

Mitochondrial Genomics in the Genus *Phytophthora* With a Focus on *Phytophthora ramorum*¹

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Abstract

The mitochondrial genomes of *Phytophthora infestans*, *P. ramorum* and *P. sojae* have been sequenced and comparative genomics has provided an opportunity to examine the processes involved with genome evolution in the genus *Phytophthora*. This approach can also be useful in assessing intraspecific genome evolution and identification of cytoplasmic markers that will be useful in population studies. Polymorphisms have been observed in the mitochondrial genomic sequences of *P. ramorum* isolates from North America and Europe (13 single base changes and an insertion of 180 bp in the European isolate). The development of seven primer pairs for amplification and sequencing of some of these polymorphic regions has identified four mitochondrial haplotypes. The two most common ones represent the North American and European lineages, but there also is a third haplotype representing the third lineage of the pathogen (from Washington State) and a fourth haplotype found in several isolates recovered from the forest ecosystem in Oregon.

Key words: Intraspecific polymorphisms, mitochondrial molecular markers.

Introduction

Due to their rates of sequence divergence, mitochondrial genomes can provide a valuable tool for studying evolutionary relationships among groups of organisms as well as provide molecular tools for isolate identification to a species, and sometimes subspecies level. The mitochondrial genomes in the genus *Phytophthora* are uniparentally inherited (from the maternal parent), in the size range of 40 kb and map to a circular orientation (reviewed in Martin and others 2007). Unlike the closely related genus *Pythium*, the mitochondrial genomes of *Phytophthora* do not have a large inverted repeat (IR; in *Pythium* the IR can represent approximately 75 percent of the genome, McNabb and others 1987). However, based on restriction mapping and Southern analysis, a short inverted repeat of 0.5 to 0.9 kb in size was observed in *P. megasperma* (Schumard-Hudspeth and Hudspeth 1990). The IR is believed to stabilize the mitochondrial genome and reduce the potential for genome rearrangements.

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The mitochondrial genomic sequences for the four mitochondrial haplotypes of *P. infestans* have been published (Paquin and others 1997, Avila-Adame and others 2006) and comparative genomics revealed that intraspecific polymorphisms were due to both single nucleotide substitutions dispersed throughout the genome as well as length variations caused by insertions/deletions that occurred primarily in two locations (Avila-Adame and others 2006). More recently, the mitochondrial genomic sequences of *P. ramorum* (California forest isolate) and *P. sojae* have become available (Martin and others 2007). These species had the same 68 coding regions that were identified in *P. infestans*, inferred to encode mitochondrial respiratory chain proteins, subunits of the mitoribosome, ribosomal RNAs, tRNAs, and unassigned ORFs that were conserved among the *Phytophthora* spp. Comparative genomics among these species indicated the gene order for *P. ramorum* and *P. sojae* were identical, but comparison with *P. infestans* revealed two inversions. It was also apparent that certain regions of the genome were more polymorphic than others and the terminal ends of the inversions in *P. infestans* corresponded to regions of greater polymorphisms in comparisons between *P. ramorum* and *P. sojae*. Furthermore, unlike *P. infestans* and *P. sojae*, there is a small inverted repeat (1,150 bp) present in *P. ramorum* that encodes a unique open reading frame and a duplicate copy of *trnR(ucu)*.

An intraspecific polymorphism in the mitochondrially encoded cytochrome oxidase 1 gene has been identified that differentiates North American from European isolates of *P. ramorum* (Kroon and others 2004), but it is possible that genome wide comparisons between these regional populations would identify further differences that could be used to classify additional mitochondrial haplotypes in this species. The objective of this project was to evaluate intraspecific variation in the mitochondrial DNA of *P. ramorum* using a whole genome approach and to design additional molecular markers that can be used to identify a greater number of haplotypes. The availability of these markers would be useful in population studies of the pathogen by providing a cytoplasmic marker that would compliment the results from nuclear markers.

Materials and Methods

To evaluate intraspecific variation in mitochondrial genomic sequences for *P. ramorum* (CBS 101553, a European isolate) the mitochondrial DNA was purified from total DNA using CsCl + bisbenzimidazole density ultracentrifugation (Martin and Kistler 1990) and sent to the production facilities at the Joint Genomic Institute where standard techniques were used to construct and sequence a clone library (detailed protocols are available at <http://www.jgi.doe.gov/sequencing>). The sequences were aligned and contigs were assembled at the JGI sequencing production facility with final verification of the consensus sequence done with Sequencher ver. 4.6 (Gene Codes, Ann Arbor, MI). Annotation of coding regions and prediction of ORFs was done with DS Gene v1.5 (Accelrys, San Diego, CA) using the universal genetic code. Identification of protein- and rRNA-encoding genes was done by comparison with sequences reported for *P. ramorum* (Martin and others 2007; DQ 832718) and BLAST analysis to other sequences in GenBank. Genes for tRNAs were confirmed using tRNAscan SE v1.1 (Lowe and Eddy 1997; <http://www.genetics.wustl.edu/eddy/tRNAscan-SE/>). Pairwise comparisons among

genomes were made using mVISTA (<http://genome.lbl.gov/vista/servers.shtml>) as described previously (Martin and others 2007).

Primers spanning the polymorphic loci were designed and the regions amplified for a total of 39 isolates representing a range of cultures recovered from nurseries and forests in California, Oregon, and Washington State, as well as European countries. Amplicons were sequenced and Sequencher or DS Gene was used to analyze the alignments.

Results and Discussion

The mitochondrial genome of the European isolate of *P. ramorum* (CBS101553) was identical to the California isolate (DQ 832718) with the exception that there were single nucleotide polymorphisms at 13 positions dispersed throughout the genome and a 180 bp insertion in the European isolate. This level of intraspecific variation is similar to what was observed in comparisons of mitochondrial haplotypes Ia and Ib for *P. infestans* (Avila-Adame and others 2006).

Primers were designed to amplify seven of these polymorphic regions (table 1). Sequence alignments of these amplicons revealed that three primer pairs identified the mitochondrial haplotype found in North America (NA) or Europe (EU).

Table 1—Molecular markers used to determine mitochondrial haplotype in *Phytophthora ramorum*

Marker	Length	# Variable bases	MtDNA Haplotype
1	540 bp	1	I – EU II – NA
2	870 bp	2	I – EU II – NA
3	1,025 bp	5	I – EU II – NA
4	370 bp	2	I – EU II – NA III – Washington State
5	347 bp	2	I – EU II – NA III – Washington State
6	740 bp	2	I – EU II – NA III – Washington State
7	865 bp	4	I – EU II – NA III – Washington State IV – Oregon forest

However, three additional primer pairs also differentiated a third haplotype representing the third lineage of the pathogen that was recovered from Washington State (Ivors and others 2006). Lastly, the seventh primer pair was able to differentiate these three mitochondrial haplotypes as well as a fourth haplotype that was present in several isolates recovered from the forest in Oregon (DNA samples provided by Nik Grünwald, United States Department of Agriculture-Agricultural Research Service (USDA ARS). What this additional haplotype represents is uncertain at this time as more samples need to be examined and compared to nuclear genotypic data.

The ability to identify mitochondrial haplotypes will provide additional markers that can be useful for monitoring specific lineages of the pathogen and when used in conjunction with nuclear markers, will provide a maternally inherited cytoplasmic marker for population genetic studies.

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