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Genome sequences of *Phytophthora* enable translational plant disease management and accelerate research

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**Abstract:** Whole and partial genome sequences are becoming available at an ever-increasing pace. For many plant pathogen systems, we are moving into the era of genome resequencing. The first *Phytophthora* genomes, *P. ramorum* and *P. sojae*, became available in 2004, followed shortly by *P. infestans* in 2006. Availability of whole genome sequences has provided rapid and immediate advances in several areas also resulting in many practical applications and critical new insights. Availability of comparative genome data facilitated discovery of new classes of effectors, such as the RxLR-dEER and crinkler effector families. Genome data also enabled development of molecular markers for population genomic approaches that provided critical new insights into the evolutionary history of species and clades of *Phytophthora*. Several select examples of advances resulting from comparative genomic approaches in a concerted effort of the Oomycete research community are reviewed.

**Keywords:** effector, emerging pathogens, exotic pathogens, genome analysis, *Phytophthora*, translational analysis

**Introduction**

Plant pathogens in the genus *Phytophthora* belong to the Oomycota currently classified within the heterokont/chromist clade of the tree of life (Cavalier-Smith & Chao, 2006; Riisberg *et al.*, 2009). *Phytophthora* is a genus which is distributed worldwide and affects many if not most commercially grown crops, forest and ornamental species. *Phytophthora* species such as *P. cinnamomi* and *P. ramorum* cause important diseases on hundreds to thousands of forest or landscape plants (Hardham, 2005; Grünwald *et al.*, 2008). Recently, *P. ramorum* was found to cause significant disease on a landscape...
scale on Japanese larch in Europe (Brasier & Webber, 2010; Grünwald et al., 2012). Other notable Phytophthora species have a small host range, such as P. infestans (Fry, 2008) which infects potato and a few other relatives, including tomato and petunia, or P. sojae infecting soybean and some lupines (Tyler, 2007). Phytophthora pathogens can be costly to manage and often cause significant crop and postharvest losses on a worldwide scale.

The whole genome sequences of several Phytophthora species have been available for several years (Tyler et al., 2006; Haas et al., 2009). The availability of these genomes has provided the opportunity to conduct novel research either on a different scale or in novel ways. This review will explore how the availability of Phytophthora whole genome sequences has enabled novel approaches leading to rapid discoveries, new applications and translational research with potential for improving disease management.

Availability of whole genome sequences can enable approaches that provide novel tools for population genomic, evolutionary and functional genomic studies that in turn provide insights into a pathogen’s plant disease epidemiology and translational tools for better disease management (Fig. 1).

**Phytophthora genomes**

Phytophthora genomes sequenced to date include P. infestans, P. ramorum and P. sojae (Tyler et al., 2006; Haas et al., 2009). More recently sequenced oomycete genomes include those of Pythium ultimum (Levesque et al., 2010) and Hyaloperonospora arabidopsidis (Baxter et al., 2010). These oomycete genomes differ drastically in genome size (43–240 Mb), although gene content (15 290–19 027) is roughly equivalent (Table 1). Comparative genomic approaches can thus unravel

![Fig. 1. Illustration of how the availability of whole genome sequences can enable approaches that provide novel tools for population genomic, evolutionary and functional genomic studies that in turn provide insights into a pathogen’s plant disease epidemiology and translational tools for better disease management.](image_url)

**Table 1. Comparison of genome sizes and estimated number of protein-coding genes in Oomycete genomes sequenced to date. The first three Oomycete genomes to be sequenced belonged to the genus Phytophthora.**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Genome size (Mb)</th>
<th>Predicted gene content</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytophthora infestans</td>
<td>240</td>
<td>17 887</td>
<td>Haas et al. (2009)</td>
</tr>
<tr>
<td>Phytophthora sojae</td>
<td>95</td>
<td>19 027</td>
<td>Tyler et al. (2006)</td>
</tr>
<tr>
<td>Phytophthora ramorum</td>
<td>65</td>
<td>15 743</td>
<td>Tyler et al. (2006)</td>
</tr>
<tr>
<td>Pythium ultimum</td>
<td>43</td>
<td>15 290</td>
<td>Levesque et al. (2010)</td>
</tr>
<tr>
<td>Hyaloperonospora arabidopsidis</td>
<td>78</td>
<td>14 453</td>
<td>Baxter et al. (2010)</td>
</tr>
</tbody>
</table>
changes in gene arrangement and synteny, changes in gene expression and identify families of genes that underwent gene expansion or contraction. Gene families showing evidence for diversifying selection are likely important for understanding the coevolutionary history of plants and pathogens.

