Keeping up with the Plant Destroyers

Sophien Kamoun
@kamounlab
"Medecine can cure you one day but plants save your life everyday" #TwitterWisdom #plantsci /via @CristobalUauy
Crop losses due to fungi and oomycetes (filamentous plant pathogens)

<table>
<thead>
<tr>
<th>Crop Host species</th>
<th>2009/2010 harvest (million tonnes)</th>
<th>Calories per 100g flour (uncooked)</th>
<th>Disease/Pathogen and variation in % losses</th>
<th>Loss of food* for x million over 1 year, given diet of 2,000 calories per day</th>
</tr>
</thead>
</table>

**TOTAL: Could feed 596 – 4,287 million mouths per annum**

Fisher et al. 2012
Armed and Dangerous

These fungi, weeds, and viruses are among the more serious biological threats to food security—so researchers are working hard on countermeasures.

BLACK SIGATOKA

Pest: Mycosphaerella fijiensis
Crops: Bananas, plantains
Whereabouts: This fungus, first detected in Fiji in 1964, is now found in 100 countries in the Americas, Africa and South Asia.

RICE BLAST

Pest: Magnaporthe oryzae
Crops: Rice, 50 species of grasses and sedges

POTATO BLIGHT

Pest: Phytophthora infestans
Crops: Potatoes; also tomatoes and other solanaceous crops

ASIAN SOYBEAN RUST

Pest: Phakopsora pachyrhizi
Crops: At least 31 legume species, notably soybeans

WHEAT STEM RUST

Pest: Puccinia graminis Ug99
Crop: Wheat
Filamentous plant pathogens (fungi and oomycetes) cause destructive plant diseases

- **Filamentous pathogens** (fungi and oomycetes) cause most destructive diseases of plants
- **Highly adaptable** - can rapidly overcome plant resistance
- **Large population sizes**; mixed asexual and sexual reproduction
The Irish potato famine pathogen *Phytophthora infestans* causes potato blight.
Phytophthora is Greek for “plant-destroyer”

Infection of potato plants by Phytophthora infestans
Phytophthora: fungus-like oomycetes
Oomycetes are heterokonts - related to brown algae and diatoms

Oomycetes are related to brown algae and diatoms. Lineages with plant pathogens are in green. Adapted from Baldauf, Science (2003).
Phytophthora is an oomycete not a fungus
Oomycetes form an ancient eukaryotic lineage

- may have been parasitic ~300 million year ago
- present in the 407 million year-old Rhynie Chert, an ecosystem of plants, fungi and oomycetes

Christine Strullu-Derrien and Paul Kenrick
@ Natural History Museum
The Irish potato famine pathogen *Phytophthora infestans* causes potato blight.
“Out of Mexico” – migration paths of *P. infestans*

Yoshida *et al.*, 2013, eLife; 2014 PLOS Pathogens
w/ Johannes Krause, Marco Thines, Detlef Weigel
and Hernan Burbano
“Genome archaeology” reveals HERB1 – the *P. infestans* lineage that triggered the Irish potato famine

Yoshida *et al.*, 2013, *eLife*

w/ Johannes Krause, Marco Thines, Detlef Weigel and Hernan Burbano
Attack of the clones - rise and fall of *Phytophthora infestans* asexual lineages in Britain

Yoshida et al., 2014 PLOS Pathogens

Adapted from David Cooke, James Hutton Inst.
Evolution of virulence in the EC1 clonal lineage

- Asexually reproducing lineage
- Appeared during early 1990s in the Andes
- Rapidly displaced older populations
- Currently dominant and widespread in Colombia, Ecuador, and Peru.

Collaboration with Matthieu Pel and Vivianne Vleeshouwers
Kentaro Yoshida with collaborators
in Ecuador, Colombia and Peru
Avr-vnt1 gene silencing resulted in gain-of-virulence

NO SNPs

Kentaro Yoshida, Marina Pais, Liliana Cano
Collaboration with Matthieu Pel and Vivianne Vleeshouwers
Attack of the Clones

Fungi have long been seen as the least interesting pathogens, but two catastrophes in the animal world have changed that view.

When Nature recently accepted a review co-authored by Sarah Gurr, the plant pathologist from the University of Oxford in the United Kingdom sent the journal a self-produced image to consider for its cover. It shows a fungus looking like one of those colossal, menacing tripods from H. G. Wells’s War of the Worlds, stalking through a field, with bats, frogs, and toads fleeing before it in a crazed panic. “Fungal Wars of the World,” Gurr called it.

The picture didn’t make it, but many scientists agree with its message: Fungi have now become a greater global threat to crops, forests, and wild animals than ever before. They have killed countless amphibians, pushing some species to extinction, and they’re threatening the food supply for billions of people. More than 125 million tons of the top five food crops—rice, wheat, maize, potatoes, and soybeans—are destroyed by fungi every year.

