Genomic characteristics of root-knot nematodes: a major group of crop pests

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Abstract

Nematodes constitute one of the most species-rich groups of animals, only paralleled by arthropods. They have a worldwide distribution being present in many biomes from deep sea sediments to deserts. Around 15% of them parasitize plants and they cause enormous damage to the global agricultural production despite the control methods deployed. Among those, the root-knot nematodes (genus *Meloidogyne*) are the most problematic. These species parasitize and stunt the roots and have a wide range of host plants. Root-knot nematodes display a variety of reproductive modes comprising obligatory and facultative sexual reproduction as well as full asexual reproduction with no functional meiosis. The most widespread and damaging species have this asexual mode of reproduction and are polyploid. Since 2008 we have been studying the genomes of these parasites with each generation of sequencing technology allowing improvement of the genome assemblies and new discoveries. The *Meloidogyne incognita* genome was the first to be sequenced and revealed a duplicated structure with high average nucleotide divergence between genome copies. This also allowed discovering *Meloidogyne*-specific genes and a whole repertoire of plant-cell wall degrading enzymes acquired by horizontal gene transfers. Second generation sequencing allowed producing more complete genomes but also suggesting that the polyploid species underwent recent hybridization events resulting in highly diverged subgenomes. Sequencing multiple populations confirmed the absence of meiotic recombination in *M. incognita* and recent activity of transposable elements. More recently, third-generation long-read sequencing technology allowed spectacular improvement in the contiguity of the assemblies and the identification of the A and B subgenomes as well as their evolutionary relations in the polyploid species. Helped by assemblies approaching chromosome-scale resolution, new discoveries were made on the intrinsic composition of the genome itself like unusual centromeres and telomeres, opening new perspectives in understanding the genome biology of these species.

INTRODUCTION

Nematodes or roundworms are usually tiny and transparent animals with an elongated body, as their name suggests, 'nema' meaning thread in Greek. As opposed to several other groups of worms such as earthworms, their body is not segmented. The phylum nematoda constitutes one of the most species-rich ones in animals with ca. 30,000 named species but an estimated total species diversity of up to 1 million species (Kiontke and Fitch 2013). Nematodes are even considered the most abundant animals on Earth and are by far the most abundant in soils (Bardgett and van der Putten 2014, Hoogen et al. 2019).

Among animals, Nematodes are only paralleled by arthropods in terms of number of species and individuals. They form with them a phylogenetic group named Ecdysozoa that also comprises tardigrades and nematomorpha. Recent discovery of a vermiform fossil from the pre-Cambrian time, suggests Ecdysozoa have been existing at least 550 - 560 millions of years before our era (Hughes et al. 2024). Although the morphological characters of this fossil are worm-like, it cannot be unambiguously attributed to nematodes.

Because of the scarcity of fossil records, the poor conservation of morphological characters, that are themselves not very discriminant, dating the origin of nematodes is complex and still controversial. Recent molecular dating efforts propose an origin between 455 and 620 Mya with an mean estimation of 531 Mya (Qing et al. 2024). These molecular estimates are consistent with previously cited ages that were more hypothetical and not based on direct evidence from fossils or modern molecular dating (Blaxter 2003, van Megen et al. 2009, Quist et al. 2015). Regardless of how ancient the Nematoda phylum exactly is, there is general agreement that they have a marine origin (van Megen et al. 2009, Ahmed et al. 2022, Qing et al. 2024).

Despite their marine origin, nematodes are now virtually present everywhere on Earth, occupying terrestrial, marine and freshwater environments as a sign of their evolutionary success and adaptability. They also present a diversity of lifestyles, with free-living as well as parasitic species. Besides the free-living model species *Caenorhabditis elegans*, parasites including nematodes that parasitize human-beings have attracted most of the attention and research efforts. It has been estimated that ca. 15% of nematode species are plant-parasitic species (Holterman et al. 2017). Fossil evidence suggests that nematodes have been forming interactions with land plants at least ca. 400 Mya (Poinar et al. 2008, Poinar 2011) and they were already present when plants colonized terrestrial environments.

