful in the initial molecular identification of species of *Meloidogyne* (see Blok and Powers, Chapter 4, this volume).

In recent years, much of the research on nematode biochemistry has been driven by molecular genetics approaches to elucidate enzymes necessary for nematode-specific functions, or secreted enzymes involved in parasitism or other aspects of the nematode–plant relationship. These molecules provide targets that are hopefully exploitable by potential control strategies (see Atkinson *et al.*, Chapter 15, this volume) and, in *Meloidogyne*, include the following: cathepsin L protease, chorismate mutase, dual oxidase (NADPH oxidase and peroxidase), β-1,4-endoglucanase, pectate lyase, polygalacturonase, serine protease and endo-1,4-β-xylanase (Lambert *et al.*, 1999: Rosso *et al.*, 1999; Doyle and Lambert, 2002, 2003; Jaubert *et al.*, 2002; Neveu *et al.*, 2003; Huang *et al.*, 2004; Bakhetia *et al.*, 2005; Fragoso *et al.*, 2005; Ledger *et al.*, 2006; Long *et al.*, 2006a,b; Mitreva-Dautova *et al.*, 2006; Shingles *et al.*, 2007). Even before the success of the *Meloidogyne* genome projects, early large-scale EST analyses revealed numerous enzymes involved in major biochemical pathways (e.g. McCarter *et al.*, 2003). Indeed, if molecular genetics is regarded as a subset of biochemistry, the entire opus of biochemical literature on *Meloidogyne* is sufficiently voluminous as to be nearly unreviewable. By contrast, the quantity of literature focusing on the analysis of specific compounds in root-knot nematodes and the elucidation of biochemical pathways involving their biosynthesis or metabolism is frighteningly scarce. Although *Meloidogyne* would be expected to share large facets of biochemical machinery with other nematode genera, extrapolation could be dangerous.

8.4.2 Other proteins

Several structural proteins have been detected in *Meloidogyne*. The collagenous nature of the nematode cuticle has been known for decades. Collagen has been localized immunologically in the cuticle of the major species of *Meloidogyne*, and several collagen genes, expression of which is often developmentally correlated, have been discovered (Van der Eycken *et al.*, 1994; Ray *et al.*, 1996a,b; Koltai *et al.*, 1997; Wang *et al.*, 1998; Abrantes and Curtis, 2002). Collagenous proteins isolated from the adult and J2 of *M. javanica* differ in size and amino acid composition: a 76-kDa protein comprises nearly half of the collagen of adult *M. incognita* yet is absent from the J2 (Reddigari *et al.*, 1986). Cuticulins, non-collagenous cuticular proteins that are not readily solubilized, are encoded by at least two distinct genes in *M. artiellia*, and the expression of at least one is highly developmentally regulated (De Georgi *et al.*, 1997).

The eggshells of root-knot nematodes are composed of three major layers: an outer vitelline membrane, a chitin layer also containing protein, and an inner lipid layer, again containing protein. Chemical analysis of eggshells revealed that protein was the most abundant component, and that proline comprised as much as 40% of the total amino acid composition of the eggshell protein of *M. incognita* (Bird and McClure, 1976). Autoradiographic studies with radiolabelled proline indicated that the proline-containing protein was incorporated into both the chitin and lipid layer of *M. javanica* (McClure and Bird, 1976).

8.4.3 Amino acids and sugars

Not unsurprisingly, numerous studies have involved the amino acid composition of root-knot nematodes, but the sole investigation to use a radiolabelled precursor was by Myers and Krusberg (1965), who reported that *M. incognita* was capable of biosynthesizing glutamic acid, glutamine, alanine, asparagine, aspartic acid, glycine, serine and tryptophan, the latter being an essential amino acid in mammals. Wang and Bergeson (1978) demonstrated the presence of 15 different amino acids and six sugars in secretions of J2 of *M. incognita*. Sadly, limited information is available on the biosynthesis of specific sugars by

Meloidogyne; most of the specific information has resulted from the several excellent lectin-based analyses of sugar residues on the *Meloidogyne* surface (e.g. Davis *et al.*, 1988; McClure and Stynes, 1988; Ibrahim, 1991; Davis and Kaplan, 1992; Spiegel *et al.*, 1995; Lin and McClure, 1996).

8.4.4 Neuropeptides

FMRFamide-like peptides (FLPs) are polypeptides containing only a few amino acids and are known neuromodulators of muscular activity in other species of nematodes (Brownlee *et al.*, 2000; Perry and Maule, 2004). Although their presence has not been demonstrated in *Meloidogyne*, numerous genes encoding at least 15 distinct FLPs have been elucidated (Fleming *et al.*, 2007; McVeigh *et al.*, 2008). Nematode FLPs are very attractive targets for development of control techniques because of their potential susceptibility to RNAi (RNA interference)-based disruption.

8.4.5 Complex carbohydrates and lipids

On a dry weight basis, J2 of *M. javanica* contained approximately 7% carbohydrate. The sugar polymer glycogen would be expected to be a major food reserve in root-knot nematodes, and electron microscopy has indicated that the major food reserves of J2 of *M. incognita* are intestinal lipids, with smaller reserves comprising hypodermal lipid and glycogen (Dropkin and Acedo, 1974). As feeding commences and the three moults to parasitic developmental stages ensue, glycogen appears to be the predominant food reserve, although adult females again contain massive quantities of lipids.

Chitin, a polymer of the amino sugar *N*-acetylglucosamine, comprises as much as 30% of the dry weight of the *M. javanica* eggshell (Bird and McClure, 1976). This is apparently the only nematode life stage where chitin exists, as a well-designed electron microscopy study clearly demonstrated that the chitin thought possibly to exist in the gelatinous matrix, if present, was likely to be a product of fungal contamination (Bird and Self, 1995).

