Multiple lateral gene transfers and duplications have promoted plant parasitism ability in nematodes

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Lateral gene transfer from prokaryotes to animals is poorly understood, and the scarce documented examples generally concern genes of uncharacterized role in the receiver organism. In contrast, in plant-parasitic nematodes, several genes, usually not found in animals and similar to bacterial homologs, play essential roles for successful parasitism. Many of these encode plant cell wall-degrading enzymes that constitute an unprecedented arsenal in animals in terms of both abundance and diversity. Here we report that independent lateral gene transfers from different bacteria, followed by gene duplications and early gain of introns, have shaped this repertoire. We also show protein immunolocalization data that suggest additional roles for some of these cell wall-degrading enzymes in the late stages of these parasites' life cycle. Multiple functional acquisitions of exogenous genes that provide selective advantage were probably crucial for the emergence and proficiency of plant parasitism in nematodes.

evolution | gene transfer | duplication | plant parasites

Lateral gene transfer (LGT) is the transmission of genes between organisms by mechanisms other than vertical inheritance from an ancestor to an offspring. Although largely documented as an important evolutionary mechanism in prokaryotes (1), LGT in animals that have a separate germline and whose genome is segregated in a nucleus is poorly explored. Although some examples have been described (2–4), most concern transfers from endosymbiotic bacteria, and none provide a clear link between the activity of the transferred gene products and the biology of the host species. Thus, arguments are lacking to support a selective advantage that would have driven fixation of transferred genes at the level of a population or species. By contrast, in plant-parasitic nematodes, a series of genes encoding plant cell wall-degrading or -modifying enzymes, which are usually absent from animals, exhibit similarity to bacteria and may thus originate from LGT. These genes are transcriptionally active, their products have been biochemically characterized, they are secreted in plant tissues, and their inactivation impairs parasitism efficiency (5). The most damaging nematodes to agriculture worldwide belong to the suborder Tylenchina in clade IV that comprises root-knot nematodes and cyst nematodes, the two most-studied lineages (*SI Appendix*, Fig. S1). These nematodes are able to penetrate and migrate into plant tissue and establish sophisticated parasitic interactions with their hosts. Invasion of the root tissues by nematodes requires degradation of the plant cell wall protective barrier, constituted mainly of cellulose and hemicelluloses as well as pectin and its branched decorations. The first plant cell wall-degrading enzymes from an animal were characterized in cyst nematodes in 1998 (6). Ten years later, analysis of the genome of *Meloidogyne incognita*, the first genome analysis for a plant-parasitic nematode, revealed that the repertoire of cell wall-degrading enzymes in a single species is diverse and abundant with more than 60 genes covering six different protein families for the degradation of cell wall oligo- and polysaccharides (7). This unprecedented repertoire in an animal includes cellulases and xylanases for the degradation of cellulose and hemicelluloses as well as polygalacturonases, pectate lyases, and candidate arabinanases for the degradation of pectins. A set of expansin-like proteins that soften the plant cell wall completes this repertoire (Table 1). Here, we have systematically investigated the evolutionary history and traced back the origin of each family of cell wall-degrading or modifying proteins in plant-parasitic nematodes. We show that these proteins most likely originate from multiple independent LGT events of different bacterial sources. Cellulases, pectate lyases, and expansin-like proteins are encoded by multigenic families, and we show that massive gene duplications after acquisition via LGT account for their abundance.