Plant-parasitism has emerged at least four times independently during the evolutionary history of nematodes (van Megen et al. 2009, Bert et al. 2011, Holterman et al. 2017). Interestingly, although all plant-parasitic nematodes bear a syringe-like stylet that serve parasitism, the ontogeny is different with at least three types of stylet that develop from different tissue during embryology (Baldwin et al. 2004, Quist et al. 2015). Therefore, independent evolution of plant parasitism has been accompanied with convergent morphological evolutionary events. It should be noted that while all known plant-parasitic nematodes have a stylet, the reverse is not true and many fungivorous nematodes also bear a stylet. This observation and the phylogenetic position of several fungivorous clades led to the hypothesis that plant-parasitic species evolved from free-living fungivorous ancestors.

Collectively, plant-parasitic nematodes are responsible for an estimated annual worldwide crop production loss of 11-14%, corresponding to a value surpassing $157 billion annually

(Lilley et al. 2024). These enormous damages are despite the continuous and diverse efforts deployed to control them, including chemical products, biocontrol solutions, resistance genes identification and introgression, crop rotations and combination of different methods through integrated pest management.

Among all plant-parasitic nematodes, the genus *Meloidogyne*, also referred to as root-knot nematodes, arguably represents the most important one in terms of plant pathology (Jones et al. 2013). All known root-knot nematodes are obligatory biotrophic and microscopic endoparasites, implying that they cannot survive without a living host and that they have to penetrate inside the roots to complete their parasitic life cycle. They penetrate the roots helped by their stylet and a cocktail of effector molecules (Vieira and Gleason 2019). Effectors secreted by the stylet and other secretory organs are also involved in the transformation of plant cells into giant multinucleated cells that serve as a feeding sites for the nematode. Formation of these giant cells is accompanied by multiplication of the surrounding cells, forming galls as symptoms visible in the roots and the reason for their name 'root-knot' nematodes. Development of the nematode inside root tissue causes rewiring of nutrients from the plant which is weakened and might eventually die.

To date, the genus contains close to 100 named species, phylogenetically organized in three main clades with a diversity of host ranges, reproductive modes, and genome structures (Castagnone-Sereno et al. 2013). Species in Clade I like the tropical root-knot nematodes *Meloidogyne incognita*, *M. arenaria* and *M. javanica* have a particularly wide range of host plants while other species are more restricted to a few or unique hosts. Three different modes of reproduction have been described within the genus, including (i) obligate outcrossing between females and males with functional meiosis giving rise to haploid gametes, (ii) facultative outcrossing occurring when males are present and with meiotic parthenogenesis (involving either the fusion of sister or non-sister chromatids) when males are absent or too rare, and (iii) obligate parthenogenesis without functional meiosis with only females genetically contributing to the offspring (Blanc et al. 2025).

Interestingly, the most economically important species belong to Clade I and reproduce by obligate parthenogenesis. Based on higher chromosome numbers these species are supposed to be 3n to 4n polyploids with instances of aneuploidy (Triantaphyllou 1985).

In this review paper, we will report how decoding and analyzing the genomes of root-knot nematodes has improved our knowledge on (i) their genome structures, idiosyncrasies and evolutionary relationships, (ii) their genomic adaptation to plant parasitism and ability to overcome their defense systems, as well as (iii) their other biological traits such as peculiar modes of reproduction.

A brief history of root-knot nematode genomics

The genome sequence of the free-living nematode and model species *Caenorhabditis elegans* was published as early as 1998 (The *C. elegans* Genome Sequencing Consortium 1998) and was the first animal genome sequenced. However, it was only ten years later, in 2008, that the first genome for a plant-parasitic nematode was published (Abad et al. 2008). This genome of the root-knot nematode *Meloidogyne incognita* was also the first for an animal parasitizing a plant. Furthermore, because of the strict asexual reproduction of this Clade I Meloidogyne species, this was also the first genome for an animal that exclusively reproduces by parthenogenesis and in the absence of complete meiosis. The main findings of this initial genome analysis included (i) a peculiar genome structure made of highly diverged duplicated regions, (ii) the presence of a whole repertoire of plant cell wall-degrading enzymes possibly acquired by horizontal gene transfers of bacterial origin and (iii) a whole set of predicted proteins that lack homology in the rest of nematodes and might be considered orphan proteins.