Perhaps because of lipid abundance in nematodes (with adult females, J2 and eggs consisting, respectively, of nearly 50%, 40% or 66% lipid on a dry weight basis; Krusberg, 1967; Reversat, 1976), the lipids of *Meloidogyne* spp. have received substantial attention. In addition to obvious roles as food reserves and structural components of membranes, the nematode surface also contains some lipids, and the lipophilicity increases in response to host root exudate (López de Mendoza *et al.*, 2000). About one-sixth of the lipid of females of *M. javanica* is phospholipid; only 2.5% is glycolipid (Chitwood and Krusberg, 1981). The major fatty acid in root-knot nematodes is vaccenic acid, an oleic acid isomer that is common in bacteria; it amounted to over 25% of the dry weight of females and 40% in eggs of *M. incognita* and *M. arenaria* (Krusberg *et al.*, 1973). At least 30 other fatty acids also exist in *Meloidogyne*, including several iso-branched acids. Although the roles of the latter are speculative, one possibility would be that they act as metabolic precursors to an as yet unidentified *Meloidogyne* analogue of the *C. elegans* dauer pheromone, which consists of a short branched-chain fatty acid attached to the sugar ascarylose (Jeong *et al.*, 2005). As much as a third of the individual phospholipids of *M. javanica* contain ether-linked alkyl moieties attached to the C-2 position of the glycerol backbone (Chitwood and Krusberg, 1981). The phospholipid alkyl groups are remarkably undiverse in structure, primarily consisting of saturated 18-carbon moieties.

8.4.6 Steroids

The major roles of sterols in organisms are to modulate membrane fluidity as components of cellular membranes, provide biochemical precursors for steroid hormones, and interact with specific proteins in the regulation of organismal development. Perhaps because *Meloidogyne* membrane phospholipids contain so much polyunsaturated fatty acid that any role of sterol to modulate fluidity would be superfluous, sterols comprise a remarkably small percentage of the nematode, only 0.02% of the eggs of *M. incognita* and *M. arenaria* (Chitwood *et al.*, 1987). In a comparison of the sterols of *M. incognita* and *M. arenaria* with those of their host plant, *Solanum melongena*, the major sterols of nematode eggs were 24-ethylcholesterol, 24-ethylcholestanol,

24-methylcholestanol, 24-ethylcholest-22-enol, cholesterol and cholestanol. The results indicated that saturation of the sterol nucleus was the major metabolic transformation of host sterols in *Meloidogyne*; additionally, the nematodes appeared to remove the C-24 methyl or ethyl substituent in the side chain of typical plant sterols. However, Hedin *et al.* (1995) did not detect the products of sterol nuclear saturation in eggs of *M. incognita* propagated on *Gossypium hirsutum*. Explanations for the analytical variation could include methodological differences, a host-mediated effect on parasite biochemistry, or a true biochemical difference between the two populations of *M. incognita* employed.

Although there have been several reports of toxicity of specific steroids to *Meloidogyne* spp., attempts to demonstrate the biosynthesis of ecdysteroids or other steroids in *Meloidogyne* have been unsuccessful (Chitwood *et al.*, 1987). Indeed, in only one case has the biosynthesis of any steroid with hormonal function in any nematode been conclusively demonstrated, in the case of the 3-ketocholest-4-en-26-oic acid and 3-ketocholest-7-en-26-oic acid, two steroid acids which are involved in the regulation of dauer larva formation in *C. elegans* (Motola *et al.*, 2006).

8.5 Sensory Perception and Neurotransmission

Of the various types of sensory perception, most research on *Meloidogyne* has focused on chemoreception, especially in relation to the amphids. Chemoreception plays an important role in the oriented movement (taxis) of nematodes. Orientation by a nematode can be achieved with one sensor, by sequentially sensing and comparing the stimulus on either side of the path of movement (klinotaxis), or with two or more sensors on different parts of the body, by simultaneously sensing different points within the stimulus field (tropotaxis). Bargmann and Horvitz (1991) demonstrated that killing neurons in either the left or the right chemosensory sensilla of *C. elegans* did not prevent chemotaxis, indicating that the nematode does not perform tropotaxis. Extrapolating this information to *Meloidogyne*, it is likely that repeated side-to-side movements of the head during sinusoidal body movement is responsible for alternating sampling of the stimulus field from either side of the head, and that klinotaxis is involved in orientation. The orientation and attraction of *Meloidogyne* to host roots and their movement in response to edaphic factors are discussed in detail by Curtis *et al.*, Chapter 6, this volume. In the following two sections, we examine the information on amphid functioning and neurotransmission in *Meloidogyne*.

8.5.1 Sensory perception

As in other nematodes, amphids are considered to be the primary chemosensilla of *Meloidogyne*. They are present as paired organs, positioned laterally and with external openings (see Eisenback and Hunt, Chapter 2, this volume). The amphidial cavity contains secretions, apparently produced by the sheath cell, that appear to have multiple roles. Trett and Perry (1985) suggested that the secretions may serve to maintain electrical continuity between the bases and tips of the dendritic processes, and they may also protect the dendritic endings of sensory nerve cells against desiccation and microbial attack (Aumann, 1993). Several investigations have centred on analysing the components of the secretions.

Bird (1966) detected esterases, enzymes that rapidly hydrolyse esters of short-chain fatty acids, in the amphidial ducts of juveniles and adults of *M. javanica* and *M. hapla*. Premachandran *et al.* (1988) found that the protein-specific dye, Coomassie Brilliant Blue R-250, bound to the amphidial secretions of a number of nematodes, including J2 of *M. incognita*, indicating that the secretions contain protein. Lectins have been used to demonstrate the presence of carbohydrate residues in amphidial secretions of species of *Meloidogyne* (McClure and Stynes, 1988). Components of amphidial secretions in J2 of *M. incognita* are thought to include *N*-acetylgalactosamine and fucose (McClure and Stynes, 1988; Spiegel and McClure, 1991).

