The complex hybrid origins of the root knot nematodes revealed through comparative genomics

Meloidogyne root knot nematodes (RKN) can infect most of the world's agricultural crop species and are among the most important of all plant pathogens. As yet however we have little understanding of their origins or the genomic basis of their extreme polyphagy. The most damaging pathogens reproduce by obligatory mitotic parthenogenesis and it has been suggested that these species originated from interspecific hybridizations between unknown parental taxa. We have sequenced the genome of the diploid meiotic parthenogen *Meloidogyne floridensis*, and use a comparative genomic approach to test the hypothesis that this species was involved in the hybrid origin of the tropical mitotic parthenogen *Meloidogyne incognita*. Phylogenomic analysis of gene families from *M. floridensis*, *M. incognita* and an outgroup species *Meloidogyne hapla* was carried out to trace the evolutionary history of these species' genomes, and we demonstrate that *M. floridensi*s was one of the parental species in the hybrid origins of *M. incognita*. Analysis of the *M. floridensis* genome itself revealed many gene loci present in divergent copies, as they are in *M. incognita*, indicating that it too had a hybrid origin. The triploid *M. incognita* is shown to be a complex double-hybrid between *M. floridensis* and a third, unidentified, parent. The agriculturally important RKN have very complex origins involving the mixing of several parental genomes by hybridization and their extreme polyphagy and success in agricultural environments may be related to this hybridization, producing transgressive variation on which natural selection can act. It is now clear that studying RKN variation via individual marker loci may fail due to the species' convoluted origins, and multi-species population genomics is essential to understand the hybrid diversity and adaptive variation of this important species complex. This comparative genomic analysis provides a compelling example of the importance and complexity of hybridization in generating animal species diversity more generally.

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Introduction

Root-knot nematodes (RKN) belong to the genus *Meloidogyne,* contain approximately 100 described species, and are globally important crop pathogens (Moens et al. 2009). The most frequent, widespread, and damaging species (*M. incognita*, *M. arenaria,* and *M. javanica*) are tropical RKN that are highly polyphagous, infecting crop species producing the majority of the world's food supply, with the damage attributable to RKN ~5% of world agriculture (Taylor & Sasser 1978; Trudgill & Blok 2001; Sasser & Carter 1985). The adaptive phenotypic diversity of these pathogens is also remarkable, with great variability observed both within and between species with respect to host range and isolate-specific vulnerability to control measures (Trudgill & Blok 2001; Castagnone-Sereno 2006). The tropical RKN typically reproduce by obligatory mitotic parthenogenesis and possess aneuploid genomes (Triantaphyllou 1982; Triantaphyllou 1985). These species have previously been suggested to be hybrid taxa, and phylogenetic analysis of nuclear loci supports this conclusion (Dalmasso & Berge 1983; Triantaphyllou 1985; Hugall et al. 1999; Castagnone-Sereno 2006; Lunt 2008).

Hybrid speciation has a long history of study in plants, with hybrid species formation having had a very significant influence on our understanding of species formation, diversity, and adaptation (Arnold 1997; P. S. Soltis & D. E. Soltis 2009). By contrast hybridization has been thought to be much less common in animals, though the utilization of multilocus genetics, and more recently genomics, has increased interest in the consequences of animal hybridization and several reviews suggest that it is much more common and important than previously thought (Mallet 2007; Mallet 2005; Bullini 1994; Nolte & Tautz 2010; Schwenk et al. 2008; Seehausen 2006). Although there have been repeated suggestions that the tropical ("Group 1") RKN might have hybrid origins, the parental species involved have never been identified. The phylogenies in Hugall *et al*. (1999) and Lunt (2008) indicate that these parents (as represented by divergent sequence clusters within the apomictic RKN) are more closely related to each other than either

is to *M. hapla*, though neither had a parental species within their sampling schemes. *Meloidogyne floridensis* is a plant pathogenic root knot nematode that was originally characterized as *M. incognita*, but has since been described as a separate species on the basis of its morphology and a unique *esterase* isozyme pattern (Jeyaprakash et al. 2006; Handoo et al. 2004). Despite both nuclear rRNA and mtDNA sequences placing it within the phylogenetic diversity of the tropical mitotic parthenogen (apomict) species (Tigano et al. 2005; Holterman et al. 2009) (Figure 1), *M. floridensis* is a meiotic parthenogen (automict) with the standard chromosome count of the meiotically reproducing RKN species (n=18), has bivalent chromosomes, and an observable meiotic division (Handoo et al. 2004). *M. floridensis* appears to suppress the second meiotic division which is a known form of automictic reproduction called first-division restitution (Bell 1982 p40). With the exception of *M. floridensis*, all of the Group 1 RKN (De Ley et al. 2002; Holterman et al. 2009) are apomicts, unable to reproduce by meiosis, lacking bivalents, and exhibiting extensive aneuploidy. This phylogenetic distribution of reproductive modes, with *M. floridensis* phylogenetically nested within the diversity of the apomict RKN (Figure 1), is unanticipated as it implies the physiologically unlikely route of re-emergence of meiosis from within the obligate mitotic parthenogens. An alternative explanation for these observations is that the observed phylogenetic relationships have not arisen from a typical ancestor-descendent bifurcating process, but instead have been shaped by reticulate evolution and transfer of genes by interspecific hybridization with *M. floridensis* a parent of the tropical apomict species.

The origins of *Meloidogyne incognita* genomic duplicates

The *M. incognita* genome revealed that many of the genes of this species are present as highly divergent copies (Abad et al. 2008), a situation that seems to apply to the other tropical apomicts too (Lunt 2008), though the origin of these divergent copies is controversial. One possible way to account for the high divergence between alleles is that they have originated by a process of 'endoduplication' (Figure 2A). Here we use endoduplication to refer to two distinct processes,

although their genomic outcomes are similar. Firstly, the entire *M. incognita* genome might have doubled to become tetraploid. The homologous chromosomes may have then diverged, and the extant pattern of partial retention of duplicated loci could be the result of gene loss. This process would leave many areas of the genome possessing divergent copies. Second, an alternative mechanism possible in apomictic species such as *M. incognita*, is that former alleles that are released from the homogenizing effects of recombination, can independently accumulate mutations over long periods of time resulting in highly divergent homologous loci ('alleles') within a diploid genome (White 1945 pg 283; Judson & Normark 1996).

Another possible explanation for a genome containing divergent homologous copies of many genes is interspecific hybridization. One (homeologous) copy is inherited from each parental species and the divergence between them derives from the divergence between the hybridizing taxa. It is likely here that all genes would be present as divergent copies, although gene conversion and related processes could homogenize some copies. If it originated by this second mechanism the resulting *M. incognita* genome would be a mosaic with genomic regions derived from both its parents.

There are several ways in which *M. incognita* and *M. floridensis* might be related through hybridization. *M. floridensis* might be one of the two parental species which hybridized to form the tropical apomicts, including *M. incognita* (Figure 2B). Alternatively, *M. floridensis* might be an independent hybrid that shares one parental taxon with *M. incognita*, and thus represents a 'sibling' hybrid taxon (Figure 2C). Finally, *M. floridensis* may itself be a hybrid, but still have played a role as a parent of *M. incognita* by a subsequent hybridization event (Figure 2D). This last option predicts three gene copies in *M. incognita* and two in *M. floridensis*.

The nuclear gene phylogenies of Lunt (2008) indicate that the parental taxa of the apomict RKN were closely related and derived from within the cluster of Group 1 *Meloidogyne* species after the divergence of *M. enterolobii* (=*M. mayaguensis*). Since this closely matches the phylogenetic position of *M. floridensis*, which is known to reproduce via sexual recombination as the parental

87 species also must have done, we set out to test by comparative genome sequencing and
88 analysis if *M. floridensis* was one of the progenitors of the tropical apomicts.

89 **Reproductive mode and *Meloidogyne* evolutionary history**

90 Given the unexpected distribution of meiosis across Group 1 *Meloidogyne* species described
91 above (Figure 1), there are several possible evolutionary pathways for the evolution of
92 reproductive modes (Figure 2): In scenario 1, *M. floridensis* has regained meiosis from an
93 apomict state. Alternatively (scenario 2), the numerous apomict species could have lost meiosis
94 many times independently. There are several additional scenarios involving hybrid origins. In
95 scenario 3, the apomicts have hybrid origins with the automict *M. floridensis* as a putative
96 parent, while in scenario 4 both *M. floridensis* and the apomicts have independent hybrid origins.
97 In scenario 5, a hybrid *M. floridensis* is in turn parental to a complex hybrid apomict.

98 Scenario 1 is very unlikely. Meiosis is an exceptionally complex system to re-evolve once it has
99 been lost (Dollo's law), and the only suggested example we are aware of in the literature is not
100 supported by robust reanalysis (see (Goldberg & Igić 2008)). In addition, the extant apomicts are
101 highly aneuploid, making it necessary for *M. floridensis* to have re-evolved 18 homologous
102 chromosome pairs, which again suggests that cytologically this route is highly unlikely. Scenario
103 2 is also not parsimonious, potentially implying very many independent major reproductive
104 transitions. Since there are already genetic data indicating that the apomicts may have hybrid
105 genomes (Lunt 2008), we focused our analyses on the much more biologically plausible
106 scenarios 3, 4 and 5 that propose hybridization drove the evolution of the apomictic RKN.