One month later the same year, the genome of *Meloidogyne hapla* was published (Opperman et al. 2008). Contrary to *M. incognita*, meiosis is complete and functional in *M. hapla*, which belongs to Clade II. This species reproduces mainly by parthenogenesis involving fusion of the products of female meiosis, but outcrossing between males and females is also present with adverse conditions generating more males. Both genomes were sequenced using 1st generation (Sanger) technology and were assembled in thousands of contigs and scaffolds. In *M. hapla*, outcrossing allowed producing a genetic map, which helped anchor the scaffolds. The study showed that the genome was relatively small (~54Mb) and the smallest by that time for an animal. The number of genes was reduced compared to *C. elegans*, with a notable reduction of the repertoire of odorant receptor proteins. Acquisition of a whole set of genes by horizontal gene transfers with probably important roles in plant parasitism was also noticed.

Availability of these two first root-knot nematode genomes opened the possibility for comparative genomics analysis (Bird et al. 2009) which revealed that the duplicated structure with diverged copies was a unique feature of *M. incognita*. On the contrary, in *M. hapla* the genome was assembled at an haploid state with the two haplotypes from homologous chromosomes being merged due to high homozygosity level. Characteristic features common to the two root-knot nematodes were also identified including (i) reduction of some gene families as compared to the free living *C. elegans* and considered as a sign of adaptation to sedentary plant parasitism and (ii) acquisition in at least an ancestor of the two species of a whole set of genes via horizontal transfers of non-animal origin that afterwards underwent duplications and diversification.

Since these two pioneering publications, continuous efforts have been deployed to improve these genomes and to decode those of other root-knot nematode species. As detailed in Table1, genome assemblies are now available for 11 different *Meloidogyne* species, with most efforts having been concentrated on Clade I which comprises the most economically important species. In the following sections of this review, we will detail (i) how technological advances in genome sequencing techniques have allowed better understanding the genome structure of this species and (ii) how the field is transitioning from comparative genomics to population genomics.

Table1:

Clade	Species	Ploidy	Mode of reproduction	First genome	Other versions
I	*M. incognita*	3n	obligatory parthenogenesis	(Abad et al. 2008)	(Blanc-Mathieu et al. 2017, Szitenberg et al. 2017, Asamizu et al. 2020, Dai et al. 2023, Mota et al. 2024)
I	*M. floridensis*	3n	parthenogenesis*	(Lunt et al. 2014)	(Szitenberg et al. 2017)
I	*M. luci*	3n	obligatory parthenogenesis	(Susič et al. 2020)	none so far
I	*M. javanica*	4n	obligatory parthenogenesis	(Blanc-Mathieu et al. 2017)	(Szitenberg et al. 2017, Asamizu et al. 2020, Dai et al. 2023, Mota et al. 2024, Winter et al. 2024)
I	*M. arenaria*	4n**	obligatory parthenogenesis	(Blanc-Mathieu et al. 2017)	(Szitenberg et al. 2017, Sato et al. 2018, Asamizu et al. 2020, Dai et al. 2023, Mota et al. 2024)
I	*M. enterolobii*	3n	obligatory parthenogenesis	(Szitenberg et al. 2017)	(Koutsovoulos et al. 2020*a*, Poullet et al. 2025)
II	*M. hapla*	2n	parthenogenesis and outcrossing	(Opperman et al. 2008)	(Shakya et al. 2025)
III	*M. graminicola*	2n	parthenogenesis and outcrossing	(Somvanshi et al. 2018)	(Phan et al. 2020, Singh Somvanshi et al. 2021, Dai et al. 2023)
III	*M. chitwoodi*	2n	parthenogenesis and outcrossing	(Bali et al. 2021)	none so far
III	*M. exigua*	2n	parthenogenesis and outcrossing	(Phan et al. 2021)	none so far
III	*M. fallax*	2n	parthenogenesis and outcrossing	(Griffin et al. 2025)	none so far

