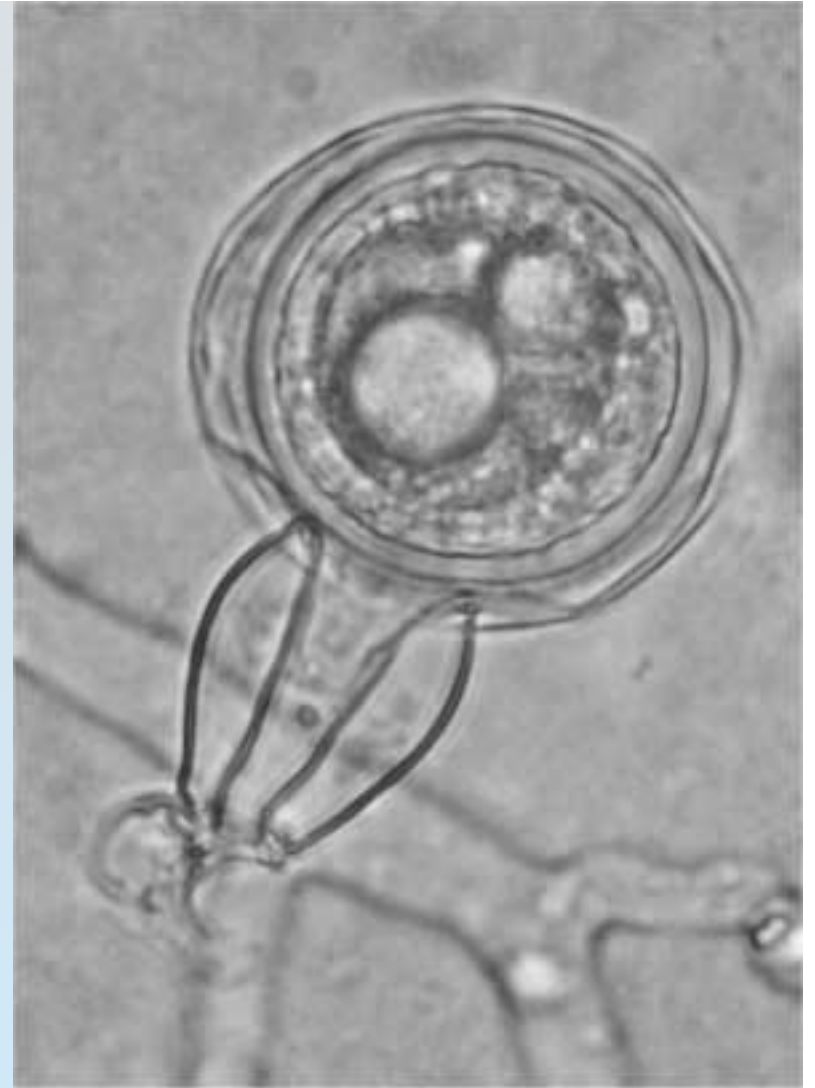
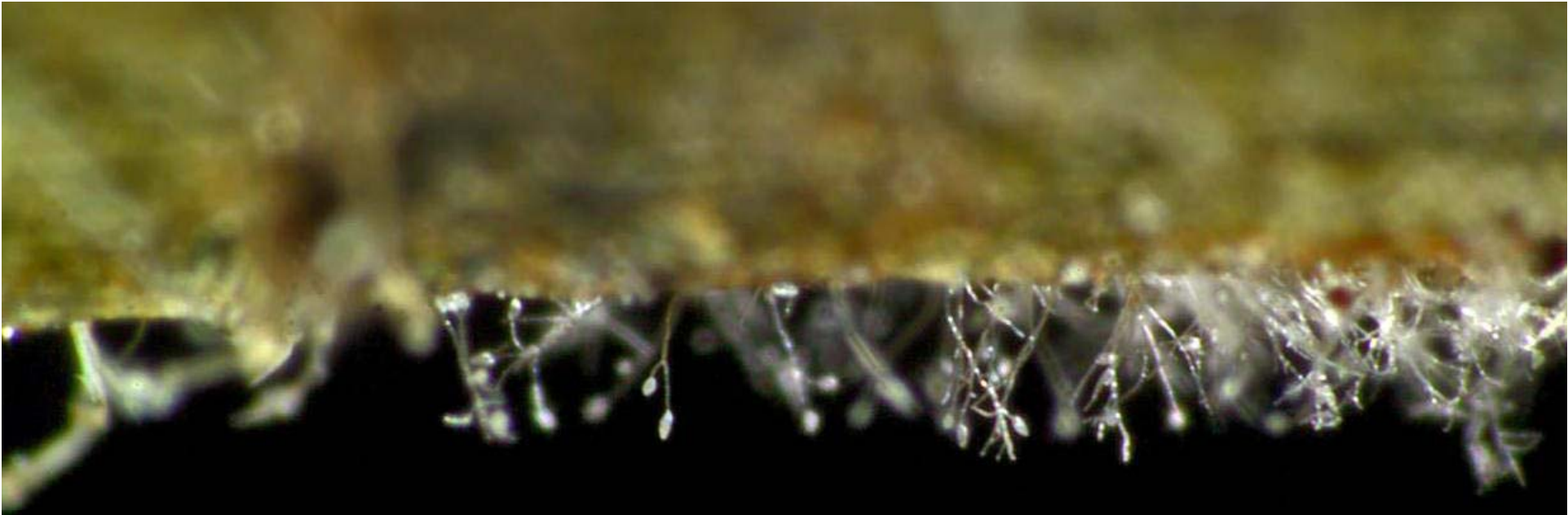