Like other infectious agents, fungi benefit from a combination of trends, such as increased global travel and trade, new agricultural practices, and perhaps global warming. But they have several unique features researchers say—including the way they can switch from asexual to sexual reproduction—that enable them to exploit these opportunities particularly effectively.

The Nature paper, published in April, was in part a cry for attention; its authors say the world isn’t fully aware of the dangers and should invest more in countermeasures. For decades, fungal diseases have been overshadowed by bacteria and viruses. “There are probably 50 or 100 bacterial experts for every fungal expert,” says Bruce McDonald, a plant pathologist at the Swiss Federal Institute of Technology in Zurich. “There has always been a sense that fungi are not that important,” adds microbiologist Arturo Casadevall of Albert Einstein College of Medicine in New York City.

That has begun to change only very recently, thanks in part to some highly publicized animal die-offs. “A few years ago people just scoffed when you thought a fungus had killed an animal such as a bat,” says Gudrun Wibbelt, a veterinary pathologist at the Leibniz Institute for Zoo and Wildlife Research in Berlin. “That is clearly changing.” In December 2010, the U.S. Institute of Medicine hosted its first-ever workshop focused exclusively on fungal diseases, which concluded that “threats posed by emerging fungal pathogens are often underappreciated and poorly understood.”

Interest in the Nature review has been huge, Gurr says. Scientists around the world have sent in articles describing other fungal diseases that could have bolstered the paper, says co-author Matthew Fisher, a molecular epidemiologist at Imperial College London. Among the wide variety of species under attack are crabs, corals, corn, and the Cavendish banana—and new fungal diseases are discovered every year. In June, Elsevier presented a new journal called Medical Mycology Case Reports, completely devoted to “unusual medical or veterinary fungal infections.”

Detective work

One of the issues that has held back research on fungi is that it’s simply very hard. Fungi are more complex, have bigger genomes, and are more difficult to characterize than viruses or bacteria. In fact, most researchers find it hard to give a good definition of the whole group. (“That’s tough. Let me have a quick look what Wikipedia says,” one of them says.)

Until the 1960s, fungi were considered the least interesting pathogens, but two catastrophes in the animal world have changed that view. One of these was the fungal disease that almost wiped out the mountain yellow-legged frog population in Kings Canyon National Park in California. Pubs he’d by AAS on August 23, 2013 www.sciencemag.org

How can pathogen clones evolve rapidly?

How does asexual reproduction affect genome evolution?

Do pathogens require sexual reproduction?

What’s the role of humans in pathogen evolution?
Why the misery? Why are oomycetes the scourge of farmers worldwide?

- *Phytophthora* are astonishing plant destroyers that can wipe out crops in days but the secret of their success is their ability to *rapidly adapt* to resistant plant varieties.

- How did *Phytophthora* and other oomycetes manage to keep on changing and adapting to ensure their uninterrupted survival over evolutionary time?
The genome sequence of *Phytophthora infestans*

with Brian Haas, Mike Zody, and Chad Nusbaum
@ Broad Institute
Pathogenomics is an emerging field of plant pathology

The genome of a rice pathogenic strain of *M. grisea*, 70-15, was sequenced through a whole-genome shotgun approach. In all, greater than sevenfold sequence coverage was produced, and a marker. In addition, 19 scaffolds (6.5%) of genome assembly contained more than one marker and could be unambiguously placed on the map. The ends of chromosomes were identified by a dinucleotide repeat motif (TTAGGG)n. Thirteen telomeric sequences were placed at the ends of scaffolds, of which six could be placed at the ends of chromosomes, whereas the remainder were associated with unanchored scaffolds (Supplementary Table S2). Genome coverage was estimated by aligning 28,682 *M. grisea* expressed sequence tags (ESTs), representing genes expressed during a range of developmental stages and environments. 94% of the ESTs were aligned many of these ESTs being from different regions of the genome. 70-15, was ordered and orientated within 159 scaffolds. The total length of all scaffolds was 144 Mbp.

**Acquisition of the *M. grisea* genome sequence**

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**The gene content of a plant pathogen**

Within the *M. grisea* genome, 1 of 2005 Nature Publishing Group

© 2005 Nature Publishing Group
*Phytophthora infestans* genome architecture - repeat-rich and gene-poor loci interrupt colinear regions

Genomes of host-specific filamentous plant pathogens – *the bigger the better!*

- Typically, larger genomes than non-parasitic relatives
- Extreme repeat-driven expansions in distinct lineages:
  - *Phytophthora infestans*: 240 Mb, 74% repeats
  - Rust fungi: 68-100 Mb, 45% repeats
  - Powdery mildew fungi: 120-160 Mb, 65% repeats
- In sharp contrast to many parasites and symbionts that tend to evolve small compact genomes
Why is bigger better in filamentous plant pathogens?