Davis *et al.* (1992) found differences in the composition of amphidial secretions of *M. incognita* using a monoclonal antibody that reacted with the amphids of adult females but not with the amphids of J2. Stewart *et al.* (1993a) demonstrated the presence of a 32 kDa glycoprotein in the amphidial duct secretions and the sheath cell of J2

of six species of *Meloidogyne*, but it was not found in representatives from eight other genera, including *Globodera* and *Heterodera*, indicating a specialized function for this protein in *Meloidogyne*. The protein was found in all stages of the *Meloidogyne* life cycle, including males of *M. javanica*, but not in the sedentary adult female, where the amphids appear to be non-functional. Electron microscopy indicated a difference in the morphology of amphidial secretions in the J2 and the adult female (Stewart *et al.*, 1993b). In agar plate behavioural assays, prior incubation of *M. javanica* J2 in the antiserum against the protein significantly retarded the ability of the J2 to orientate to host roots (Stewart *et al.*, 1993b). Thus, there are indications that at separate stages of the life cycle the amphids may have a different function or a different combination of functions. Lima *et al.* (2005) used antibodies to immunolocalize secreted–excreted products of species of *Meloidogyne*. The antibodies reacted with antigens present in the amphids of *M. incognita* and *M. arenaria*, and one antibody recognized secretions in the amphidial and phasmidial glands of *M. arenaria*. The functions of the proteins from the amphids identified using antibodies are still unknown. Two of the polyclonal antibodies used by Lima *et al.* (2005) bound to the surface coat of the cuticle as well as to the amphids of *M. incognita* and *M. arenaria*; surface coat components are reviewed by Curtis *et al.*, Chapter 6, this volume.

Semblat *et al.* (2001) cloned a cDNA (complementary DNA) encoding a secretory protein from the amphids of *M. incognita*. The protein (MAP-1) expressed by the *map-1* gene was restricted to the three species of *Meloidogyne*, *M. arenaria*, *M. incognita* and *M. javanica*, controlled by the *Mi* resistance gene, suggesting a specialized function for this protein. The authors speculate that the MAP-1 protein might be involved in the early steps of recognition between (resistant) plants and (avirulent) nematodes.

8.5.2 Neurotransmission

Neurotransmission in nematodes has been summarized by Wright and Perry (1998) and Perry and Maule (2004), and reviewed in detail for *C. elegans* by Rand and Nonet (1997), but there is only fragmentary information on *Meloidogyne*. Typical synaptic transmission involves the arrival of an action potential at a presynaptic nerve ending, causing the opening of voltage-gated Ca^{2+} ion channels and the influx of Ca^{2+} ions into the nerve cell. This results in the secretion of neurotransmitter molecules, which diffuse across the synaptic cleft and bind reversibly to specific receptor proteins on the post-synaptic membrane of a nerve or muscle cell. This causes a conformational change in the receptor proteins that are linked to ion channels. Whether the response is excitatory or inhibitory depends on the type of receptor and, thus, which ion channel is activated. Hence, the same neurotransmitter can be excitatory and inhibitory.

Classical transmitters include acetylcholine, probably the primary excitatory transmitter, several amino acids and various biogenic amines. The amino acid transmitter, γ-amino butyric acid (GABA), and the biogenic amine, dopamine, have been reported in J2 of *M. incognita* (Stewart *et al.*, 1994, 2001).

An essential feature of all neurotransmitter systems is a mechanism for the rapid removal of neurotransmitter from the synaptic cleft. Removal of acetylcholine is enzymatic, and inhibition of acetylcholinesterase is the target for the control of plant-parasitic nematodes, including *Meloidogyne*, by organophosphate and carbamate nematicides. In *C. elegans*, four acetylcholinesterase genes have been isolated, *ace-1* to *ace-4*, coding for biochemical classes of acetylcholinesterase. *Meloidogyne arenaria* and *M. incognita* J2 contain several molecular forms of acetylcholinesterase, which vary in sedimentation coefficient, substrate affinity, thermal inactivation profiles, and/or inhibitor and detergent sensitivity (Nordmeyer and Dickson, 1990; Chang and Opperman, 1991). At least two acetylcholinesterase genes have been identified in *Meloidogyne*. A single gene homologous to the *ace-1* gene of *C. elegans* has been isolated from *M. incognita* and *M. javanica* (Piotte *et al.*, 1999), and Laffaire *et al.* (2003) isolated a new acetylcholinesterase-encoding gene, named *Mi-ace-2*, from *M. incognita*, which is transcribed in J2 before and after hatching, and in females and males.

8.6 Conclusions and Future Directions

There have been rapid advances in experimental techniques, especially in genome sequencing,

proteomics and metabolomics, which have generated large amounts of data relating to helminth physiology and biochemistry (Barrett, 2009). Proteomics is the large-scale analysis of proteins in a single cell or tissue, whereas metabolomics is the analysis of the low molecular weight metabolites in the tissue or cell (Barrett, 2009). Instead of focusing on a single gene or protein, proteomics can reveal the relative amounts of protein, the degree of protein modification and turnover, and the interactions between proteins (Barrett *et al.*, 2005; Barrett, 2009). The information that will be generated by the various nematode genome projects, including those of *M. incognita* and *M. hapla* (Abad *et al.*, 2008, Opperman *et al.*, 2008), presages an exciting future for elucidating nematode biological systems. Annotation of the sequences will provide a vast amount of data relating to the physiology and biochemistry of *Meloidogyne*. Although the ability to identify candidate parasitism genes is one obvious outcome of this research, rapid insight should accrue with respect to the basic biochemical mechanisms that, for example, permit reproductive success through varied reproductive strategies mediated by differing cytogenetics.

Thus, genome annotation, proteomics and metabolomics herald an era of enormous opportunities for research workers. However, it will be necessary to interpret and understand the biological relevance of these data and to clarify details of the biological functioning of *Meloidogyne* and other nematodes. The current lack of information on important aspects of physiology and biochemistry reflects, in part, the worrying paucity of researchers trained to use physiological, biochemical and behavioural techniques to investigate gene functioning. The need to interpret components of the host–parasite system is predicated on the existence of these research skills. This is a vital component of future progress, especially as the information gained could lead to much-needed environmentally benign, novel control approaches.

8.7 References

Abad, P., Gouzy, J., Aury, J.-M. *et al.* (2008) Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology* 8, 909–915.