107 Scenario 3 restricts the hybrid taxa to the apomict Group 1 species, and places *M. floridensis* as
108 one of the hybridizing parental species (Figure 2B). This model makes predictions that, where
109 divergent homeologous sequences are detected in the *M. incognita* genome, *M. floridensis*
110 would possess two alleles closely related to one of these homeologues. The *M. floridensis*
111 genome itself would also be substantially different from that of *M. incognita*, not possessing

divergent homeologous blocks but rather displaying normal allelic variation, perhaps more similar to that observed in the *M. hapla* genome.

In scenarios 4 (Figure 2C) and 5 (Figure 2D) *M. floridensis* would also be a product of an interspecific hybridization, as are the apomicts. Both these scenarios predict that the *M. floridensis* genome will, like *M. incognita*, show substantial sequence divergence between homeologues throughout its genome, although it may also possess some regions where one parental copy has been eliminated, and remaining diversity is simple allelism. In scenario 4, the parents of *M. incognita* need not be the same as those of the apomicts, although the phylogenetic position of *M. floridensis* implies that at least one of them may have been identical or very closely related. The different putative hybrid origins of *M. incognita* predict two (scenario 4, Figure 2C) or three (scenario 5, Figure 2D) homeologous copies, potentially modified by subsequent loss events.

Here, we generate a *de novo* assembled genome for *M. floridensis*, identify and analyse a large number of sets of homologous sequences in *M. floridensis*, *M. incognita* and *M. hapla*, and use both gene copy number distributions and gene phylogenies to test the predictions of the different scenarios outlined in Figure 2.

Materials and Methods

Nematode materials

DNA from female egg mass cultures of *Meloidogyne floridensis* isolate 5 was generously sourced and provided from culture by Dr Tom Powers (University of Lincoln, Nebraska, USA) and Dr Janete Brito (Florida Department of Agriculture and Consumer Services, Gainesville, USA).

Sequencing and draft genome assembly

Meloidogyne floridensis DNA was prepared for sequencing using standard Illumina protocols by the GenePool Genomics Facility of the University of Edinburgh. A 260 bp insert library was sequenced using one lane of an Illumina HiSeq2000 (v2 reagents) with 101 base paired-end sequencing. 14.5 gigabases (Gb) of raw sequence data were adapter trimmed and quality filtered using perl and bash scripts to yield 70.2 M pairs totaling 13.2 Gb. The raw read data have been submitted to the Short Read Archive as accession ERP001338.

The genomic DNA sample derived from nematodes isolated from plant roots, and surrounded, therefore, by the bacterial communities of the rhizosphere. Egg masses of RKN are known to be associated with microbial taxa. To identify potential contaminants, we performed a preliminary assembly of all the trimmed reads ignoring pairing information. We then estimated read coverage of each assembled contig by mapping all reads back to the assembly, and annotated 10,000 randomly sampled contigs with the taxonomic order of their best megablast (BLAST+ version 2.2.25+ (Zhang et al. 2000)) match to the NCBI nt database (Benson et al. 2011). A taxon-annotated scatter plot of the GC% and coverage of each contig was used to visualize the contaminants present in the data (Supplementary Figure S1) (Kumar & Blaxter 2012). Distinct GC%-coverage clusters in this plot were annotated with distinct taxonomic matches. A major cluster annotated as nematode was clearly dominant. Additional minor clusters were annotated

as deriving from the bacterial orders Bacillales, Burkholderiales, Pseudomonadales and Rhizobiales. These all either had much lower coverage or much higher GC content than the nematode cluster. We conservatively removed contigs that matched the GC content and coverage of the identified contaminant blobs. To ensure optimal contamination removal, a second round of megablast searches was performed and any contigs that matched Bacterial databases were removed. Only reads mapping to the remaining, putatively nematode contigs and their pairs were retained for the next step. The true insert size distribution of these reads was also estimated by mapping the pairs back to the preliminary assembly.

A stringent reassembly of the cleaned read set (11.1 Gb) was performed using reliable coverage information estimated from the preliminary assembly GC%-coverage plot. Velvet v1.1.04 (Zerbino 2010; Zerbino & Birney 2008) was used with a k-mer value of 55 and the parameters -exp_cov 45, -cov_cutoff 4.5, and -ins_length 260. Other parameters and assemblers were also tried but this assembly had the best contig length optimality scores (e.g. N50, the contig length at which 50% of the assembly is in contigs of that length or greater) and the highest CEGMA values (using CEGMA version 2.3, (Parra et al. 2007)). Redundant contigs likely to derive from independent assembly of allelic copies were removed using CD-HIT-EST (version 4.5.5, (Li & Godzik 2006)) with -c 0.97 (removing all contigs that were more than 97% identical over their entire length to another, longer contig). The final assembly file is available as a blast database and fasta download at www.meloidogyne.org and meloidogyne.nematod.es.

Protein predictions and comparisons

A full annotation of the *M. floridensis* draft genome was not carried out, because no transcriptome data for the species was available. Instead, because we were interested in comparing coding sequences conserved with *M. hapla* and *M. incognita*, we used the protein2genome model in exonerate v2.2.0, (Slater & Birney 2005) to align all *M. hapla* and *M. incognita* proteins, derived from the published genome sequences, to the *M. floridensis* draft genome. We extracted coding sequences (CDSs) that aligned to at least 50% of the length of

the query protein sequences. If multiple *M. hapla* or *M. incognita* query protein sequences aligned to overlapping loci on the *M. floridensis* genome, only the longest locus was chosen as a putative *M. floridensis* CDS. The CDSs for all three species were trimmed after the first stop codon, and only sequences with a minimum of 50 amino acids were retained for further analysis.

To assess the level of self-identity among CDSs in each species, a BLASTn (version 2.2.25+, (Altschul et al. 1990)) search (with a sensitive E-value cutoff of 1e-5) was performed and the top scoring hit for each sequence to a CDS (other than itself) was selected if the length of the alignment was longer than 70% of the query sequence. The transcriipts of *M. incognita* were compared to the genomes of *M. floridensis* and *M. hapla* to identify levels of between species similarity using the same strategy.

Clustering

We used Inparanoid (version 4.1, (Ostlund et al. 2010)) and QuickParanoid (Kim n.d.) with default settings to assign proteins from the three *Meloidogyne* species to orthology groups. While assessing the level of duplication within the CDS sets (Figure 3), we noted that several *M. incognita* CDS sequences were identical or nearly identical (>98% identity). These are most likely derived from allelic variants rather than gene duplications (which show a separate peak between 95 and 97% identity). To simplify the construction of orthologous gene clusters, we reduced these near identical sequences in each species using CD-HIT-EST, removing any CDSs that were at least 98% identical across their whole length to another CDS.

Phylogenetic analyses

For each InParanoid cluster, Clustal Omega v1.0.3, (Sievers et al. 2011) was first used to align the protein sequences. Tranalign (from the Emboss suite, v6.2.0, (Rice et al. 2000)) was then used along with the protein alignment as a guide to align the nucleotide CDS sequences. Finally, RAxML v7.2.8, (Stamatakis 2006) was used to create maximum likelihood trees for each set of aligned CDS sequences in three steps: (i) finding the best ML tree by running the GTRGAMMA

model for 10 runs using the command “raxmlHPC-PTHREADS-SSE3 -m GTRGAMMA -s $a -# 10 -n $a -T 2”; (ii) getting the bootstrap support values for this tree by running the same model until the autoMRE convergence criterion was satisfied employed the command “raxmlHPC-PTHREADS-SSE3 -m GTRGAMMA -s $a -# autoMRE -n $a.b -T 2 -b 12345”; (iii) using the bootstrap trees to draw bipartitions on the best ML tree used the command “raxmlHPC-PTHREADS-SSE3 -m GTRCAT -f b -t RAxML_bestTree.$a -z RAxML_bootstrap.$a.b -n $a.l -T 2 -o mh”. Gene trees with a BP support of 70% or more were included in the analysis. The resulting trees were imported into the R Ape package v2.8, (Paradis et al. 2004) to count the number of trees with the same topology. Treefiles and scripts for processing trees can be obtained from DataDryad accession [to be advised].

Results

The genome of *Meloidogyne floridensis*

The *M. floridensis* genome was assembled using 11.1 Gb of cleaned data (see Supplementary Figure S1) from 116 M reads (an estimated ~100X coverage), using Illumina HiSeq2000 100 base paired-end sequencing of 250 bp fragments.

Intra-genomic comparisons reveal high numbers of duplicate genes in *M. incognita* and *M. floridensis*

Analysis of the distribution of within-genome CDS matches (Figure 3A) identified an unexpected excess of apparent duplication in *M. floridensis*. While the CDS set of *M. hapla* had a relatively low rate of duplication, and no excess of duplicates of any particular divergence level, both *M. incognita* and *M. floridensis* had many more duplicates and a peak of divergence between duplicates at 95 to 97% identity. *M. incognita* showed an additional peak at ~100% identity likely due to a failure to collapse allelic copies of some genes by the original authors (Abad et al. 2008). Because of the way we constructed our draft genome assembly, collapsing high-identity assembly fragments before analysis, *M. floridensis* lacked a near complete identity peak. The very high frequency of intragenomic duplicate copies with a consistent divergence level strongly suggest that either *M. floridensis*, like *M. incognita*, is a hybrid species, with contributions from two distinct parental genomes, or that it has undergone a whole genome duplication. These distinct possibilities are addressed below. Comparing CDS between species we identified a high frequency of near-100% identity between *M. incognita* and its best match in the *M. floridensis* genome (Figure 3B). This pattern was not evident when *M. incognita* was compared to *M. hapla*.

