Food crisis: The silent tsunami

- Food and Ag commodity prices at all time highs
- Famine, hunger, food riots
- Chemical control: expensive, environmental damage...
- Crop diseases caused by plant pathogens are a major constraint for food production
Food Export Prices Rise

An index showing what countries around the world pay to import basic foodstuffs rose in January for the seventh consecutive month.

MONTHLY FOOD PRICE INDEX
(2002-04 = 100)

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<th>'95</th>
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*The adjusted food price index is the unadjusted index deflated by the World Bank Manufactures Unit Value Index (MUV).

Source: Food and Agriculture Organization of the United Nations

February 4, 2011
Diverting Food to Fuel

More than ever, grain is being used for biofuels rather than food consumption. This trend started to take off in 2004; by 2010, 6 percent of all grain went into making biofuels. The diversion is a contributing factor in rising food prices. (The grains factored in here include barley, corn, millet, mixed grains, oats, milled rice, rye, sorghum, wheat and durum wheat.)

World grain consumption

Biofuels 1%
2000
Food and other uses 99%
2010
Biofuels 6%

Change in world grain use since 2000

 Grain for biofuels
+350 million metric tons
+300
+250
+200
+150
+100
+50

 Grain for food and other uses

Sources: United States Department of Agriculture; Food and Agricultural Policy Research Institute

The New York Times
April 6, 2011
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.

**BIG 7**

**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

*12 FEBRUARY 2010  VOL 327  SCIENCE*
Infection of potato plants by *Phytophthora infestans*
Filamentous plant pathogens (fungi and oomycetes) cause destructive crop diseases

- Often host-specialized biotrophs - require living plant cells
- Highly adaptable - can rapidly overcome plant resistance
- Large population sizes, mixed asexual and sexual reproduction
- ~30 genome sequences described to date
Oomycetes are fungus-like filamentous microbes: a unique group of eukaryotic plant pathogens


Animal parasites in red
Plant pathogens in green
Expanded filamentous plant pathogen genomes are enriched in noncoding DNA
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?
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.
Bakanae (バカナエ) – “foolish seedling”
disease caused by *Gibberella fujikuroi*
Suppression of post-translational gene silencing (PTGS) by plant virus effectors

Hamilton and Baulcombe
Science 1999

Viral protein p19 dimer complexed with ds siRNA

www.proteopedia.org
AY-WB phytoplasma induces witches’ broom symptoms in Arabidopsis

MacLean et al. Plant Phys 2011; Saskia Hogenhout’s lab
The phytoplasma effector protein SAP54 induces shoot formation from flowers.

Phytoplasma-infected Arabidopsis

35S:SAP54

MacLean et al. Plant Phys 2011; Saskia Hogenhout’s lab
Xanthomonas TAL effectors: DNA binding proteins with an amino acid to nucleotide specificity code

TAL effectors – Designer DNA binding proteins

Hypervariable residues: NI HD HG NG
Target base: A C G T

Moscou et al.; Boch et al. Science 2009
Microbes alter plant cell processes by secreting a diversity of effector molecules.
Some effectors “trip on the wire” and activate immunity in particular plant genotypes.
Surface receptors mediate basal immunity – often suppressed by effectors

Pathogen-associated molecular patterns (PAMPs) stimulate cell surface immune receptors, leading to effector-triggered immunity. Effectors from pathogens suppress basal immunity, which is mediated by surface receptors. Intracellular immune receptors are also activated by PAMPs, leading to PAMP-triggered immunity. The figure illustrates the interaction between a bacterium, haustorium, and plant cell, with oomycetes and fungi also depicted. 

Dodds and Rathjen 2010 Nature Reviews Genetics
*P. infestans* delivers effectors inside host cells to suppress or activate immunity.
The diverse effectors of *Phytophthora infestans*

**Protease inhibitors**

- **EPI1**: ~38
- **EPI10**: ~38

**Apoplastic**

**RXLR**

- **AVR3a**: ~550
- **Avr1b-1**: ~550

**Host-translocated**

**Crinklers**

- **CRN2**: ~200
- **CRN8**: ~250
Modular structure of RXLR effectors

AVR3a

NUK10

IPIO1

PEX-RD3

Targeting

Function
The C-terminal region of Avr3a is sufficient for triggering R3a dependent HR.
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
Questions driving oomycete effector research

- How do effectors traffic inside host cells?
- How do they vary within and between pathogen species? How do they evolve?
- How do they function? What are their host targets? How do they perturb plant processes?
- How are effectors recognized by plant immune receptors? How can this be exploited to develop resistant crops?
The genome sequence of *Phytophthora infestans*
Brian Haas, Mike Zody, and Chad Nusbaum @ Broad Institute
Oomycete genome sequences from divergent species

