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Plant Parasitic Nematodes: Habitats, Hormones, and Horizontally-Acquired Genes

David McKenzie Bird* and Hinanit Koltai

Plant Nematode Genetics Group, Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695, USA

Abstract

Plant parasitic nematodes are ubiquitous and cosmopolitan pathogens of vascular plants and exploit all parts of the roots and shoots, causing substantial crop damage. Nematodes deploy a broad spectrum of feeding strategies, ranging from simple grazing to the establishment of complex cellular structures (including galls) in host tissues. Various models of feeding site formation have been proposed, and a role for phytohormones has long been speculated, although whether they perform a primary or secondary function is unclear. On the basis of recent molecular evidence, we present several scenarios involving phytohormones in the induction of giant cells by root-knot nematode. The origin of parasitism by nematodes, including the acquisition of genes to synthesize or modulate phytohormones also is discussed, and models for horizontal gene transfer are presented.

Key words: *Anguina*; Cytokinin; Gall; Giant cell; *Heterodera*; KNOX; *Meloidogyne*; PHAN; Syncytium

Introduction

The unsegmented round-worms (Figure 1) that comprise the phylum Nematoda are the most successful metazoans. Numerically, they represent 80–90% of all animals, and random sampling techniques imply that there may be as many as 10^5–10^8 species yet to be described (Boucher and Lambshead 1994). Nematodes occupy all ecological niches, including being parasites of probably all other complex animals and plants (Blaxter and Bird 1997). The impact of nematodes on humans is felt through yield reductions in food and fiber crops, through debilitation of livestock and companion animals, and by direct infection; nematodes such as hookworm and Ascaris each infect more than a billion people worldwide, and nematodes are responsible for exotic diseases such as elephantiasis and river-blindness.

Like insects with which nematodes share a common ancestor as members of the high-level taxon, Ecdysozoa (Aguinaldo and others 1997), their complex animal anatomy (Bird and Bird 1991) includes a well-developed nervous system. Consequently, nematodes can integrate a wide range of environmental cues to effect developmental and behavioral outcomes, and these flexible life strategies play an important role in the success of nematodes as parasites. Also like insects, certain plant-parasitic nematode species induce cellular modifications in host tissues, including the formation of galls, implicating a role for phytohormones. Thus it seems likely that nematodes and insects have acquired the ability to manipulate very fundamental aspects of their host's

*Corresponding author; e-mail: david_bird@ncsu.edu

biology, also no doubt contributing to the success of these parasites.

The high level of sophistication parasitic nematodes exhibit distinguishes them from other plant pathogens (including viruses, bacteria, and fungi) and probably contributes to the observation that most individual plants (in wild and agricultural settings) are infected with nematodes. Nevertheless, plants have evolved defenses against nematodes, and the molecules involved are of the same type(s) that plants use to defend themselves against other pathogens (Williamson 1999). This suggests that at the molecular level, nematodes use strategies conceptually similar to other microorganisms in interacting with plants. Strikingly, the tomato *Mi* gene that confers resistance to root-knot nematode *Meloidogyne incognita* also appears to condition resistance to aphids (Rossi and others 1998), further emphasizing the similarities between insect-plant and nematode-plant interactions. Plant-parasitism by insects and nematodes has arisen independently and on multiple occasions (Blaxter and others 1998), and recent evidence points to an ancient acquisition of microbial genes by horizontal gene-transfer as being one evolutionary origin of parasitism genes by nematodes (Keen and Roberts 1998). Host genes too might prove to be a source of parasitism functions in nematodes. It will be interesting to determine whether nematodes and insects share sequences perhaps independently acquired from microbes. Genetic analysis of plant-parasitic nematodes is in its infancy, but several nematode genes that condition resistance-breaking have been identified and mapped (see Opperman and Bird 1998). Analysis of such genes will likely reveal clues to understanding the host-parasite interaction. It is increasingly clear that understanding nematode-plant interactions will reveal more generally applicable paradigms for host-parasite interactions and will likely provide insights into normal plant developmental and physiologic processes.

Despite the biologic complexity of nematodes, one species, *Caenorhabditis elegans,* has become the best understood animal (Riddle and others 1997; Wood 1988). The sheer volume of information obtained for *C. elegans,* along with its mature genome project (*C. elegans* Sequencing Consortium 1998) and suite of research tools (Epstein and Shakes 1995) serve as essential resources to underpin the burgeoning deployment of genomics in studies of parasitic nematode biology (Bird and Opperman 1998; Bird and others 1999; Blaxter 1998; Blaxter and Bird 1997; Opperman and Bird 1998).

The Impact of Plant-Parasitic Nematodes

As parasites, nematodes exploit all parts of vascular plants, yet their net impact is difficult to establish accurately. On the basis of an extensive international survey (Sasser and Freckman 1987), it has been estimated that overall yield loss averages 12.3%, with this figure approaching 20% for some crops. In monetary terms losses certainly exceed $100 billion annually. Most of the damage is caused by a relatively small number of the dozens of nematode genera that attack crops (Nickle 1991), principally the sedentary root-knot (*Meloidogyne* spp.) and cyst (*Globodera* and *Heterodera* spp.) nematodes, as well as several migratory nematodes (including *Pratylenchus* and *Radopholus* spp.).

Another way to consider the impact of plant-parasitic nematodes is through the management strategies used in their control. In 1982, 109 million pounds of nematicide active ingredient were applied to crops in the United States, at a cost exceeding $1 billion (Landels 1989). Between 1986 and 1990 in the Netherlands, nematicide application was more than three times the combined total of chemicals needed to combat insects, fungi, and weeds on experimental farms (Lewis and others 1997). However, in recent decades, issues such as ground water contamination, mammalian and avian toxicity, and residues in food have caused much tighter restrictions on the use of agricultural chemicals, and in many countries, effective nematicides have been, and continue to be, deregistered (Thomason 1987).

Until environmentally safe nematicides are developed, host resistance remains the most sound nematode management approach, and in those crops where resistance is available, it has proved to be an extremely valuable commodity. For example, soybean growers in the southern United States saved more than $400 million during a 5-year period after introduction of the *Heterodera glycines*–resistant cultivar "Forrest" (Bradley and Duffy 1982). Regrettably, nematode resistance is yet to be identified for many crop plants, although several naturally occurring resistance genes have recently been cloned (Williamson 1999). The potential use of these dominant loci to construct transgenic plants to circumvent breeding difficulties is an appealing approach. Transfer of cloned $Hs1^{pro\text{-}1}$ from a wild relative of sugar beet was shown to confer resistance to beet cyst nematode to susceptible sugar beet roots (Cai and others 1997) and also to *Arabidopsis* (D. Cai, personal communication). However, experiments to transfer resistance from tomato into tobacco using the cloned *Mi* gene have so far, for reasons that re-

main unclear, been unsuccessful (Williamson 1998). Other approaches to make transgenic, nematode-resistant crop plants based on an understanding of the host-parasite interaction have been proposed (for example, see Bird 1996) and are reviewed in detail by Atkinson and others (1998).