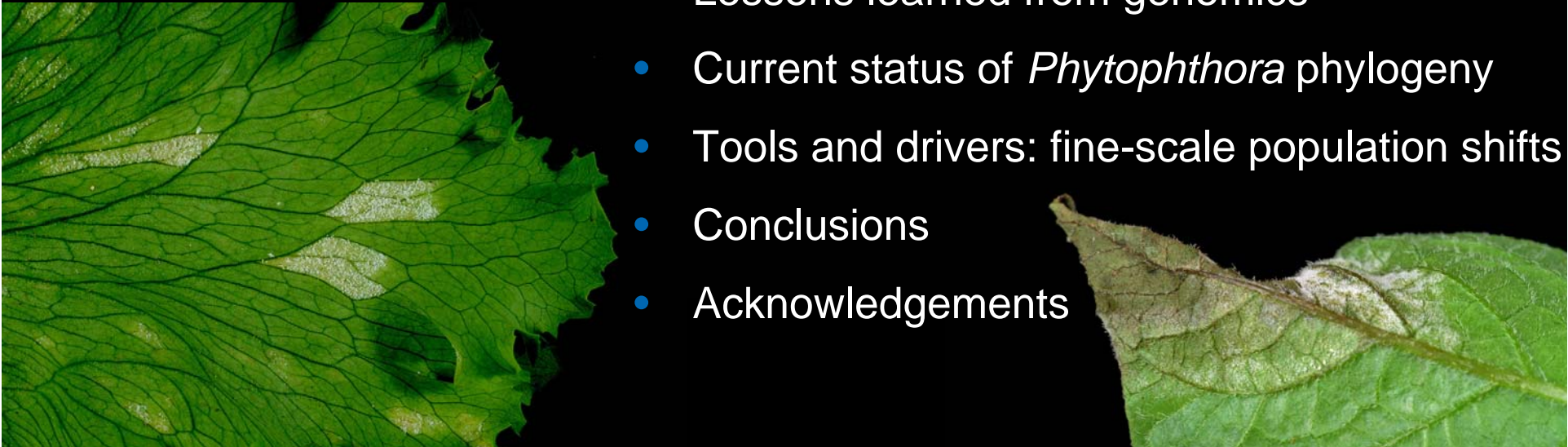


