ORPHAN GENES: THEIR IDENTIFICATION AND EVOLUTION IN PLANT PARASITIC NEMATODES

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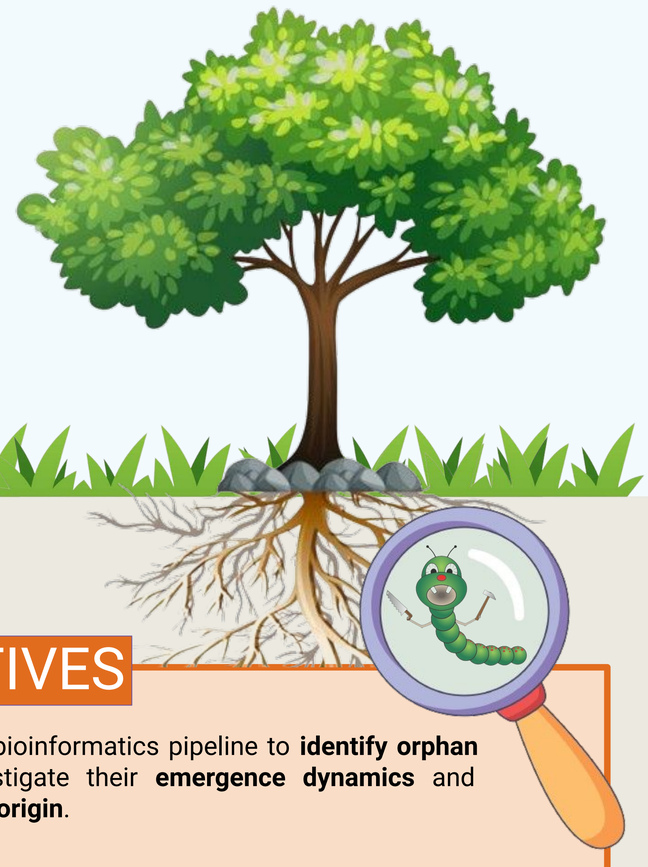

INTRODUCTION

- **Orphan genes** are genes that have no identifiable homologs. They are systematically found in eukaryotic genomes, and represent up to 30% of the genes [1].
- The **emergence** of such genes is an important mechanism of functional acquisition during evolution. They may originate from duplication or horizontal transfer events followed by **rapid divergence**. Another possibility is that they may emerge from non-genic regions, which is known as ***de novo* gene birth** [2]. Hence, there is a debate on the real origin of orphan genes [3].
- The most common method for **identifying** orphan genes involves the search for homologues (identification of their absence). Then, their origin can be studied by syntenic approaches. However, these approaches cannot always distinguish between *de novo* genes and the ones that diverged significantly from their ancestor.

OBJECTIVES

- We developed a bioinformatics pipeline to **identify orphan genes** and investigate their **emergence dynamics** and possible ***de novo* origin**.
- The biological purpose is to apply the built pipeline to the genus ***Meloidogyne*** of **root-knot nematodes**, which cause a huge global crop loss. The species of this genus secrete parasitism effector, proteins, many of which are encoded by genes having no identifiable homologues [4].

METHOD & RESULTS

PIPELINE

Identification of homology relationships in *nematoda*

Proteome completeness analysis with *BUSCO [5]* and *OMArk [6]* for all the *nematoda* proteomes present on *WormBase Parasite [7]* database or predicted by the GAME team

Selection of 85 *nematode* proteomes (including 8 *Meloidogyne* species) and 1 outgroup of *nematodes (BUSCO completeness > 60%)*

Comparative proteome analysis and establishment of homology relationships

Clustering of best reciprocal hits to orthogroups by alignments between all proteomes with *OrthoFinder [8]*

Clustering of best reciprocal hits to orthogroups by kmer approach between all proteomes with *SonicParanoid [9]*