Which evolutionary tradeoffs counterbalance the cost of the larger genomes?
Fungal mimicry of plants - “fake” flowers triggered by the rust *Puccinia monoica* on *Boechera stricta*

- Rust infected plant with pseudoflowers
- Healthy plant with normal flowers
**Effectors** – secreted pathogen molecules that perturb plant processes

- **Effectors** – described in parasitic bacteria, oomycetes, fungi, nematodes, and insects

- Encoded by genes in pathogen genomes but function in (inside) plant cells – **operate as plant proteins**

- **Target of natural selection** in the context of coevolutionary arms race between pathogen and plant

- **Current paradigm** – effector activities are key to understanding parasitism
Microbes alter plant cell processes by secreting a diversity of effector molecules.
Some effectors “trip the wire” and activate immunity in particular plant genotypes.
The diverse effectors of *Phytophthora infestans*

### Protease inhibitors

**EPI1**

- **Genome region:** ~38

**EPI10**

- **Genome region:** Apoplastic

### RXLR

**AVR3a**

- **Genome region:** ~550

**Avr1b-1**

- **Genome region:** Host-translocated

### Crinklers

**CRN2**

- **Genome region:** ~200

**CRN8**

- **Genome region:** ~250ψ
Positive selection has targeted the C terminal domain of RXLR effectors (ML method in paml)

- Consistent with the view that RXLR effectors are modular

RXLR effector proteins have conserved but adaptable structures

Mark Banfield Lab @ John Innes Centre
Boutemy et al. JBC 2011
Win et al. PLoS Pathogens 2012
WY-fold of *Phytophthora* RXLR-WY effectors

- Insertion/deletions in loop regions between α-helices
- Extensions to the N- and C-termini
- Amino acid replacements in surface residues
- Tandem domain duplications
- Oligomerization

• A structural template for rapid biochemical diversification?
Why the misery? Why are oomycetes the scourge of farmers worldwide?

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- How did *Phytophthora* and other oomycetes manage to keep on changing and adapting to ensure their uninterrupted survival over evolutionary time?
Phytophthora infestans genome architecture - repeat-rich and gene-poor loci interrupt colinear regions

P. sojae

P. infestans

P. ramorum

The diverse effectors of *Phytophthora infestans*

**Protease inhibitors**

<table>
<thead>
<tr>
<th>Protease Inhibitor</th>
<th>Length</th>
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<tbody>
<tr>
<td>EPI1</td>
<td>~38</td>
</tr>
<tr>
<td>EPI10</td>
<td>~550</td>
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</table>

**RXLR**

<table>
<thead>
<tr>
<th>RXLR Domain</th>
<th>Length</th>
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<tbody>
<tr>
<td>AVR3a</td>
<td>~550</td>
</tr>
<tr>
<td>Avr1b-1</td>
<td>~200</td>
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**Crinklers**

<table>
<thead>
<tr>
<th>Crinkler</th>
<th>Length</th>
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<tbody>
<tr>
<td>CRN2</td>
<td>~250ψ</td>
</tr>
<tr>
<td>CRN8</td>
<td>~599</td>
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</table>
Phytophthora infestans effectors typically occur in the expanded, repeat-rich and gene-poor loci.

The “two-speed genome” of *P. infestans* underpins high evolutionary potential

- Gene-sparse regions of genome show highest rates of structural and sequence variation, signatures of adaptive selection
- Gene-sparse regions underpin rapid evolution of virulence (effector) genes and host adaptation
Oomycete and fungal plant pathogens independently evolved “two-speed” genomes

Phytophthora infestans >>> Oomycete

Leptosphaeria maculans >>> Fungus

Sylvain Raffaele
Genome biology: the peculiar architecture of filamentous plant pathogen genomes

- Effector genes populate specific (repeat-rich) compartments of filamentous pathogen genomes

- Repeat-rich genome compartments contribute to the emergence of new virulence traits > “two-speed genome”
“...it would be advantageous for a species if the genes for biochemical diversity [in disease resistance] were particularly mutable, provided that this could be achieved without increasing the mutability of other genes whose mutation would give lethal or sublethal genotypes.”

Haldane, J.B.S. (1949). Disease and evolution. La Ricerca Scientifica, 19, 2–11.
How does the two-speed genome accelerate evolution? …drive new virulence traits?

- **Structural genome variation** — increased genome instability and structural variation, deletions, duplications etc.
- **Horizontal gene/chromosome transfer** — mobile effectors
- **Increased local mutagenesis** — RIP mutation leakage
- **Epigenetics** — heterochromatin leakage?

…more to be discovered
Why the “two-speed” genome? Jump or die!

