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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

Des séquences entières ou partielles de génomes deviennent de plus en plus accessibles. En ce qui concerne plusieurs systèmes plante-agent pathogène, nous abordons l'ère du recéquençage du génome. Les premiers génomes de *Phytophthora*, *P. ramorum* et *P. sojae*, ont été disponibles à partir de 2004, suivis de près par celui de *P. infestans* en 2006. L'accessibilité à des séquences génomiques entières a favorisé des progrès rapides dans plusieurs domaines, ce qui a engendré des applications pratiques et ouvert de nouvelles perspectives stimulantes. La disponibilité des données comparatives sur le génome a facilité la découverte de nouvelles classes d'effecteurs comme le RxLR-dEER et les familles d'effecteurs crinkler. Les données sur le génome ont aussi permis le développement de marqueurs moléculaires particuliers aux approches en génomique des populations qui fournissent de nouvelles idées séduisantes sur l'histoire évolutive des espèces et des variantes de *Phytophthora*. Plusieurs exemples typiques de progrès, consécutifs aux approches en génomique comparative et à l'action concertée du milieu de la recherche sur les Oomycètes, sont passés en revue.

Mots clés: agents pathogènes émergents, agents pathogènes exotiques, analyse génomique, effecteur, *Phytophthora*, recherche translationnelle

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

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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

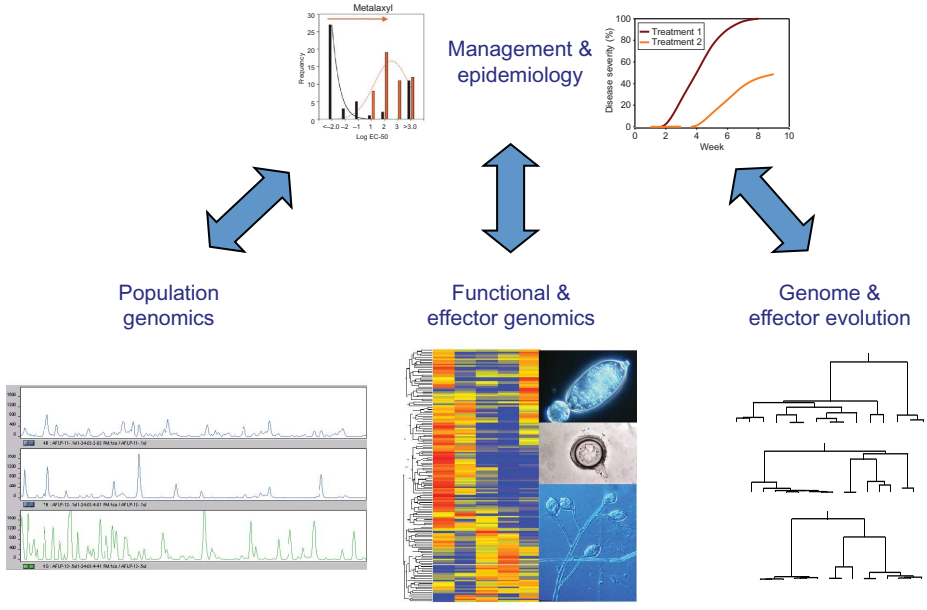

Fig. 1. Illustration of how the availability of whole genome sequences can enable approaches that provide novel tools for population genomic, evolutionary and functional genomic studies that in turn provide insights into a pathogen's plant disease epidemiology and translational tools for better disease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

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*.

Organism	Genome size (Mb)	Predicted gene content	Source
Phytophthora infestans	240	17 887	Haas *et al.* (2009)
Phytophthora sojae	95	19 027	Tyler *et al.* (2006)
Phytophthora ramorum	65	15 743	Tyler *et al.* (2006)
Pythium ultimum	43	15 290	Levesque *et al.* (2010)
Hyaloperonospora arabidopsidis	78	14 453	Baxter *et al.* (2010)

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.*, 2009*a*; 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.*, 2009*a*). 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 *P. ramorum* (Ivors *et al*., 2004, 2006; Grünwald *et al*., 2008, 2009).

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

Fig. 2. Global migration patterns of the sudden oak death pathogen *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.*, 2009*a*, 2009*b*, 2011; Grünwald & Goss, 2009; Vercauteren *et al.*, 2010). Clonal lineage EU1 was introduced to Europe and likely moved from Europe to North America (Goss *et al.*, 2009*a*, 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.*, 2009*a*).

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.*, 2009*b*). 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.*, 2001) 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 Washington, USA, by nursery imports from Europe (Goss *et al.*, 2011; Grünwald *et al.*, 2012).

It has now become clear that *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 *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 *Avr1b-1* from *P. sojae* (Shan *et al.*, 2004), *Avr3a* from *P. infestans* (Armstrong *et al.*, 2005) and *ATR1* (Rehmany *et al.*, 2005) and *ATR13* (Allen *et al.*, 2004) from *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 domain (Tyler *et al.*, 2006). The RxLR-dEER motif is now known to mediate entry of oomycete effectors into host plant cells (Whisson *et al.*, 2007; Dou *et al.*, 2008*b*). The genome sequences of *Phytophthora* and *Hyaloperonospora* encode large numbers of genes in several families that have the RxLR domain. For example, *P. sojae*, *P. ramorum* and *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

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.*, 2008*a*). 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 that was genome-enabled is that of the crinklers. Crinkler genes trigger crinkling and necrosis of leaves when overexpressed in *Nicotiana benthamiana* (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. arabidopsidis* on *Arabidopsis* versus broad host ranges such as those found in *P. ramorum* or *Pythium* species.

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