Comparison of orthogroups from both tools

Meloidogyne **orphan gene candidates** in *nematoda*

Double check against nr database with *Diamond* [10] *(e-value 0.001)*

Expression validation by using transcriptomic data

FPKM > defined threshold for at least 1 gene of an orthogroup

Expressed orphan genes (figure 2)

Study of origin

MSA for each sequence of an orthogroup with *MAFFT* [11]

Gene tree reconstruction for each orthogroup by *IQTREE* [12] and reconciliation of gene trees with species tree by *TreeRecs* [13]

Ancestral sequence reconstruction (ASR) with *ARPIP* [14]

species tree of the genus of interest with the closest outgroup species

Emergence dynamics with *Bridge* [16] (figure 4)

Emergence position on the species tree for each orthogroup

Alignment of ASR sequences to the genomes of corresponding outgroups (according to phylogeny in figure 3) with *Exonerate* [15] (figure 4A)

***de novo* gene**
if aligned with the presence of a mutated splicing site, stop codon or indels (not multiple of 3) on non-coding region in the outgroup

orphan from pre-existing gene
if aligned on a position at which there is a coding gene in the outgroup

Verification of orphan regions via syntenic approaches (figure 4B)

Figure 1: Developed bioinformatics pipeline to identify orphan genes and to study their evolution in Meloidogyne.

RESULTS

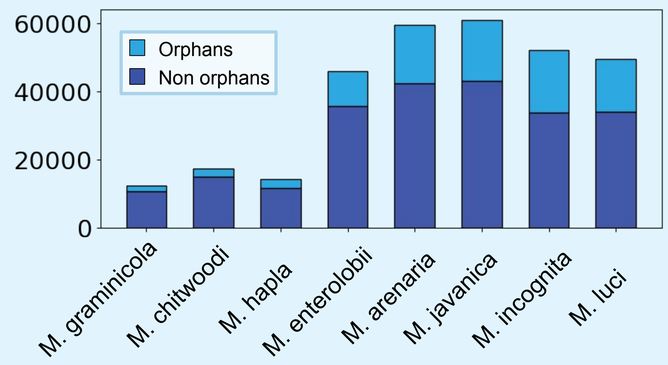

Figure 2: Number of orphan and non orphan genes for 8 Meloidogyne species studied
Barplot representing the number of orphan genes identified for each of the 8 Meloidogyne species. Each bar corresponds to the total number of genes of each species. Light blue represents orphans and dark blue represents non orphan genes.

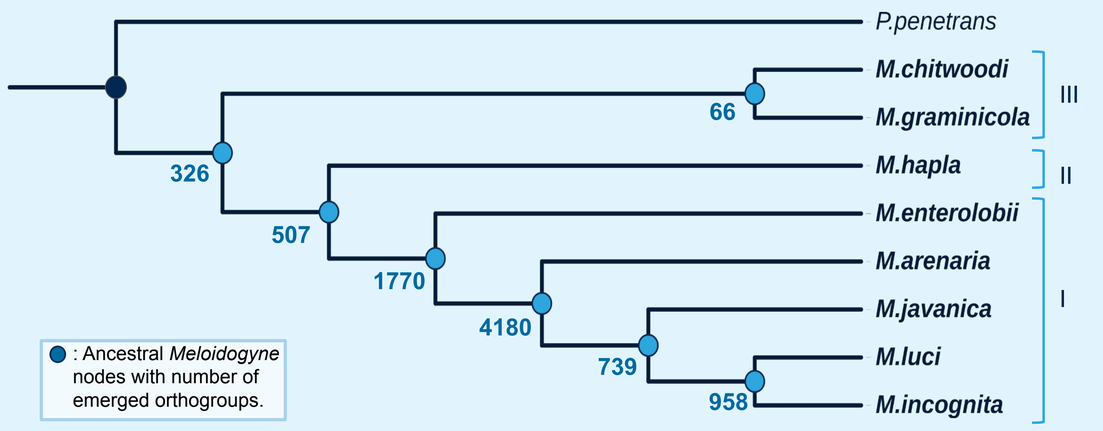

Figure 3: Species tree of Meloidogyne species with their closest outgroup species P.penetrans.
Species tree with 8 Meloidogyne species corresponding to three different clades (represented at the right of the species) of Meloidogyne genus along with P. penetrans, which is the closest outgroup to this genus. Each ancestral node is represented by a dot. Number of orphan orthogroups emerged in each node is given by a value next to it.