* only one division of meiosis is described (Handoo et al. 2004)

** at least one 3n populations has been described (Dai et al. 2023)

Genome structure of polyploid Clade I Meloidogyne: origin and consequences

As soon as 2008, with the first version of the *M. incognita* genome, a peculiar structure was identified (Abad et al. 2008). Indeed, most of the biggest scaffolds in the genome assembly could be aligned with other collinear scaffolds covering most of their length and presenting an elevated 7-8% average nucleotide divergence. This suggested most of the genome was present as at least two highly-diverged copies.

By this time, because most regions were present in two copies and only a few as three copies, it was hypothesized that the genome was mainly diploid with high divergence between former homologous chromosomes. The high divergence was supposed to be the result of progressive accumulation of mutations in the absence of constraint for pairing of homologous chromosomes during abnormal meiosis, a process known as Meselson-White effect (Mark Welch and Meselson 2001).

In 2014, with the help of 2nd generation sequencing technology (Illumina short reads), the genome of *M. floridensis* was sequenced and compared to that of *M. incognita* to shed light on its evolutionary origin (Lunt et al. 2014). *M. floridensis* is a particularly interesting species since it belongs to the same clade as *M. incognita* (Álvarez-Ortega et al. 2019) and is the only one in that clade described as having at least one complete meiotic division, although it seems that only one of the two expected meiosis divisions is present (Handoo et al. 2004). Even though the *M. floridensis* genome assembly was highly fragmented with >80,000 contigs compared to ca. 2,800 in *M. incognita*, it was still possible to predict genes and perform a phylogenomic analysis of the gene copies. The comparative phylogenomic study allowed hypothesizing that *M. incognita* is a polyploid hybrid and that one of the parental species was likely *M. floridensis*, which was itself considered as a hybrid with a diploid genome. Therefore, these Meloidogyne species were supposed to have undergone complex interconnected hybridization events.

To further investigate the origin and consequences of the peculiar genome structure originally identified in *M. incognita* and whether it was a feature conserved in other ameiotic root-knot nematodes, its genome was re-sequenced using 2nd generation sequencing technology (Illumina and 454), and for the first time those of the related species *M. javanica* and *M. arenaria* (Blanc-Mathieu et al. 2017). Specific methods previously used to assemble the tetraploid genome of the bdelloid rotifer *Adineta vaga* (Flot et al. 2013) were employed to separate as extensively as possible the different genome copies in the three Meloidogyne species. Consequently, the new *M. incognita* genome assembly was bigger than the previous one (184 vs. 86 Mb) and consistent with flow cytometry estimation of total DNA content (189 +/- 15Mb). The genome assemblies of *M. javanica* and *M. arenaria* were even bigger with respectively 236 and 256Mb and was also not far from flow cytometry estimates (297 +/- 27 and 304 +/- 9 Mb). Analysis of gene copies concluded that *M. incognita* was most likely triploid (3n) while *M. javanica* and *M. arenaria* were most likely tetraploid (4n). Phylogenomics analysis of collinear duplicated regions and mitochondrial genomes suggested that polyploidy in these species resulted from complex hybridization events. Very high similarity between mitochondrial genomes of the three species suggested they recently diverged from a common or closely related maternal ancestor. Therefore, the high (7-8%) nucleotide divergence between nuclear genome copies within a species was hypothesized to be mostly the result of hybridizations between quite diverged paternal lineages (i.e. allo-polyploidy). Finally, the

study also showed in *M. incognita* that most gene copies resulting from hybridization events had different expression patterns and a substantial part showed signs of positive selection. Consequently, it was hypothesized that this allo-polyploid genome structure represented a possible evolutionary advantage and might be involved in the wider range of host plant species of the three above-mentioned polyploid species as compared to the diploid species *M. hapla*.

