Genomic Structures and Phylogenetic Relationships of Plant-Parasitic Nematodes: Insights into Evolutionary Adaptations and Parasitism Mechanisms

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Abstract. The objective of this project was to investigate the genomic structures and phylogenetic relationships of plant-parasitic nematode (PPN) species to understand their evolutionary history and parasitic traits. Genome assemblies, gene duplication events, and parasitism modes were analyzed to identify relationships between genomic features and parasitic adaptations. A total of 18 plant-parasitic nematode species were identified and analyzed for a phylogenetic tree construction. Statistical analyses indicated a correlation between genome size and number of gene duplication events. The findings suggest that gene duplications might have supported evolutionary adaptations in PPNs by enabling their diverse parasitism strategies and ecological niches.

Keywords: Plant-Parasitic Nematodes · Genome Size · Gene Duplication Events · Parasitism · Evolution.

1 Introduction

Nematodes, also called roundworms, are classified under the phylum Nematoda. The phylum is highly diverse and contains about 25,000 species that can inhabit terrestrial, marine, and freshwater environments in high numbers [1, 2]. Usually being microscopic, most nematode species reach a maximum length of approximately 2.0 mm [3]. While the majority of nematodes are free-living, some species are parasitic, affecting humans, plants, and most animals as either endoparasites or ectoparasites [3, 4]

Parasitism within the phylum Nematoda is thought to have evolved independently at least seven times, with four events resulting in animal parasitism and three to plant parasitism [5]. Among the parasitic nematodes, more than 4,000 species are known to parasitize plants [6]. Collectively, these plant-parasitic nematodes cause agricultural and economic damage, with global effects estimated to reach $80 billion annually [7]. Although they share anatomical similarities, PPNs exhibit significant diversity in their modes of host interaction, including

the development of specialized feeding structures and various mechanisms of parasitism [8].

The evolution of parasitic mechanisms in PPNs is driven by various genetic changes, including the development of genes utilized when interacting with hosts and the regulation of parasitism-specific genes [9]. Studying these processes is important to understand how these species have adapted to parasitize plants.

This study focuses on analyzing the genomic structures and phylogenetic relationships of PPNs. By examining these features, the research seeks to provide insights into how different parasitism mechanisms might have evolved in these nematodes and enhance our understanding of their adaptations to plant hosts.

2 Methodology

2.1 Obtaining Data

There are 160 Nematode species belonging to 6 clades (C, I, II, III, IV, V) in the WormBase ParaSite website. Genomic sequence, protein, and CDS transcript files of these species were downloaded in FASTA format.

N50 values, genome size, number of scaffolds, number of coding genes, feeding styles, assembly platform/method, and genome coverage data of each species were obtained from NCBI genome database, European Nucleotide Archive (EBI-ENA), and UniProt.

An Excel table was created based on these data and the species were classified based on their clades.

Conda was used as the environment management tool for the command line programs.

2.2 Quality Check

BUSCO v5.8.0 [10] was used on CDS and protein files for quality assessment. The results were added to the table. The code below was used to run BUSCO.

```
busco -i [SEQUENCE\_FOLDER] -m [MODE] --lineage\_dataset eukaryota\_odb10
     --cpu 4
```

Here, [MODE] was set to [genome] for CDS files and [proteins] for protein files.

OMAmer [11] and OMArk [12] command line programs were used to further assess the quality of the downloaded protein files. The results were added to the table.

The OMAmer and OMArk codes used for a single input file:

```
omamer search --db LUCA.h5 --query *.protein.fa --out *.omamer

omark -f *.omamer -d LUCA.h5 -o *\_output/
```

These codes run the programs with a single protein input file and then they stop. Considering the need for running them for all 160 species, a shell script was written for OMAmer and OMArk to accelerate the process and run all input files in a folder at a time:

```
#!/bin/bash
db="LUCA.h5"
output_folder="omamer_combined_output"
mkdir -p "$output_folder"

for file in *.protein.fa; do
    base_name=$(basename "$file" .fa)

    omamer search --db "$db" --query "$file" --out "$output_folder/${
        base_name}.omamer"

    omark -f "$output_folder/${base_name}.omamer" -d "$db" -o "
        $output_folder/${base_name}_output"
done

echo "Processing␣complete.␣Results␣saved␣in␣$output_folder"
```