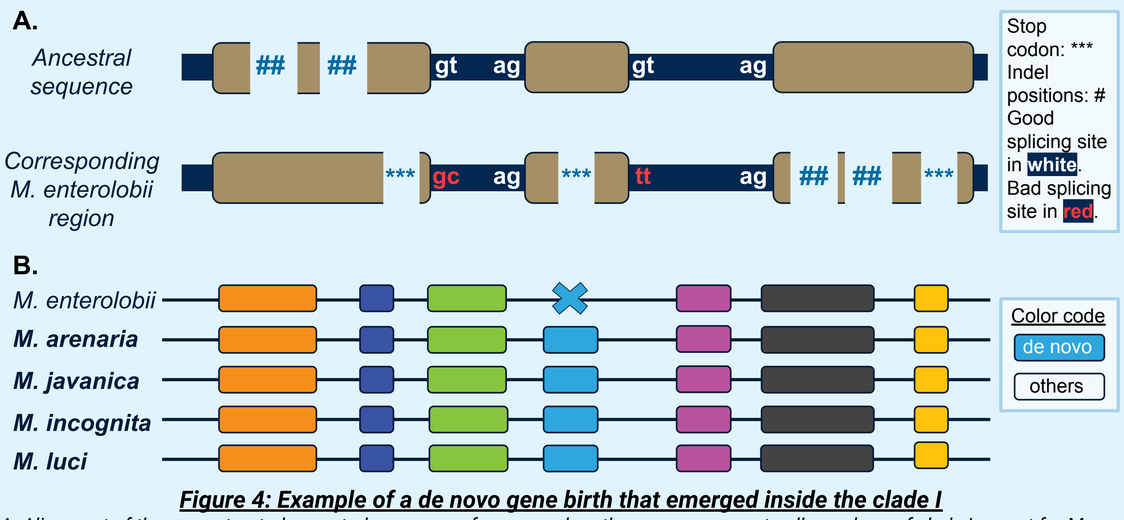

Figure 4: Example of a de novo gene birth that emerged inside the clade I
*A. Alignment of the reconstructed ancestral sequence of an example orthogroup common to all members of clade I except for M. enterolobii to the genome of M.enterolobii by Exonerate [15]. Brown represents exons and dark blue represents introns. Stop codons are represented by ***. Insertion-deletion positions (not a multiple of 3) are represented by #. Good splicing sites are in white, mutated ones in red. B. Verification of the syntenic conservation of the identified de novo gene. For the position where ancestral sequence aligns to M.enterolobii, closest 3 genes in both directions are studied to determine the conservation of the region. Lines corresponds to introns and boxes corresponds to genes. Blue boxes corresponds to the de novo gene and blue cross is where it aligns on M.enterolobii. The color code indicates where the same genes align on different species.*

CONCLUSION & DISCUSSION

- This study introduces a pipeline that combines homology, transcriptomics, and ancestral sequence reconstruction to identify and investigate the origin of orphan genes. This makes it possible to correctly distinguish between orphan genes that have diverged from pre-existing genes and those that have emerged *de novo*. To date, results indicate that **24%** of *Meloidogyne* genomes consist of **orphan genes**. At least **7% of these** are the result of ***de novo* gene birth**. Yet, it should be kept in mind that the results depend highly on the quality of genomes in disposition.
- Despite the fact that some effectors in *Meloidogyne* are coded by non-homologous genes, there is still no connection between the orphan genes and their functions in *Meloidogyne*. Thus, we are exploring structural approaches to better characterize these gene-structure-functions associations.

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