**Informative molecular markers and phylogenetic loci**

The most immediate applications emerging from the availability of genomes were development of diagnostic, molecular markers particularly for *P. ramorum* that was previously relatively unknown to science. Martin et al. (2004) developed a PCR protocol based on mitochondrial sequences of the *cox I* and *cox II* genes for detection of *Phytophthora* spp., and discrimination among *P. ramorum*, *P. nemorosa* and *P. pseudosyringae* in particular. Shortly thereafter, the same group developed a real-time PCR assay for detection of *P. ramorum* (Tooley et al., 2006). In parallel, Hayden and colleagues (2004, 2006) developed *P. ramorum* detection methods based on real-time PCR using a nested protocol for the internal transcribed spacer (ITS) region. All these parallel efforts culminated in a large study where 11 reported diagnostic techniques were compared using a standardized DNA library for 315 isolates for over 60 described *Phytophthora* spp. to develop protocols for routine diagnosis by state and federal agencies in nursery environments (Martin et al., 2009).

Another set of diagnostic markers was developed for analysis of populations. Several groups developed simple sequence repeats (SSR) to understand the clonality and structure of populations of *P. ramorum*. Garnica et al. (2006) mined SSRs from the *P. ramorum* genome and provided bioinformatically predicted primer sequences. Similarly, Ivors et al. (2006) and Vercauteren et al. (2010, 2011) developed and applied SSR markers for *P. ramorum* while Dorrance & Grünwald (2009) developed SSRs for *P. sojae*. Finally, Grünwald & Goss (2009) determined frequency and density of SSRs in the genomes of both *P. ramorum* and *P. sojae*.

Meanwhile, several groups investigated single nucleotide polymorphisms as diagnostic markers. Bilodeau et al. (2007) discovered SNPs in two genes, β-tubulin and cellulose binding elicitin lectin (CBEL), that can distinguish the European NA1 and the North American EU1 *P. ramorum* clonal lineages. Analogous work was conducted by Kroon and colleagues (2004) using the cytochrome c oxidase subunit 1 (*CoxI*) gene to distinguish the same lineages. Similarly, Elliott et al. (2008) used PCR-RFLP to distinguish clonal lineages of *P. ramorum*.

Another unique effort based on genome availability was provided by Blair and colleagues (2008) who compared the genomes of *P. sojae* and *P. ramorum* for sequence loci that are phylogenetically informative at the genus level. They used seven nuclear loci to revise definition of phylogenetic clades in the genus *Phytophthora*. Availability of genome sequences and the seven loci identified by Blair et al. (2008) also provided opportunity for developing diagnostic websites for the genus *Phytophthora*: (1) http://www.phytophthoradb.org/ (Park et al., 2008), and (2) http://phytophthora-id.org/ (Grünwald et al., 2011).

**Evolutionary and demographic history**

Contemporary analytical tools for the analysis of population genetic variation are powerful resources to infer the evolutionary history of populations of pathogens (Grünwald & Goss, 2011). These novel tools allow for the intricate reconstruction of the demographic history of pathogens. Understanding of the evolutionary history of exotic pathogens can, in turn, inform management of emerging epidemics. Plant pathogens in the genus *Phytophthora* have a long history as exotic and reemerging pathogens that continue to cause significant damage to agriculture and natural ecosystems. Availability of a whole genome sequence for an exotic pathogen such as *P. ramorum* provided unprecedented opportunities for unravelling the evolutionary history of this pathogen (Grünwald et al., 2012).