Niches Occupied by Plant-Parasitic Nematodes

The first recorded observation of a plant parasitic nematode was of *Anguina tritici* (Needham 1743), a flower and seed pathogen of wheat and other small grains, but in fact nematodes occupy all parts of vascular plants including leaves (*Aphelenchoides* spp. on strawberries), stems (*Bursaphalenchus xylophilus* in the xylem of conifers), tubers (*Globodera rostochiensis* on potato), bulbs (*Ditylenchus dipsaci* on onions and ornamentals), corms (*Radopholus similis* on banana), and roots (*Heterodera glycines* on soybean; *Meloidogyne* spp. on many plants). To date, most attention has been focused on the root-parasitic species, and various classification schemes based on the site of feeding within the root have been developed (Dropkin 1969; Hussey and Grundler 1998; Wyss 1997). In the simplest versions, nematodes are considered to be migratory or sedentary, and endo- or ecto-parasites. Additional categories recognize classes such as "ecto-endoparasite." In some classification schemes, additional criteria are considered, such that the most elaborate versions recognize 14 "modes" of root-parasitism (Hussey and Grundler 1998). However, although they serve as a useful tool for describing individual host-parasite interactions, these classification schemes provide no information on mechanisms of the host-parasite interaction, nor do they give any clues to the evolution of parasitism. It is often argued (for example, Wyss 1997) that parasitic interactions exhibiting the most extensive or most elaborate feeding sites in the host reflect the evolutionarily most advanced form of parasitism. However, all extant plant-parasitic nematodes should be considered to be equally evolved. Differences between parasitic strategies reflect adaptations to exploit different ecological niches within the host, and this is particularly true of feeding behavior and the nature of the feeding site induced.

Case Studies: The Biology of *Anguina*, *Heterodera* and *Meloidogyne*

The feeding cells induced by root-knot and cyst nematodes, termed giant cells and syncytia, respectively, have been the subject of numerous studies using both light and electron microscopes (EM) (for example, Bird 1961; Christie 1936; Jones and Northcote 1972; Jones and Payne 1978), and their anatomy and cytology is well established. Furthermore, the ontogeny of syncytia (Golinowski and others 1997) and giant cells (Bleve-Zacheo and Melillo 1997) and the physiology of both types (Grundler and Böckenhoff 1997) are the subject of recent comprehensive reviews. The emphasis on giant cells and syncytia is partly reflective of the great economic importance of the nematodes that induce them but also because these large feeding sites are amenable to microscopic studies and to biochemical and molecular analyses. However, EM studies on the supposedly simple feeding sites induced by the "primitive" ecto-endoparasite *Scutellonema brachyurum* (Schuerger and McClure 1983) and the ectoparasite *Criconemella xenoplax* (Hussey and others 1992) revealed surprisingly complex morphologic responses in the host; much insight might be gained from careful examination of even the seemingly simplest interactions.

Anguina: ***A shoot parasite.*** *Anguina* spp. establish a large gall inside a grass seed, in which the nematodes are able to survive essentially indefinitely in an anhydrobiotic state. After rain, *Anguina funesta,* a parasite of annual ryegrass (*Lolium rigidum*), emerges from the seed gall as infective second-stage (L2) larvae that make their way to the young plant. L2s locate the developing inflorescence, where galls are induced in tissues that would normally become the ovules or sometimes stamens, glumes, or the rachis (Stynes and Bird 1982). Changes take place in a large number of host cells. The cytoplasm becomes dense and granular, the nuclei enlarge, and gradually the cell contents become vacuolated and the cells empty and collapse. This process ultimately leads to a gall with a cavity that becomes filled with nematodes. Each gall typically is established by several individuals of each sex that undergo a single round of reproduction resulting in hundreds of L2s, which subsequently enter anhydrobioisis (without molting) as the host senesces (Riddle and Bird 1985). It seems reasonable that, like *C. elegans, Anguina* larvae sense population density based on the concentration of a nematode-produced pheromone and use this information to regulate initiation of anhydrobiosis. An alternative possibility is that some host cue is used, permitting developmental processes in the nematode to be intimately coupled to those of the host.

In certain grasses, including the important forage crop annual ryegrass, the *Anguina* seed galls can become toxic to grazing animals when a bacterium (*Clavibacter toxicus*) is brought into the developing seed heads attached to the cuticle of the invading

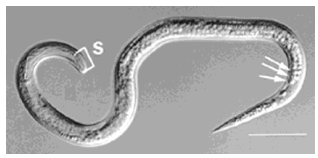

Figure 1. Newly hatched *Meloidogyne incognita* L2(J2) larva (juvenile). The feeding stylet (*S*) is fully retracted. A large number of lipid granules that provide energy reserves until the host root is located and feeding is initiated are apparent (*arrows*). Scale bar: 50 µm.

nematode. Intriguingly, the toxin is encoded by a *C. toxicus* bacteriophage (Ophel and others 1993). This unique association of microorganisms is dependent on the nematode providing both transport and a niche in the seed gall that it initiates.

Meloidogyne *and* heterodera: ***Root parasites***

These species hatch in the soil as an L2 larva (Figure 1), which penetrates and migrates within a host root to establish permanent feeding sites that are characterized by extensive modifications to host cells. The nematodes undergo dramatic developmental and morphologic changes and adopt a sedentary lifestyle. Eggs are either released in masses on to the surface of the root gall (root-knot) or encased in the body of the female, thus forming a cyst. Depending on the particular nematode and host, as well as environmental conditions, there are typically between one and four generations per year.

Giant cells arise by expansion of individual parenchyma cells in the vascular cylinder. The developing cells undergo rounds of synchronous nuclear division uncoupled from cytokinesis, and individual nuclei become highly polyploid (Figure 2). The cell wall is extensively remodeled, with the development of fingerlike projections into the cell, and a marked reduction in plasmodesmatal connections with cells other than other neighboring giant cells. These events are tightly coupled to the developmental status of the nematode, and the giant cells, which serve as the sole nutritive source for the nematode, reach maximal size and activity at the onset of egg-laying (Bird 1971). Interestingly, the transition from a parenchyma cell to a fully differentiated giant cell occurs early in the parasitic association; once the giant cells have been initiated, their characteristics do not change appreciably throughout the period of nematode feeding (apart from getting bigger, having more nuclei, and so on). In many hosts (but not all) cortical and pericycle cells around the giant cells expand and divide, resulting in the formation of a gall or knot, which can lead to highly disfigured and functionally compromised roots (Figure 3). It is important to emphasize the distinction between the giant cells and the surrounding gall; the former is