Abrantes, I.M. de O. and Curtis, R.H.C. (2002) Immunolocalization of a putative cuticular collagen protein in several developmental stages of *Meloidogyne arenaria*, *Globodera pallida* and *G. rostochiensis*. *Journal of Helminthology* 76, 1–6.

Aumann, J. (1993) Chemosensory physiology of nematodes. *Fundamental and Applied Nematology* 16, 193–198.

Bakhetia, M., Charlton, W., Atkinson, H. J. and McPherson, M. J. (2005) RNA interference of dual oxidase in the plant nematode *Meloidogyne incognita*. *Molecular Plant–Microbe Interactions* 18, 1099–1106.

Bargmann, C.I. and Horvitz, H.R. (1991) Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. *Neuron* 7, 729–742.

Barrett, J. (2009) Forty years of helminth biochemistry. *Parasitology* 136, 1–10.

Barrett, J., Brophy, P.M. and Hamilton, J.V. (2005) Analysing proteomic data. *International Journal for Parasitology* 35, 543–553.

Baxter, R.I. and Blake, C.D. (1969) Oxygen and the hatch of eggs and migration of larvae of *Meloidogyne javanica*. *Annals of Applied Biology* 63, 191–203.

Bird, A.F. (1966) Esterases in the genus *Meloidogyne*. *Nematologica* 12, 359–361.

Bird, A.F. and McClure, M.A. (1976) The tylenchid (Nematoda) egg shell: structure, composition and permeability. *Parasitology* 72, 19–28.

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

Bird, A.F. and Self, P.G. (1995) Chitin in *Meloidogyne javanica*. *Fundamental and Applied Nematology* 18, 235–239.

Bird, A.F., Bonig, I. and Bacic, A. (1988) A role for the 'excretory' system in secernentean nematodes. *Journal of Nematology* 20, 493–496.

Blaxter, M.L. and Robertson, W.M. (1998) The cuticle. In: Perry, R.N. and Wright, D.J. (eds) *The Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes*. CAB International, Wallingford, UK, pp. 25–48.

Brownlee, D., Holden-Dye, L. and Walker, R. (2000) The range and biological activity of FMRFamide-related peptides and classical neurotransmitters in nematodes. *Advances in Parasitology* 45, 109–180.

Carneiro, R.M.D.G., Carneiro, R.G., Abrantes, I.M.O., Santos, M.S.N.A. and Almeida, M.R.A. (1996) *Meloidogyne paranaensis* n. sp. (Nemata: Meloidogynidae), a root-knot nematode parasitizing coffee in Brazil. *Journal of Nematology* 28, 177–189.

Carneiro, R.M.D.G., Randig, O., Almeida, M.R.A. and Gomes, A.C.M.M. (2004) Additional information on *Meloidogyne ethiopica* Whitehead, 1968 (Tylenchida: Meloidogynidae), a root-knot nematode parasitising kiwi fruit and grape-vine from Brazil and Chile. *Nematology* 6, 109–123.

Carneiro, R.M.D.G., Almeida, M.R.A., Gomes, A.C.M.M. and Hernandez, A. (2005) *Meloidogyne izalcoensis* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitising coffee in El Salvador. *Nematology* 7, 819–832.

Carneiro, R.M.D.G., Mendes, M.L., Almeida, M.R.A., dos Santos, M.F.A., Gomes, A.C.M.M. and Karssen, G. (2008) Additional information on *Meloidogyne inornata* Lordello, 1956 (Tylenchida: Meloidogynidae) and its characterisation as a valid species. *Nematology* 10, 123–136.

Castagnone-Sereno, P. (2006) Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes. *Heredity* 96, 282–289.

Chang, S. and Opperman, C.H. (1991) Characterization of acetylcholinesterase molecular forms of the root-knot nematode, *Meloidogyne. Molecular and Biochemical Parasitology* 29, 205–214.

Charchar, J.M., Eisenback, J.D. and Hirschmann, H. (1999) *Meloidogyne petuniae* n. sp. (Nemata: Meloidogynidae), a root-knot nematode parasitic on petunia in Brazil. *Journal of Nematology* 31, 81–91.

Chitwood, D.J. and Krusberg, L.R. (1981) Diacyl, alkylacyl, and alkenylacyl phospholipids of *Meloidogyne javanica* females. *Journal of Nematology* 13, 105–111.

Chitwood, D.J., McClure, M.A., Feldlaufer, M.F., Lusby, W.R. and Oliver, J.E. (1987) Sterol composition and ecdysteroid content of eggs of the root-knot nematodes, *Meloidogyne incognita* and *M. arenaria. Journal of Nematology* 19, 352–360.

Coghlan, A. (2005) Nematode genome evolution. In: The *C. elegans* Research Community (eds) *WormBook*, http://www.wormbook.org/chapters/www_genomevol/genomevol.html.

Dalmasso, A. and Berge, J.B. (1978) Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp.: application to the taxonomy of *Meloidogyne. Journal of Nematology* 10, 323–332.

Davies, K.G., Rowe, J. and Williamson, V.M. (2008) Inter- and intra-specific cuticle variation between amphimictic and parthenogenetic species of root-knot nematode (*Meloidogyne* spp.) as revealed by a bacterial parasite (*Pasteuria penetrans*). *International Journal for Parasitology* 38, 851–859.

Davis, E.L. and Kaplan, D.T. (1992) Lectin binding to aqueous-soluble and body wall proteins from infective juveniles of *Meloidogyne* species. *Fundamental and Applied Nematology* 15, 243–250.

Davis, E.L., Kaplan, D.T., Permar, T.A., Dickson, D.W. and Mitchell, D.J. (1988) Characterization of carbohydrates on the surface of second-stage juveniles of *Meloidogyne* spp. *Journal of Nematology* 20, 609–619.

Davis, E.L., Aaron, L.M., Pratt, L.H. and Hussey, R.S. (1992) Novel immunization procedures used to develop antibodies that bind to specific structures in *Meloidogyne* spp. *Phytopathology* 82, 1244–1250.

De Giorgi, C., De Luca, F., Di Vito, M. and Lamberti, F. (1997) Modulation of expression at the level of splicing of *cut-1* RNA in the infective second-stage juvenile of the plant parasitic nematode *Meloidogyne artiellia. Molecular and General Genetics* 253, 589–598.