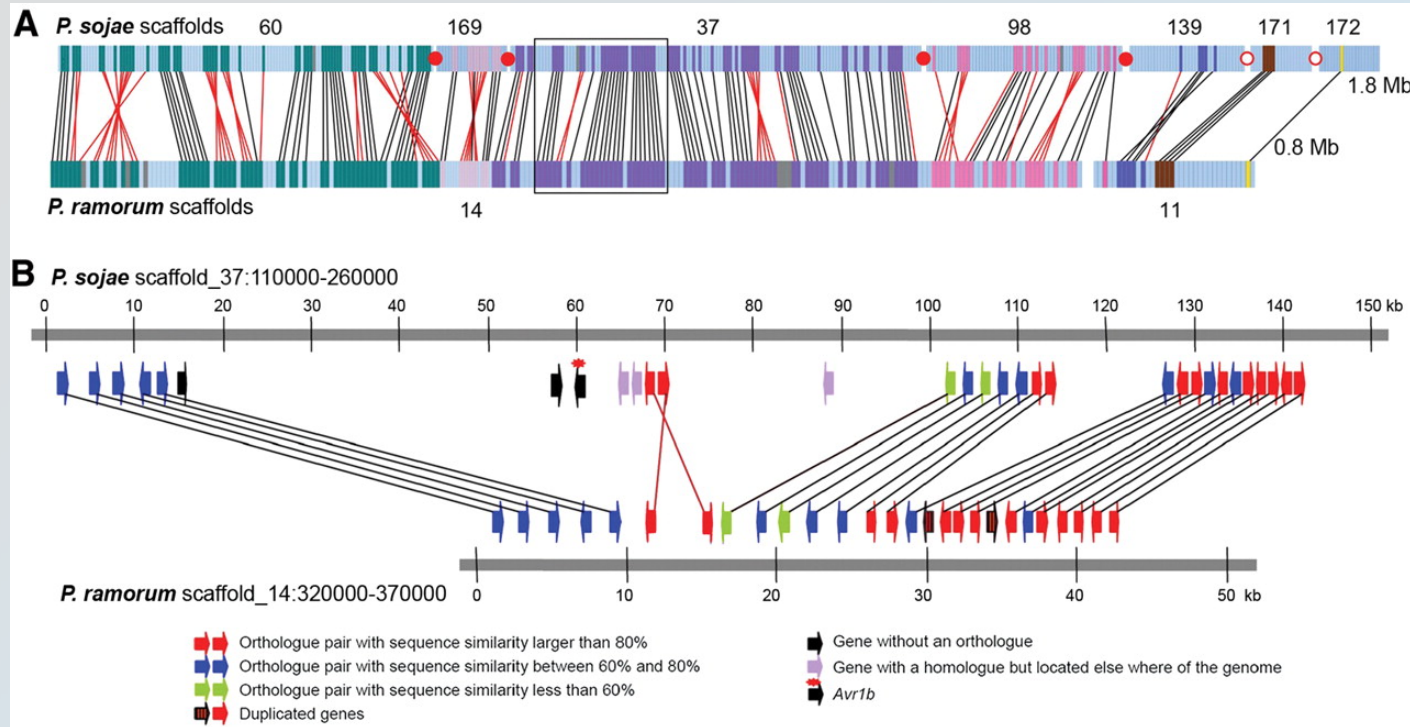
**A clearing picture of
Phytophthora evolution: from
the wide-angle to the zoom lens
for optimal phylogenetic focus**