To be used in a further study, a threshold of 75% was set for BUSCO protein completeness and OMArk protein completeness values to eliminate low-quality assemblies. R (v4.4.1) code below was used to select species with both BUSCO and OMArk values of more than 75%. A total of 114 species remained after applying the threshold.

```
table <- read_excel("~/Desktop/species_Nematoda.xlsx")

busco_protein_c <- as.numeric(gsub('"', '', as.character(unlist(table[,
    10]))))

busco_more_75 <- busco_protein_c[!is.na(busco_protein_c) & busco_protein_
    c > 75]

omark <- as.numeric(gsub('"', '', as.character(unlist(table[, 21]))))

omark_more_75 <- omark[!is.na(omark) & omark > 75]

filtered_table <- table[!is.na(busco_protein_c) & busco_protein_c > 75 &
                      !is.na(omark) & omark > 75, ]
```

Based on their feeding styles, a total of 18 plant parasitic nematodes were identified as shown below. All belong to Clade IV.

1. *Aphelenchoides besseyi*
2. *Aphelenchoides bicaudatus*
3. *Aphelenchoides fujianensis*
4. *Bursaphelenchus xylophilus*

5. *Ditylenchus destructor*
6. *Ditylenchus dipsaci*
7. *Globodera pallida*
8. *Globodera rostochiensis*
9. *Heterodera gylcines*
10. *Heterodera schahtii*
11. *Meloidogyne arenaria*
12. *Meloidogyne chitwoodi*
13. *Meloidogyne enterolobii*
14. *Meloidogyne floridensis*
15. *Meloidogyne graminicola*
16. *Meloidogyne hapla*
17. *Meloidogyne incognita*
18. *Meloidogyne javanica*

2.3 Phylogenetic Analysis

OrthoFinder v3.0.1b1 [13] was used with protein sequences of these plant parasitic species. It is a command-line program that infers gene trees and rooted species trees based on orthologues and orthogroups. The code below was used to run OrthoFinder.

```
orthofinder -M msa -S diamond -f [SEQUENCE_FOLDER]
```

Here, -M msa option was used to infer gene trees for each orthogroup based on a multiple sequence alignment by using MAFFT [14]. -S diamond option was used to utilize DIAMOND [15] protein sequence alignment algorithm, which runs faster than the default BlastP algorithm while keeping the match sensitivity.

Among numerous OrthoFinder results, SpeciesTree_rooted.txt file, which contains the data to create a rooted species tree, shown below was used to construct a phylogenetic tree with FigTree (v1.4.4).
The Duplications_per_Species_Tree_Node file was used to interpret gene duplication events for each species.

2.4 Statistical Analysis and Visualization

Number of gene duplications and genome size of the species were compared and checked to see if there are any correlations. To do so, first, species names, genome size, and duplication numbers were stored in R as shown below.

```
species <- c("A.besseyi", "A.bicaudatus", "A.fujianensis", "B.xylophilus"
    , "D.destructor", "D.dipsaci", "G.pallida", "G.rostochiensis", "H.
    glycines", "H.schachtii", "M.arenaria", "M.chitwoodi", "M.enterolobii
    ", "M.floridensis", "M.graminicola", "M.hapla", "M.incognita", "M.
    javanica")
```

```
genome_size <- c(46759715, 46428382, 143834322, 74561461, 111138200,
    227234012, 123625196, 92682755, 157978452, 179246932, 258067405,
    47477280, 240054310, 96673063, 38184958, 53017507, 183531997,
    235798407)

gene_duplications <- c(1554, 1331, 7771, 4304, 2330, 11231, 4509, 6718,
    7549, 12948, 14849, 1810, 7806, 3615, 1073, 2186, 3988, 13915)
```