Dickson, D., Huisingh, D. and Sasser, J.N. (1971). Dehydrogenases, acid and alkaline phosphatases, and esterases for chemotaxonomy of selected *Meloidogyne*, *Ditylenchus*, *Heterodera* and *Aphelenchus* spp. *Journal of Nematology* 3, 1–16.

Doyle, E.A. and Lambert, K.N. (2002) Cloning and characterization of an esophageal-gland-specific pectate lyase from the root-knot nematode *Meloidogyne javanica*. *Molecular Plant–Microbe Interactions* 15, 549–556.

Doyle, E.A. and Lambert, K.N. (2003) *Meloidogyne javanica* chorismate mutase 1 alters plant cell development. *Molecular Plant–Microbe Interactions* 16, 123–131.

Dropkin, V.H. and Acedo, J. (1974) An electron microscopic study of glycogen and lipid in female *Meloidogyne incognita* (root-knot nematode). *Journal of Parasitology* 60, 1013–1021.

Eisenback, J.D., Yang, B. and Hartman, K.M. (1985) Description of *Meloidogyne pini* n. sp., a root-knot nematode parasitic on sand pine (*Pinus clausa*), with additional notes on the morphology of *M. megatyla. Journal of Nematology* 17, 206–219.

Eisenback, J.D., Bernard, E.C. and Schmitt, D.P. (1994) Description of the Kona coffee root-knot nematode, *Meloidogyne konaensis* n. sp. *Journal of Nematology* 26, 363–374.

Esbenshade, P.R. and Triantaphyllou, A.C. (1990) Isozyme phenotypes for the identification of *Meloidogyne* species. *Journal of Nematology* 22, 10–15.

Evans, A.A.F. (1998) Reproductive mechanisms. In: Perry, R.N. and Wright, D.J. (eds) *The Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes*. CAB International, Wallingford, UK, pp. 133–154.

Fleming, C.C., McKinney, S., McMaster, S., Johnston, M.J.G., Donnelly, P., Kimber, M.J. and Maule, A.G. (2007) Getting to the root of neuronal signalling in plant-parasitic nematodes using RNA interference. *Nematology* 9, 301–315.

Fragoso, R. da R., Batista, J.A.N., Neto, O.B.O. and Grossi de Sa, M.F. (2005) Isolation and characterization of a cDNA encoding a serine proteinase from the root-knot nematode *Meloidogyne incognita. Experimental Parasitology* 110, 123–133.

Goldstein, P. and Triantaphyllou, A.C. (1982) The synaptonemal complexes of *Meloidogyne*: relationship of structure and evolution of parthenogensis. *Chromosoma* 87, 117–124.

Handoo, Z.A., Nyczepir, A.P., Esmenjaud, D., van der Beek, J.G., Castagnone-Sereno, P., Carta, L.K., Skantar, A.M. and Higgins, J.A. (2004) Morphological, molecular, and differential-host characterization of *Meloidogyne floridensis* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitizing peach in Florida. *Journal of Nematology* 36, 20–35.

Hedin, P.A., Callahan, F.E., Dollar, D.A. and Creech, R.G. (1995) Total sterols in root-knot nematode *Meloidogyne incognita* infected cotton *Gossypium hirsutum* (L.) plant roots. *Comparative Biochemistry and Physiology* 111B, 447–452.

Huang, G., Dong, R., Maier, T., Allen, R., Davis, E.L., Baum, T.J. and Hussey, R.S. (2004) Use of solid-phase subtractive hybridization for the identification of parasitism gene candidates from the root-knot nematode *Meloidogyne incognita. Molecular Plant Pathology* 5, 217–222.

Hugall, A., Stanton, J. and Moritz, C. (1999) Reticulate evolution and the origins of ribosomal internal transcribed spacer diversity in apomictic *Meloidogyne. Molecular Biology and Evolution* 16, 157–164.

Hussey, R.S., Sasser, J.N. and Huisingh, D. (1972) Disc-electrophoretic studies of soluble proteins and enzymes of *Meloidogyne incognita* and *M. arenaria. Journal of Nematology* 4, 183–189.

Ibrahim, S.K. (1991) Distribution of carbohydrates on the cuticle of several developmental stages of *Meloidogyne javanica. Nematologica* 37, 275–284.

Ishibashi, N. (1970) Variations of the electrophoretic protein patterns of Heteroderidae (Nematodea: Tylenchida) depending on the developmental stages of the nematode and on the growing conditions of the host plants. *Applied Entomology and Zoology* 5, 23–32.

Jaubert, S., Laffaire, J.-B., Abad, P. and Rosso, M.-N. (2002) A polygalacturonase of animal origin isolated from the root-knot nematode *Meloidogyne incognita. FEBS Letters* 522, 109–112.

Jeong, P.-Y., Jung, M., Yim, Y.-H., Kim, H., Park, M., Hong, E., Lee, W., Kim, Y.H., Kim, K. and Paik, Y.-K. (2005) Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. *Nature* 433, 541–545.

Jones, J.T. and Robertson, W.M. (1997) Nematode secretions. In: Fenoll, C., Grundler, F.M.W. and Ohl, S.A. (eds) *Cellular and Molecular Aspects of Plant–Nematode Interactions*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 98–106.

Karssen, G. and Moens, M. (2006) Root-knot nematodes. In: Perry, R.N. and Moens, M. (eds) *Plant Nematology*. CAB International, Wallingford, UK, pp. 59–90.

Karssen, G., Bolk, R.J., van Aelst, A.C., van den Beld, I., Kox, L.F.F., Korthals, G., Molendijk, L., Zijlstra, C., van Hoof, R. and Cook, R. (2004) Description of *Meloidogyne minor* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode associated with yellow patch disease in golf courses. *Nematology* 6, 59–72.

Koltai, H., Chejanovsky, N., Raccah, B. and Spiegel, Y. (1997) The first isolated collagen gene of the root-knot nematode *Meloidogyne javanica* is developmentally regulated. *Gene* 196, 191–199.