- Lineages with less adaptable genomes suffer higher extinction rates, thus a macroevolutionary disadvantage.
- Lineages that have adaptable genomes end up dominating the biota.
- **Clade selection** – opposes short term advantages conferred by smaller compact genomes.

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GC Williams (1992) Oxford Uni Press
# EvoMPMI – Evolutionary Plant-Microbe Interactions

- Comparative studies within a **phylogenetically and ecologically robust framework** to test **specific hypotheses** about how evolution has tweaked mechanisms of pathogenicity and immunity
- Alternative to genetic approaches – genetic screens have taken place in nature throughout evolution

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ancestral state

<table>
<thead>
<tr>
<th>phenotype</th>
<th>gene</th>
<th>process</th>
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Species in the *Phytophthora infestans* lineage (clade 1c) evolved by host jumps

- Diverged ~1300 years ago; 99.9% identical in ITS
- Three species naturally co-occur in Toluca, Mexico
- Specialized on their respective hosts

multilocus phylogeny of *Phytophthora* from Blair et al. 2008 Fungal Genet Biol
Host jumps must have a dramatic impact on effector evolution

P = parasite
H = host

Time

P1

H1

P2

H2

Effector

Target

Purifying or neutral selection dN <= dS

Pseudogenization $\Psi$

Adaptive selection dN > dS
Impact of host jumps on genome and effector evolution

...and positive selection. These Lead are enriched in genes induced in planta, implicating host adaptation in genome evolution. Unexpectedly, genes involved in epigenetic processes formed another class of evolving residents of the gene-sparse regions. These results demonstrate that dynamic repeat-rich genome compartments underpin accelerated gene evolution following host jumps in this pathogen lineage.

Phytophthora infestans is an economically important specialized pathogen that causes the destructive late blight disease on Solanum plants, including potato and tomato. In central Mexico, P. infestans naturally co-occurs with two extremely closely related species, Phytophthora ipomoeae and Phytophthora mirabilis, that specifically infect plants as diverse as morning glory (Ipomoea longipesculata) and four-o’clock (Mirabilis jalapa), respectively. Elsewhere in North America, a fourth related species, Phytophthora phaseoli, is a pathogen of lima beans (Phaseolus lunatus). Altogether these four Phytophthora species form a very tight clade of pathogens that share ~99.9% identity in their ribosomal DNA internal transcribed spacer regions (1). Phenotypic inferences clearly indicate that species Phytophthora clade 1e (nomenclature of (2)) could be separated through host jumps followed by adaptive specialization on plants belonging to four different botanical families (2, 3). Adaptation to these plants most likely involves mutations in threed of disease effector genes that populate poor and repeat-rich regions of the 240–me pair genome of P. infestans (4). However, comparative genome analyses of specialized sister species have indicated that the genome, in contrast to core ortholog genes, architecture evolved at different rates. We used 614 differentially expressed genes in 2572 genes (14.2% of the whole genome) with dN/dS in genes from gene-dense regions (GDRs) and gene-sparse regions (GSRs). Statistical significance was assessed by unpaired t test assuming unequal variance (CVN, dN/dS); assuming equal variance (SNP frequency); or by Fisher’s exact test (P/A) (P < 0.1; ***P < 10^-4). Whiskers show first value outside 1.5 times the interquartile range.

We detected 345 in planta induced genes under positive selection in P. mirabilis relative to P. infestans (Raffaele et al. 2010)

What is the biochemical basis of adaptive selection? 

\( \rightarrow P. \) mirabilis protease inhibitor PmEPIC1 on Mirabilis jalapa
Evolution of EPIC1 protease inhibitor effector family

Suomeng Dong, Remco Stam, Liliana Cano et al. Science 2014
Protease inhibitor PmEPIC1 is under positive selection

ω = nonsynonymous (dN)/ synonymous (dS)
ω ratio calculated per branch using two-ratio model of codeml

Suomeng Dong, Remco Stam, Liliana Cano et al. Science 2014
Effector adaptation and specialization to host target following jump?

Suomeng Dong, Remco Stam, Liliana Cano et al. Science 2014
A resurrected ancestral EPIC1 can inhibit potato RCR3 but not Mirabilis MRP2.
EPIC1/RCR3 predicted contact residues are polymorphic.

Suomeng Dong with Mark Banfield and Renier van der Hoorn
Single residues in EPIC1 and RCR3 affect inhibition

- **Gln to Arg substitution in PmEPIC1** increased MRP2 inhibition but carries a **trade off**, impairs RCR3 inhibition

- **Antagonistic pleiotropy** - an effector that evolved higher activity on new target performs poorly on the ancestral host; leads to specialization!
Mo Farah challenges Usain Bolt to a charity race over an intermediate distance of 600 or 800 metres

Double Olympic champion Mo Farah has challenged Usain Bolt, the world's fastest man, to a charity race over an intermediate distance.