- **Phytophthora infestans** (240 Mbp) - *Solanum* spp.
- **P. capsici** (65 Mbp) - pepper, tomato, cucurbits
- **P. sojae** (95 Mbp) - soybean
- **P. ramorum** (65 Mbp) - various woody plants
- **Hyalop. arabidopsidis** (100 Mbp) - Arabidopsis
- **Pythium ultimum** (50 Mbp) - various dicots

multilocus phylogeny of *Phytophthora* from Blair et al. 2008 Fungal Genet Biol
## Major features of the genome of *P. infestans*

<table>
<thead>
<tr>
<th></th>
<th><em>P. infestans</em></th>
<th><em>P. sojae</em></th>
<th><em>P. ramorum</em></th>
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</thead>
<tbody>
<tr>
<td><strong>Estimated genome size</strong></td>
<td>240 Mbp</td>
<td>95 Mbp</td>
<td>65 Mbp</td>
</tr>
<tr>
<td><strong>Number of genes</strong></td>
<td>17,887</td>
<td>16,988</td>
<td>14,451</td>
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<tr>
<td><strong>Orthologous genes</strong></td>
<td>11,893</td>
<td>12,427</td>
<td>12,136</td>
</tr>
<tr>
<td><strong>Colinear blocks</strong></td>
<td>85 Mbp</td>
<td>52 Mbp</td>
<td>37 Mbp</td>
</tr>
<tr>
<td><strong>Repeats</strong></td>
<td>74%</td>
<td>39%</td>
<td>28%</td>
</tr>
<tr>
<td><strong>Repeats in colinear blocks</strong></td>
<td>57%</td>
<td>28%</td>
<td>13%</td>
</tr>
<tr>
<td><strong>Repeats outside colinear</strong></td>
<td>86%</td>
<td>60%</td>
<td>56%</td>
</tr>
</tbody>
</table>

- Repeat driven expansion of the *P. infestans* genome
Significant 1:1:1 orthology and colinearity between 
*P. infestans*, *P. sojae* and *P. ramorum*

RXLR effectors typically occur in expanded, repeat-rich and gene-poor loci

AVR4: a single gene in a repeat-rich expanded ~100 kb locus

P. sojae

P. infestans

P. ramorum

RFLR DEKNEER

1 24 42 55 287
*P. infestans* genome shows an unusual variability in intergenic region length (gene density)

**P. infestans** (16442 genes)

- Number of genes per bin
- 3' Intergenic border length (kb)
- 5' Intergenic border length (kb)

Core orthologs (7580)
RXLR effectors (520)

Sylvain Raffaele, Brian Haas
Effector genes populate plastic regions of filamentous plant pathogen genomes.
Sylvain Raffaele; with thanks to Thierry Rouxel
Effector genes populate plastic regions of filamentous plant pathogen genomes
Salisapiliaceae: a new family of salt march saprophytes

Hulvey et al. Persoonia 2010
Salisapilia genomes are significantly reduced relative to other oomycetes.
Why is bigger better in filamentous plant pathogens?

Which evolutionary tradeoffs counterbalance the cost of the larger genomes?
How do pathogens adapt to environmental change?
How does environmental change impact genome evolution?
Models of host-parasite evolution

coevolution / cospeciation

host-jumps / host-shifts

BA Roy, Evolution 2001
Species in the *Phytophthora infestans* lineage (clade 1c) evolved by host jumps

- “Recently” diverged: 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

Effector

Target

Purifying or neutral selection \( \text{dN} \leq \text{dS} \)

Pseudogenization \( \Psi \)

Adaptive selection \( \text{dN} > \text{dS} \)
Genes in repeat-rich regions are more likely to be missing in sister species: 4X faster turnover

Length of intergenic regions (Kb) % of gene class with 0% coverage

Rhys Farrer, Sylvain Raffaele
Genes in repeat-rich regions are more likely to be under positive selection ($\omega = dN/dS > 1$).
Repeat-rich regions are highly enriched in genes induced during colonization of tomato and potato

Average Induction Fold (Log2 T/T₀) in bins (n=3 min)

Sporangium  Zoospore  Tomato 2dpi  Tomato 5dpi

Potato 2dpi  Potato 3dpi  Potato 4dpi  Potato 5dpi

P. infestans intergenic distance

Sylvain Raffaele, Liliana Cano
Summary - The two-speed genome of *Phytophthora infestans*

- **The core genome** - high gene density, low repeat content, carries the core ortholog genes.
- **The ‘plastic’ genome** - low gene density, high repeat content, highly enriched in secreted protein and effector genes.
- Higher rates of gene turnover and positive selection in the ‘plastic’ genome.
- Niches in the genome that enable rapid effector evolution and adaptation to host plants.
Why bigger is better?