Results and Discussion

In plant-parasitic nematodes, polygalacturonases and pectate lyases participate in pectin degradation. Polygalacturonase activity is known in the glycoside hydrolase GH28 family, frequently found in bacteria, fungi, oomycetes, and plants (www.cazy.org; ref. 8). Although generally absent from animals, this enzyme has been characterized in two phytophagous insects: *Sitophilus oryzae* (9), for which an acquisition via LGT from fungi has been proposed (10), and *Phaedon cochleariae*, in which the enzyme may be encoded by a gut digestive symbiont (11). In nematodes, polygalacturonase activity has been suspected in *Ditylenchus dipsaci* since the 1970s (12), and GH28 enzymes have been isolated and biochemically characterized in *M. incognita* (13). We identified polygalacturonase genes only in root-knot nematodes. Our phylogenetic analysis (Fig. 1*A* and *SI Appendix*, Fig. S2) shows that these nematode GH28 enzymes form a highly supported group with a series of bacterial orthologs. A cluster of GH28 enzymes from the bacterium *Ralstonia solanacearum* is positioned in the middle of root-knot nematode GH28 enzymes. Interestingly, *R. solanacearum* is a plant-pathogenic soil bacterium that shares plant hosts with root-knot nematodes. Our tree topology suggests that at least one LGT event occurred between bacteria that are probably closely related to *Ralstonia* and these nematodes. Possibly, a second LGT occurred with a different bacterial donor species. As an outgroup to the root-knot nematodes/*Ralstonia* cluster, we observed a series of other bacteria. The closest relatives in eukaryotes are from plants but are much more distant. Homologs from fungi and oomycetes and from the two reported insects were too distantly related to be included in a phylogenetic analysis, suggesting distinct origins.

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Table 1. Plant cell wall-modifying proteins in plant-parasitic nematodes

Family	Activity	Presence in nematodes	Closest relative
GH28	Polygalacturonase (EC 3.2.1.15)	RKN*	Ralstonia: *Ralstonia solanacearum*†
PL3	Pectate lyase (EC 4.2.2.2)	RKN,* CN,* Aphelenchoidea*	Actinomycetales: *Clavibacter michiganensis*,† *Frankia* sp.,‡ *Actinosynnema mirum, Cellulomonas flavigena, Jonesia denitrificans, Streptomyces avermitilis, S. coelicolor,*
GH43	Putative arabinanase (EC 3.2.1.99)	RKN, CN	Actinomycetales: *Streptomyces coelicolor, Thermomonospora curvata, Kineococcus radiotolerans*
GH5 (cel)	Cellulase (EC 3.2.1.4)	RKN,* CN,* Pratylenchidae, Anguinidae, Radopholinae, Aphelenchoidea	Coleoptera: *Apriona germari*, *Psacothea hilaris*, Bacteroidetes: *Cytophaga hutchinsonii*
GH5 (xyl)	Endo-1,4-β-xylanase (EC 3.2.1.8)	RKN* and Radopholinae*	Firmicutes: *Clostridium acetobutylicum*
EXPN	Loosening of plant cell wall (EC N/A)	RKN, CN,* Anguinidae, Aphelenchoidea, Dorylaimida (*clade I*)	Actinomycetales: *Amycolatopsis mediterranei*, *Actinosynnema mirum*, *Streptomyces lavendulae*

Spectrum of the presence in nematodes is indicated according to the taxonomy in *SI Appendix*, Fig. S1. Species possessing the genes most closely related to those of plant-parasitic nematodes are in the last column. CN, cyst nematode; RKN, root-knot nematode.
*For nematodes, species in which activity has been experimentally shown; details on functional characterization and corresponding bibliographic references are indicated in *SI Appendix*, Table S1.
†Plant-pathogenic bacteria.
‡Plant-symbiotic bacteria.

In contrast to polygalacturonases, pectate lyases cleave α-1,4-galacturonan, the major component of pectin backbone, via β-elimination instead of hydrolysis. All pectate lyases characterized in plant-parasitic nematodes belong to polysaccharide lyase (PL) family 3. In root-knot nematodes, PL3s are present as multigenic families in both *M. incognita* and *Meloidogyne hapla* (7, 14). Functional PL3's have also been isolated in cyst nematodes (15, 16) and in Aphelenchoidea. Nematodes that belong to this last