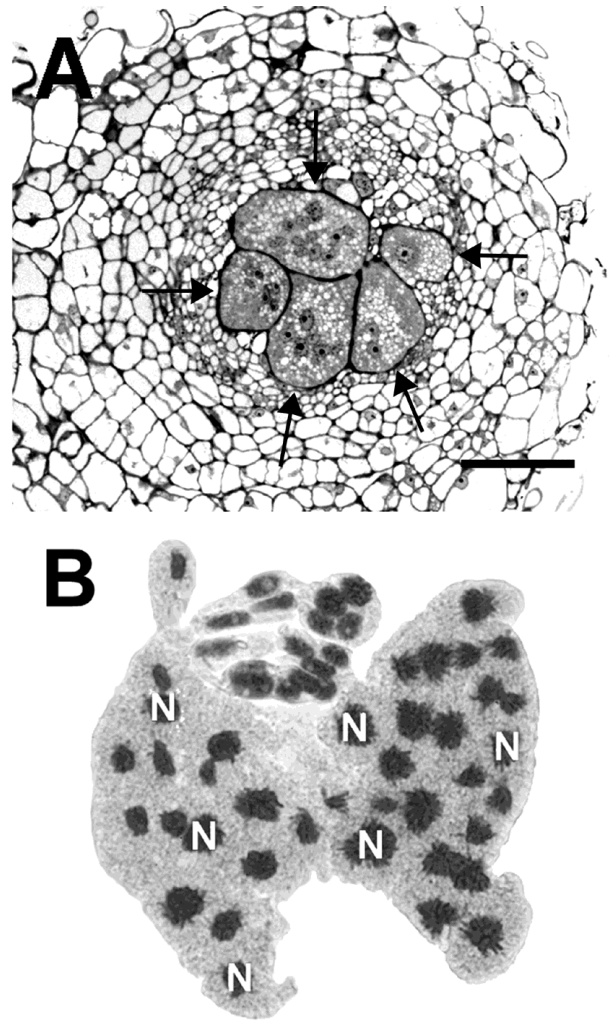

Figure 2. Giant cells induced in tomato (*Lycopersicon esculentum*) roots by *Meloidogyne incognita.* **(A).** Toluidine blue stained, transverse paraffin section of a mature gall. Five giant cells are apparent (*arrows*). Scale bar: 100 µm. **(B)** A single, Feulgen-stained, dissected giant cell, with at least 51 mitotic nuclei (N) visible.

central to the parasitic interaction, and the latter is presumably a secondary response.

In contrast to giant cells, the syncytia induced by cyst nematodes arise by coalescence of adjacent cells, resulting in a multinucleate cell in the absence of mitosis. Thus, despite superficially resembling each other, giant cells and syncytia apparently have different ontogenies. However, at the molecular level many genes induced in giant cells also are induced in syncytia (D. Bird unpublished results).

Genes Expressed in Feeding Cells

Nematode-induced feeding cells are unique cell types and presumably have unique gene expression

Figure 3. Celery roots heavily infected with root-knot nematode **(A)** show the characteristic root knots (galls) that are clearly absent on an uninfected plant **(B).** Image courtesy of K. Barker, NCSU.

profiles. Various strategies to identify these genes have been used, and these have recently been extensively reviewed (Fenoll and others 1997). The most productive approach to identify transcripts that are expressed in giant cells and are not expressed in spatially or temporally equivalent healthy cells has been a subtractive cDNA cloning approach, which defined hundreds of genes (Bird and Wilson 1994; Wilson and others 1994). These genes have been extensively characterized (Bird 1996; A. Green and D. Bird personal communication), and their sequences are available from GenBank. The greatest challenge with these (and indeed, all differentially-expressed genes) is to relate their expression specifically to feeding site function, and ultimately this requires functional tests (for example, inactivation in a transgenic plant). However, some inference can be made from sequence identity and/or spatial expression patterns (Bird 1996), and the role of two such genes, *Le-phan* (Thiery and others 1999) and *Le-ubc4* (Bird and Wilson 1994), in giant cell formation is discussed in the following.

Another productive experimental approach to understand giant cell formation has been to focus on the cell cycle events in feeding sites. On the basis of their cytogenetics (Figure 2), it can be surmised that giant cells exhibit differences from typical mitotic cells in at least three points of the cell cycle (1) giant cells re-enter the cycle (that is, pass the G1 to S phase transition) without prior cell division; (2) the metaphase to anaphase transition is perturbed, resulting in endo-reduplication; and (3) the anaphase to telophase step is disrupted, leading to giant cells becoming multinucleate. Obviously, for any individual nucleus, once mitosis is initiated, the result will be either endoreduplication or nuclear division, but not both. Recent work in yeast has shown that these three points are major sites of cell cycle control. Because the cell cycle has been intensively studied in Arabidopsis, it has proven possible to probe giant cells and syncytia by blocking various stages of the cycle using genetic and chemical inhibitors, and the results of these experiments have been recently reviewed (Gheysen and others 1997). Importantly, it was found that blocking the cell cycle also arrests development of giant cells.

Feeding Site Induction

Linford (1937) and numerous investigators since then have speculated that feeding cells form in response to an inductive signal that emanates from the parasite (Bird 1962; Hussey 1989), and more specifically, from one or all of the pharyngeal glands (Bird 1967, 1968, 1969; Hussey 1989; Linford 1937). The role of other secretory organs, such as the amphids (chemosensory structures in the nematode's head) also has been formally discussed (Bird 1992). There is no doubt that proteinaceous secretory products play a critical role in the host-parasite interaction, and this is discussed further later. However, although some models go as far as to postulate a physical interaction between pharyngeal gland proteins and host genes (for example, Hussey and Grundler 1998; Williamson and Hussey 1996), there is as yet, neither evidence to support nor deny a direct role for such proteins as the inductive signal. Induction of giant cells by root-knot nematodes is perhaps the most studied of the feeding sites, and although this process is still far from understood, a conceptual model in which giant cell formation is initiated by an incompletely executed, host developmental program, has been proposed (Bird 1996).

The genus *Meloidogyne* is very cosmopolitan, inducing stereotypical giant cells in a vast range of vascular plants. This implies that the process leading to giant cell formation involves some fundamental and widely conserved aspect(s) of plant biology. Because of their central role in mediating developmental processes in plants, it is likely that phytohormones play a role in feeding site formation and, indeed, may be the key factors in modulating this aspect of the host-parasite interaction. Direct biochemical methods have shown that root-knot nematode-induced galls have elevated levels of auxin and its precursors (Balasubrama and Rangaswami 1962; Viglierchio and Yu 1968). In addition, cytokinin levels were found to be increased in nematode-infected roots, although the systemic cytokinin levels (as measured by the amount in xylem exudate)

were not appreciably elevated (Bird and Loveys 1980). Importantly, root-knot nematodes have been shown to produce biologically active cytokinin (Bird and Loveys 1980).

Using reporter constructs (*GH3::gusA*) in transgenic plants, Hutangura and colleagues (1999) mapped auxin levels in *Meloidogyne*-infected roots. Although this is an indirect assay and may, for example, reflect altered sensitivity to auxin (such as can be induced by cytokinin), induction of the *GH3* auxin-responsive promoter was observed in the parenchyma cells destined to become giant cells. Auxin levels declined over several days, accompanied by apparent disruption of polar auxin flow at feeding sites. Auxin accumulated basipetally and was reduced acropetal to the forming gall. Because flavonoids can affect auxin levels directly by interaction with auxin-degrading enzymes (Stenlid 1963) and indirectly by serving as auxin transport inhibitors (Jacobs and Rubery 1988), it was proposed that auxin transport inhibition in the presence of the nematode is mediated through flavonoid pathway activation (Hutangura and others 1999). Indeed, promoters from three members of the chalcone synthase gene-family, which encode the first enzyme of this pathway, were activated on nematode infection (Hutangura and others 1999). A similar induction of the flavonoid pathway has been observed in formation of lateral roots (Pelosi and others 1995) and in *Rhizobium* nodules (Mathesius and others 1998).