Krusberg, L.R. (1967) Analysis of total lipids and fatty acids of plant-parasitic nematodes and host tissues. *Comparative Biochemistry and Physiology* 21, 83–90.

Krusberg, L.R., Hussey, R.S. and Fletcher, C.L. (1973) Lipid and fatty acid composition of females and eggs of *Meloidogyne incognita* and *M. arenaria. Comparative Biochemistry and Physiology* 45B, 335–341.

Laffaire, J.-B., Jaubert, S., Abad, P. and Rosso, M.-N. (2003) Molecular cloning and life stage expression pattern of a new acetylcholinesterase gene from the plant-parasitic nematode *Meloidogyne incognita. Nematology* 5, 213–217.

Lambert, K.N., Allen, K.D. and Sussex, I.M. (1999) Cloning and characterization of an esophageal-gland-specific chorismate mutase from the phytoparasitic nematode *Meloidogyne javanica. Molecular Plant–Microbe Interactions* 12, 328–336.

Ledger, T.N., Jaubert, S., Bosselut, N., Abad, P. and Rosso, M.-N. (2006) Characterization of a new [beta]-1,4-endoglucanase gene from the root-knot nematode *Meloidogyne incognita* and evolutionary scheme for phytonematode family 5 glycosyl hydrolases. *Gene* 382, 121–128.

Lee, D.L. and Atkinson, H.J. (1976) *The Physiology of Nematodes,* 2nd edn. MacMillan Press, London.

Lima, L.M., Grossi-de-Sa, M.F., Pereira, R.A. and Curtis, R.H.C. (2005) Immunolocalisation of secreted–excreted products of *Meloidogyne* spp. using polyclonal and monoclonal antibodies. *Fitopatologia Brasileira* 30, 629–633.

Lin, H.-J. and McClure, M.A. (1996) Surface coat of *Meloidogyne incognita. Journal of Nematology* 28, 216–224.

Liu, Q.L. and Williamson, V.M. (2006) Host-specific pathogenicity and genome differences between inbred strains of *Meloidogyne hapla. Journal of Nematology* 38, 158–164.

Liu, Q.L., Thomas, V.P. and Williamson, V.M. (2007) Meiotic parthenogenesis in a root-knot nematode results in rapid genomic homozygosity. *Genetics* 176, 1483–1490.

Long, H., Wang, X. and Xu, J. (2006a) Molecular cloning and life-stage expression pattern of a new chorismate mutase gene from the root-knot nematode *Meloidogyne arenaria. Plant Pathology* 55, 559–563.

Long, H., Wang, X., Xu, J.H. and Hu, Y.J. (2006b) Isolation and characterization of another cDNA encoding a chorismate mutase from the phytoparasitic nematode *Meloidogyne arenaria. Experimental Parasitology* 113, 106–111.

López de Mendoza, M.E., Modha, J., Roberts, M.C., Curtis, R. and Kusel, J. R. (2000). Changes in the lipophilicity of the surfaces of *Meloidogyne incognita* and *Haemonchus contortus* during exposure to host signals. *Parasitology* 120, 203–209.

Lunt, D.H. (2008) Genetic tests of ancient asexuality in root knot nematodes reveal recent hybrid origins. *BMC Evolutionary Biology* 8, 194.

Marais, M. and Kruger, J.-C. de W. (1991) The cytogenetics of some South African root-knot nematodes (Heteroderidae: Nematoda). *Phytophylactica* 23, 265–272.

Marwah, R. and Khera, S. (1988) Histochemical localization of nucleic acids and some enzymes in females of *Meloidogyne incognita. Helminthologia* 25, 49–60.

McCarter, J.P., Mitreva, M.D., Martin, J. *et al.* (2003) Analysis and functional classification of transcripts from the nematode *Meloidogyne incognita. Genome Biology* 4, R3.

McClure, M.A. and Bird, A.F. (1976) The tylenchid (Nematoda) egg shell: formation of the egg shell in *Meloidogyne javanica. Parasitology* 72, 29–39.

McClure, M.A. and Stynes, B.A. (1988) Lectin binding sites on the amphidial exudates of *Meloidogyne. Journal of Nematology* 20, 321–326.

McVeigh, P., Alexander-Bowman, S., Veal, E., Mousley, A., Marks, N. and Maule, A.G. (2008) Neuropeptide-like protein diversity in phylum Nematoda. *International Journal for Parasitology* 38, 1493–1503.

Mitreva-Dautova, M., Roze, E., Overmars, H., de Graaff, L., Schots, A., Helder, J., Goverse, A., Bakker, J. and Smant, G. (2006) A symbiont-independent endo-1,4-β-xylanase from the plant-parasitic nematode *Meloidogyne incognita. Molecular Plant–Microbe Interactions* 19, 521–529.

Molinari, S., Lamberti, F., Crozzoli, R., Sharma, S.B. and Sanchez Portales, L. (2005) Isozyme patterns of exotic *Meloidogyne* spp. populations. *Nematologia Mediterranea* 33, 61–65.

Motola, D.L., Cummins, C.L., Rottiers, V. *et al.* (2006) Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans. Cell* 124, 1209–1223.

Muniz, M. de F., Campos, V.P., Almeida, M.R.A., Gomes, A.C.M.M., dos Santos, M.F. and Carneiro, R.M.D.G. (2009) Additional information on an atypical population of *Meloidogyne exigua* Göldi 1887 (Tylenchida: Meloidogynidae) parasitising rubber trees in Brazil. *Nematology* 11, 95–106.

Myers, R.F. and Krusberg, L.R. (1965) Organic substances discharged by plant-parasitic nematodes. *Phytopathology* 55, 429–437.

Navas, A., Castagnone-Sereno, P., Blazquez, J. and Esparrago, G. (2001) Genetic structure and diversity within local populations of *Meloidogyne* (Nematoda: Meloidogynidae). *Nematology* 3, 243–253.