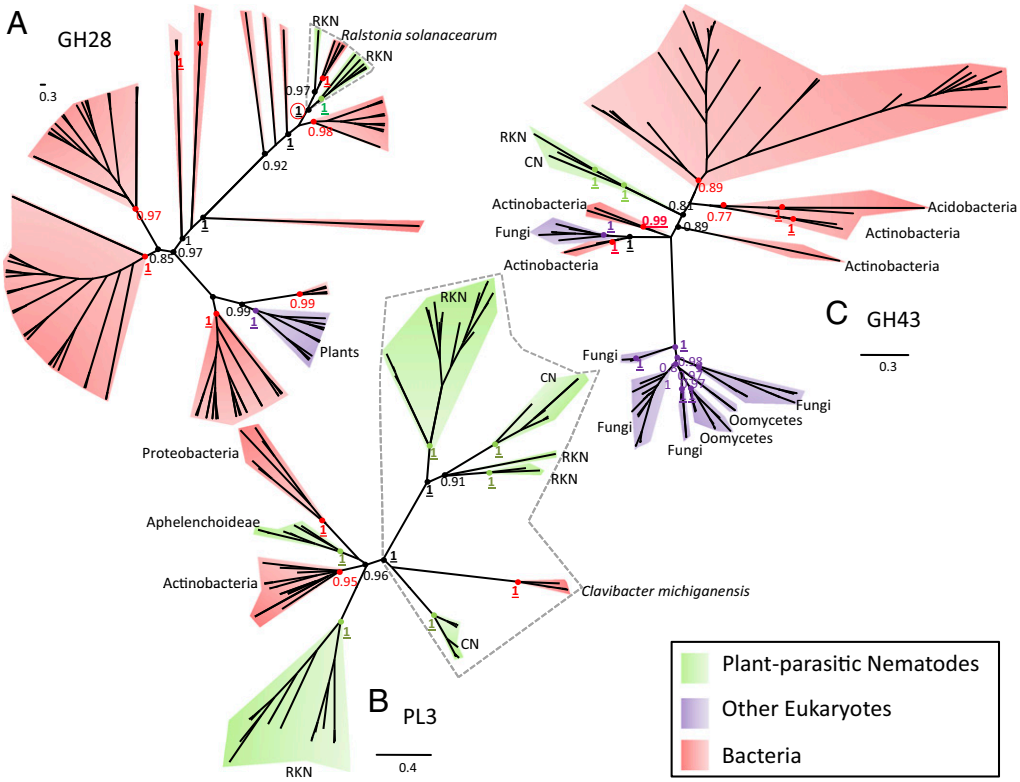

Fig. 1. Phylogenetic trees of pectin-modifying proteins. (*A*) GH28 polygalacturonases. (*B*) PL3 pectate lyases. (*C*) GH43 candidate arabinanases. Phylogenetic groups are color-coded according to their taxonomy. Posterior probability (PP) support values are indicated at corresponding nodes, and those supported by bootsrap values higher than 75 in maximum likelihood trees are underlined and in boldface type. RKN, root-knot nematode; CN, cyst nematode. Dashed lines delineate phylogenetic groupings of bacterial and plant-parasitic nematode genes; the corresponding PP value is circled.

group are plant-associated and hold an outgroup position relative to root-knot and cyst nematodes (17, 18) (*SI Appendix*, Fig. S1). Our phylogenetic reconstructions with homologs from nematodes, bacteria, oomycetes, and fungi provided strong support for separation of PL3's in two main clusters: one cluster grouping bacteria and nematodes and another cluster containing fungi and oomycetes *(SI Appendix*, Fig. S3). Inside the bacteria/nematode cluster, nematode PL3's are interspersed by two clusters of Actinobacteria. To gain a deeper insight into the bacteria/nematode cluster, we performed a phylogenetic analysis using only bacterial and nematode PL3's because they form a distinct monophyletic group. This tree (Fig. 1*B*) revealed evolutionary relations between the different plant-parasitic nematodes and bacterial clusters. Remarkably, *Clavibacter michiganensis*, the bacterium that possesses PL3's most closely related to the majority of those of root-knot and cyst nematodes, is a notorious plant parasite that shares host plants with these nematodes. This group of plant-parasitic nematodes may have acquired PL3's from an ancestor or a close relative of this bacterium. The non-monophyly of root-knot and cyst nematode PL3's suggests that a few independent LGT events gave rise to the different subfamilies. These distinct root-knot and cyst nematode clusters show that, in both lineages, a series of duplications followed the likely acquisition of ancestral PL3's via LGT and account for the abundance of this family.