Rapid, genome-enabled discovery of microsatellite markers allowed for extensive demographic analysis of *P. ramorum* populations. It soon became clear that there exist three distinct clonal lineages named NA1, NA2 and EU1 (Grünwald et al., 2009) (Table 2). Detailed analysis by several groups has now clearly demonstrated that these three lineages are exotic to both Europe and North America, yet the origin of these lineages remains unknown (Grünwald et al., 2008; Goss et al., 2009a; Grünwald & Goss, 2009) (Fig. 2). Surprisingly, coalescent analysis and dating of mutations that are lineage-specific versus those that are shared among lineages indicated that the three *P. ramorum* clonal lineages were anciently diverged, that is they diverged long before the emergence of modern agriculture some 150–400,000 years ago (Goss et al., 2009a). Thus, the three clonal lineages shown in Fig. 2 likely were introduced from three separate, reproductively isolated populations.

The demographic history of *P. ramorum* since its emergence is now well documented (Grünwald et al., 2012). NA1 established itself in California, USA probably in the
Table 2. Documented emergence of clonal lineages of the sudden oak death pathogen \textit{P. ramorum} (Ivors et al., 2004, 2006; Grünwald et al., 2008, 2009).

<table>
<thead>
<tr>
<th>Clonal lineage</th>
<th>Distribution</th>
<th>Mating type</th>
<th>First report</th>
<th>References documenting first report</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA1</td>
<td>USA, Canada</td>
<td>A2</td>
<td>CA, USA</td>
<td>Rizzo et al. (2002); Ivors et al. (2004)</td>
</tr>
<tr>
<td>NA2</td>
<td>USA, Canada</td>
<td>A2</td>
<td>WA, USA; BC, Canada</td>
<td>Ivors et al. (2006); Elliott et al. (2008); Goss et al. (2011)</td>
</tr>
<tr>
<td>EU1</td>
<td>Europe, USA, Canada</td>
<td>A1</td>
<td>Germany</td>
<td>Werres et al. (2001); Werres &amp; De Merlier (2003); Vercauteren et al. (2010)</td>
</tr>
</tbody>
</table>

Fig. 2. Global migration patterns of the sudden oak death pathogen \textit{Phytophthora ramorum} as supported by research conducted to date. The global population consists of three clonal lineages of unknown origin that were introduced to North America and Europe (Grünwald et al., 2008, 2009; Goss et al., 2009a, 2009b, 2011; Grünwald & Goss, 2009; Vercauteren et al., 2010). Clonal lineage EU1 was introduced into Europe and likely moved from Europe to North America (Goss et al., 2009a, 2011). NA1 was introduced into California (Ivors et al., 2006; Mascheretti et al., 2008) and NA2 was introduced into either British Columbia, Canada or Washington, US (Goss et al., 2011). The source populations for NA1, NA2 and EU1 populations remain unknown and were reproductively isolated long before introduction to Europe or North America (Goss et al., 2009a).

Washington, USA, by nursery imports from Europe (Goss et al., 2011; Grünwald et al., 2012).

It has now become clear that \textit{P. ramorum} has been introduced into North America at least three times, and thus has migrated globally at least four times given the introduction into Europe. All these analyses were critically enabled by availability of whole genome sequences.

Effector discovery

Availability of two simultaneously sequenced \textit{Phytophthora} genomes was a coup de maître that facilitated comparative genomic approaches and resulted in rapid discovery of new gene families (Tyler et al., 2006). The most notable discoveries were made regarding effectors (Kamoun, 2006; Tyler, 2009). Effectors are small, secreted proteins produced by the pathogen that target host plant molecules and/or alter host plant processes.