David Cooke, SCRI

- 
- Introduction – aims of paper
 - Early evolution of oomycetes
 - Lessons learned from genomics
 - Current status of *Phytophthora* phylogeny
 - Tools and drivers: fine-scale population shifts
 - Conclusions
 - Acknowledgements
- 

Genomics in the oomycetes

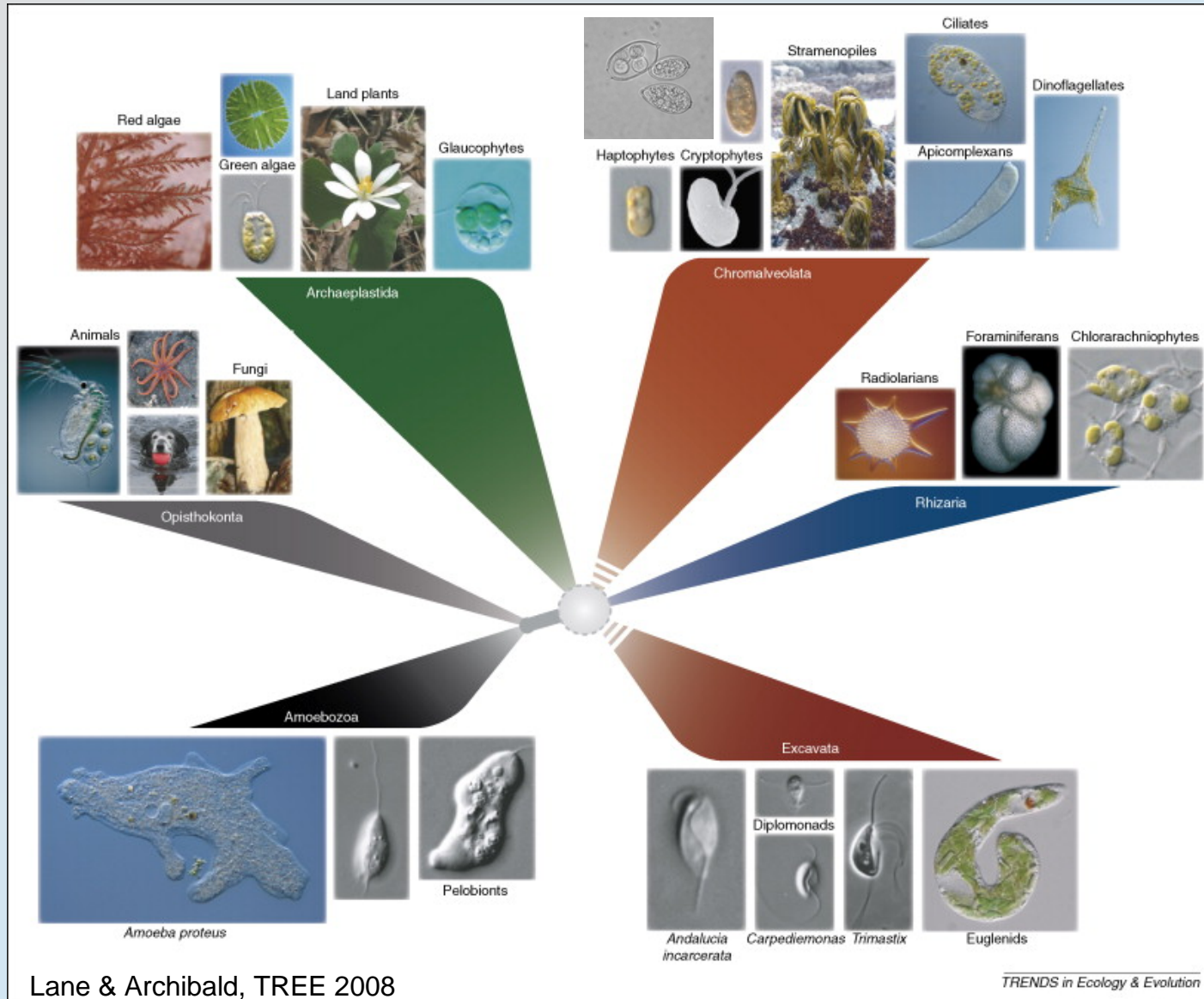


P. infestans
P. ramorum
P. sojae
H. parasitica
P. capsici

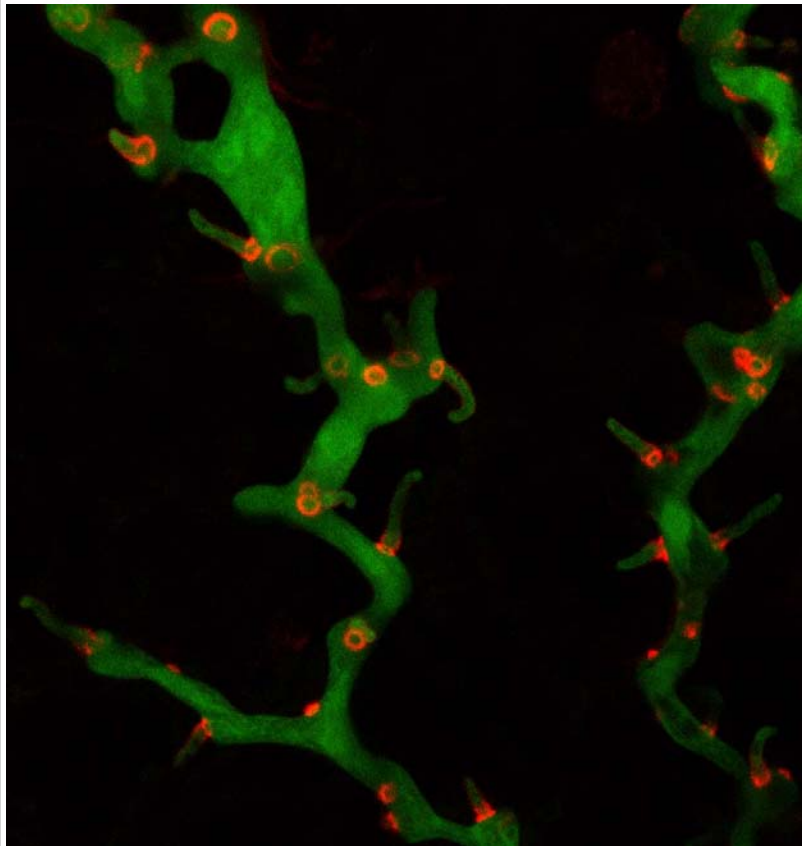
P. andina
P. mirabilis
P. phaseoli
Albugo
Pythium?
Saprolegnia?
Aphanomyces

High throughput sequencing technologies
providing many new opportunities

Eukaryote tree of life



Contributions of genomics



Lane & Archibald, TREE 2008

(b)

Tyler *et al.*, 2006 *Science*
P. ramorum and *P. sojae* genomes
Found 855 *Phytophthora*
genes with similarity to red
algae or cyanobacteria

Whisson *et al* 2007 *Nature*
Shared mechanisms of
effector transport into host
cells – oomycete and
Apicomplexans

phyte plastid ○ Plastid loss? ● Reduced plastid or partial loss
in lineage

TRENDS in Ecology & Evolution

RxLR class of effectors considered key to pathogenicity: a reduced repertoire noted in *Hy. parasitica* genome (Warwick HRI).

Also an absence of genes involved in zoospore formation...