Arabinans and arabinogalactans are the main components of pectin side chains. These chains can prevent access to the pectin backbone for cleavage by polygalacturonases or pectate lyases. We identified candidate arabinanases of the family GH43 in the root-knot nematode genomes and in the draft genomes of the cyst nematodes *Heterodera glycines* and *Globodera pallida*. No other significant similarity was found in animals, but a series of candidate homologs was identified in bacteria, oomycetes, and fungi. Root-knot and cyst nematode GH43 enzymes form a monophyletic group in our phylogenies (Fig. 1*C* and *SI Appendix*, Fig. S4). This suggests that a GH43 enzyme was present in the last common ancestor of these nematodes. Thus, the possibility of finding an enzyme from this family in other Tylenchina remains open. Actinomycetales GH43 enzymes appear to be the most closely related to nematode GH43 enzymes. This suggests that GH43 genes have been acquired in nematodes via LGT of bacterial origin (probably an ancestral or relative of Actinomycetales). Interestingly, a putative arabinogalactan endo-1,4-β-galactosidase (EC 3.2.1.89) from family GH53, unrelated to GH43, was found in ESTs from the cyst nematode *Heterodera schachtii*, and we identified only a homolog in the draft genome of *H. glycines* (19, 20), suggesting that it is restricted to cyst nematodes.

Cellulose is the most abundant biopolymer on earth, and cellulases secreted by plant-parasitic nematodes allow its breakdown during root invasion (5, 21). In clade IV nematodes, cellulases from a subfamily of family GH5 (8) and those from family GH45 have been characterized. GH5 cellulases have been reported in Tylenchina and in one Aphelenchoidea species (18), whereas GH45 cellulases were reported only in Aphelenchoidea (*SI Appendix*, Fig. S1). No nematode species has been found to harbor both families of cellulases, suggesting that they are mutually exclusive. We identified candidate homologs of GH5 cellulases in