-Convergent evolution of large genomes infested with repetitive elements in deep lineages of host-specific plant pathogens

-Which trade-offs drive this evolutionary trend and counterbalance the cost of maintaining these large genomes?

-TEs are thought to enhance plasticity and evolutionary potential of pathogens, but this creates a conundrum because natural selection cannot maintain genes for future use

-Conundrum is solved by the evolutionary concept of clade selection (species selection) put forward by Georges C. Williams
Clade selection

- Lineages that produce new species at a high frequency and, therefore, are better at avoiding extinction, will dominate the biota compared to lineages that are prone to extinction.
- Explains major evolutionary trends (sexual reproduction etc.).
- Our model is that clade selection opposes the advantages conferred by smaller, compact genomes and underlies the evolutionary trend towards larger plastic genomes.
- Lineages with compact genomes have an increased probability of extinction, they suffer a macroevolutionary disadvantage.
Jump or die! Lineages with less adaptable genomes suffer higher extinction rates.
Prospects for blight resistance
Understanding and Exploiting Late Blight Resistance in the Age of Effectors

Vivianne G.A.A. Vleeshouwers,¹ Sylvain Raffaele,² Jack Vossen,¹ Nicolas Champouret,¹ Ricardo Oliva,² Maria E. Segretin,² Hendrik Rietman,¹ Liliana M. Cano,² Anoma Lokossou,¹ Geert Kessel,³ Mathieu A. Pel,¹ and Sophien Kamoun²

¹Wageningen UR Plant Breeding, 6700 AJ, Wageningen, The Netherlands; email: vivianne.vleeshouwers@wur.nl, jack.vossen@wur.nl, nicolas.champouret@sainsbury-laboratory.ac.uk, hendrik.rietman@wur.nl, Anoma.Lokossou@syngenta.com, m.pel@enzazaden.nl

²The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, United Kingdom; email: sylvain.raffaele@sainsbury-laboratory.ac.uk, ricardo.oliva@sainsbury-laboratory.ac.uk, maria.segretin@sainsbury-laboratory.ac.uk, liliana.cano@sainsbury-laboratory.ac.uk, sophien.kamoun@sainsbury-laboratory.ac.uk

³Plant Research International, 6700 AA, Wageningen, The Netherlands; email: geert.kessel@wur.nl
Recent potato and tomato blight epidemics
Genome sequencing of *P. infestans* epidemic strains: “blue 13” asexual lineage in the UK

Associated with severe epidemics, metalaxyl resistant, more aggressive

Liliana Cano; with David Cooke @ JHI
Tomato (and potato) epidemics caused by US22 clonal lineage in North America

- Emerged in Northeast US in summer 2009
- Moved to Canada in 2010

- A2 mating type
- Susceptible to mefenoxam and metalaxyl
Core effectors as targets for resistance

>550 RXLR effector genes in *P. infestans*

- By focusing on *R* genes that recognize “core” *P. infestans* effectors, we maximize the potential for resistance durability in the field
Late blight resistance in *Solanum* germplasm

V. Vleeshouwers, E. van der Vossen *et al.* Wageningen

- Resistant accessions
- Segregation for resistance
- Positional cloning

<table>
<thead>
<tr>
<th>Species</th>
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<th>Genotypes</th>
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<tr>
<td>181</td>
<td>1000</td>
<td>5000</td>
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Effectoromics for late blight resistance

Vleeshouwers et al. Annu Rev Phytopathol 2011
Effectoromics for late blight resistance

Vleeshouwers et al. Annu Rev Phytopathol 2011
Accelerates cloning and profiling of \( R \) genes


Co-segregation of late blight resistance and effector response

Cloning of \( Rpi-blb1 \) (=RB) homologs from \textit{S. stoloniferum} and \textit{S. papita}
Effectoromics for late blight resistance

Vleeshouwers et al. Annu Rev Phytopathol 2011
Nicotiana benthamiana: The ‘HeLa cells’ system of plant biology

Agroinfiltration
- Virus vectors
- Gene co-expression
- Gene silencing
- Cell biology
- Protein biochemistry
- Protein complexes
- High-throughput screens
Exploiting effectors in breeding and deployment of resistance

- Accelerate cloning of disease resistance (R) genes – *effectoromics*: R gene activity screens, R gene allele mining

- Profiling R gene specificities – classify germplasm/R genes, avoid redundant breeding/cloning

- **Synthetic R genes** – Expand effector recognition

- Monitoring pathogen populations – population status in different geographic regions, effector allelic diversity
AVR effectors of *P. infestans*

- AVR1 and AVR4 are dispensable
- AVR2, AVR3a, and AVRblb2 are always present and expressed; polymorphic families

Vleeshouwers *et al.* Annu Rev Phytopathol 2011
The RXLR effector AVR3a is recognized by the NB-LRR protein R3a.