In 2017, as an effort to better understand the evolutionary origin of polyploid Clade I Meloidogyne species, short read Illumina data were produced for 19 geographical isolates of five species in total, *M. incognita*, *M. javanica*, *M. arenaria*, *M. enterolobii* and *M. floridensis* (Szitenberg et al. 2017). After genome assembly, phylogenomic analysis of the gene copies allowed confirming a complex scenario of whole genome duplications via hybridization. Improvement of the *M. floridensis* genome assembly and addition of all the other sequenced Meloidogyne species in the analysis also allowed concluding that this species is not one of the direct parents of *M. incognita* but rather a sibling species that shares a common parent.

The *M. incognita*, *M. javanica* and *M. arenaria* genome assemblies produced in this study were all substantially smaller than previous ones and that those expected according to flow cytometry estimates (Blanc-Mathieu et al. 2017). This highlights the limitation of short read sequencing and standard genome assembly software in resolving complex polyploid genomes.

However, thanks to the high accuracy of 2nd generation Illumina short-read data and methodological developments using the distribution of words of size k (k-mer), predicting several genome features directly from the raw reads became possible. Indeed, decomposition of these genome reads in such k-mers and statistical analysis of their distribution and coverage allows predicting their size, ploidy and heterozygosity levels (Ranallo-Benavidez et al. 2020). A broad analysis of the genomes of various parthenogenetic species, including five belonging to the Meloidogyne genus, allowed a more accurate prediction of their genome structures (Jaron et al. 2021). For instance, *M. floridensis*, *M. incognita* and *M. enterolobii* were predicted to have AAB triploid genomes with two relatively closer A subgenomes and one more distantly related B subgenome. This finding also reinforced the idea that *M. floridensis* as a triploid species could not be one of the diploid parents of *M. incognita*. Similarly, *M. javanica* and *M. arenaria* were predicted to have AABB tetraploid genomes with high divergence between A and B but less divergence between AA or between BB subgenomes.

Despite the first genome for a root-knot nematode being sequenced since 2008, basic essential features of chromosomes such as centromeres and telomeres had been so far overlooked, as most attention focused on parasitism and polyploidy. Further bioinformatics analysis of Meloidogyne genomes, combined with cytogenetics immunolocalization of centromere-specific histones in *M. incognita* revealed chromosomes with clustered holo-centromeres (Despot-Slade et al. 2021). Six types of chromosomes could be defined in *M. incognita* according to the distribution of these clusters. Furthermore, a Chip-seq analysis allowed identifying different kinds of tandem repeats associated with centromere-specific histones. Although a diversity of repeats was identified, a 19bp box was found to be conserved not only in *M. incognita* but also in *M. javanica* and *M. arenaria*. The study also showed the population of *M. incognita* studied had 46 tiny chromosomes, consistent with previous gigantic efforts to characterize the number of chromosomes in various Meloidogyne populations and species (Triantaphyllou 1985).

More recently, with the advent of 3rd generation long-read sequencing technology (i.e. PacBio and Oxford Nanopore), huge improvement in the contiguity of Meloidogyne genome assemblies was achieved, unlocking new discoveries and improving our understanding of their genome structure. The first Meloidogyne genome assembly to reach a megabase-scale median contig size (N50) was the one of *M. luci* with ca. 1.7 Mb (Susič et al. 2020), another polyploid parthenogenetic species closely related to *M. incognita* and belonging to Clade I. All the previous genome assemblies reached at best N50 values of a few kilobases. This was followed by the genomes of three populations of *M. chitwoodii* whose average N50 value reached ca. 2.4Mb (Bali et al. 2021). However, these two studies were published as genome announcement papers which presented the genomes as resources and were not accompanied by study of genome structure or comparative analysis.