Can the changes in phytohormone levels associated with feeding site formation be linked with transcriptional events inside giant cells? Molecular and genetic approaches have begun to unravel the auxin-response pathways, and transcription factors and other downstream functions have been identified (Walker and Estelle 1998), but as yet, no role for these specific components in giant cell formation has been established. Of more interest are transcriptional regulators such as the class I knotted (*KNOX*) homeodomain genes, which are required for normal meristem maintenance and function and for development of lateral organs in plants (Goliber and others 1999; Waites and others 1998). Disruption of auxin transport was found to phenocopy overexpression of *KNOX* genes, suggesting that aberrant polar auxin transport might result from ectopic *KNOX* expression (Tsiantis and others 1999a). Furthermore, a strict correlation between *KNOX* expression and elevated cytokinin levels has been observed, suggesting either that cytokinins may regulate *KNOX* expression or be a secondary signal regulated by *KNOX* (Frugis and others 1999; Rupp and others 1999). Significantly, the *Tkn2 KNOX* gene was found to be expressed in tomato giant cells (Koltai and Bird 2000).

Based largely on genetic studies, it has been proposed that *KNOX* expression is transcriptionally suppressed by a specific Myb called *PHANTASITCA* in *Antirrhinum* (Waites and others 1998), and *rough sheath2* in maize (Timmermans and others 1999; Tsiantis and others 1999b). The canonical *PHANTASITCA* gene was first identified as a tomato cDNA expressed in giant cells (Bird and Wilson 1994; Thierry and others 1999). In tomato, *in situ* localization studies established that *KNOX* expression is coincidental with *PHAN* expression (Koltai and Bird 2000), implying that expression of *Le-phan* alone is insufficient for repression of *Tkn2*. In giant cells, *Tkn2* and *Le-phan* appear to be co-induced (Koltai and Bird 2000), and although the precise temporal relationship of this co-induction is yet to be determined, transgenic roots in which *Le-phan* transcripts are absent also fail to express *Tkn2* (H. Koltai, J. Schaff, and D. Bird, unpublished results). This result implies that in tomato, *Le-phan* expression may in fact be necessary for *Tkn2* expression.

The trigger for the perceived initial spike of auxin at the feeding sites remains unknown, but at least two scenarios can be envisaged. In the first, auxin levels initially do not increase *per se,* but rather the sensitivity of cells to auxin is enhanced by exogenous cytokinin emanating from the nematode. However, although it has been demonstrated *in vitro* that root-knot nematodes produce biologically active cytokinin (Bird and Loveys 1980), its role remains to be demonstrated *in planta.* In the second scenario, a signal from the nematode directly (or indirectly) induces *PHAN/KNOX* expression, which in turn activates the flavonoid pathway, resulting in altered auxin distribution. Whether either of these events are adequate to establish a sufficiently unique hormonal signature in the plant to induce the unique cell type that becomes the giant cell remains unknown, and neither scenario rules out the requirement for an additional component(s).

The rapid, but transient, accumulation of auxin during the formation of giant cells is consistent with the model in which a "developmental switch" is thrown (Bird 1996). In this highly simplified model where signals are broadcast from the nematode, all cells in the vicinity might be expected to respond and, indeed, experiments using the *GH3::gusA* constructs showed strong reporter activity centered around the nematode in many (perhaps all) cell types at 96 hours after infection, although expression was transient (Hutangura and others 1999). Importantly, only that subset of cells in the vicinity that were competent to initiate a developmental

program were observed to respond further. Pericycle cells, especially those outside the xylem poles and which are the origin of lateral root meristems, were seen to divide, and vascular parenchyma cells began to develop into giant cells. In this sense, feeding site induction is typical of certain other hormonally mediated, developmental events such as lateral root initiation and nodule growth.

Auxin alone is sufficient to promote DNA synthesis (John and others 1993; Zhang and others 1996) and the inactivation of cyclin-dependent kinase (CDK); CDK inactivation alone is sufficient to promote resetting of the replication origins to initiate DNA synthesis and re-entry into the cell cycle (Noton and Diffley 2000). However, nuclear proliferation continues unabated in giant cells (Bird 1971) long after the *GH3::gusA* experiments (Hutangura and others 1999) imply that auxin levels have dropped. This may reflect a refractory period for the *GH3* promoter; auxin levels might actually remain above a threshold sufficient to potentiate CDK inactivation. Alternatively, once down-stream compounds are in an on-state, a primary auxin signal may no longer be necessary. Of course, ongoing karyokinesis in giant cells might involve some mechanism not directly based on auxin-mediated CDK-inactivation, and it is not inconceivable that a constant signal from the nematode is required to maintain nuclear proliferation.

We have emphasized changes in the cytokinin/auxin ratio, because the levels of these hormones were altered in the host-parasite interaction, and numerous studies have examined the role of cytokinin and auxin in activation and completion of the cell cycle (John and others 1993; Riou-Khamlichi and others 1999; Zhang and others 1996). However, there is considerable interplay between various phytohormones, and any or all could be exerting influence in feeding site induction. Interestingly, a pattern of auxin transport inhibition, similar to that in galls, was described in root nodules induced by the symbiotic bacteria *Rhizobium* (Hirsch 1992; Mathesius and others 1998). *Rhizobium* produces a lipochitooligosaccharide called "Nod factor" that functions as an external mitogenic signal to induce cell proliferation in the root cortex, leading to the formation of a nodule meristem (Crespi and Gálvez, 2000). Cells continuously exit from the persisting nodule meristem in indeterminate nodules and enter the nodule differentiation program by arrest of division, followed by several rounds of endoreduplication resulting in elevated ploidy and cell enlargement. Although there is no direct evidence that nematodes can produce Nod factor, EST sequencing (McCarter and others 2000; D. Bird, unpublished data) has revealed both *chitin synthase* and *nodL* homologues in *M. incognita.* The bacterial homologues of these enzymes are required for synthesis of the oligosaccharide backbone and *O*-acetylation of Nod factor, respectively. A role for Nod factor in induction of giant cells has previously been proposed (Bird 1996), and what is perhaps the best characterized gene known to be expressed in giant cells, namely, *Tob-RB7* (Opperman and others 1994) encodes the orthologue of soybean Nod26 (Yamamoto and others 1990).

The *Rhizobium*-nodule differentiation program is regulated by *ccs52,* which is highly expressed in the differentiating cells (Cebolla and others 1999) and functions as a substrate-specific activator of the anaphase-promoting complex (APC). APC functions as a molecular ratchet to control progression through mitosis by means of specific, ubiquitin-mediated proteolysis of APC components (Townsley and Ruderman 1998). Although there is no direct evidence to implicate misregulation of the APC as a factor in giant cell endoreduplication and karyokinesis, indirect evidence suggests that the entire ubiquitination system might be disrupted in giant cells. In particular, *Le-ubc4* (Bird 1996), which encodes a ubiquitin-conjugating (E2) enzyme, is highly up-regulated in giant cells, accounting for up to 3% of the total mRNA (D. Bird, unpublished data). Intriguingly, ubiquitin-mediated proteolysis also is required for cellular responses to auxin (Walker and Estelle 1998), and so high levels of *Le-ubc4* also might perturb this process.

The temporal requirement for a specific inductive signal is unknown. Temperature shift experiments have shown that the induction of resistance mediated by the *Mi* locus in tomato is restricted to the first 24 to 48 h after infection by *Meloidogyne* L2, showing that one aspect of the host-parasite interaction at least is temporally restricted (Dropkin 1969). In the "developmental switch" model (Bird 1996), a transient induction is sufficient, but it is clear that some ongoing interaction between parasite and giant cells is required, because removal of the nematode leads to feeding site dissolution (Bird 1962). Whether this constitutive stimulus is simply a physiologic effect caused by the metabolic sink of feeding (Bird 1996; Jones and Northcote 1972) or something more specific, such as a nematode-synthesized ligand, remains unknown.