Distinguishing sibling from parent-child species relationships

We identified several models that might explain the observed levels of within-genome divergent duplicates in *M. incognita* and *M. floridensis* (Figure 3A). Expectations of relative numbers of (homeologous) gene copies per species, and the phylogenetic relationships of these homeologue sets differ and allow us to distinguish between the models. Thus for example under scenario 3 (Figure 2B) we test to determine if *M. incognita* has two divergent homeologous gene copies, one of which is phylogenetically very closely related to the (collapsed) allelic copies in *M. floridensis*. We therefore clustered the CDS of the three species using InParanoid, after removing all CDS encoding peptides less than 50 amino acids in length.

We defined 11,587 clusters that contained CDS from more than one species, and 4,018 that had representatives from all three species (Supplementary Figure S2). These represent a number and proportion similar to comparisons between other nematode species with complete genomes (e.g. 2,501 clusters were previously identified containing representatives from four nematode genomes (Mitreva et al. 2011)). As *M. hapla* is not expected to have undergone whole genome duplication, and we find no evidence of an excess of diverged duplicates in the *M. hapla* genome, we selected homologous gene sets where the ancestral gene was likely to have been single-copy by excluding clusters with more than one *M. hapla* member, and those lacking *M. hapla* members. We classified these clusters by the numbers of *M. incognita* and *M. floridensis* genes they contained (Table 2; Figure 4). The trees generated and the scripts used to parse them into the categories represented in Figure 4 are available through DataDryad.

The process of idiosyncratic gene loss (or failure to capture a gene in the draft sequencing and assembly) is evident in the numbers of genes that have one *M. hapla* representative and no members from either *M. incognita* (column 1 of Table 2) or *M. floridensis* (row 1 of table 2). Here it is striking that the clusters that contain only one *M. hapla* and one *M. floridensis* member (Mh1:Mf1:Mi0) outnumber by approximately two to one clusters that have one *M. hapla* and one *M. incognita* member (Mh1:Mf0:Mi1). This suggests that the *M. floridensis* genome draft is a good substrate for these analyses (it contains homologues of many conserved genes apparently

lost from, or missing in the draft assembly of, the *M. incognita* genome), and that the *M. incognita* draft is either incomplete or has experienced greater rates of gene loss.

The numbers of genes present in clusters that have two or more members, but lack one of *M. floridensis* or *M. incognita* (for example the 226 Mh1:Mf2:Mi0 clusters) reveal the potential extent of within-lineage duplication and divergence (and a component of stochastic loss of several homeologues in the missing species). There is no excess of these classes of cluster in *M. incognita*, arguing against a within-lineage, whole-genome duplication (i.e. against scenarios 1 or 2; Figure 2A).

The striking feature of the membership of clusters (Table 2) is the number of cases where *M. incognita* has more cluster members than does *M. floridensis*. Thus there are 920 clusters in the class Mh1:Mf1:Mi2, but only 257 in the class Mh1:Mf2:Mi1, and 102 clusters in the class Mh1:Mf1:Mi3 compared to 17 in the class Mh1:Mf3:Mi1. This finding argues for the presence in *M. incognita* of at least one more genome copy than in *M. floridensis*, i.e. that *M. incognita* is likely to be a degenerate triploid hybrid (scenario 5, Figure 2D). It is possible that some of the clusters in the Mh1:Mf1:Mi0 and Mh1:Mf0:Mi1 sets arise from *M. floridensis* and *M. incognita* being derived from different, divergent parents.

Phylogenomic analysis of homologue relationships

A second set of predictions from the models in Figure 2 concerns the phylogenetic relationships of the resulting sets of homologous gene sequences. Each model predicts a particular set of relationships between gene copies in each species. We therefore analyzed each informative set of clusters represented in Table 2 to identify which alternate topology was supported, assuming in each case that the single *M. hapla* representative was the outgroup. These phylogenomic results are summarized in Figure 4. For each informative set of clusters, the majority topology supported scenario 5 (Figure 2D), i.e. that *M. floridensis* is a hybrid, and was one of the parent species in a hybridization event that gave rise to a triploid *M. incognita*. Thus for the 902 Mh1:Mf1:Mi2 clusters, the topology in which one *M. incognita* CDS groups with the *M. floridensis*

CDS to the exclusion of the other *M. incognita* sequence was favoured in 79% of the clusters, while in only 201 clusters (21%) the two *M. incognita* genes instead appeared to have arisen by duplication within *M. incognita*. In the Mh1:Mf2:Mi2 cluster set, one third of the clusters supported the topology where there were two independent sister relationships between *M. incognita* and *M. floridensis* genes. A further 48% of the trees were congruent with a triploid status for *M. incognita* where gene loss (or lack of prediction) had removed one *M. incognita* representative. The other classes of clusters could be interpreted in the same manner, and displayed trends that supported scenario 5.

Discussion

The genome structure and content of tropical *Meloidogyne* is revealed by our analyses to have had complex origins. It is likely that hybridization, ploidy change, and subsequent aneuploidy have all played a role in the evolution of the diversity in this genus. The molecular evolutionary patterns revealed by comparative genomics however give us tools to conduct detailed analysis of these histories. This approach allows us to interpret the evolution of different reproductive strategies in terms of genome change, and better understand the evolution of these polyphagous pathogens.

The *M. floridensis* genome reveals its hybrid origins

Our draft assembly of the genome of *M. floridensis* reveals a relatively typical nematode genome. The base haploid genome size for Meloidogyninae is likely to be ~50 Mb. Both the sequenced genome of *M. hapla* (Opperman et al. 2008), and independent measurement of its genome size from densitometry (Pableo & Triantaphyllou 1989), yield estimates of 50-54 Mb. The sequenced genome estimate is unlikely to be inflated through issues of uncollapsed haploid contigs, as *M. hapla* is expected to have reduced heterozygosity through its automictic reproductive mode (Liu et al. 2007), and the sequenced strain was inbred (Opperman et al. 2008). Hybrid taxa, containing homeologous chromosomes from more than one parental lineage, would be expected to have genome assembly sizes that are the sum of the parental genomes, albeit modified by idiosyncratic post-hybridization gene loss and repeat copy change. Thus the ~100 Mb genome size estimated for *M. floridensis* is in keeping with a base Meloidogyninae genome of ~50 Mb, with homeologous sequences assembled independently. The divergence between inferred homeologous genes in our genome (~4-8%) would preclude coassembly of homeologous coding sequences, and the higher divergence found in intergenic and intronic sequences would make them even less likely to be coassembled. The published *M. incognita genome* is 86 Mb, but ongoing revision of the assembly suggests a true value of ~130

Mb Mb (Etienne Danchin, personal communication), as might be expected for a hypo-triploid species.

The *M. floridensis* genome assembly is less contiguous than those of *M. hapla* and *M. incognita* (reflected in the lower contiguity and content of conserved eukaryotic genes). Such fragmentation is a known limitation of using a single small-insert paired-end library, and refinement of the assembly using larger-insert mate pair, or long single molecule reads, would undoubtedly improve the biological completeness of the product. Our primary aim however was not to produce a highly contiguous assembly, but rather to identify protein-coding sequences (CDS) for use in comparative genomic analyses. Despite the fragmentation we were able to identify over 15,000 CDS segments to address the possible hybrid status of *M. floridensis* and *M. incognita*, making it more than sufficient for this study.

We note that both the *M. incognita* and the *M. floridensis* genomes have low scores (60-75%) when assessed by the Core Eukaryotic Genes Mapping Approach (CEGMA), compared to the 94% scored by the *M. hapla* assembly (and assemblies of other nematode genomes). However, the published *M. incognita* genome, while having much better assembly statistics (only 9,538 scaffolds, and a contiguity ~4 times that achieved for *M. floridensis*), has similarly poor scores in CEGMA analysis. Whether this is a reflection of shared divergent biology, or, as we suspect, a poor fragmented assembly, will require additional sequencing data, reassembly and reassessment.

The phylogenetic position of the automictic *M. floridensis* suggest that this species, or an immediate ancestor, was parental to the tropical apomicts, i.e. being one partner in the hybrid origins of the group (scenarios 3 and 5, Figure 2B, D). It is also possible however that *M. floridensis* is not directly parental to the apomicts, but rather a hybrid sibling, also arising by interspecific hybridization (scenario 4, Figure 2C). In this case one parent of *M. floridensis* is very likely to also have been involved in the hybrid origins of *M. incognita* as very many loci were found to be nearly identical between *M. incognita* and *M. floridensis* (Figure 3B). In order to

distinguish between scenario 3 (diploid parent), scenario 4 (hybrid sibling) and scenario 5 (hybrid parent) we examined the sequence diversity within each species' genome.

Intra-genomic Divergence of Coding Loci

Information concerning the hybrid status of *M. floridensis* can be gained from comparing the pattern of gene duplication within its genome to that of other RKN species, since *Meloidogyne incognita* has been suggested previously to have hybrid origins (Dalmasso & Berge 1983; Triantaphyllou 1985; Hugall et al. 1999; Castagnone-Sereno 2006; Lunt 2008) whereas *M. hapla* never has. An interspecific hybrid would be expected to have an excess of divergent intra-genomic duplicates compared to a non-hybrid, due to its homeologous chromosome pairs. The genome of *M. hapla*, a closely related species without a hybrid origin, represents the normal intra-genomic duplication pattern without homeologous chromosomes. In *M. hapla* there was a very much lower number of divergent duplicates compared to the other species, and these had a wide range of divergences rather than a frequency peak at any divergence value. While there was a slight excess of duplicates with high identity in *M. hapla*, the distribution overall is consistent with an ongoing rare process of stochastic duplication followed by gradual divergence (Figure 3A).