The first public release of new results concerning the genome structure of root-knot nematode genomes based on long-read assemblies was posted as a preprint in 2023 (Mota et al. 2023). The study, later published in Nature Communications (Mota et al. 2024), revealed new kinds of candidate telomeric repeats in the genomes of *M. incognita*, *M. javanica* and *M. arenaria*. Indeed, in most species, telomeres are made of simple short repetitive sequences associated with a complex of proteins that protect chromosome ends. This is also the case in *C. elegans* and many other nematode species. However, simple telomeric repeats were not found neither in the three above-mentioned species nor in any other root-knot nematode so far. Instead, in *M. incognita*, *M. javanica* and *M. arenaria*, longer and complex degenerate repeats were identified at contig ends and their presence mostly at one end of chromosomes was shown by DNA FISH analysis. These complex repeats share several features that are typical of telomeric repeats such as being transcribed and predicted to be able to form G-quadruplex structures. Interestingly, while they share some sequence homology in the closely related species *M. luci* and *M. floridensis*, no homology is found neither in other Meloidogyne nor any other species genome so far. This suggests clade-specific novel telomeric repeats. This discovery will not only guide efforts to further scaffold the genome assemblies but opens many new perspectives towards understanding genome structure and integrity in these species. The study also confirmed an AAB triploid genome for *M. incognita* and AABB tetraploid genomes for *M. javanica* and *M. arenaria*. Based on this asymmetric distribution of sequence divergence between the subgenomes, it was possible to assign some of the contigs of these species to either an A or B subgenome.

Another major study of long-read based genomes of *M. incognita*, *M. javanica* and *M. arenaria* (Dai et al. 2023) re-visited the evolutionary origin and consequences of the genome structures of these species. Indeed, in the light of nearly chromosome-scale assemblies of these three ameiotic polyploid species as well as of the diploid mitotic species *M. graminicola* (Clade III), used as an outgroup, a more comprehensive phylogenomics analysis of genome copies could be undergone at a much higher resolution than previously (Lunt et al. 2014, Blanc-Mathieu et al. 2017, Szitenberg et al. 2017). The study allowed identifying the A and B subgenomes in each of the three species as well as their phylogenetic relationships. A and B were confirmed to be highly diverged even within a species while the different copies of A or different copies of B were more closely related even between different species. The two most closely related subgenomes were the two A copies of *M. incognita*. The study confirmed with higher resolution, previous findings suggesting the most likely hypothesis for the observed genome structures in the polyploid species was a complex scenario involving inter-species

hybridizations. It was also confirmed that most gene copies in *M. incognita* showed different expression patterns, suggesting functional divergence between subgenomes with a possible associated selective advantage. Furthermore, the long-range organization of the subgenomes allowed determining that there was no systematic dominance of one subgenome over the other ones in *M. incognita*. Subgenome dominance is when a subgenome tends to have its gene copies more expressed than the other subgenomes, to lose less gene copies and to accumulate less transposable elements. Depending on the AAB triplet of contigs considered, all the different possible cases were observed in *M. incognita*, with AAB codominance, AA dominance over B, B dominance over AA, or one A over another. Finally, this study also identified the same complex telomeric repeats as in (Mota et al. 2024) in the three polyploid species and different ones in *M. graminicola*.

In another recently published analysis (Winter et al. 2024), a combination of PacBio HiFi, ultra-long read Nanopore and Hi-C data, was produced to try to resolve the genome structure of *M. javanica*. So far, the previous assembly efforts based on long reads had tried to assemble the four AABB subgenomes separately, although the task is complicated by some long regions of low heterozygosity that tend to collapse in one single region (Dai et al. 2023, Mota et al. 2024). In this new study, the authors tried to produce a purged diploid genome with one single A and one single B consensus subgenome from the tetraploid AABB genome. One original aspect of the study was to compare relative abundance of some repetitive sequences to phase and tell apart the A from B subgenomes. However, the task is complicated by the high persistent nucleotide divergence between AA and between BB.