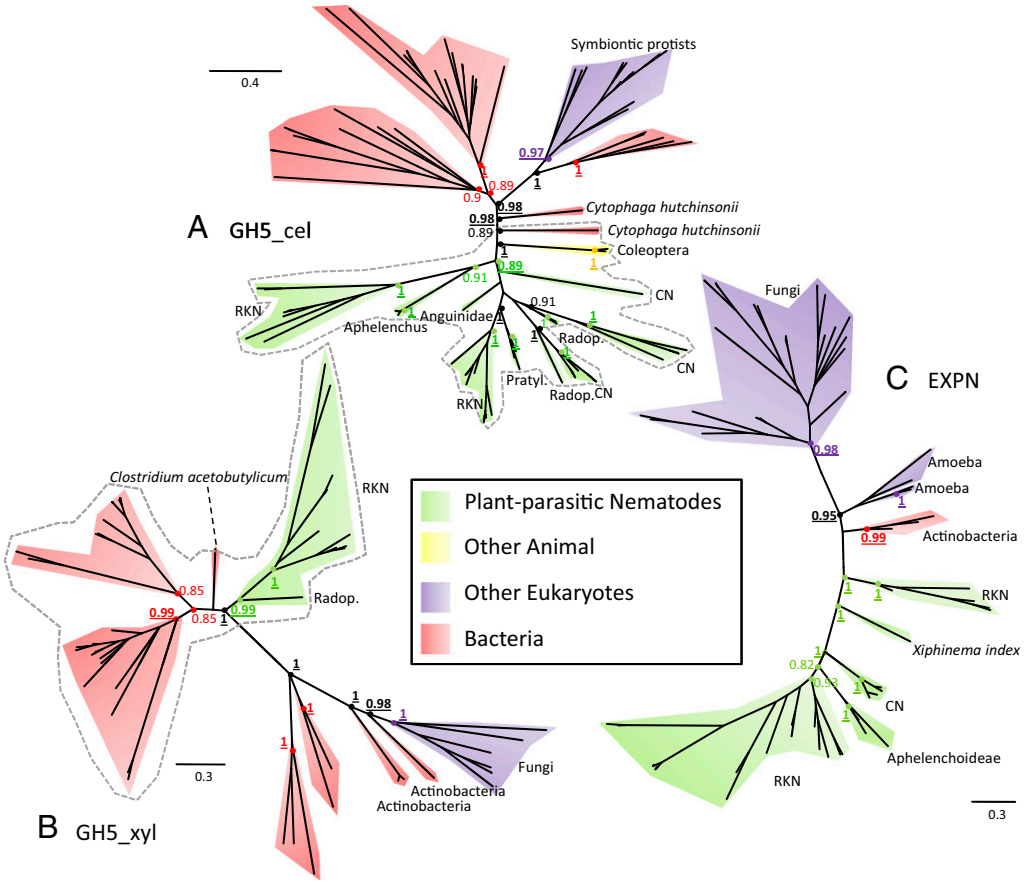

Fig. 2. Phylogenetic trees of cellulose/hemicellulose-modifying proteins and expansin-like proteins. (*A*) GH5 cellulases. Radop. stands for Radopholinae, Pratyl. for Pratylenchidae. (*B*) GH5 xylanases. (*C*) Expansin-like proteins. Phylogenetic groups are color-coded according to their taxonomy. Posterior probability (PP) support values are indicated at corresponding nodes, and those supported by bootstrap values higher than 75 in maximum likelihood trees are underlined and in boldface type. Groups supported by PP values higher than 0.8 were collapsed. RKN, root-knot nematode; CN for cyst nematode.

bacteria, in two insects that feed on plants, *Psacothea hilaris* (22) and *Apriona germari* (23), and in gut digestive symbionts of termites. Interestingly, as opposed to nematode cellulase genes, those found in insects are intronless. GH5 cellulases from clade IV nematodes form a highly supported monophyletic group (Fig. 2*A* and *SI Appendix*, Fig. S5). Their closest orthologs are found in the two insects, in *Cytophaga hutchinsonii*, and in other bacteria. Assuming that the two insect cellulase genes are endogenous, this topology suggests two hypotheses: (*i*) two LGT events occurred from a similar bacterial source in these insects and in clade IV nematodes or (*ii*) a cellulase gene was already present in the last common ancestor of these animals. The second hypothesis appears unlikely because their otherwise general absence in nematodes and insects would require many independent gene losses. An origin in the last common ancestor of eukaryotes is even less likely because GH5 enzymes found in plants and fungi belong to subfamilies distinct from that found in nematodes and are more distantly related than those of bacteria. In a set of nematode cellulases, a cellulose-binding CBM2 module is appended at the C-terminal end (*SI Appendix*, Fig. S5). This module is found neither in plants nor in fungi although association of CBM2 modules with GH5 modules is frequent in bacteria. This observation reinforces the hypothesis of the acquisition of nematode GH5 cellulases via LGT of bacterial origin. Notably, none of the two reported insect cellulases bear a CBM2 module.

Xylanases catalyze the degradation of xylose, the main constituent of hemicellulose. All xylanases reported in nematodes belong to a subfamily of family GH5 that is clearly distinct from that of the cellulases discussed above. We found candidate nematode xylanase only in root-knot nematodes and *Radopholus similis*, and our phylogenetic analysis showed that they form a monophyletic group (Fig. 2*B* and *SI Appendix*, Fig. S6). Nematode xylanases are nested among clusters of bacterial xylanases, and their closest ortholog is a protein from the soil bacteria *Clostridium acetobutylicum*. This suggests that an ancestral xylanase was acquired via LGT at least in the common ancestor of root-knot nematodes and *R. similis*. As the common ancestor of these species is also the ancestor of cyst nematodes (*SI Appendix*, Fig. S1), the absence of GH5 xylanase in these nematodes suggests that they were either secondarily lost in this lineage or not yet identified. Alternatively, enzymes from another family may perform the same function in cyst nematodes. This is the case for cellulases discussed previously that belong to different and mutually exclusive families (GH5, GH45) but perform the same enzymatic activity in nematodes.

Expansins (EXPNs) are not enzymes per se but loosen the noncovalent interactions between the constituents of the plant cell wall (24). They are typically plant proteins, but a functional EXPN has been characterized in cyst nematodes (25). Candidate EXPNs are present as multigenic families in root-knot nematodes (7, 26) and have also been found in other plant-associated nematodes (27, 28). Interestingly, we identified a homolog in *Xiphinema index*, a clade I plant-parasitic nematode (*SI Appendix*, Fig. S1). Our phylogenetic analysis shows that EXPNs from all plant-parasitic nematodes, including *X. index*, are grouped in a highly supported monophyletic group (Fig. 2*C* and *SI Appendix*, Fig. S7). Two hypotheses can be formulated concerning their origin. The first hypothesis says that a single acquisition occurred in the last common ancestor of clade IV and clade I nematodes with subsequent losses in the numerous nematode taxa not associated with plants. This appears unlikely and in contradiction with the current hypothesis of three independent emergences of plant parasitism in nematodes (29). The second hypothesis suggests that two LGT events from similar bacterial donor species occurred independently in clade I and clade IV nematode lineages. This is further supported by the noncongruence between the relative position of plant-parasitic nematode lineages in the EXPN phylogeny and the actual taxonomy (*SI Appendix*, Fig. S1).