Many of the events that occur during giant cell induction also occur in meristems. In particular, polar auxin transport is repressed (Tsiantis and others 1999a), and both *KNOX* and *PHAN* are induced (Koltai and Bird 2000). Like giant cells, meristems lack plasmodesmatal connections with surrounding, nonmeristematic cells (Gisel and others 1999). Per-

haps an absence of homeostatic regulation from the rest of the plant is necessary for establishment and maintenance of both giant cells and meristems. However, giant cells are clearly different from meristems. This may reflect some unique contribution from the nematode, or it may reflect the altered developmental potential of vascular parenchyma cells vis-à-vis meristem cells. A further and perhaps attractive possibility is that, because they lack plasmodesmatal connections with their neighbors, giant cells lack the potential for the types of cell-cell signaling experienced by cells within the meristem and that play a central role in establishing specific cellular identity of meristematic cells (Gisel and others 1999).

Nematode Secretions

Nematodes have a number of secretory systems, and there is little doubt that secretions play numerous roles in the host-parasite interaction (Blaxter and Bird 1997). In particular, all plant parasitic nematodes have an extensible stylet (Figure 1) that is connected to a well-developed pharynx containing three or five gland cells. Microscopy studies have revealed marked changes in the shape and volume of the pharyngeal glands that appeared to correlate with key events in establishment of the parasitic interaction. In root-knot and cyst nematodes, the subventral glands seem to be more active before host penetration, with the reduction of secretory activity coordinated with the onset of parasitism (Endo 1987; Endo and Wergin 1988) at which time activity of the dorsal gland increases (Bird 1983).

Various enzymatic functions for the secretions have been proposed, and convincing biochemical evidence exists at least for the secretion of root-knot nematode-encoded cellulase (Bird and others 1975). However, not until the recent cloning of genes encoding gland proteins has the nature of the secretion products been discerned with confidence. Lambert and colleagues (1999) demonstrated expression of a gene encoding chorismate mutase (an enzyme typically associated with the biosynthesis of the essential amino acid phenylalanine) in the pharyngeal glands of *M. javanica,* but secretion of the enzyme was not formally shown. Monoclonal antibodies to subventral gland antigens demonstrated true secretion of enzymes (de Boer and others 1996), and genes defining a small family of endoglucanases were isolated from the potato cyst nematode (*Globodera rostochiensis*) and from the soybean cyst nematode (Smant and others 1998; Yan and others 1998). These *eng* genes, which encode cellulases used during migration and perhaps also host penetration, appear to be widely present in plant-parasitic nematodes, having been isolated from root-knot nematodes (McCarter and others 2000; Rosso and others 1999) and detected in plant-nematodes with diverse parasitic habits, including *Pratylenchus agilis, Paratrichodorus minor, Bursaphalenchus xylophilus, Rotylenchulus reniformis,* and *Ditylenchus dipsaci* (Y. Yan and E.L. Davis, personal communication). Understanding the specific role of each family member in each of the various nematode-plant interactions will likely shed considerable light on the infection process.

Evolution of Parasitism: An Ancient Symbiosis?

The fossil record for nematodes is poor (Poinar 1983), and not until recently have molecular methods permitted reliable linking of groups of nematode species into phylogenetic clades (Blaxter and others 1998). Parasitism is an acquired trait and one unlikely to have evolved before evolution of the host, which for vascular plants is 400 million years ago. Presumably most of the genes in extant parasites share a common origin with most of the genes in extant free-living forms. Indeed, analysis of deduced proteins from a large number of randomly generated cDNA sequences from the filarial nematode *Brugia malayi* compared with the entire suite of *C. elegans* predicted-proteins revealed a match with 86% of the genes (Bird and others 1999). It is possible that a subset of the 14% of sequences with no clear *C. elegans* homologue, such as the *eng* loci (Smant and others 1998), either have diverged from an ancient ancestor gene such that no homology can be detected, or they have evolved independently. Although the cellulase genes (Smant and others 1998), the chorismate mutase gene (Lambert and others 1999), and the *M. incognita nodL* gene (D. Bird, unpublished data) exhibit typical eukaryotic gene structure, their deduced protein sequences are not similar to these enzymes from other eukaryotes but rather have strong homologies to bacterial proteins. Thus it seems likely that these genes were acquired from microbes by means of horizontal gene transfer (Keen and Roberts 1998; Yan and others 1998). Significantly, the homology between the ENG proteins and these enzymes from plant sources is low, suggesting that plants were not the source of the nematode genes. Cursory examination of the *M. incognita* EST dataset (McCarter and others 2000) reveals

other candidates for horizontally transferred genes, including a transcript encoding pectate lysae.

The discovery of horizontal gene transfer of parasitic functionality from microbes to nematodes is probably the most significant finding in plant nematology in the past quarter century, and we predict that the small suite of nematode genes with apparent bacterial or fungal origins identified thus far will prove not to be unusual or rare anomalies. Evolution of parasitism by means of a horizontal gene transfer mechanism does help explain how plant parasitism has apparently arisen on multiple, independent occasions, and it will be especially interesting to examine in detail the candidate cellulase (and other) genes identified in other plant-nematode species. For example, *Bursaphelenchus xylophilus,* a clade IV member (Blaxter and others 1998), is a fungal feeder; will its *eng* loci resemble bacterial or fungal genes? *Paratrichodorus minor* is a member of clade II; what will its *eng* loci most resemble? Placing these and other genes into the context of the ever-developing nematode phylogeny will undoubtedly further our understanding of the origins of plant-parasitism. It also will be interesting to examine the genomic environs of the nematode loci with presumed microbial origins. Can the boarders of the ancient recombination events be discerned, and will that suggest clues as to the mechanism of transfer? Are these genes grouped into clusters (pathogenicity islands), perhaps reflective of the organization of their microbial cognates?

Presumably one requirement for a horizontal gene transfer event is a physical interaction between the organisms involved. Numerous interactions have been identified between bacteria and members of clade IV, which include the genera *Anguina, Globodera,* and *Meloidogyne* (Blaxter and others 1998). The simplest interaction is where a free-living nematode, such as *Acrobeloides nanus,* eats bacteria, including *C. toxicus* (Bird and Ryder 1993). By contrast, the plant parasite *Anguin funesta* is unable to use *C. toxicus* as a food source, but this same bacterium can adhere to the surface of the *Anguina* cuticle as a mild pathogen and is carried by the nematode into the plant. Thus, what is food for one group of nematodes (*Acrobeloides-Clavibacter*) becomes a pathogen for another (*Anguina-Clavibacter*). The most intimate of the bacterial-nematode associations involves *Wolbachia,* a genus of rickettsia-like, alpha-proteobacteria found in obligate intracellular association with a wide variety of arthropods, and an increasing number of nematodes, including *Brugia malayi* (Slatko and others 1999). Although not formally identified as *Wolbachia,* the presence of rickettsia-like organisms has been observed in *Globodera* females (Shepherd and others 1973) and males (Walsh and others 1983) and in *Heterodera* larvae (Endo 1979).