In contrast to the pattern observed in *M. hapla*, the intra-genomic comparisons of both *M. incognita* and *M. floridensis* revealed many more divergent duplicated CDS (Figure 3A). We observed a peak of high-identity duplicates in *M. incognita* that was absent in *M. floridensis*. This is most likely because we stringently collapsed high identity segments (as putative allelic copies) during assembly of *M. floridensis* whereas the *M. incognita* genome assembly may still contain some of these alleles. Most striking however was the presence in both species of a frequency peak of more diverged duplicates showing ~96% identity. Such duplicates have been described in *M. incognita* (Abad et al. 2008; Lunt 2008) although the scale of these diverged loci and their presence in *M. floridensis* has not been reported previously. Ongoing individual gene duplication events - which we propose has generated the *M. hapla* distribution - could not have produced

these patterns. Instead, the distributions are congruent with either a single major past event of genome duplication followed by divergence, or else hybridization to bring together pre-diverged homeologous chromosome copies that had been evolving independently since the last common ancestor of the parental species. On top of these processes differences in the rates of evolution of individual loci has resulted in variation in observed identity in the extant genomes, producing a distribution around a single peak of divergence. While these two alternative scenarios (endoduplication and homeologous chromosomes) cannot be distinguished on the basis of duplicate divergence data alone, the analysis does suggest that the genome content of both *M. floridensis* and *M. incognita* have been shaped in very similar ways by major duplication or divergence events.

Integrating Phylogenomic Analyses

To distinguish between endoduplication and hybrid origins of these CDS divergences, we examined the phylogenetic histories of sets of homologous loci from the three *Meloidogyne* genomes. By selecting CDS clusters with only a single member from the *M. hapla* genome we have likely restricted our analyses to loci that were single copy in the last common ancestor of the three species, and thus do not show the complexities of turnover in large multigene families.

We compared support on a gene-by-gene basis for tree topologies that would support or refute the hybrid *versus* endoduplication scenarios (Figure 2, Table 2 and Figure 4). Using this approach we could robustly exclude scenario 1, endoduplication of the *M. incognita* genome, as a source of duplicate CDS since we frequently observed that these *M. incognita* sequences were not monophyletic with respect to *M. floridensis*. If *M. incognita* had duplicated its own genome we would expect these duplicate CDS to share a recent origin and be each other's closest relatives. We could similarly exclude scenarios 2 and 3, since intra-genomic comparisons of CDS in the *M. floridensis* genome revealed that it also possesses divergent duplicates, and phylogenetic analyses indicated that these, just like the *M. incognita* sequences, are not monophyletic by species.

Thus we suggest that the most parsimonious explanation of the duplicate divergence and phylogenetic data is that both *M. floridensis* and *M. incognita* are hybrid species, and the duplicate CDS are homeologues rather than within-species paralogues. We can distinguish between scenario 4 (independent hybrid origins: the two species are step-sisters) and scenario 5 (*M. floridensis* represents one of the parents of a triploid hybrid *M. incognita*) by phylogenetic analyses of the clustered CDS. We observed an excess of clusters where there were more *M. incognita* members than there were *M. floridensis* members, as would be expected from a triploid species, whether or not it was now losing duplicated genes stochastically. In these clusters, the extra *M. incognita* CDS was less likely to be sister to one of the other *M. incognita* CDS than it was to be a sister to a *M. incognita - M. floridensis* pair. Based on these data we suggest that the triplicate loci in *M. incognita* are the three homeologues that have resulted from a hybridization event between the hybrid *M. floridensis* and an unidentified second, likely non-hybrid, parent (scenario 5, Figure 2D). For clusters containing two *M. floridensis* homeologues and two *M. incognita* homeologues, the topology supporting shared hybrid ancestry was again more frequently recovered than topologies supporting independent hybridization events.

Hybrid speciation and adaptive novelty

Animal hybrids have been characterized as rare, unfit, and adversely affected by both competition and gene flow from their parents (Mayr 1963; Barton 2001). There is now an increasing awareness in the literature however of animal hybridization as both a speciation mechanism and a route to the generation of novel phenotypic diversity on which natural selection may act (Bullini 1994; Arnold 1997; Mavarez & Linares 2008; P. S. Soltis & D. E. Soltis 2009; Abbott et al. 2013). There are a growing number of cases in which animal species have a hybrid origin, i.e.: it is known that all vertebrate constitutive parthenogens, and gynogenetic species have hybrid origins (Avise 2008); the Italian sparrow (*Passer italiae*) has been shown to be a nascent hybrid species (Hermansen et al. 2011); hybridization between two species of *Rhagoletis* tephritid fruitflies has led to expansion into a novel ecological niche (host plant) in the

hybrid, and also reproductive isolation from both parents since mating is confined to the host plant (Schwarz et al. 2005). The genetic basis of hybridization in generating adaptive diversity has been revealed in a number of studies: the *Heliconius melpomene* genome demonstrates that hybridization and introgression has been important for the adaptive radiation of these butterflies, by sharing protective colour-pattern genes among co-mimics (Dasmahapatra et al. 2012); the Northern European freshwater 'invasive sculpin' fish are hybrids between two geographically isolated *Cottus* species and they have colonized a novel niche consisting of the extensively human-altered lower reaches of the rivers Rhine and Scheldt (Czypionka et al. 2012); The cichlid adaptive radiation in Lake Malawi involves the evolution of more than 400 species, over a period of only 4.6 million years (Genner et al. 2007), which have colonized and adapted to many diverse lacustrine habitats. Recent genetic studies indicate that this radiation, and cichlid diversification in general, has been strongly influenced by interspecific hybridization (Joyce et al. 2011; Schwarzer et al. 2012; Loh et al. 2013; Genner & Turner 2012).

It has been suggested that hybrid animal taxa are most likely to succeed where new habitats open up, and such events may have played a significant role in several classic examples of adaptive radiation (Seehausen 2006; Abbott et al. 2013; Seehausen 2013; Kearney 2005). The tropical RKN are exceptionally successful globally-distributed pathogens of diverse agricultural crops (Moens et al. 2009; Trudgill & Blok 2001). These species have colonized a novel habitat, show extensive functional diversity, and have adapted to crop host-plants in the very brief evolutionary timeframe that agriculture has existed (a few thousand years). This is a situation similar to other animal adaptive radiations where hybridization may also have played a significant role (Seehausen 2006; Seehausen 2013; Abbott et al. 2013).

Although the adaptive consequences of hybridization are being increasingly recognized as important for biodiversity, ecology and evolution, the origin of novel traits, colonization of new ecological niches, and adaptive evolution can lead to serious problems if the organisms concerned are pathogens of humans, livestock, or crops (Bisharat et al. 2005; Brasier 2001;

Stukenbrock et al. 2012; Inderbitzin et al. 2011; Goss et al. 2011). It is particularly important therefore to understand the genetic basis of adaptive diversification in relation to existing or emerging pathogens.

The tropical apomictic RKN, exemplified by *M. incognita*, *M. arenaria* and *M. javanica*, possess host ranges that may include practically all agriculturally important species overlapping their distribution, causing *M. incognita* to be described as the “single most damaging crop pathogen in the world” (Trudgill & Blok 2001). Such extreme polyphagy is not typically encountered in *Meloidogyne* species outside of the radiation of tropical apomicts, although some do exploit multiple hosts. The origins and mechanisms of this greatly expanded host range are not only interesting from an evolutionary genomics perspective but also important to our understanding of the mode of action of these globally important crop pathogens. The demonstration of the hybrid origins of *M. incognita* and *M. floridensis*, and by implication *M. javanica* and *M. arenaria* also, suggests transgressive segregation of adaptive variation might have played an important role in determining host range. Transgressive segregation is when the absolute values of traits in some hybrids exceed the trait variation shown by either parental lineage. Such transgressive phenotypes are common in hybrid offspring in both animals and plants, and particularly so where the parents derive from inbred but divergent lineages (Rieseberg, Archer, et al. 1999a). Transgressive phenotypes have played a significant role in plant breeding, where crossing of inbred parental lineages can lead to extreme offspring variation onto which artificial selection is imposed, and similar processes are likely to act on hybrid swarms resulting from natural selection acting on inter-species crosses in the wild (Rieseberg, Archer, et al. 1999a; Stelkens & Seehausen 2009; Genner & Turner 2012). We do not yet know whether transgressive phenotypes in hybrid apomict RKN have been shaped by natural selection, but given our increasing awareness of its importance in adaptive radiations, and the frequency with which hybrid plant pathogens are detected in other systems (Stukenbrock et al. 2012; Stukenbrock & McDonald 2008; Inderbitzin et al. 2011; Brasier 2001), it may be an important direction for future research allowing us to detect likely pathogens at early stages.

Although we have not yet identified the parental taxa of *M. floridensis*, or the second parent of the tropical apomict RKN, it is likely that they were facultatively sexual meiotic parthenogens, as this is the most common reproductive mode within *Meloidogyne* (Triantaphyllou 1982; Triantaphyllou 1985; Chitwood & Perry 2009). This breeding system can fuse the products of a single meiotic division in order to regain diploidy, making these taxa more similar to the inbred lineages of plants highlighted as frequent sources of transgressive segregation and extreme phenotypes (Rieseberg, Whitton, et al. 1999b) than to the typical (amphimictic) species of hybridizing animals. If this "polyphagy as transgressive segregation" hypothesis were correct then we would predict that the parents of the polyphagous RKN would most likely be automicts with considerably smaller host ranges.

