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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





*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

The New York Times

February 4, 2011



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

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

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

12 FEBRUARY 2010 VOL 327 SCIENCE

BLACK SIGATOKA

Crops: Bananas, plantains Whereabouts: This fungus, first detected in Fiji in 1964, is now found in 100 countries in the Americas, Africa and

Pest: Mycosphaerella

fijiensis

Pest: Puccinia graminis Ug99 Crop: Wheat

WHEAT STEM RUST

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





obligate biotroph; 🛞 biotroph;

A hemibiotroph; A necrotroph.

gene/pathways losses
gene gain or gene families expansion non-repetitive
repetitive

Expanded filamentous plant pathogen genomes are enriched in noncoding DNA



Sylvain Raffaele

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

Reduction and Compaction in the Genome of the Apicomplexan Parasite Cryptosporidium parvum

Parasites genomes are often considered to be "reduced" or "degenerate," but exactly what do these terms mean? How various are the forces that affect genome size and density, and how do their effects differ in different parasites?

The genome of *Tetranychus urticae* reveals herbivorous pest adaptations

At 90 megabases *T. urticae* has the smallest sequenced arthropod genome.

Extreme genome reduction in symbiotic bacteria

John P. McCutcheon¹ and Nancy A. Moran²

Sequence and genetic map of *Meloidogyne hapla*: A <u>compact</u> nematode genome for plant parasitism

Charles H. Opperman^{a,b,c}, David M. Bird^{a,b}, Valerie M. Williamson^d, Dan S. Rokhsar^e, Mark Burke^a, Jonathan Cohn^a, John Cromer^a, Steve Diener^{a,†}, Jim Gajan^a, Steve Graham^a, T. D. Houfek^{a,g}, Qingli Liu^{d,h}, Therese Mitros^I, Jennifer Schaff^{a,J}, Reenah Schaffer^a, Elizabeth Scholl^a, Bryon R. Sosinski^{k,J}, Varghese P. Thomas^d, and Eric Windham^a

- 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



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



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



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



Modular structure of RXLR effectors



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)



	20	40	60	80	100	120
PiPGG1_3	MRISYALTVTVATLLVPSNALVNSKPAMLS	SPPGEP SQ RHLR SHDT PVL ⁱ	VDDY NAD <mark>EE R</mark> GLD KAAMKT MWB	DGMSAAGYAKKLGITDKIAL	AEKSAGVLQQLMQTRRYEKYQ	QYENYEAKKNKKKKPDEIYES
PiPGG1_4	T	E		т	S	
PiPGG1_1	.CAS.K	E.L	N.KSK	WG.DSPD.A.DH	тАНКК	TITN.V.Y.
PiPGG1_2	AS.K	E	N.KSk	WDNPA.DH	TATHKK	TITQ.N.V.Y.

• Consistent with the view that RXLR effectors are modular

Win et al. Plant Cell, 2007

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?



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The genome sequence of *Phytophthora infestans* Brian Haas, Mike Zody, and Chad Nusbaum @ Broad Institute







Oomycete genome sequences from divergent species



Major features of the genome of *P. infestans*

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

• 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





B. Haas, S. Kamoun et al. Nature, 2009

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





P. infestans genome shows an unusual variability in intergenic region length (gene density)

Core orthologs (7580)

P. infestans (16442 genes)



Effector genes populate plastic regions of filamentous plant pathogen genomes



P. Infestans gene-sparse vs L. maculans isochore-like regions



Leptosphaeria maculans



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



Species	Total No contigs	Average length	Longest contig	Number of bases assembled (Mb)	N50
S. sapeloensis LT6440	4363	5305.1	176061	23.1	29926
S. nakagirii LT6456	2020	13812.4	281703	27.9	71080
S. sterilis LT6471	2430	8544.3	283395	20.8	47392

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How do pathogens adapt to environmental change? How does environmental change impact genome evolution?



Models of host-parasite evolution



Species in the *Phytophthora infestans* lineage (clade 1c) evolved by host jumps

Clade 9

Clade 10

Pythium undulatun



multilocus phylogeny of Phytophthora from Blair et al. 2008 Fungal Genet Biol

Host jumps must have a dramatic impact on effector evolution



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 ($\omega = dN/dS > 1$)



* Normalized ω for genes in bin is: sum of ω values for genes in bins / sum of bases at least covered twice for genes in bin

Rhys Farrer, Sylvain Raffaele

Repeat-rich regions are highly enriched in genes induced during colonization of tomato and potato

Average Induction Fold (Log2 T/T_0) in bins (n=3 min)



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

pathogen with plastic genome frequency Host plant lineages of host jump Lineage extinction Pathogen jumps to susceptible or new host



Raffaele and Kamoun, submitted GC WIliams (1992) Oxford Uni Press





Understanding and Exploiting Late Blight Resistance in the Age of Effectors

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Recent potato and tomato blight epidemics



"Growers need to be aware that we are dealing with a different type of blight these days," advises Potato Council blight specialist Gary Collins. "It is more aggressive, it is fitter and it will come into crops earlier. So consult an agronomist to ensure you correctly interpret the risk of blight, indicated by Smith Periods, and choose the right control strategy."

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



Aggressive on tomato

- 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



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 V. Vleeshouwers *et al.* (2008) PLoS One



Co-segeregation of late blight resistance and effector response

Cloning of *Rpi-blb1* (=*RB*) homologs from *S. stoloniferum* and *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




Functional cloning of AVRblb1and AVRblb2

RpiBlb1

RpiBlb2



Exploiting effectors in breeding and deployment of resistance

breeding, biotech

- 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 deployment

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



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

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

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

• Armstrong *et al.*, *PNAS*, 2005; Bos *et al.*, Plant J., 2006; Bos *et al.*, PNAS 2010

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



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

R3a mutants that recognize AVR3a^{EM} 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)





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



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?