One large effector family discovered after availability of whole genome sequences is the RxLR-dEER family. Members of this gene class had been cloned and validated to be avirulence genes corresponding to host R genes, including \textit{Avr1b-1} from \textit{P. sojae} (Shan et al., 2004), \textit{Avr3a} from \textit{P. infestans} (Armstrong et al., 2005) and \textit{ATRI} from \textit{Hyaloperonospora} (Allen et al., 2004) from \textit{H. arabidopsidis}. Once genomes were available, it became clear that these four cloned genes all had a conserved gene structure and included the RxLR-dEER motif. The RxLR-dEER motif is now known to mediate entry of oomycete effectors into host plant cells (Whisson et al., 2007; Dou et al., 2008b). The genome sequences of \textit{Phytophthora} and \textit{Hyaloperonospora} encode large numbers of genes in several families that have the RxLR domain. For example, \textit{P. sojae}, \textit{P. ramorum} and \textit{P. infestans} have anywhere from 400–600 RxLR effectors depending on the bioinformatic criteria used for mining them out of the available genomes (Tyler et al., 2006; Jiang et al., 2008). Once the RxLR motif was recognized, rapid progress

early 1990s (Mascheretti et al., 2008). Subsequently, the pathogen migrated up (and down) the West coast and West to East via nursery shipments (Prospero et al., 2007, 2009; Goss et al., 2009b). Recently, the NA1 pathogen migrated among East coast states (California Oak Mortality Taskforce reports).

NA2 in contrast, migrated to the Pacific Northwest and it is currently not clear if it was introduced into British Columbia, Canada or Washington, USA (Ivors et al., 2006; Elliott et al., 2008; Goss et al., 2011).

EU1 was first discovered in Germany (Werres et al., 2004) but has since spread throughout Europe (Brasier et al., 2004; Grünwald et al., 2009; Vercauteren et al., 2010). Most recently, this pathogen was shown to cause extensive landscape-level disease on Japanese larch (Brasier & Webber, 2010). EU1 was most likely moved from Europe to either British Columbia, Canada, or
was made in cloning other RxLR effectors, including identification of Avr4 (van Poppel et al., 2008) and AvrBlb1 (Vleeshouwers et al., 2008) from P. infestans and Avr4/6 from P. sojae (Dou et al., 2008a). Predicted RxLR effector genes have been used for high throughput screening of germplasm to rapidly identify novel functional host genes (Vleeshouwers et al., 2008). This effector-based genomic screening now enables discovery of host genes that promise to accelerate the engineering of Phytophthora-resistant hosts.

Another notable effector group discovered was genome-enabled is that of the crinklers. Crinkler genes trigger crinkling and necrosis of leaves when overexpressed in Nicotiana benthamiiana (Torto et al., 2003). Similar to RxLR effectors, crinklers are modular and feature an N-terminal secretory signal and a domain defined by the LxLFLAK amino acid motif (Haas et al., 2009).

Conclusions

Availability of whole genome sequences has led to significant, novel discoveries and applications, and thus opened doors for approaches that were previously either inconvenient or cost-prohibitive. Many of the translational results that will emerge out of the genome sequences are still to occur. Immediate applications derived from genome-enabled science occurred mostly in the areas of molecular marker development, population genetics, evolution and effector discovery. The case of P. ramorum exemplifies how this genome provided federal regulatory agencies insights into the migration pathways that led to multiple introductions of this pathogen.

One exciting possibility is that the convergence of decreasing sequencing costs and increased parallel computing resources will provide opportunities for sequencing of all Phytophthora species and resequencing of populations of single species. These approaches might answer long-standing questions about the evolution of sex in the genus, where homothallism and heterothallism are subject to convergent evolution or occasional reversal of traits. Similarly, identification of genes responsible for important traits such as mefenoxam resistance, mating type or host adaptation in Phytophthora will soon be possible, thanks to the novel genomic tools and resources available. Genome-wide association analysis will enable discovery of novel genes responsible for distinct phenotypic traits. Finally, it will further our understanding of what genome features provide narrow host ranges such as those found in P. sojae on Glycine or H. arabidopsisidis on Arabidopsis versus broad host ranges such as those found in P. ramorum or Pythium species.

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