Neveu, C., Semblat, J-P., Abad, P. and Castagnone-Sereno, P. (2002) Characterization of a cDNA related to K–Cl cotransporters in the root-knot nematode *Meloidogyne incognita. DNA Sequence – the Journal of Sequencing and Mapping* 13, 117–121.

Neveu, C., Abad, P. and Castagnone-Sereno, P. (2003) Molecular cloning and characterization of an intestinal cathepsin L protease from the plant-parasitic nematode *Meloidogyne incognita*. *Physiological and Molecular Plant Pathology* 63, 159–165.

Nordmeyer, D. and Dickson, D.W. (1989) Effect of carbamate, organophosphate and avermectin nematicides on oxygen consumption by three *Meloidogyne* spp. *Journal of Nematology* 21, 472–476.

Nordmeyer, D. and Dickson, D.W. (1990) Molecular forms of cholinesterase in the plant-parasitic nematodes *Meloidogyne incognita* and *Radopholus similis*. *Revue de Nématologie* 13, 311–316.

Opperman, C.H., Bird, D.McK., Williamson, V.M. *et al*. (2008) Sequence and genetic map of *Meloidogyne hapla*: a compact nematode genome for plant parasitism. *Proceedings of the National Academy of Sciences of the USA* 105, 14802–14807.

Papadopoulou, J. and Triantaphyllou, A.C. (1982) Sex differentiation in *Meloidogyne incognita* and anatomical evidence of sex reversal. *Journal of Nematology* 14, 549–566.

Perry, R.N. and Maule, A.G. (2004) Physiological and biochemical basis of behaviour. In: Gaugler, R. and Bilgrami, A.L. (eds) *Nematode Behaviour*. CAB International, Wallingford, UK, pp. 197–238.

Piotte, C., Arthaud, L., Abad, P. and Rosso, M.-N. (1999) Molecular cloning of an acetylcholinesterase gene from the plant parasitic nematodes, *Meloidogyne incognita* and *Meloidogyne javanica*. *Molecular and Biochemical Parasitology* 99, 247–256.

Plantard, O., Valette, S. and Gross, M.F. (2007) The root-knot nematode producing galls on *Spartina alterniflora* belongs to the genus *Meloidogyne*: rejection of *Hypsoperine* and *Spartonema* spp. *Journal of Nematology* 39, 127–132.

Premachandran, D., von Mende, N., Hussey, R.S. and McClure, M.A. (1988) A method for staining nematode secretions and structures. *Journal of Nematology* 20, 70–78.

Qi, Z.Q., Chen, C.J., Wang, J.X. and Zhou, M.G. (2008) Mode of action of methylene bisthiocyanate and its decomposition products to *Meloidogyne* spp. juveniles. *Acta Phytopathologica Sinica* 38, 420–424.

Rammah, A. and Hirschmann, H. (1988) *Meloidogyne mayaguensis* n. sp. (Meloidogynidae), a root-knot nematode from Puerto Rico. *Journal of Nematology* 20, 58–69.

Rammah, A. and Hirschmann, H. (1990) *Meloidogyne morocciensis* n. sp. (Meloidogynidae), a root-knot nematode from Morocco. *Journal of Nematology* 22, 279–291.

Rand, J.B. and Nonet, M.L. (1997) Synaptic transmission. In: Riddle, D.L., Blumenthal, T., Meyer, B.J. and Priess, J.R. (eds) *C. elegans II*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 611–643.

Ray, C., Wang, T.Y. and Hussey, R.S. (1996a) Identification and characterization of the *Meloidogyne incognita col*1 cuticle collagen gene. *Molecular and Biochemical Parasitology* 83, 121–124.

Ray, C., Reddigari, S.R., Jansma, P.L., Allen, R. and Hussey, R.S. (1996b) Immunocytochemical analysis of the stage-specific distribution of collagen in the cuticle of *Meloidogyne incognita*. *Fundamental and Applied Nematology* 19, 71–78.

Reddigari, S.R., Jansma, P.L., Premachandran, D. and Hussey, R.S. (1986) Cuticular collagenous proteins of second-stage juveniles and adult females of *Meloidogyne incognita*. *Journal of Nematology* 18, 294–302.

Reversat, G. (1976) Etude de la composition biochimique globale des juvéniles des nématodes *Meloidogyne javanica* et *Heterodera oryzae*. *Cahiers ORSTOM, Série Biologie* 11, 225–234.

Ritz, K. and Trudgill, D.L. (1999) Utility of nematode community analysis as an integrated measure of the functional state of soils: perspectives and challenges. *Plant and Soil* 212, 1–11.

Robinson, A.F. and Carter, W.W. (1986) Effects of cyanide ion and hypoxia on the volumes of second-stage juveniles of *Meloidogyne incognita* in polyethylene glycol solutions. *Journal of Nematology* 18, 563–570.

Rosso, M.-N., Favery, B., Piotte, C., Arthaud, L., De Boer, J.M., Hussey, R.S., Bakker, J., Baum, T.J., and Abad, P. (1999) Isolation of a cDNA encoding a β-1,4-endoglucanase in the root-knot nematode *Meloidogyne incognita* and expression analysis during plant parasitism. *Molecular Plant–Microbe Interactions* 12, 585–591.

Semblat, J.-P., Rosso, M.-N., Hussey, R.S., Abad, P. and Castagnone-Sereno, P. (2001) Molecular cloning of a cDNA encoding an amphid-secreted putative avirulence protein from the root-knot nematode *Meloidogyne incognita*. *Molecular Plant–Microbe Interactions* 14, 72–79.

Sharon, E. and Spiegel, Y. (1993) Glycoprotein characterization of the gelatinous matrix in the root-knot nematode *Meloidogyne javanica*. *Journal of Nematology* 25, 585–589.

Shingles, J., Lilley, C.J., Atkinson, H.J. and Urwin, P.E. (2007) *Meloidogyne incognita*: molecular and biochemical characterisation of a cathepsin L cysteine proteinase and the effect on parasitism following RNAi. *Experimental Parasitology* 115, 114–120.

Soriano, I.R., Riley, I.T., Potter, M.J. and Bowers, W.S. (2004) Phytoecdysteroids: a novel defense against plant-parasitic nematodes. *Journal of Chemical Ecology* 30, 1885–1899.