A feature common to several GH5 cellulases and EXPNs is the presence of an appended CBM2 cellulose-binding module. In nematodes and bacteria, proteins only made of a single CBM2 module can be found. In root-knot and cyst nematodes, these proteins are called cellulose-binding proteins (CBPs). A CBP from the cyst nematode *H. schachtii* has been shown to interact with a plant pectin-methylesterase, promoting degradation of its cell wall (30). CBM2 modules are frequently present in bacteria and otherwise found only in a few mollusks associated with a GH9 cellulase module. Our similarity searches using nematode CBM2 modules as queries returned a series of bacterial CBM2's but none from mollusks, indicating that they are distantly related and probably have a distinct origin. All CBM2's found in nematodes form a monophyletic group, and most closely related CBM2 modules are those of actinobacteria (*SI Appendix*, Fig. S8). This suggests that, in plant-parasitic nematodes, CBM2's appended to other different modules have a common bacterial origin. We observed no clearly distinct groups separating CBM2's appended to EXPN modules from those appended to GH5 modules, suggesting that domain shuffling occurred. In root-knot nematodes, CBPs form a monophyletic group closely related to a group of CBM2's appended to GH5 cellulases. In contrast, CBPs of cyst nematodes are more closely related to CBM2's appended to EXPN modules. This suggests that root-knot and cyst nematode CBPs derive from CBM2-bearing cellulases and EXPNs, respectively. The secretion of CBM2-bearing cellulases in plants has been demonstrated along the migratory path of cyst nematodes that migrate intracellularly and destroy the walls of the cells that they travel through (31, 32). No *in planta* localization data have been published for these proteins in root-knot nematodes that migrate intercellularly without damaging plant cells. Our immunolocalization studies on tomato roots infected with *M. incognita* showed the secretion of CBM2-bearing proteins by root-knot nematodes during migratory stages (*SI Appendix*, Fig. S9). Interestingly, we also detected these proteins in later sedentary parasitism stages in eggs about to be extruded by the female and at its vulva region (Fig. 3 and *SI Appendix*, Fig. S10). This suggests a role for these proteins, probably in successful egg laying, a process necessary for spreading the offspring of nematodes to surrounding roots, which is crucial for their parasitic life cycle.

Although our phylogenetic analyses show that bacterial cell-wall–modifying proteins are the most closely related to those of nematodes, suggesting acquisition via LGT, the alternative hypothesis of descent from a common ancestor in eukaryotes, cannot be totally ruled out. To test the likelihood of this alternative hypothesis, we have compared constrained trees in which all eukaryotic homologs were put together in monophyletic groups to the unconstrained trees that we obtained. Statistical testing allowed us to reject with high confidence the topologies presenting monophyly of eukaryotes in all families except the EXPNs for which monophyly of fungal and nematode genes is only slightly less likely (*SI Appendix*, Table S2 *A–F*).

Our tree topologies show that the abundance of multigenic families (cellulases, pectate lyases, and EXPNs) in plant-parasitic nematodes is due to a series of duplications that started after acquisition by LGT events and before the separation of the different clade IV nematode lineages. Duplications that pursued independently after the separation of root-knot and cyst nematodes contibuted the most to this abundance. From the available plant-parasitic nematode genomes, only a few duplications appear to have continued at a species-specific level. All families of cell wall-modifying proteins feature gene structures with multiple introns that contradict the hypothesis of bacterial contamination. In families that are present in several clade IV nematode lineages, at least one intron position could be identified as shared between all lineages, suggesting that these introns were gained early after LGT and before the separation of these different lineages (*SI Appendix*, Fig. S11). In *M. incognita*, considering GC content and codon usage, LGT-acquired genes are indistinguishable from the other *M. incognita*