Then, the normality of these data was checked in R using the shapiro.test() function to perform the Shapiro-Wilk test of normality. After that, Spearman's rank correlation test was applied to the data. The code used is shown below.

```
shapiro.test(genome_size)

shapiro.test(gene_duplications)

cor.test(genome_size, gene_duplications, method = "spearman")
```

Next, the Python (v3.12.4) code below was used to plot genome size vs. gene duplications. Matplotlib, seaborn, and pandas libraries were used.

```
import matplotlib.pyplot as plt
import seaborn as sns
import pandas as pd

species = "A.besseyi", "A.bicaudatus", "A.fujianensis", "B.xylophilus", "
    D.destructor", "D.dipsaci", "G.pallida", "G.rostochiensis", "H.
    glycines", "H.schachtii", "M.arenaria", "M.chitwoodi", "M.enterolobii
    ", "M.floridensis", "M.graminicola", "M.hapla", "M.incognita", "M.
    javanica"]

genome_size = [46759715, 46428382, 143834322, 74561461, 111138200,
    227234012, 123625196, 92682755, 157978452, 179246932, 258067405,
    47477280, 240054310, 96673063, 38184958, 53017507, 183531997,
    235798407]

gene_duplications = [1554, 1331, 7771, 4304, 2330, 11231, 4509, 6718,
    7549, 12948, 14849, 1810, 7806, 3615, 1073, 2186, 3988, 13915]

categories = ["Diploid"] * 10 + ["Polyploid", "Diploid", "Polyploid", "
    Diploid", "Diploid", "Diploid", "Polyploid", "Polyploid"]

genome_size_mbp = [i / 1e6 for i in genome_size]

data = pd.DataFrame({
    'GenomeSizeMbp': genome_size_mbp,
    'GeneDuplications': gene_duplications,
    'Species': species,
    'Ploidy': categories
})
```

```
plt.figure(figsize=(10, 8))
sns.scatterplot(
    x='GenomeSizeMbp',
    y='GeneDuplications',
    hue='Ploidy',
    data=data,
    palette={'Diploid': 'blue', 'Polyploid': 'orange'},
    s=50,
    edgecolor='black'
)

sns.regplot(
    x='GenomeSizeMbp',
    y='GeneDuplications',
    data=data,
    scatter=False,
    line_kws={'color': 'brown'}
)

for i in range(len(data)):
    plt.text(
        x=data['GenomeSizeMbp'][i] + 1.5,
        y=data['GeneDuplications'][i],
        s=data['Species'][i],
        fontsize=10,
        color='black'
    )

plt.grid(True)
plt.ylim(bottom=0, top=16000)
plt.title('Genome␣Size␣vs.␣Gene␣Duplications', fontsize=14)
plt.xlabel('Genome␣Size␣(Mbp)', fontsize=12)
plt.ylabel('Number␣of␣Gene␣Duplications', fontsize=12)
plt.legend(title='Ploidy', bbox_to_anchor=(1.05, 1), loc='upper␣left')
plt.tight_layout()
plt.show()
```