Spiegel, Y. and McClure, M. (1991) Stage-specific differences in lectin binding to the surface of *Anguina tritici* and *Meloidogyne incognita*. *Journal of Nematology* 23, 259–263.

Spiegel, Y., Inbar, J., Kahane, I. and Sharon, E. (1995) Carbohydrate-recognition domains on the surface of phytophagous nematodes. *Experimental Parasitology* 80, 220–227.

Starr, J.L. (1981) Beta-glycosidases from *Meloidogyne incognita* and *M. javanica*. *Journal of Nematology* 13, 413–414.

Stewart, G.R., Perry, R.N., Alexander, J. and Wright, D.J. (1993a) A glycoprotein specific to the amphids of *Meloidogyne* species. *Parasitology* 106, 405–412.

Stewart, G.R., Perry, R.N. and Wright, D.J. (1993b) Studies on the amphid specific glycoprotein gp32 in different life cycle stages of *Meloidogyne* species. *Parasitology* 107, 573–578.

Stewart, G.R., Perry, R.N. and Wright, D.J. (1994) Immunocytochemical studies on the occurrence of gamma-aminobutyric acid in the nervous system of the nematodes *Panagrellus redivivus*, *Meloidogyne incognita* and *Globodera rostochiensis*. *Fundamental and Applied Nematology* 17, 433–439.

Stewart, G.R., Perry, R.N. and Wright, D.J. (2001) Occurrence of dopamine in *Panagrellus redivivus* and *Meloidogyne incognita*. *Nematology* 3, 843–848.

Thompson, D.P. and Geary, T.G. (2002) Excretion/secretion, ionic and osmotic regulation. In: Lee, D.L. (ed.) *The Biology of Nematodes*. Taylor and Francis, London, pp. 291–320.

Trett, M.W. and Perry, R.N. (1985) Functional and evolutional implications of the anterior sensory anatomy of species of root-lesion nematodes (genus *Pratylenchus*). *Revue de Nématologie* 8, 106–110.

Triantaphyllou, A.C. (1962) Oogenesis in the root-knot nematode *Meloidogyne javanica*. *Nematologica* 7, 105–113.

Triantaphyllou, A.C. (1963) Polyploidy and reproductive patterns in the root-knot nematode *Meloidogyne arenaria*. *Journal of Morphology* 113, 489–499.

Triantaphyllou, A.C. (1966) Polyploidy and reproductive patterns in the root-knot nematode *Meloidogyne hapla*. *Journal of Morphology* 118, 403–413.

Triantaphyllou, A.C. (1971). Genetics and cytology. In: Zuckerman, B.M., Mai W.F. and Rohde, R.A. (eds) *Plant Parasitic Nematodes*, Vol. II. Academic Press, New York, pp. 1–34.

Triantaphyllou, A.C. (1981) Oogenesis and the chromosomes of the parthenogenetic root-knot nematode *Meloidogyne incognita*. *Journal of Nematology* 13, 95–104.

Triantaphyllou, A.C. (1984) Polyploidy in meiotic parthenogenetic populations of *Meloidogyne hapla* and a mechanism of conversion to diploidy. *Revue de Nématologie* 7, 65–72.

Triantaphyllou, A.C. (1985) Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. In: Sasser, J.N. and Carter, C.C. (eds) *An Advanced Treatise on* Meloidogyne. *Vol. I, Biology and Control*. North Carolina State University Graphics, Raleigh, North Carolina, pp. 113–126.

Triantaphyllou, A.C. (1990) Cytogenetic status of *Meloidogyne kikuyensis* in relation to other root-knot nematodes. *Revue de Nématologie* 13, 175–180.

Triantaphyllou, A.C. and Hirschmann, H. (1997) Evidence of direct polyploidization in the mitotic parthenogenetic *Meloidogyne microcephala*, through doubling of its somatic chromosome number. *Fundamental and Applied Nematology* 20, 385–391.

Trudgill, D.L and Blok, V.C (2001) Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annual Review of Phytopathology* 39, 53–77.

van der Beek, J.G. and Karssen, G. (1997) Interspecific hybridization of meiotic parthenogenetic *Meloidogyne chitwoodi* and *M. fallax*. *Phytopathology* 87, 1061–1066.

van der Beek, J.G. and Pijnacker, L.P. (2008) Maintenance of genetic variation in automictic root-knot nematodes. *Nematology* 10, 313–321.

Van der Eycken, W., de Almeida Engler, J., Van Montagu, M. and Gheysen, G. (1994) Identification and analysis of a cuticular collagen-encoding gene from the plant-parasitic nematode *Meloidogyne incognita*. *Gene* 151, 237–242.

Van Gundy, S.D. and Stolzy, L.H. (1961) Influence of soil oxygen concentrations on the development of *Meloidogyne javanica*. *Science* 134, 665–666.

Wang, E.L.H. and Bergeson, G.B. (1978) Amino acids and carbohydrates secreted by *Meloidogyne incognita*. *Journal of Nematology* 10, 367–368.

Wang, T., Deom, C.M. and Hussey, R.S. (1998) Identification of a *Meloidogyne incognita* cuticle collagen gene and characterization of the developmental expression of three collagen genes in parasitic stages. *Molecular and Biochemical Parasitology* 93, 131–134.

Wright, D.J. (1998) Respiratory physiology, nitrogen excretion and osmotic and ionic regulation. In: Perry, R.N. and Wright, D.J. (eds) *The Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes*. CAB International, Wallingford, UK, pp. 103–131.

Wright, D.J. and Perry, R.N. (1998) Musculature and neurobiology. In: Perry, R.N. and Wright, D.J. (eds) *Free-living and Plant-parasitic Nematodes*. CAB International, Wallingford, UK, pp. 49–73.

Wright, D.J. and Perry, R.N. (2006) Reproduction, physiology and biochemistry. In: Perry, R.N. and Moens, M. (eds) *Plant Nematology*. CAB International, Wallingford, UK, pp. 187–209.