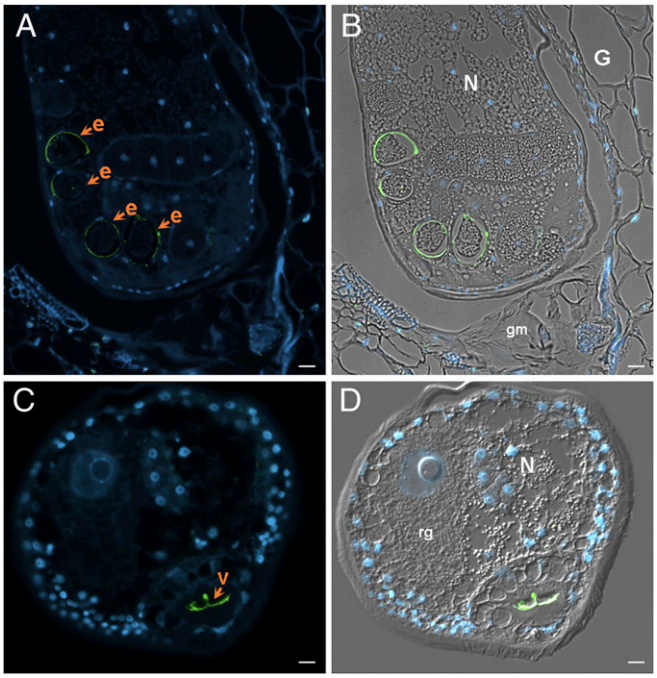

Fig. 3. Immunodetection of CBM2-bearing proteins within adult sedentary females of *M. incognita* during parasitism of tomato roots. (*A* and *B*) Gall containing an adult female, displaying CBM2-bearing proteins accumulated in the eggs (green and indicated by orange arrows) within the ovary. (*C* and *D*) Localization of CBM2-bearing proteins accumulated in the vagina of adult females (green and indicated by orange arrow in *C*). (*A* and *C*) Overlay images of CBM2-bearing proteins (green) and DAPI-stained nuclei (blue); (*B* and *D*) Overlay images of CBM2-bearing proteins (green), DAPI-stained nuclei (blue), and differential interference contrast (gray). N, nematode; e, egg; G, gall; gm, gelatinous matrix; v, vagina; rg, rectal gland. Scale bar: 10 μm. Control images are available in the *SI Appendix*, Fig. S10.

genes (*SI Appendix*, Fig. S12, and Tables S3 and S4). This ensemble of observations reinforces the hypothesis of ancient transfer.

Duplication events that led to multigenic families could have been under positive selective pressure as proposed for other species (33). Such duplications can promote subfunctionalization and neo-functionalization or increase the level of transcribed genes. The dual protein localization pattern of CBM2-bearing proteins, during both migratory and late sedentary stages of infection, may reflect neo- or subfunctionalization.

Our analysis shows that LGT events from several independent bacterial sources most likely gave rise to the six different gene families involved in plant cell-wall modification in clade IV plant-parasitic nematodes. Consistent with this hypothesis is the absence of genomic clusters grouping members of different families that could be a sign of cotransfer from an identical source (*SI Appendix*, Fig. S13). Four different groups of bacteria can be viewed as potential donors for these six cell-wall–modifying gene families. Three of these soil bacteria are notorious plant pathogens or are associated with symbiotic interactions within plant roots (Table 1 and *SI Appendix*, Fig. S14). Being sympatric with plant-parasitic nematodes, these soil bacteria satisfy the most elemental criterion to make LGT possible. However, how the genes are successfully transferred to the germline and then fixed in populations and species is not evident. Gene transfers from algae to the nuclear genome of their predator, a sea slug, have recently been shown (34). At least one example of a nematode that spends part of its life cycle in plant tissue and feeds on plant-symbiotic bacteria is known (35). The bacteria that they feed on possess plant cell-wall degradation genes, and this nematode belongs to the Cephalobidae family, a lineage closely related to clade IV plant-parasitic nematodes (*SI Appendix*, Fig. S1). Thus, acquisition through feeding can be hypothesized. An alternative hypothesis is gene transfers from endosymbiotic bacteria. Such events have been shown in several other animals, including nematodes, probably aided by physical proximity of endosymbionts and germline cells (2). Endosymbionts have been reported in several plant-parasitic nematodes (36–38), all at the vicinity of gametes or eggs. Although transfers appear more evident for endosymbionts, the presence of plant cell wall degradation genes is less likely in these bacteria.