Hybridization and molecular genetic approaches to *Meloidogyne* diversity

Molecular approaches to understanding the diversity of apomictic RKN have a long history and include studies of isozymes, mitochondrial DNA (mtDNA), ribosomal internal transcribed spacer (ITS), ribosomal RNA genes (rDNA), random amplified polymorphic DNA markers (RAPDs), amplified fragment length polymorphisms (AFLPs), and other marker systems (see Blok and Powers (2009) for a review). However, if some *Meloidogyne* species are in fact hybrids, this presents particular problems for the standard molecular approaches used to characterize diversity. These typically assume that species or isolates have diverged following a bifurcating, tree-like, evolutionary pathway. Hybridization violates this assumption and produces more complex evolutionary histories that can either be misrepresented by single locus markers, or else produce intermediate or equivocal signal from multi-locus approaches. For example, a major reason that mtDNA and rDNA sequencing have been useful in evolutionary ecology is that they are effectively haploid, and hybrid taxa, which often retain just one of their parental species' genotypes at these loci, present particular problems for these approaches (Seehausen 2006; Hailer et al. 2012; Meyer et al. 2012). While carefully benchmarked marker approaches may still have utility in diagnostics, they will not be able to accurately reflect the complex evolutionary

pathway of hybrid *Meloidogyne* species where different loci are likely to have experienced very different histories. Incongruence between markers is therefore to be expected as a true reflection of history, rather than due to a lack of analytical power. We are currently in the early stages of *Meloidogyne* comparative genomics and current estimates of the complex phylogenetic relationships between hybrid taxa will need to be constantly refined as more species are added.

Genomic approaches to the RKN system hold many advantages, including documenting the genomic changes associated with host-specialization, extreme polyphagy, and interaction with plant defense systems. An interesting and important question now is whether the main apomictic RKN species have a single origin, with species divergence perhaps related to aneuploidy, or are instead the result of repeated hybridizations of the same or similar parental lineages. Different patterns of origin may determine the extent to which control strategies may be broadly or only locally applicable. Finally, if transgressive segregation is a cause of extreme and unique diversity, including polyphagy and novel resistance breaking isolates, then monitoring of new hybrid lineages may be an agricultural necessity. We are now close to the time where RKN isolates can be characterized not only with a trivial name (e.g. *M. incognita* race X) but instead a detailed list of genome wide variants and their known association with the environment, response to nematicides, and virulence against a range of plant host species and genotypes - an approach that will surely be extremely valuable in optimizing agricultural success. We caution therefore that although traditional genetic approaches may be valuable for rapid diagnostics, population genomics must be embraced in order to really advance our understanding of these important pathogens and maximize our ability to successfully intervene.

Conclusions

Here we have used whole genome sequencing and evolutionary comparative genomics to demonstrate the complex hybrid origins of key Root Knot Nematode species. Understanding the evolutionary history of *Meloidogyne* species is a priority since only by this route can the evolution

of pathogenicity and resistance, the emergence of new pathogenic strains, horizontal transfer of genes, and geographic spread of one of the world's most important crop pathogens be properly understood. The importance of animal hybridization to speciation and adaptation is being increasingly recognized, driven by new insights from genome sequencing. *Meloidogyne incognita* is shown to be an unusual double-hybrid, suggesting that hybridization may be a common and complex process in the history of this group. The *Meloidogyne* system, with its very recent expansion to fill numerous agricultural ecological niches, shows interesting parallels to natural adaptive radiations that may also have been greatly influenced by hybridization. Further work elucidating whether hybridization contributes adaptively to polyphagy will be important not just in the context of root knot nematodes, but also in determining the interplay of evolutionary forces generating organismal adaptive divergence more generally.

Acknowledgements

We thank Tom Powers and Janete Brito for sourcing and supplying *M. floridensis* materials, Etienne Danchin for access to *M. incognita* genome data and Marian Thomson and members of the GenePool Genomics Facility for sequencing support. We thank Africa Gómez, Amir Szitenberg, Steve Moss, Richard Ennos and Karim Gharbi for comments on the manuscript and the project.

References

Abad, P. et al., 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature biotechnology*, 26(8), pp.909–915. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18660804.

Abbott, R. et al., 2013. Hybridization and speciation. *Journal of Evolutionary Biology*, 26(2), pp.229–246.

Altschul, S.F. et al., 1990. Basic local alignment search tool. *Journal of molecular biology*, 215(3), pp.403–410.

Arnold, M.L., 1997. *Natural Hybridization and Evolution*, New York: Oxford University Press.

Avise, J., 2008. *Clonality:The Genetics, Ecology, and Evolution of Sexual Abstinence in Vertebrate Animals*, Oxford University Press, USA.

Barton, N.H., 2001. The role of hybridization in evolution. *Molecular Ecology*, 10(3), pp.551–568.

Bell, G., 1982. The Masterpiece of Nature: The Evolution and Genetics of Sexuality, University of California Press ISBN 0520045831

Benson, D.A. et al., 2011. GenBank. *Nucleic Acids Research*, 39(Database issue), pp.D32–7.

Bisharat, N. et al., 2005. Hybrid *Vibrio vulnificus*. *Emerging infectious diseases*, 11(1), pp.30–35.

Blok, V.C. & Powers, T.O., 2009. Biochemical and molecular identification. In *Root-knot nematodes*. Wallingford, UK: CABI Publishing, pp. 98–118.

Brasier, C.M., 2001. Rapid evolution of introduced plant pathogens via interspecific hybridization. *Bioscience*, 51(2), p.123.

Bullini, L., 1994. Origin and evolution of animal hybrid species. *Trends In Ecology & Evolution*, 9(11), pp.422–426.

Castagnone-Sereno, P., 2006. Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes. *Heredity*, 96(4), pp.282–289.

Chitwood, D.J. & Perry, R.N., 2009. Reproduction, Physiology and Biochemistry. In R. N. Perry, M. Moens, & J. L. Starr, eds. *Root-knot nematodes*. CAB International, pp. 182–200.

Czypionka, T. et al., 2012. Transcriptome changes after genome-wide admixture in invasive sculpins (*Cottus*). *Molecular Ecology*, 21(19), pp.4797–4810.

Dalmasso, A. & Berge, J.B., 1983. Enzyme polymorphism and the concept of parthenogenetic species, exemplified by *Meloidogyne*. In S. AR, P. HM, & K. LF, eds. *Concepts in nematode systmatics*. Academic Press.

Dasmahapatra, K.K. et al., 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature*, 487, pp.94–98.

De Ley, I.T. et al., 2002. Phylogenetic Analyses of *Meloidogyne* Small Subunit rDNA. *Journal of*

Nematology, 34(4), pp.319–327.

Genner, M.J. & Turner, G.F., 2012. Ancient hybridization and phenotypic novelty within Lake Malawi's cichlid fish radiation. *Molecular Biology and Evolution*, 29(1), pp.195–206.

Genner, M.J. et al., 2007. Age of cichlids: new dates for ancient lake fish radiations. *Molecular Biology and Evolution*, 24(5), pp.1269–1282.

Goldberg, E.E. & Igić, B., 2008. On phylogenetic tests of irreversible evolution. *Evolution; international journal of organic evolution*, 62(11), pp.2727–2741.

Goss, E.M. et al., 2011. The plant pathogen *Phytophthora andina* emerged via hybridization of an unknown *Phytophthora* species and the Irish potato famine pathogen, *P. infestans*. *PLoS ONE*, 6(9), p.e24543.

Hailer, F. et al., 2012. Nuclear Genomic Sequences Reveal that Polar Bears Are an Old and Distinct Bear Lineage. *Science (New York, NY)*, 336(6079), pp.344–347.

Handoo, Z.A. et al., 2004. Morphological, Molecular, and Differential-Host Characterization of *Meloidogyne floridensis* n. sp. (Nematoda: Meloidogynidae), a Root-Knot Nematode Parasitizing Peach in Florida. *Journal of Nematology*, 36(1), pp.20–35.

Hermansen, J.S. et al., 2011. Hybrid speciation in sparrows I: phenotypic intermediacy, genetic admixture and barriers to gene flow. *Molecular Ecology*, 20(18), pp.3812–3822.

Holterman, M. et al., 2009. Small Subunit rDNA-Based Phylogeny of the Tylenchida Sheds Light on Relationships Among Some High-Impact Plant-Parasitic Nematodes and the Evolution of Plant Feeding. *Phytopathology*, 99(3), pp.227–235.

Hugall, A., Stanton, J. & Moritz, C., 1999. Reticulate evolution and the origins of ribosomal internal transcribed spacer diversity in apomictic *Meloidogyne*. *Molecular Biology and Evolution*, 16(2), pp.157–164.

Inderbitzin, P. et al., 2011. The ascomycete *Verticillium longisporum* is a hybrid and a plant pathogen with an expanded host range. *PLoS ONE*, 6(3), p.e18260.

Jeyaprakash, A. et al., 2006. Differentiation of *Meloidogyne floridensis* from *M. arenaria* using high-fidelity PCR amplified mitochondrial AT-rich sequences. *Nematropica*, 36(1), pp.1–12. Available at: http://brokert10.fcla.edu/DLData/NM/NM00000014/NM00995444/1_12.pdf.