3 Results and Discussion

Table 1. *Excel table of the 18 plant-parasitic species.*

									BUSCO Protein					BUSCO CDS					OMARK Proteins	
Clade	Species Name	N50 (kb)	Genome Size (bp)	No. Scaffolds (k)	No. Coding Genes	Feeding Style	Platform	G. Coverage	Complete	Single	Duplicated	Fragmnt.	Missing	Complete	Single	Duplicated	Fragmnt	Missing	Complete	Single
Clade IV	**Aphelenchoides besseyi**	16,068,654	46,759,715	32	11,239	plant pathogenic nematode	ONT	188x	81.2	77.3	3.9	4.7	14.1	82.0	78.4	3.5	4.7	13.3	77.39	74.28
Clade IV	**Aphelenchoides bicaudatus**	11,905,581	46,428,382	106	10,675	arasite of mushroom and plants	ONT	422.3x	79.8	78.4	1.2	5.5	14.9	80.4	78.8	1.8	5.1	14.5	80.27	77.29
Clade IV	**Aphelenchoides fujianensis**	553,027	143,834,322	780	17,477	plant parasitic nematode	ONT	197.5x	68.2	48.2	20.0	9.8	22.0	68.2	48.2	20.0	9.4	22.4	75.29	57.47
Clade IV	**Bursaphelenchus xylophilus**	949,83	74,561,461	5,527	17,704	parasite of Pine trees	BGISEQ-500	?	83.9	82.7	1.2	7.1	9.0	83.9	82.7	1.2	7.1	9.0	86.44	81.13
Clade IV	**Ditylenchus destructor**	555,026	111,138,200	1,761	13,931	endoparasitic plant nematode	Illumina + Pacbio	200x	79.6	77.6	2.0	5.9	14.5	77.8	75.7	2.0	7.8	14.5	79.51	74.82
Clade IV	**Ditylenchus dipsaci**	287,009	227,234,012	1,394	26,428	plant pathogenic nematod	Illumina + Pacbio	110x	88.6	82.0	6.7	15.7	15.7	69.0	62.4	6.7	16.1	14.9	84.53	64.21
Clade IV	**Globodera pallida**	120,163	123,625,196	6,873	16,403	plant pathogen	?	145x	61.6	57.6	3.9	14.5	23.9	59.8	55.7	3.9	16.5	23.9	68.32	56.87
Clade IV	**Globodera rostochienais**	3,274,970	92,682,755	88	17,856	plant pathogenic nematode	Illumina + Pacbio	119x	88.8	74.5	14.1	5.1	6.3	89.0	75.3	13.7	5.1	5.9	78.42	64.84
Clade IV	**Heterodera glycines**	17,907,690	157,978,452	9	22,465	plant-parasitic nematode	Pacbio	110.7x	77.3	68.2	9.0	8.6	14.1	78.0	68.6	9.4	8.2	13.7	73.37	56.87
Clade IV	**Heterodera schachtii**	1,273,070	179,246,932	395	26,739	plant pathogenic parasite	Pacbio	160x	87.5	79.8	8.8	6.3	6.3	86.3	78.0	8.2	6.3	7.5	81.28	65.68
Clade IV	**Meloidogyne arenaria**	16,417	258,067,405	26,196	101,269	plant pathogenic nematode	454 + Illumina	100x	90.6	14.5	76.1	4.3	5.1	90.6	12.9	77.6	3.9	5.5	84.86	13.42
Clade IV	**Meloidogyne chitwoodi**	2,451,023	47,477,280	30	12,295	endoparasite of potato	Pacbio	172x	60.8	59.6	1.2	16.1	23.1	61.2	60.0	1.2	16.1	22.7	66.04	60.28
Clade IV	**Meloidogyne enterolobii**	143,364	240,054,310	4,437	82,586	root-knot nematode	Illumina	?	67.8	40.0	27.8	17.3	14.9	68.7	38.4	28.2	17.6	15.7	79.08	25.89
Clade IV	**Meloidogyne floridensis**	3,688	96,673,063	58,696	49,938	root-knot nematode	?	200x	54.5	50.6	3.9	30.2	15.3	52.2	49.0	3.1	30.2	17.6	81.72	36.63
Clade IV	**Meloidogyne graminicola**	20,427	38,184,958	4,304	10,895	root-knot nematode	Pacbio	90x	71.0	70.6	0.4	14.1	14.9	71.4	71.0	0.4	13.7	14.9	76.68	67.87
Clade IV	**Meloidogyne hapla**	37,501	53,017,507	3,452	14,419	plant-parasitic nematode	ABI3730	10.4x	79.2	76.9	2.4	10.2	10.6	78.4	76.1	2.4	10.2	11.4	78.7	71.44
Clade IV	**Meloidogyne incognita**	38,543	183,531,997	12,091	43,718	root-knot nematode	454 + Illumina	100x	89.4	18.4	71.0	5.9	4.7	88.2	18.0	70.2	6.3	5.5	84.1	14.32
Clade IV	**Meloidogyne javanica**	10,373	235,798,407	31,341	97,208	root-knot nematode	454 + Illumina	100x	87.5	20.4	67.1	7.8	4.7	87.5	18.0	69.4	7.1	5.5	85.23	13.76

N50 values in kilobases (kb), genome size in base pairs (bp), number of scaffolds in thousands (k), number of coding genes, feeding styles, assembly platform(s), genome coverage, BUSCO results for protein and CDS files, and OMArk results for protein files were indicated. Missing data were indicated by a (?) symbol.