Plant-parasitic insects support microbial endosymbionts in addition to *Wolbachia.* All aphids appear to have symbiotic bacteria of the genus *Buchnera,* believed to have entered an aphid ancestor as a free-living form some 250 million years ago (Baumann and others 1995), and tephritid flies host an *Erwinia* endosymbiont (Drew and Lloyd 1991). What makes these interactions particularly interesting is that the insects involved induce galls on the plants from which they feed. A good example is that of the galls produced on grape roots by the phylloxera aphid *Daktulosphaira vitifoliae.* Whether induction of galls by insects involves the same host pathways as feeding site induction by nematodes is unknown, but it is a reasonable hypothesis that phytohormones play a role. *Buchnera* are capable of synthesizing large quantities of tryptophan, an auxin precursor, and most aphids are known or thought to contain auxins (Baumann and others 1995). Similarly, the tephritid endosymbiont is capable of synthesizing cytokinin (Drew and Lloyd 1991). It is an intriguing possibility that the insect galling genes have a bacterial origin, and perhaps this is true also for the cytokinin synthesis genes in *Meloidogyne.* The fact that similar associations exist between bacteria and gall-forming insects may be coincidence, or it may reflect some underlying universal mechanism(s) involving host-plant modification.

On the basis of extant nematode-microbe relationships, various obviously highly conjectural models for the transfer of bacterial genes to nematodes can be proposed. In the simplest instance, a bacterivorous nematode acquired the enzymes required to invade vascular plants directly by ingesting phytopathogenic bacteria. It is not too big a conceptual leap to envision an endosymbiotic relationship evolving, with the ingested bacteria entering the ancient nematode's subventral pharyngeal glands. Alternatively, microbial genes might have entered the nematode by way of an invasive organism such as *Wolbachia.* In each instance, extreme reduction of the bacterium would need to be envisaged to the point where the only remaining remnants were a small suite of genes, the size of which will ultimately be revealed as plant-parasitic nematodes that are subjected to extensive DNA sequence analyses, a process now underway. Revealing the ancient relics

of the evolution of plant-parasitism by nematodes will likely shed light on how the extant forms function as parasites.

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REFERENCES

Aguinaldo AMA, Turbeville JM, Linford LS, Rivera MC, Garey JR, Raff RA, Lake JA. 1997. Evidence for a clade of nematodes, arthropods and other moulting animals. Nature 387:489–493.

Atkinson HJ, Lilley CJ, Urwin PE, McPherson MJ. 1998. Engineering resistance to plant-parasitic nematodes. In: RN Perry, Wright DJ, editors. The physiology and biochemistry of free-living and plant-parasitic nematodes. Wallingford: CABI Publishing. p 381–413.

Balasubrama M, Rangaswami G. 1962. Presence of indole compound in nematode galls. Nature 194:774–775.

Baumann P, Baumann L, Lai CY, Rouhbakhsh D, Moran NA, Clark MA. 1995. Genetics, physiology, and evolutionary relationships of the genus *Buchnera:* intracellular symbionts of aphids. Annu Rev Microbiol 49:55–94.

Bird AF. 1961. The ultrastructure and histochemistry of a nematode-induced giant cell. J Biophys Biochem Cytol 11:701–715.

Bird AF. 1962. The inducement of giant cells by *Meloidogyne javanica.* Nematologica 8:1–10.

Bird AF. 1967. Changes associated with parasitism in nematodes. I. Morphology and physiology of preparasitic and parasitic larvae of *Meloidogyne javanica.* J Parasitol 53:768–776.

Bird AF. 1968. Changes associated with parasitism in nematodes. IV. Cytochemical studies on the ampulla of the dorsal esophageal gland of *Meloidogyne javanica* and on exudations from the buccal stylet. J Parasitol 54:879–890.

Bird AF. 1969. Changes associated with parasitism in nematodes. V. Ultrastructure of the stylet exudation and dorsal esophageal gland contents of female *Meloidogyne javanica.* J Parasitol 55:337–345.

Bird AF. 1971. Quantitative studies on the growth of syncytia induced in plants by root knot nematodes. Int J Parasitol 2:157–170.

Bird AF. 1983. Changes in the dimensions of the oesophageal glands in root-knot nematodes during the onset of parasitism. Int J Parasitol 13:343–348.

Bird AF, Bird J. 1991. The Structure of Nematodes, 2nd ed. San Diego: Academic Press. 316 p

Bird AF, Downton JS, Hawker JS. 1975. Cellulase secretion by second stage larvae of the root-knot nematode (*Meloidogyne javanica*). Marcellia 38:165–169.

Bird AF, Loveys BR. 1980. The involvement of cytokinins in a host-parasite relationship between the tomato (*Lycopersicon esculentum*) and a nematode (*Meloidogyne javanica*). Parasitol 80:497–1505.

Bird AF, Ryder MH. 1993. Feeding of the nematode *Acrobeloides nanus* on bacteria. J Nematol 25:493–499.

Bird DM. 1992. Mechanisms of the *Meloidogyne*-host interaction. In: Gommers FJ, Maas PWT, editors. Nematology: From molecule to ecosystem. Dundee: ESN Inc. p 51–59.

Bird DM. 1996. Manipulation of host gene expression by root-knot nematodes. J Parasitol 82:881–888.

Bird DM, Opperman CH. 1998. *Caenorhabditis elegans:* A genetic guide to parasitic nematode biology. J Nematol 30:299–308.

Bird DM, Opperman CH, Jones SJM, Baillie DL. 1999. The *Caenorhabditis elegans* genome: A guide in the post genomics age. Annu Rev Phytopathol 37:247–265.

Bird DM, Wilson MA. 1994. DNA sequence and expression analysis of root-knot nematode elicited giant cell transcripts. Mol Plant-Microbe Interact 7:419–424.

Blaxter M. 1998. *Caenorhabditis elegans* is a nematode. Science 282:2041–2046.

Blaxter M, Bird DM. 1997. Parasitic nematodes. In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR, editors. *C. elegans* II. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. p 851–878.

Blaxter ML, DeLey P, Garey J, Liu LX, Scheldeman P, Vierstraete A, Vanfleteren J, Mackey LY, Dorris M, Frisse LM, Vida JT, Thomas WK. 1998. A molecular evolutionary framework for the phylum Nematoda. Nature 392:71–75.

Bleve-Zacheo T, Melillo MT. 1997. The biology of giant cells. In: Fenoll C, Grundler FMW, Ohl SA, editors. Cellular and molecular aspects of plant-nematode interactions. Dordrecht: Kluwer Academic Publishers. p 65–79.

Boucher G, Lambshead PJD. 1994. Ecological biodiversity of marine nematodes in samples from temperate, tropical, and deep-sea regions. Conserv Biol 9:1594–1604.

Bradley EB, Duffy M. 1982. The value of plant resistance to the soybean cyst nematode: A case study of Forrest soybean (AGES820929). Washington: USDA-ARS Staff Report.

C. elegans Sequencing Consortium. 1998. Genome sequence of the nematode *Caenorhabditis elegans:* A platform for investigating biology. Science 282:2012–2018.

Cai D, Kleine M, Kifle S, Harloff HJ, Sandal NN, Marcker KA, Klein-Lankhorst RM, Salentijn EMJ, Lange W, Stiekema WJ, Wyss U, Grundler FMW, Jung C. 1997. Positional cloning of a gene for nematode resistance in sugar beet. Science 275:832–834.