A major achievement that will undoubtedly help resolving and understanding the genome structure of polyploid Meloidogyne genomes is the availability of chromosome-scale assemblies for diploid species in Clade II. Indeed, these outgroup genomes will help better identify the A and B subgenomes as well as their evolutionary relationship through conserved synteny analysis. These synteny analyses will also guide scaffolding of the contigs. Such a resource is now available with the genome sequence of *M. hapla* resolved in 16 complete chromosomes (Shakya et al. 2025). Besides being of great interest for comparative genomics with Clade I, this chromosome-level genome assembly revealed several interesting features. First, the possibility of outcrossing in this species allowed defining the recombination landscape at the chromosome level, with regions of higher recombination being enriched in effector genes. Second, analysis of the genome structure revealed yet other candidate telomeric repeats in this species, suggesting recruitment of different satellite DNA to serve as protective structure in each Meloidogyne Clade.

From comparative genomics to population genomics

The first efforts to study and compare the genomes of different populations of root-knot nematodes were undertaken in 2017 with the Illumina short-read sequencing of 19 geographical isolates for 5 different Meloidogyne species, *M. incognita*, *M. floridensis*, *M. javanica*, *M. arenaria* and M*. enterolobii* (Szitenberg et al. 2017). The study identified very low divergence at the nucleotide level (SNP) between the isolates of a species, even between the most geographically distant ones. This suggested a recent spread of a few isolates due to agricultural practices.

Besides point mutations, a study published in 2019 researched and analyzed the gene copy number variations in *M. incognita* (Castagnone-Sereno et al. 2019). A comparative genomic hybridization (CGH) array was conducted to identify gene copy number variations between four isolates of *M. incognita* with different virulence phenotypes. The goal was to assess whether some gene copy gains or losses were associated with the transition from an avirulent to a virulent phenotype. Two populations, one from Kursk in Russia and one from Morelos in Mexico that were initially avirulent and thus controlled by the resistance gene Mi-1 in tomato (Ho et al. 1992), were used as a starting point. Using experimental evolution in controlled conditions, virulent sub-populations that overcame the resistance gene in tomato plants were obtained after a few generations. The CGH array allowed identifying some convergent gene copy losses in the two virulent subpopulations as compared to their parental avirulent populations. This suggested that structural variations (here copy number variations) in the genome could be involved in the adaptive evolution of root-knot nematodes to resistance in plants or to other adverse conditions.

Although the two above-mentioned studies were pioneering, they resembled more comparative genomics at the scale of different populations / isolates and did not really include classical population genetics metrics and analysis at the scale of whole genomes. The first such population genomics study in root-knot nematodes was conducted in *M. incognita* (Koutsovoulos et al. 2020*b*). Here, 11 geographical isolates of *M. incognita* presenting different ranges of host plant compatibility and collected on different crops were studied. Their genomes were sequenced at high-coverage with Illumina short reads and SNPs as well as short indels were detected and studied. Statistical analysis of the distribution of SNPs allowed performing analysis of linkage disequilibrium and 4-gamete tests as a function of the inter-SNP distance. These analyzes confirmed the lack of characteristic signatures of meiotic recombination in *M. incognita* for the first time at the genetic level. The study also showed that, as expected according to the theory, the lack of recombination was associated with a lower efficiency of selection as measured by the ratio of the diversity at nonsynonymous and synonymous sites. Overall, very few variations in terms of SNPs were identified in the *M. incognita* genome across the 11 populations and the addition of other populations from previous study (Szitenberg et al. 2017) did not substantially increase the number of identified variable sites. Yet, clustering of the populations according to these few point variations showed interesting results. First, grouping the populations according to their genetic distance revealed three main clusters that neither correlated with the geographical distance nor the crop on which they had been collected. Second, a phylogeny of the populations based on their genetic distance showed that the different ranges of host compatibility, originally termed as 'host races' had no underlying phylogenetic support. In contrast, it seems that these different

host ranges resulted from multiple independent transitions and adaptations. Therefore, the wide host range observed at the species level is probably not due to a single general-purpose genotype but to multiple independent adaptations.