In the OrthoFinder analysis of all 18 species, a total of 551,558 genes were identified. Of these, 92% were assigned to 33,202 orthogroups, while the remaining genes were unassigned.

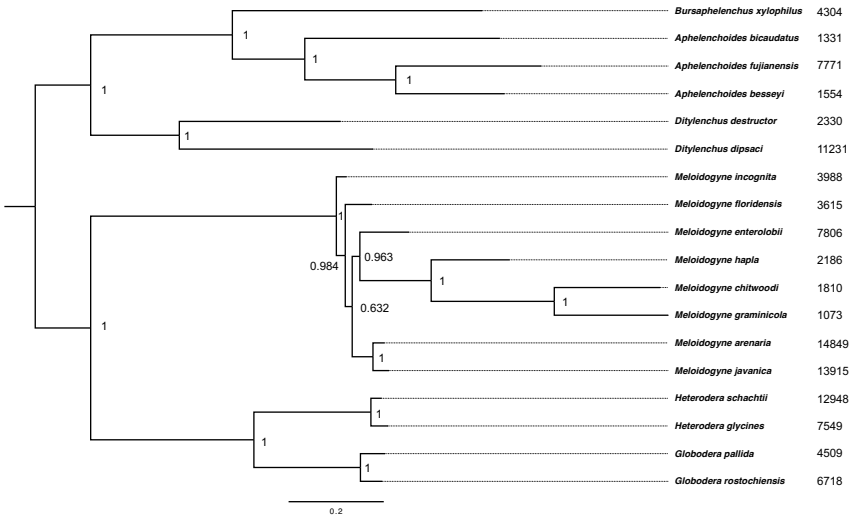

Fig. 1: The phylogenetic relationships among the 18 plant-parasitic nematode species and associated gene duplication events.

Among the 18 PPNs shown in Fig. 1, 4 of them are polyploid species, *M. incognit*a, *M. enterolobii*, *M. arenaria*, and *M. javanica* [16].

In the phylogenetic tree, the 18 plant-parasitic nematode species are divided into two primary groups, further classified based on their parasitism modes.

The first group includes species from the genera *Bursaphelenchus*, *Aphelenchoides*, and *Ditylenchus*. Within this group, species from *Bursaphelenchus* and *Aphelenchoides* are facultative migratory endoparasites, which invade plant roots through penetration [16, 17]. *Ditylenchus* species, on the other hand, are obligate plant and fungi endoparasites [18]. *B. xylophilus* serves as an outgroup to the *Aphelenchoides* species, which form a monophyletic group. Additionally, *A. fujianensis* and *A. besseyi* are identified as sister species, a pattern also observed between the two *Ditylenchus* species.

The second group includes species from the genera *Meloidogyne*, *Heterodera*, and *Globodera*. *Meloidogyne* species, also called root-knot nematodes, are sedentary obligate endoparasites [19]. These nematodes form a distinct and sizable monophyletic group, separate from the other two genera. Additionally, all polyploid species belong to the *Meloidogyne* genus. In contrast, species from *Heterodera* and *Globodera*, also called cyst nematodes, are migratory obligate parasites [19]. Within these genera, pairs of sister species are identified as *H. schachtii* and *H. glycines* in the *Heterodera*, and *G. pallida* and *G. rostochiensis* in the *Globodera* genus. These results indicate that the evolutionary history of PPNs is largely shaped by their feeding behavior and parasitism modes. Differences in these features may contribute to their diversification by enabling adaptation to various hosts, environments, and ecological niches.