Cebolla A, Vinardell JM, Kiss E, Oláh B, Roudier F, Kondorosi A, Kondorosi E. 1999. The mitotic inhibitor *ccs52* is required for endoreduplication and ploidy-dependent cell enlargement in plants. EMBO J 18:4476–4484.

Christie JR. 1936. The development of root-knot nematode galls. Phytopathology 26:1–22.

Crespi M, Gálvez S. 2000. Molecular mechanisms in root nodule development. J Plant Growth Reg 19:155–166.

de Boer JM, Smant G, Goverse A, Davis EL, Overmars HA, Pomp H, van Gent-Pelzer M, Zilverentant JF, Stokkermans JPWG, Hussey RS, Gommers FJ, Bakker J, Schots A. 1996. Secretory granule proteins from the subventral esophageal glands of the potato cyst nematode identified by monoclonal antibodies to a protein fraction from second-stage juveniles. Mol Plant-Microbe Interact 9:39–46.

Drew RAI, Lloyd AC. 1991. Bacteria in the life cycle of tephritid

fruit flies. In: Barbosa P, Krischik VA, Jones CG, editors. Microbial mediation of plant-herbivore interactions. New York: John Wiley & Sons. p 441–465.

Dropkin VH. 1969. Cellular responses of plants to nematode infections. Annu Rev Phytopathol 7:101–122.

Endo BY. 1979. The ultrastructure and distribution of an intracellular bacterium-like microorganism in the tissues of larvae of the soybean cyst nematode *Heterodera glycines.* J Ultrastruct Res 67:1–14.

Endo BY. 1987. Ultrastructure of the esophagus of larvae of the soybean cyst nematode, *Heterodera glycines.* J Nematol 19:469–483.

Endo BY, Wergin WP. 1988. Ultrastructure of the second stage juvenile of the root knot nematode, *Meloidogyne incognita.* Proc Helminthol Soc Wash 55:286–316.

Epstein HF, Shakes DC. 1995. *Caenorhabditis elegans:* Modern biological analysis of an organism. San Diego: Academic Press. 659 p.

Fenoll C, Aristizabal FA, Sanz-Alferez S, del Campo FF. 1997. Regulation of gene expression in feeding sites. In: Fenoll C, Grundler FMW, Ohl SA, editors. Cellular and molecular aspects of plant-nematode interactions. Dordrecht: Kluwer Academic Publishers. p 133–149.

Frugis G, Giannino D, Mele G, Nicolodi C, Innocenti AM, Chiappetta A, Bitonti MB, Dewitte W, van Onckelen H, Mariott D. 1999. Are homeobox knotted-like genes and cytokinins the leaf architects? Plant Physiol 119:371–374.

Gheysen G, de Almeida Engler J, van Montague M. 1997. Cell cycle regulation in nematode feeding sites. In: Fenoll C, Grundler FMW, Ohl SA, editors. Cellular and molecular aspects of plant-nematode interactions. Dordrecht: Kluwer Academic Publishers. p 120–132.

Gisel A, Barella S, Hempel FD, Zambryski PC. 1999. Temporal and spatial regulation of symplastic trafficking during development in *Arabidopsis thaliana* apices. Development 126:1879–1889.

Goliber T, Kessler S, Chen J-J, Bharathan G, Sinha N. 1999. Genetic, molecular and morphological analysis of compound leaf development. Curr Top Develop Biol 43:259–290.

Golinowski W, Sobczak M, Kurek W, Grymaszewska G. 1997. The structure of syncytia. In: Fenoll C, Grundler FMW, Ohl SA, editors. Cellular and molecular aspects of plant-nematode interactions. Dordrecht: Kluwer Academic Publishers. p 80–97.

Grundler FMW, Böckenhoff A. 1997. Physiology of nematode feeding and feeding sites. In: Fenoll C, Grundler FMW, Ohl SA, editors. Cellular and molecular aspects of plant-nematode interactions. Dordrecht: Kluwer Academic Publishers. p 107–119.

Hirsch AM. 1992. Tansley Review No. 40. Developmental biology of legume nodulation. New Phytol 122:211–237.

Hussey RS. 1989. Disease-inducing secretions of plant-parasitic nematodes. Annu Rev Phytopathol 27:123–141.

Hussey RS, Grundler FMW. 1998. Nematode parasitism of plants. In: Perry RN, Wright DJ, editors. The physiology and biochemistry of freeliving and plant-parasitic nematodes. Wallingford: CABI Publishing. p 213–243.

Hussey RS, Mims CW, Westcott SWI. 1992. Ultrastructure of root cortical cells parasitized by the ring nematode *Criconemella xenoplax.* Protoplasma 167:55–65.

Hutangura P, Mathesius U, Jones MGK, Rolfe BG. 1999. Auxin induction is a trigger for root gall formation caused by root-knot nematodes in white clover and is associated with the activation of the flavonoid pathway. Aust J Plant Physiol 26:221–231.

Jacobs M, Rubery PH. 1988. Naturally-occurring auxin transport regulators. Science 241:346–349.

John PCL, Zhang K, Dong C, Diederich L, Wightman F. 1993. $p34^{cdc2}$ related proteins in control of cell cycle progression: the switch between division and differentiation in tissue development and stimulation of division by auxin and cytokinin. Aust J Plant Physiol 20:503–526.

Jones MGK, Northcote DH. 1972. Nematode-induced syncytium-a multinucleate transfer cell. J Cell Sci 10:789–809.

Jones MGK, Payne HL. 1978. Early stages of nematode-induced syncytium giant-cell formation in roots of *Impatiens balsamina.* J Nematol 10:70–84.

Keen NT, Roberts PA. 1998. Plant parasitic nematodes: digesting a page from the microbe book. Proc Natl Acad Sci USA 95:4789–4790.

Koltai H, Bird DM. 2000. Epistatic repression of *PHANTASTICA* and class 1 *KNOTTED* genes uncoupled in tomato. Plant J 22:455–459.

Lambert KN, Allen KD, Sussex IM. 1999. Cloning and characterization of an esophageal-gland-specific chorismate mutase from the phytoparasitic nematode *Meloidogyne javanica.* Mol Plant-Microbe Interact 12:328–336.

Landels S. 1989. Fumigants and nematicides. Chemical Economics Handbook. California: Stanford Research Institute International.

Lewis WJ, van Lenteren JC, Phatak SC, Tumlinson III JH. 1997. A total system approach to sustainable pest management. Proc Natl Acad Sci USA 94:12243–12248.

Linford MB. 1937. The feeding of the root-knot nematode in root tissue and nutrient solution. Phytopathology 27:824–835.

Mathesius U, Schlaman HRM, Spaink HP, Sautter C, Rolfe B, Djordjevic MA. 1998. Auxin transport inhibition precedes root nodule formation in white clover roots and is regulated by flavonoids and derivatives of chitin oligosaccharides. Plant J 14:23–34.

McCarter J, Bird DMcK, Rao U, Kloek A, Clifton S, Pape D, Martin J, Wylie T, Eddy S, Waterston and the Washington University GSC EST Team R. 2000. Progress toward high throughput gene discovery in parasitic nematodes: Initial findings from *Meloidogyne incognita*EST sequencing. J Nematol 32: (in press).

Needham JT. 1743. Concerning certain chalky concretions, called malm; with some microscopical observations on the farina of Red Lilly, and worms discovered in Smutty Corn. Phil Transact Royal Soc Lond 42:634

Nickle WR. 1991. Manual of agricultural nematology. New York: Marcel Dekker, Inc. 1035 p.