Joyce, D.A. et al., 2011. Repeated colonization and hybridization in Lake Malawi cichlids. *Current Biology*, 21(6), p.526.

Judson, O.P. & Normark, B.B., 1996. Ancient asexual scandals. *Trends In Ecology & Evolution*, 11(2), pp.41–46.

Kearney, M., 2005. Hybridization, glaciation and geographical parthenogenesis. *Trends In Ecology & Evolution*, 20(9), pp.495–502.

Kim, T., QuickParanoid - A Tool for Ortholog Clustering. *pl.postech.ac.kr*.

Kumar, S. & Blaxter, M.L., 2012. Simultaneous genome sequencing of symbionts and their hosts. *Symbiosis*, 55(3), pp.119–126.

Li, W. & Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics (Oxford, England)*, 22(13), pp.1658–1659.

Liu, Q.L., Thomas, V.P. & Williamson, V.M., 2007. Meiotic parthenogenesis in a root-knot nematode results in rapid genomic homozygosity. *Genetics*, 176(3), pp.1483–1490. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1931544\&tool=pmcentrez\&rendertype=abstract.

Loh, Y.-H.E. et al., 2013. Origins of Shared Genetic Variation in African Cichlids. *Molecular Biology and Evolution*.

Lunt, D., 2008. Genetic tests of ancient asexuality in root knot nematodes reveal recent hybrid origins. *BMC Evolutionary Biology*, 8(1), p.194.

Mallet, J., 2007. Hybrid speciation. *Nature*, 446(7133), pp.279–283.

Mallet, J., 2005. Hybridization as an invasion of the genome. *Trends In Ecology & Evolution*, 20(5), pp.229–237.

Mavarez, J. & Linares, M., 2008. Homoploid hybrid speciation in animals. *Molecular Ecology*, 17(19), pp.4181–4185.

Mayr, E., 1963. *Animal species and evolution*, Harvard University Press.

Meyer, M. et al., 2012. A High-Coverage Genome Sequence from an Archaic Denisovan Individual. *Science (New York, NY)*, 338(6104), pp.222–226.

Mitreva, M. et al., 2011. The draft genome of the parasitic nematode *Trichinella spiralis*. *Nature Genetics*, 43(3), pp.228–235.

Moens, M., Perry, R.N. & Starr, J.L., 2009. *Meloidogyne* species – a Diverse Group of Novel and Important Plant Parasites. In *Root-knot nematodes*. CAB International, pp. 1–17.

Nolte, A.W. & Tautz, D., 2010. Understanding the onset of hybrid speciation. *Trends in Genetics*, 26(2), pp.54–58.

Opperman, C.H. et al., 2008. Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. *Proceedings of the National Academy of Sciences of the United States of America*, 105(39), pp.14802–14807.

Ostlund, G. et al., 2010. InParanoid 7: new algorithms and tools for eukaryotic orthology analysis. *Nucleic Acids Research*, 38(Database issue), pp.D196–203.

Pableo, E.C. & Triantaphyllou, A.C., 1989. DNA complexity of the root-knot nematode (*Meloidgyne* spp.) genome. *Journal of Nematology*, 21, pp.260–263.

Paradis, E., Claude, J. & Strimmer, K., 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics (Oxford, England)*, 20(2), pp.289–290.

Parra, G., Bradnam, K. & Korf, I., 2007. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. *Bioinformatics (Oxford, England)*, 23(9), pp.1061–1067.

Rice, P., Longden, I. & Bleasby, A., 2000. EMBOSS: the European Molecular Biology Open Software Suite. *Trends in Genetics*, 16(6), pp.276–277.

Rieseberg, L.H., Archer, M.A. & Wayne, R.K., 1999a. Transgressive segregation, adaptation and speciation. *Heredity*, 83 (Pt 4), pp.363–372.

Rieseberg, L.H., Whitton, J. & Gardner, K., 1999b. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics*, 152(2), pp.713–727.

Sasser, J.N. & Carter, C.C., 1985. Overview of the International Meloidogyne Project 1975-1984. In K. R. Barker, C. C. Carter, & J. N. Sasser, eds. *An Advanced treatise on Meloidogyne*. Dept. of Plant Pathology. North Carolina State Univ.

Schwarz, D. et al., 2005. Host shift to an invasive plant triggers rapid animal hybrid speciation. *Nature*, 436(7050), pp.546–549.

Schwarzer, J. et al., 2012. Repeated trans-watershed hybridization among haplochromine cichlids (Cichlidae) was triggered by Neogene landscape evolution. *Proceedings Biological sciences / The Royal Society*, 279(1746), pp.4389–4398.

Schwenk, K., Brede, N. & Streit, B., 2008. Introduction. Extent, processes and evolutionary impact of interspecific hybridization in animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1505), pp.2805–2811.

Seehausen, O., 2013. Conditions when hybridization might predispose populations for adaptive radiation. *Journal of Evolutionary Biology*, 26(2), pp.279–281.

Seehausen, O., 2006. Hybridization and adaptive radiation. *Trends In Ecology & Evolution*, 19(4), pp.198–207.

Sievers, F. et al., 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular systems biology*, 7, p.539.

Slater, G.S.C. & Birney, E., 2005. Automated generation of heuristics for biological sequence comparison. *BMC Bioinformatics*, 6, p.31.

Soltis, P.S. & Soltis, D.E., 2009. The role of hybridization in plant speciation. *Annual review of plant biology*, 60, pp.561–588.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics (Oxford, England)*, 22(21), pp.2688–2690.

Stelkens, R. & Seehausen, O., 2009. Genetic distance between species predicts novel trait expression in their hybrids. *Evolution; international journal of organic evolution*, 63(4), pp.884–897.

Stukenbrock, E.H. & McDonald, B.A., 2008. The origins of plant pathogens in agro-ecosystems. *Annual Review of Phytopathology*, 46, pp.75–100.

Stukenbrock, E.H. et al., 2012. Fusion of two divergent fungal individuals led to the recent emergence of a unique widespread pathogen species. *Proceedings of the National Academy of Sciences of the United States of America*, 109(27), pp.10954–10959.

Taylor, A.L. & Sasser, J.N., 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). *International Meloidogyne Project,Raleigh, North*.

692 Tigano, M.S. et al., 2005. Phylogeny of *Meloidogyne* spp. based on 18S rDNA and the intergenic
693 region of mitochondrial DNA sequences. *Nematology*, 7(6), pp.851–862.

694 Triantaphyllou, A.C., 1982. Cytogenetics and sexuality of root-knot and cyst nematodes. In R. D.
695 Riggs, ed. *Nematology in the southern region of the United States*. Arkansas Agricultural
696 Experiment Station, Fayetteville, AK: Southern Cooperative Series Bulletin, pp. 71–76.

697 Triantaphyllou, A.C., 1985. Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes.
698 In J. N. Sasser & C. C. Carter, eds. *An Advanced Treatise on Meloidogyne*. North Carolina
699 State University, Raleigh.

700 Trudgill, D.L. & Blok, V.C., 2001. Apomictic, polyphagous root-knot nematodes: Exceptionally
701 successful and damaging biotrophic root pathogens. *Annual Review of Phytopathology*, 39,
702 pp.53–77.

703 White, M.J.D., 1945. *Animal Cytology & Evolution* 1st ed, Cambridge University Press.

704 Zerbino, D.R., 2010. Using the Velvet de novo assembler for short-read sequencing
705 technologies. *Current protocols in bioinformatics*, Chapter 11, p.Unit 11.5.

706 Zerbino, D.R. & Birney, E., 2008. Velvet: algorithms for de novo short read assembly using de
707 Bruijn graphs. *Genome Research*, 18(5), pp.821–829.

708 Zhang, Z. et al., 2000. A greedy algorithm for aligning DNA sequences. *Journal of computational
709 biology : a journal of computational molecular cell biology*, 7(1-2), pp.203–214.

Figure 1

The relationships of tropical apomict *Meloidogyne*

This cartoon summarizes the relationships of the tropical apomict *Meloidogyne* root knot nematodes ("Group 1") to other *Meloidogyne*. *Meloidogyne floridensis* is a Group 1 species that can reproduce by meiotic parthenogenesis (blue colouration) while all other Group 1 species are obligate mitotic parthenogens (red colouration). *Meloidogyne hapla* is a meiotic parthenogenic species in Group 2. We have not used bifurcating trees to represent the relationships within the Group 1 and 2 species because of issues (highlighted in this paper) concerning possible hybrid origins of some taxa.