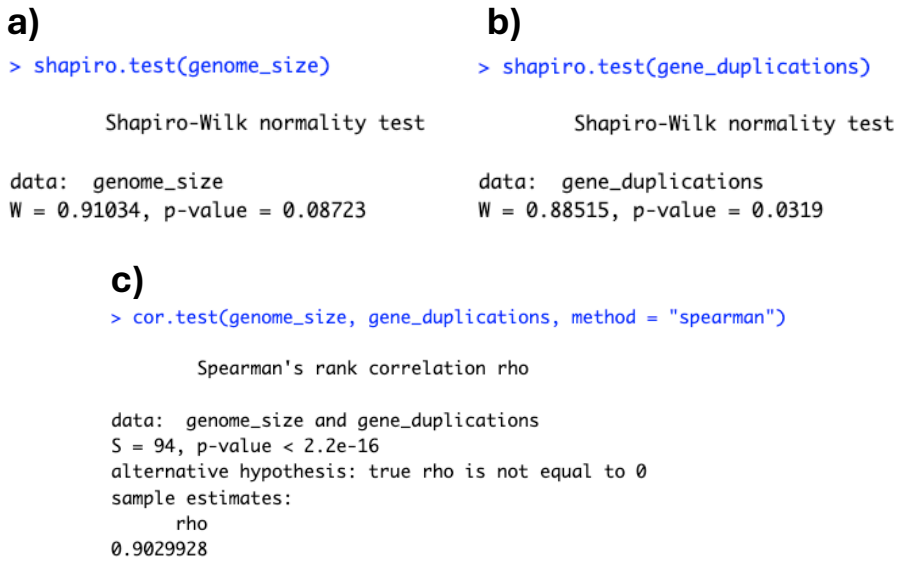

Fig. 2: Result of Shapiro-Wilk normality test of genome sizes **(a)** and number of gene duplications **(b)** in plant parasitic nematodes, and Spearman's rank correlation test **(c)**.

The Shapiro-Wilk normality test yielded a p-value of 0.087 for genome sizes and 0.032 for the number of gene duplications. Based on these results, the null hypothesis of normal distribution cannot be rejected for genome sizes data. How-

ever, for the number of gene duplications data, the null hypothesis must be rejected, showing that this dataset does not follow a normal distribution.

Given that the gene duplication data is not normally distributed, Spearman's rank correlation test, which does not assume normality, was employed to assess the relationship between genome sizes and gene duplications and to determine whether they are correlated [20]. The test yielded a p-value of <2.2e-16, leading to the rejection of the null hypothesis that there is no correlation between the two variables [20]. Furthermore, the Spearman's rho value exceeded 0.9, demonstrating a strong positive correlation between genome size and the number of gene duplications [21].