Noton E, Diffley JFX. 2000. CDK inactivation is the only essential function of the APC/C and the mitotic exit network proteins for origin resetting during mitosis. Mol Cell 5:85–95.

Ophel KM, Bird AF, Kerr A. 1993. Association of bacteriophage particles with toxin production by *Clavibacter toxicus,* the causal agent of annual ryegrass toxicity. Phytopathology 83:676–681.

Opperman CH, Bird DM. 1998. The soybean cyst nematode, *Heterodera glycines:* A genetic model system for the study of plant-parasitic nematodes. Current Opin Plant Biol 1:342–346.

Opperman CH, Taylor CG, Conkling MA. 1994. Root-knot nematode-directed expression of a plant root-specific gene. Science 263:221–223.

Pelosi A, Lee MCS, Chandler SF, Hamill JD. 1995. Hormonal control of root primordia differentiation and root formation in

cultured explants of *Eucalyptus globulus* seedlings. Aust J Plant Physiol 22:409–415.

Poinar Jr. GO. 1983. The natural history of nematodes. Prentice Hall: Englewood Cliffs. 323 p.

Riddle DL, Bird AF. 1985. Responses of *Anguina agrostis* to detergent and anesthetic treatment. J Nematol 17:165–168.

Riddle DL, Blumenthal T, Meyer BJ, Priess JR. 1997. *C. elegans* II. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. 1222 p.

Riou-Khamlichi C, Huntley R, Jacqmard A, Murray JA. 1999. Cytokinin activation of Arabidopsis cell division through a D-type cyclin. Science 283:1541–1544.

Rossi M, Goggin F, Milligan SB, Kaloshian I, Ullman D, Williamson VM. 1998. The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proc Natl Acad Sci USA 95:9750–9754.

Rosso MN, Favery B, Piotte C, Arthaud L, de Boer JM, Hussey RS, Bakker J, Baum TJ, Abad P. 1999. Isolation of a cDNA encoding a beta-l,4-endoglucanase in the root-knot nematode *Meloidogyne incognita* and expression analysis during plant parasitism. Mol Plant-Microbe Interact 12:585–591.

Rupp HM, Frank M, Werner T, Strnad M, Schmulling T. 1999. Increased steady state mRNA levels of the *STM* and *KNAT1* homeobox genes in cytokinin overproducing *Arabidopsis thaliana* indicate a role for cytokinins in the shoot apical meristem. Plant J 18:557–563.

Sasser JN, Freckman DW. 1987. A world perspective on nematology: The role of the society. In: Veech JA, Dickson DW, editors. Vistas on nematology. Hyattsville: Society of Nematology, Inc. p 7–14.

Schuerger AC, McClure MA. 1983. Ultrastructural changes induced by *Scutellonema brachyurum* in potato roots. Phytopathology 73:70–81.

Shepherd AM, Clark SA, Kempton A. 1973. An intracellular micro-organism associated with tissues of *Heterodera* spp. Nematologica 19:31–34.

Slatko BE, O'Neill SL, Scott AL, Werren JL, Blaxter ML. 1999. The *Wolbachia* genome consortium. Microb Comp Genomics 4:161–165.

Smant G, Stokkermans JPWG, Yan Y, de Boer JM, Baum TJ, Wang X, Hussey RS, Gommers FJ, Henrissat B, Davis EL, Helder J, Schots A, Bakker J. 1998. Endogenous cellulases in animals: Isolation of β-1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. Proc Natl Acad Sci USA 95:4906–4911.

Stenlid G. 1963. The effect of flavonoid compounds on oxidative phosphorylation and on the enzymatic destruction of indoleacetic acid. Physiol Plant 16:110–l21.

Stynes BA, Bird AF. 1982. Development of galls induced in *Lolium rigidum* by *Anguina agrostis.* Phytopathology 72:336–346.

Thiery MG, Greene AE, Bird DM. 1999. The *Lycopersicon esculentum* orthologue of *PHANTASTICA/rough sheath2* (Accession No. AF148934) is expressed in feeding sites induced by root-knot nematodes (PGR99-099). Plant Physiol 120:934

Thomason IJ. 1987. Challenges facing nematology: Environmental risks with nematicides and the need for new approaches. In: Veech JA, Dickson DW, editors. Vistas on nematology. Hyattsville: Society of Nematology, Inc. p 469–476.

Timmermans MCP, Hudson A, Becraft PW, Nelson T. 1999. *ROUGH SHEATH2:* A Myb protein that represses knox homeobox genes in maize lateral organ primordia. Science 284:151–153.

Townsley FM, Ruderman JV. 1998. Proteolytic ratchets that control progression through mitosis. Trends Cell Biol 8:238–244.

Tsiantis M, Brown MIN, Skibinski G, Langdale JA. 1999a. Disruption of auxin transport is associated with aberrant leaf development in maize. Plant Physiol 121:1163–1168.

Tsiantis M, Schneeberger R, Golz JF, Freeling M, Langdale JA. 1999b. The maize *rough sheath2* gene and leaf development programs in monocot and dicot plants. Science 284:154–156.

Viglierchio DR, Yu PK. 1968. Plant growth substance and plant parasitic nematodes II. Host influence on auxin content. Expt Parasitol 23:88–95.

Waites RH, Selvadurai RN, Oliver IR, Hudson A. 1998. The *PHANTASTICA* gene encodes a myb transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum.* Cell 93:779–789.

Walker L, Estelle M. 1998. Molecular mechanisms of auxin action. Current Opin Plant Biol 1:434–439.

Walsh JA, Lee DL, Shepherd AM. 1983. The distribution and effect of intracellular rickettsia-like micro-organisms infecting adult males of the potato cyst nematode, *Globodera rostochiensis.* Nematologica 29:227–239.

Williamson VM. 1998. Root-knot nematode resistance genes in tomato and their potential for future use. Annu Rev Phytopathol 36:277–293.

Williamson VM. 1999. Nematode resistance genes. Curent Opin Plant Biol 2:327–331.

Williamson VM, Hussey RS. 1996. Nematode pathogenesis and resistance in plants. Plant Cell 8:1735–1745.

Wilson MA, Bird DM, van der Knaap E. 1994. A comprehensive subtractive cDNA cloning approach to identify nematode-induced transcripts in tomato. Phytopathology 84:299–303.

Wood WB. 1988. The Nematode *Caenorhabditis elegans.* Cold Spring Harbor Laboratory Press: Cold Spring Harbor. 667 p.

Wyss U. 1997. Root parasitic nematodes: An overview. In: Fenoll C, Grundler FMW, Ohl SA, editors. Cellular and molecular aspects of plant-nematode interactions. Kluwer Academic Publishers.: Dordrecht. p 5–22.

Yamamoto Y, Cheng C-L, Conkling ME. 1990. Root-specific genes from tobacco and *Arabidopsis* homologous to an evolutionarily conserved gene family of membrane channel proteins. Nuc Acids Res 18:7449

Yan Y, Smant G, Stokkermans J, Qin L, Helder J, Baum T, Schots A, Davis E. 1998. Genomic organization of four β-1,4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications. Gene 220:61–70.

Zhang K, Letham DS, John PCL. 1996. Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of $p34^{cdc2}$-like H1 histone kinase. Planta 200:2–12.