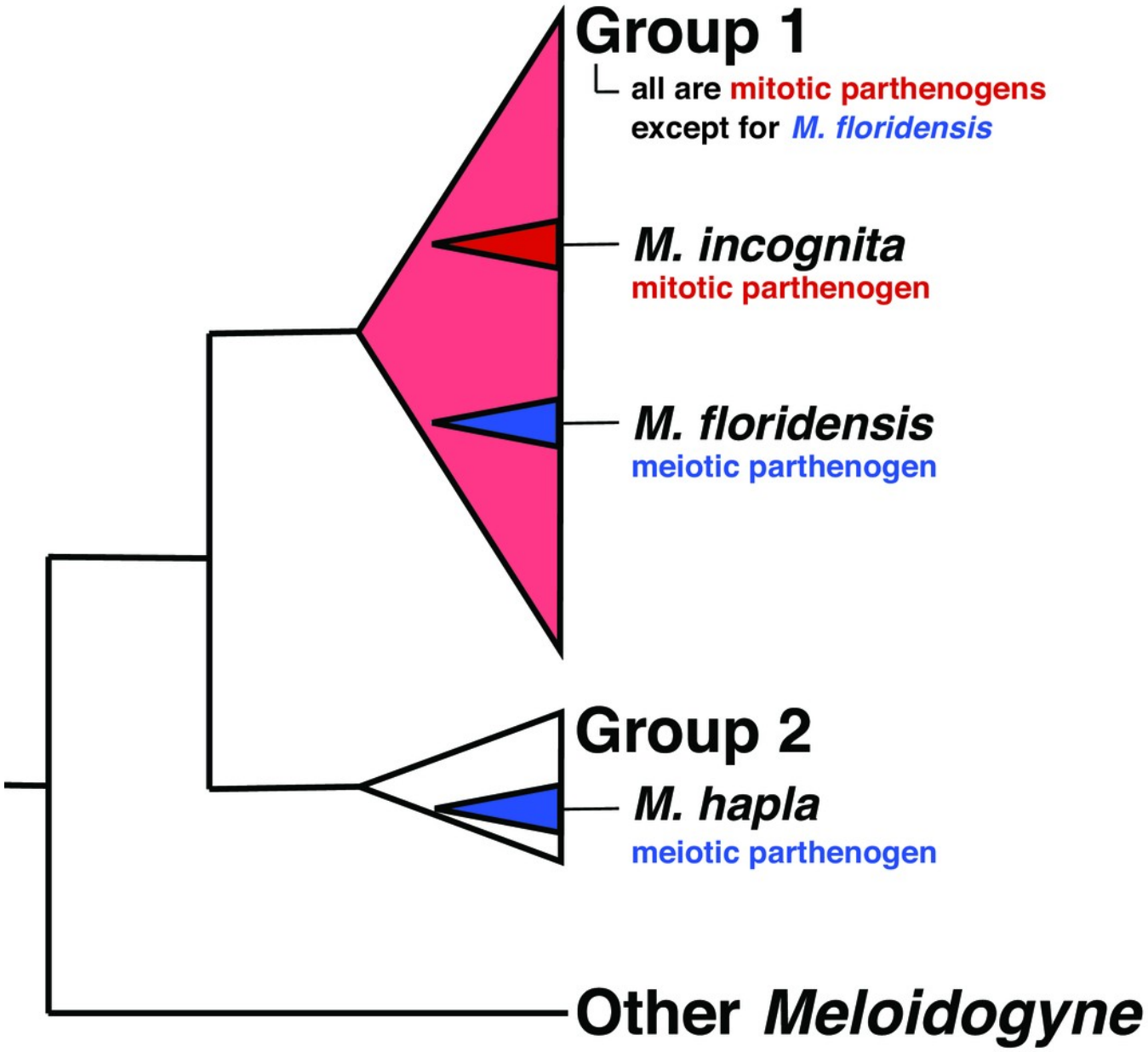
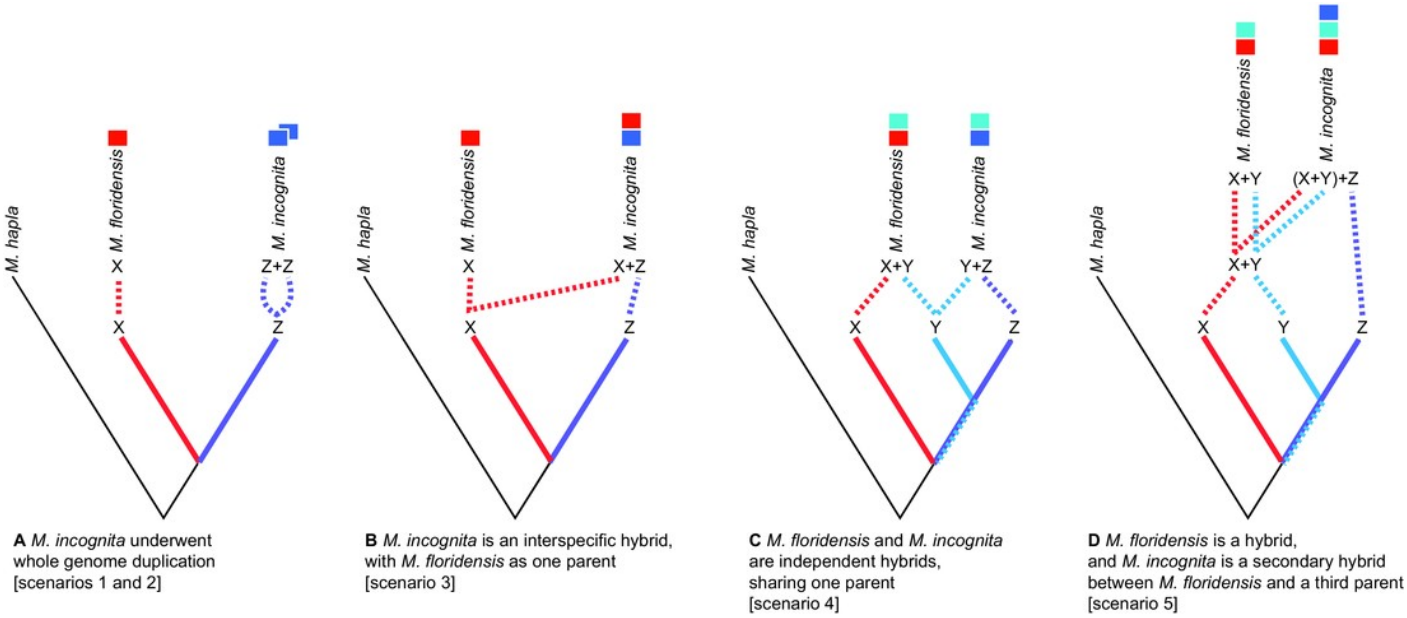


Figure 2

Scenarios of the possible relationships between *Meloidogyne floridensis*, *Meloidogyne incognita* and *Meloidogyne hapla*, and the origins of duplicated gene copies

M. hapla is a diploid species in a different sub-generic group to that of *M. incognita* and *M. floridensis*. Species “X”, “Y” and “Z” are postulated ancestral parents that could have given rise to *M. incognita* and *M. floridensis*. A. Scenarios 1 and 2: Here *M. floridensis* is a diploid sister species to *M. incognita* and possesses the “X” genome. Scenario 1 postulates reacquisition of apomixis in *M. floridensis* from an apomict ancestor, while Scenario 2 postulates that the apomicts repeatedly lost meiosis independently. Under both these scenarios, the presence of significant duplications in *M. incognita* suggests that it has undergone whole genome endoduplication. The duplicated genomes (“Z+Z”) in *M. incognita* are diverging under Muller’s ratchet. B. Scenario 3: Ancestor “X” gave rise to the diploid species *M. floridensis*, and also interbred with “Z” to yield *M. incognita*, which thus carries two divergent copies of each gene (“X+Z”). In this model only *M. incognita*, not *M. floridensis*, is predicted to carry two homeologues of many genes. C. Scenario 4: Both *M. floridensis* (“X+Y”) and *M. incognita* (“Y+Z”) are hybrid species, and share one parent (“Y”). In this model both *M. incognita* and *M. floridensis* are predicted to carry two homeologues of many genes. D. Scenario 5: Both *M. floridensis* (“X+Y”) and *M. incognita* (“X+Y+Z”) are hybrid species, but *M. incognita* is a triploid hybrid between the hybrid *M. floridensis* ancestor (“X+Y”) and another species (“Z”). In this model *M. incognita* is predicted to carry three, and *M. floridensis* is predicted to carry two, homeologues of many genes.



A *M. incognita* underwent whole genome duplication [scenarios 1 and 2]

B *M. incognita* is an interspecific hybrid, with *M. floridensis* as one parent [scenario 3]

C *M. floridensis* and *M. incognita* are independent hybrids, sharing one parent [scenario 4]

D *M. floridensis* is a hybrid, and *M. incognita* is a secondary hybrid between *M. floridensis* and a third parent [scenario 5]

Figure 3

Inter- and intra-genomic identification of duplicated protein-coding regions

A Each coding sequence from each of the three target genomes (*M. hapla*, *M. incognita* and *M. floridensis*) was compared to the set of genes from the same species. The percent identity of the best matching (non-self) coding sequence was calculated, and is plotted as a frequency histogram. Both *M. incognita* and *M. floridensis* show evidence of the presence of many duplicates, while *M. hapla* does not. **B** The *M. incognita* gene predictions were compared to the *M. floridensis* genome and the *M. hapla* gene set. For each *M. incognita* gene, the similarity of the top matches in each genome was assessed. *M. incognita* has many genes that are highly similar to those of *M. floridensis* (similarity >98%). This contrasts with the matches to *M. hapla*, where the modal similarity is ~92%, and there is no peak of high-similarity matches.