At least one other example of gene transfer from bacteria to nematodes has been reported. Interestingly, this also involved a gene coding for an enzyme, although in this case the transfer probably took place in the last common ancestor of all nematodes (39) and appears not specifically linked to a given lifestyle. In animals other than nematodes, a similar case of massive gene transfers has been reported from bdelloid rotifers (4). Intriguingly, these transfers also concerned genes involved in the degradation of polysaccharides, and gains of introns were also reported. As for the nematode transfers, several transferred genes in these rotifers were indistinguishable from the endogenous genes, suggesting ancient acquisition. Gene transfers in bdelloid rotifers appear to have been fixed preferentially in telomeric regions, and transposable elements may play a role in successful transfer. These features were not observed in nematode transfers. Conversely, the massive duplications that we observed after transfer in nematodes have not been noted in bdelloid rotifers. Bacteria appear as common candidate donors in both reports, but fungi and plants are also candidate donors for bdelloid rotifers. In contrast to plant-parasitic nematodes, in bdelloid rotifers, transfers may have been facilitated by their peculiar life cycle during which they undergo desiccation involving DNA fragmentation and dispersal of membranes, which allows foreign DNA to be incorporated during recovery. Regardless of the possible mechanisms, both these cases highlight the potential importance of LGT in animals and suggest that this phenomenon may be more frequent than usually considered.

Conclusions

We have shown that in plant-parasitic nematodes, a whole set of genes encoding proteins involved in the plant cell wall degradation was most likely acquired by LGT of bacterial origin. The function of the transferred gene products is directly linked to the capacity of these nematodes to parasitize plants. Selective advantage associated with transfer of these genes probably has driven their duplications and facilitated fixation in the different populations and species of plant-parasitic nematodes. Far from being negligible, these LGT events certainly have radically remolded evolutionary trends in recipient organisms, and similar roles in other animals can be expected to be discovered.

Materials and Methods

Phylogenetic Analyses. Homologs of nematode plant cell-wall–degrading proteins were searched in public databases and checked for significance using the approach described in *SI Appendix*. Multiple alignments were done with MUSCLE (40). Phylogenetic analyses were performed using two approaches: a Bayesian method with mrBayes (41) using a mixture of models and a bootstrapped maximum-likelihood approach with RAxML (42) using the evolutionary models returning the highest posterior probabilities in Bayesian analyses. For both methods, we used an evaluation of the proportion of invariable rates as well as of the shape of the γ-distribution of evolutionary rates. The procedure is detailed in *SI Appendix*.

Tree Selection Topology Tests. We statistically tested the significance of the obtained tree topologies in comparison with alternative trees in which all eukaryotic homologs were constrained to form monophyletic groups using the program CONSEL (43). Details of the procedure are available in *SI Appendix*.

GC Content, Codon Use, and Gene Localization. We used the EMBOSS (44) software suite to calculate the codon usage and GC content of plant cell-wall

degradation genes and compared these values to those calculated for the rest of the protein-coding genes. Details of these analyses are available in the *SI Appendix*. Genome localization of plant cell wall degradation genes in *M. incognita* were obtained from GFF files of the genome sequence (7).

Exon/Intron Structure Determination. Information about the intron/exon structures was extracted from the literature and from the National Center for Biotechnology Information's GenBank. For sequences resulting from genome-sequencing projects, intron/exon structures were deduced from the alignment of protein models with the corresponding genome sequences using the procedure detailed in *SI Appendix*.

Immunolocalizations. Antibodies were raised against a peptide from the CBM2 module of *M. incognita* ENG1 cellulase. Dissected tomato roots infected with *M. incognita* were fixed and cut in sections. Slides harboring the nematode feeding sites were immunolabeled with a serum containing the anti-ENG1 antibodies. Slides were observed with a microscope equipped for epifluorescence and differential interference contrast optics. Full details of the procedure used are available in *SI Appendix*.

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