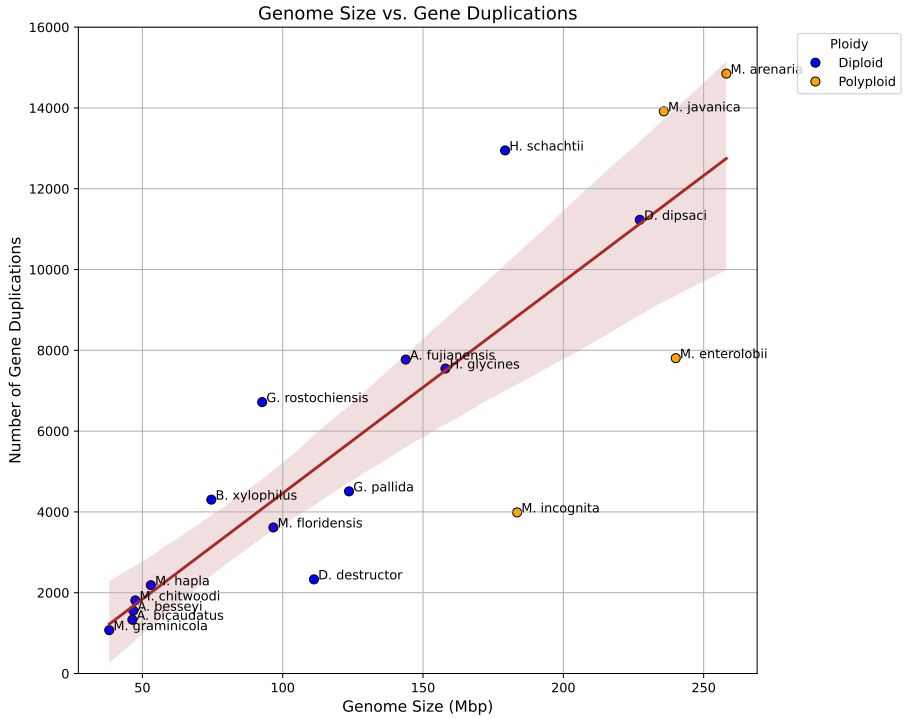

Fig. 3: Genome size in million base pairs (x-axis) vs. number gene duplications per species (y-axis) plot of the 18 plant parasitic species. The red line indicates the linear regression line, and 95% confidence interval is shaded around it. Diploid species are shown with blue dots and polyploid species are shown with orange dots.

As shown in Fig.3, certain species, such as those in the genus *Meloidogyne*, have both high levels of genome size and high number of gene/genome duplications. On the other hand, *Aphelenchoides* and *Bursaphelenchus* species tend to have lower numbers of each. These differences might reflect distinct evolutionary pressures that lead to differentiation of parasitism modes of these species, such as being obligate or facultative parasites, as discussed previously.

Gene duplication events can be a result of various genomic alterations, such as whole-genome duplications (WGDs) as a form of large-scale gene duplication [22]. WGD is also known as polyploidization in which both coding and non-coding parts of the genome is doubled, resulting in a more complex and an expanded genome [23, 24]. Both WGDs and individual gene duplication events are among the drivers of evolution and morphological and physiological diversity in which they provide extra genetic material that can change over time through mutation, drift and natural selection [25, 26]. Specifically, in some nematode species, gene and genome duplications are known to increase developmental plasticity, further providing phenotypic diversity [27]. WGD events can also increase transposable element (TE) accumulation [23]. In fact, even though most nematode species contain TEs, the polyploid ones have higher number of them [16].

The graph in Fig. 3 illustrates a positive correlation between genome size and the number of gene duplication events across PPNs. This suggests that as gene/genome duplications increase, the genome size also tends to increase. This result is in correlation with the previous studies discussed above suggesting that gene/genome duplications increase genome size. Furthermore, 3 of the 4 polyploid PPNs are among the species that have both high genome size and high number of duplications, highlighting the relationship with polyploidy and genome size.

Meloidogyne species are known to be able to parasitize a wide variety of plant hosts [19]. They also tend to have higher genome size and duplications, especially the polyploid ones. Therefore, it is possible that their high versatility of parasitism might be supported by their genomic structure where larger genomes may allow keeping higher number of parasitism-specific genes and thus increase their adaptation through selection.

However, in some cases, parasitism is known to reduce host population size [28] and since parasitic nematode population structure and size is dependent on that of the host [29], parasitism can reduce the niche and population size (and effective population size) of the parasite itself [30]. As the effective population size decreases, the rate of genetic drift increases [31]. Therefore, in species with small (effective) population sizes, purifying selection would not be effective to eliminate various genomic features, such as non-coding DNA, and the genome size would remain higher [32]. Additionally, TE content and variation is also known to be under the control of genetic drift [33]. In conclusion, contrary to the adaptation and selection model discussed above, effects of genetic drift might also be the driver of evolution of the PPNs with high genome size, considering their increased parasitism variety.

In summary, the correlation between genome size and gene/genome duplication numbers suggests that the latter have played a key role in the evolution and differentiation of plant-parasitic nematodes. However, to completely understand whether selection or genetic drift is the main reason of high genome size, high duplication numbers and increased parasitism, more research is needed. In general, results of this study might help explaining how these species have developed their parasitic abilities.

4 Conclusion

The result of this study reveals the role of gene duplication events in the evolution and diversification of plant-parasitic nematodes. The strong correlation between genome size and duplication events highlights their contribution to genetic adaptability and parasitism. Variations in parasitism modes of the species correspond to their genomic traits and phylogenetic relationships, offering insights into how evolutionary pressures have shaped their diversity. These findings improve our understanding of the genomic foundations of parasitism in nematodes, with possible future implications for developing control strategies against PPNs in agriculture.

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