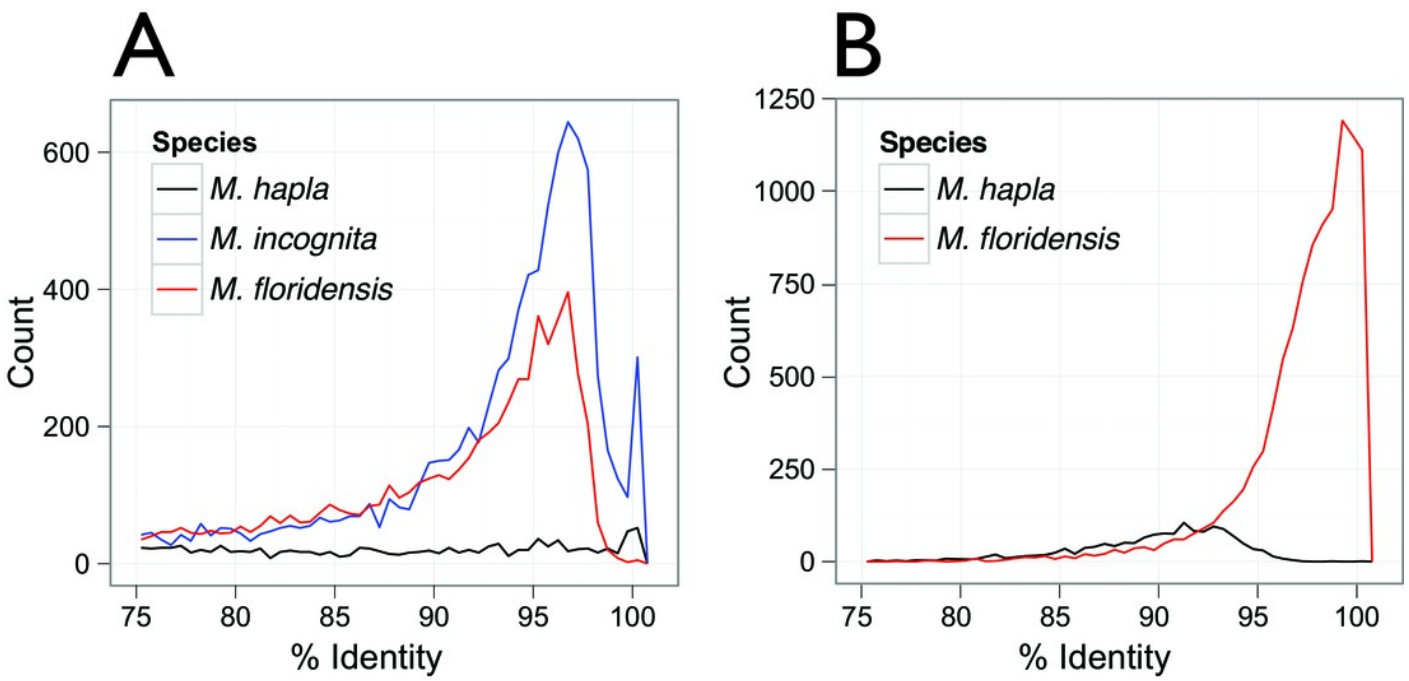


Figure 4

Phylogenomic analyses of clustered gene sets

For cluster sets represented in Table 2 that had representation of both *M. floridensis* and *M. incognita*, more than three members (i.e. where there was more than one possible topology), and fewer than five total members (i.e. where the number of possible topologies was still reasonably low and close to the number of clusters to be analyzed), we generated an estimate of the relationships between the sequences using RAxML. The resultant trees were bootstrapped, and rooted using the *M. hapla* representative. For each cluster set, the topologies were summarized by the different unique patterns possible. Within each figure cell, each cladogram in the figure is scaled by the number of clusters that returned that topology, with terminal nodes coloured by the origin of the sequences (black representing *M. hapla*, blue *M. incognita*, and red *M. floridensis*). The number of clusters congruent with each cladogram is given above the trees. The numbers of clusters contributing to each cell in the figure is represented by the grey box, which is scaled by the number of clusters summarized (e.g. the box in the central cell represents 902 trees, while the box in the bottom left cell represents 17 trees).

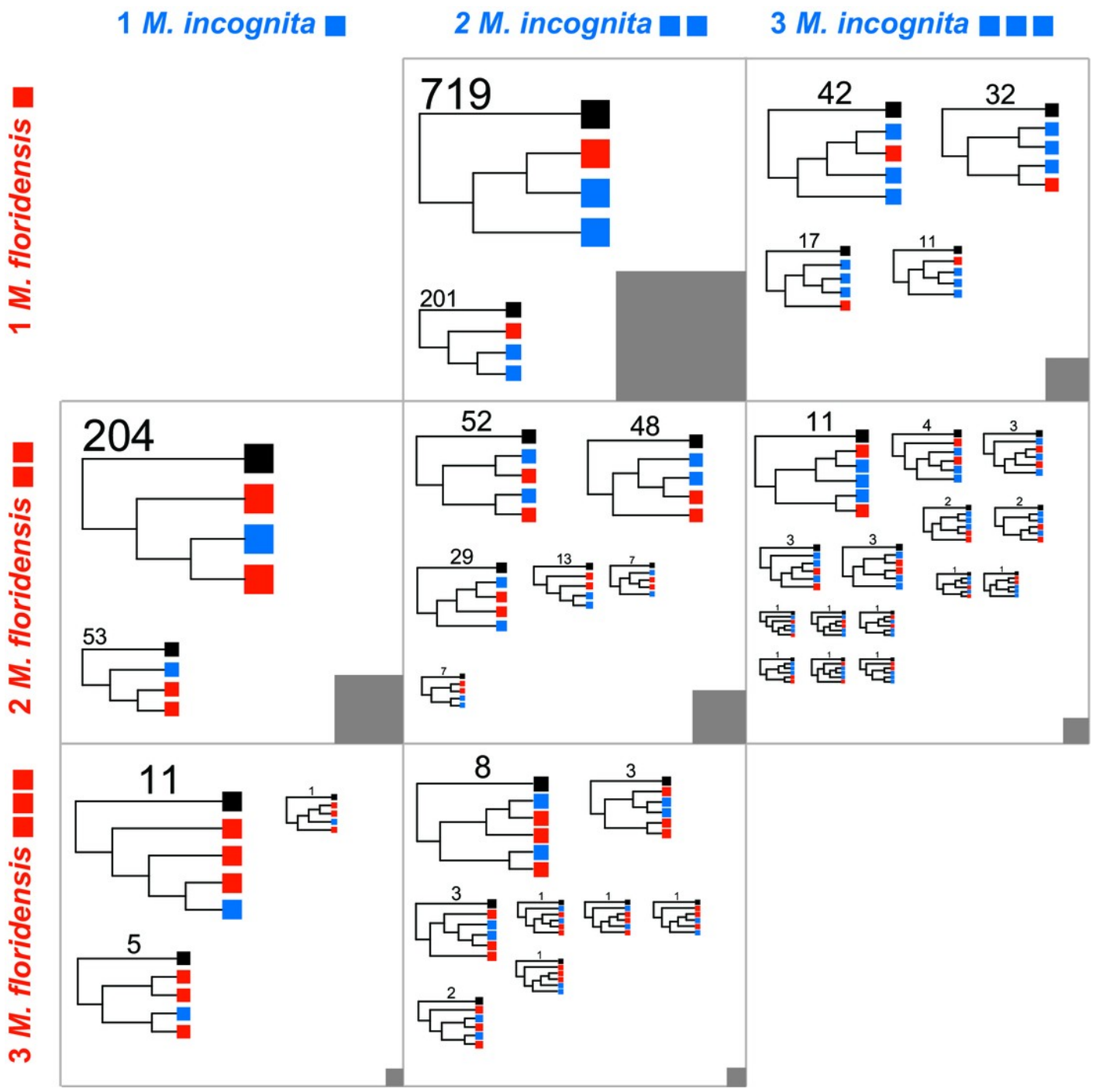


Table 1(on next page)

Summary statistics describing genome assemblies of *Meloidogyne*

Species	*Meloidogyne hapla*	*Meloidogyne incognita*	*Meloidogyne floridensis*
Source	NCSU / WormBase WS227	INRA / WormBase WS227	959 Nematode Genomes Project
Data URL	ftp://ftp.wormbase.org/pub/wormbase/species/m_hapla/	ftp://ftp.wormbase.org/pub/wormbase/species/m_incognita/	http://downloads.nematodegenomes.org
Citation	(Opperman et al. 2008)	(Abad et al. 2008)	this work
Maximum scaffold length	360,446	154,116	40,762
Number of scaffolds	3,452	9,538	81,111
Assembled size (bp)	53,017,507	82,095,019	99,886,934
Scaffold N50* (bp)	37,608	12,786	3,516
GC%	27.4	31.4	29.7
CEGMA** completeness Full / Partial	92.74 / 94.35	75.00 / 77.82	60.08 / 72.18
Predicted proteins (used for clustering***)	13,072 (12,229)	20,359 (17,999)	15,327 (15,121)

* N50: weighted median contig length; the contig length at which 50% of the assembled genome is present in contigs of that or greater length.

** CEGMA: Core Eukaryotic Genes Mapping Approach (Parra et al. 2007).

*** Predicted proteins used for clustering and inferring phylogenies after filtering for length >50 amino acids (see Methods).

Table 2(on next page)

Numbers of *Meloidogyne floridensis* and *Meloidoigyne incognita* members in homeologue gene sets that have one *Meloidogyne hapla* member

	0 *M. incognita* members	1 *M. incognita* member	2 *M. incognita* members	3 *M. incognita* members	>3 *M. incognita* members
0 *M. floridensis* members	0	907	327	44	17
1 *M. floridensis* member	2196	2189	920	102	40
2 *M. floridensis* members	226	257	156	36	21
3 *M. floridensis* members	17	17	20	7	14
>3 *M. floridensis* members	8	11	6	4	21