A similar study of SNPs was conducted on 48 Japanese populations of *M. incognita* that were collected on different geographical sites and that presented different compatibility with five different cultivars of sweetpotato (Asamizu et al. 2020). The authors identified a ca. 1Mb region in the genome that showed substantial variations between the populations. Interestingly, the genomic variations correlated with the different ranges of compatibilities regarding the five cultivars of sweetpotato. This is in contrast with results of a previous analysis which identified no correlation between the pattern of host compatibility across four different plants and the 11 studied populations (Koutsovoulos et al. 2020*b*). Interestingly, addition of the 48 new Japanese populations to the previous clustering of *M. incognita* populations revealed that all of them fell in one single cluster together with some populations from Brazil and the USA. The second cluster was composed of African and Brazilian populations while the third cluster was only composed of Brazilian populations.

Collectively, these results suggest that most *M. incognita* field or greenhouse populations sampled so far across the world are genetically very similar and most probably result from the recent spread of one or a few original isolates via global agricultural exchanges. Moreover, so far, the hotspot of genetic diversity seems to be in Brazil although many locations across the world have been unsampled to date and all from the field or greenhouse. Therefore, this observation might be related to oversampling in Brazil and lack of sampling in other locations.

Transposable elements (TEs) are known to cause structural variations in genomes either actively by their transposition or passively with their repetitive nature prone to forming loops or recombinations. Furthermore, polyploidy, hybridization and asexual reproduction are factors that can influence the proliferation and activity of transposable elements. Therefore, the contribution of TEs to the genomic variations across populations of *M. incognita* was investigated (Kozlowski et al. 2021). In this analysis, the Illumina data of the 11 populations studied in (Koutsovoulos et al. 2020*b*) were re-used. The *M. incognita* repeatome was annotated and analyzed as well as its variations in the populations. According to the high similarity of several TE *loci* to the consensus sequences, it was estimated that *M. incognita* underwent recent TE bursts. Furthermore, *de novo* insertions of TEs were identified in some populations, including within coding or potential regulatory regions. Finally, clustering and phylogenetic analysis of the populations according to their pattern of TE *loci* presence / absence recapitulated the same classification as the SNP analysis of (Koutsovoulos et al. 2020*b*). This showed that TE movements participated in the genome diversification and accompanied the divergence between populations.

CONCLUSION

Genome sequencing and its technological progress have unlocked the analysis of the genome structure of root-knot nematodes as well as their characteristic features. With each new generation of genome sequencing technology, new discoveries could be made. These ensembles of genome analyses revealed some genomic signatures of adaptation to a plant-parasitic lifestyle and also particularly complex genome structures in Clade I parthenogenetic species. These complex polyploid genome structures with non-homogenous distribution of divergence level between subgenomes probably explain why there is not yet a genome truly resolved at the chromosome level for species in this Clade I. The situation is complicated by the fact that the parental species at the origin of the hybridization remain unknown.

Naturally, with the availability of good quality genomes, the field of Meloidogyne genomics is transitioning from comparative genomics, which revealed ancient adaptations to plant parasitism and genomic idiosyncrasies, to population genomics. This transition offers a potential to unlock the genomic mechanisms underlying more contemporary adaptation, structures of the populations, their age and phylogeography. Indeed, contrary to cyst nematodes, for which population geneticists have reached conclusions concerning the origin and routes of dispersion, these important elements are still completely unknown for root-knot nematodes (Montarry et al. 2020).

Further theoretical, methodological, and bioinformatics developments will be needed to pave the way towards fully resolving genome assemblies of polyploid parthenogenetic nematodes, and help the assembly of other species with complex genomes. Similarly, because most methods and software for population genetics and genomics have been developed and optimized for diploid genomes and for outcrossing species, the field is still in its infancy for polyploid and obligate parthenogenetic nematodes and further methodological developments are needed.

These limitations do not apply to diploid and outcrossing root-knot nematodes. Recently, application of modern long-read genome sequencing technology with Hi-C has allowed producing genome assemblies for some diploid species at the chromosome-scale resolution.

Population genomics study on these diploid species readily benefit from the methods and software currently available, promising recent major results. Furthermore, these near-complete genome assemblies will undoubtedly be of invaluable interest to guide the assembly of the polyploid genomes and help tell apart the subgenomes and reconstruct their evolutionary history.

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