Avirulent on R3a

Leu  Thr  E/K  Arg

Avirulent on R3a

Leu  Thr  E  Arg

virulent on R3a

AVR3a\textsuperscript{KI}  AVR3a\textsuperscript{EM}

- R3a

+ R3a
Balancing selection results in maintenance of both AVR3a alleles in *P. infestans* populations

- *P. infestans* strains always carry an intact AVR3a gene (AVR3a\textsuperscript{KI} and/or AVR3a\textsuperscript{EM})
- AVR3a knock-down mutants have markedly reduced virulence
- AVR3a\textsuperscript{EM} also recognized by an (uncloned) Solanum *R* gene
- Both AVR3a\textsuperscript{KI} & AVR3a\textsuperscript{EM} are predicted to have virulence activities

An R3a mutant that recognizes both AVR3a\textsuperscript{KI} and AVR3a\textsuperscript{EM} is expected to be effective against all *P. infestans* isolates

Artificial evolution to extend R3a recognition: 
Experimental design (Maria Eugenia Segretin)

Random mutagenesis on R3a
1,37 > num. mut/1000bp > 1,92
75-80% positive clones
Library (19x384w plates)

Growth @28°C (O.N.):
pGR106-AVR3aEM (23-147, N-FLAG)
pCB302-3/AVR3aKI

Centrifuge 5min @3400rpm
Resuspend in Infiltration buffer to have a final OD of 0.15 when mixed

Mix
pGR106-AVR3aEM 1:2 R3A mutant clones
Evaluate phenotypes at 5 d.p.i.

Re-inoculate in L-agar plates
(4x96colonies/plate)

Inoculate 96 deep wells plates
(4x96colonies/plate)

Growth @28°C 2 days
Measure OD of 1 column
Centrifuge 5min @3400rpm
Resuspend in Infiltration buffer to have a final OD of 0.3 when mixed

Confirm positive clones co-infiltrating again with pGR106-AVR3aEM and pGR106-DGFP (to exclude autoactivation). Also with pCB302-3/AVR3aEM
R3a mutants that recognize AVR3aEM recovered

Co-infiltrate the candidate clones with pGR106-AVR3aEM, pGR106-DGFP or pGR106-AVR3aKI.
Analyze the phenotype day by day (starting at 2 d.p.i.). 12 spots per clone.
Establish and “HR index” from 0 (nothing visible) to 10 (confluent necrosis)
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<td>1B/A10-N</td>
<td>1B/A10-C</td>
</tr>
<tr>
<td>1B/A10-N</td>
<td>1B/A10-C</td>
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R3a+ mutants that sense AVR3a homologs from other *Phytophthora* species

*P. capsici* AVR3a11  
vector control
Next generation resistance breeding

• R3a+ predicted to confer resistance to all strains of *Phytophthora infestans* and some other *Phytophthora* spp.

• Single amino acid mutations expand effector recognition

• Recognition of effectors from diverse species

• Basic knowledge of pathogen effectors essential

• Non-GM solutions?
Targeted genome mutagenesis and editing

TAL effectors – Designer DNA binding proteins

TAL hypervariable amino acids: \text{NI HD HG NG}

Target base: \text{A C G T}

Moscou et al.; Boch et al. Science 2009
Marton et al. Plant Physiol 2010
Targeted genome mutagenesis to engineer disease resistant crops

- TALN (TAL-nuclease) technology greatly facilitates genome engineering
- Mutant plants are recombinant DNA-free (no transgenic sequences, indistinguishable from naturally occurring mutations)
- Opportunity to further integrate biotechnology with plant breeding
An arms race between the biotechnologist and the pathogen?

- Ability of the pathogen to adapt is astounding
- “Never bet against the pathogen” – silver bullet solutions unlikely to be durable
- Our vision: Framework to rapidly generate new resistance specificities and introduce these traits into crop genomes
- Can we generate and deploy new resistance traits faster than the pathogen can evolve?