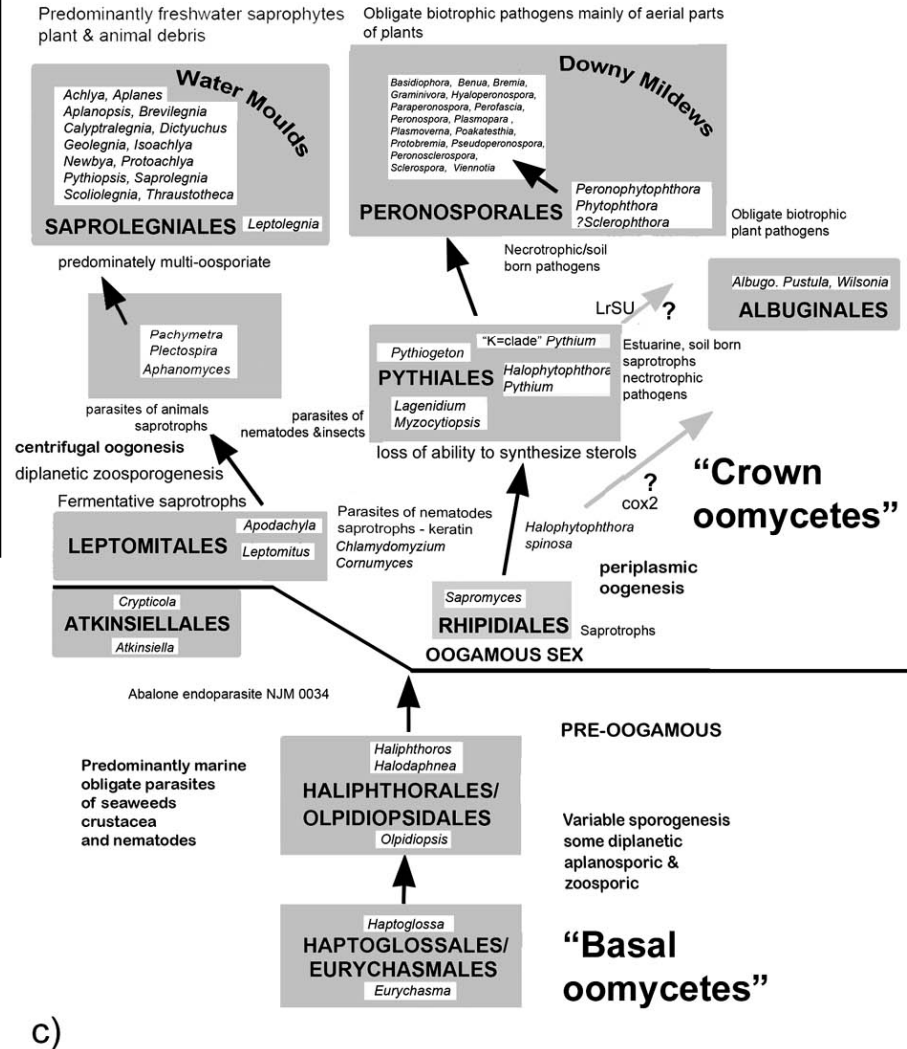
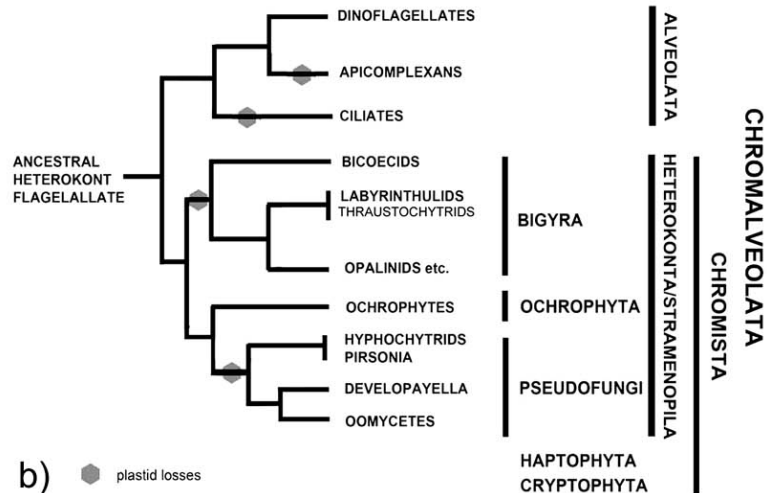
Long history of parasitism in oomycetes



Gordon Beakes – Chapter in “Oomycete Genetics and Genomics: Diversity, Plant and Animal Interactions, and Toolbox” (in press, 2008)

Long history of parasitism in oomycetes – diverse and elegant range of infection devices & abundance of marine species.

Tree of Life upgrade planned

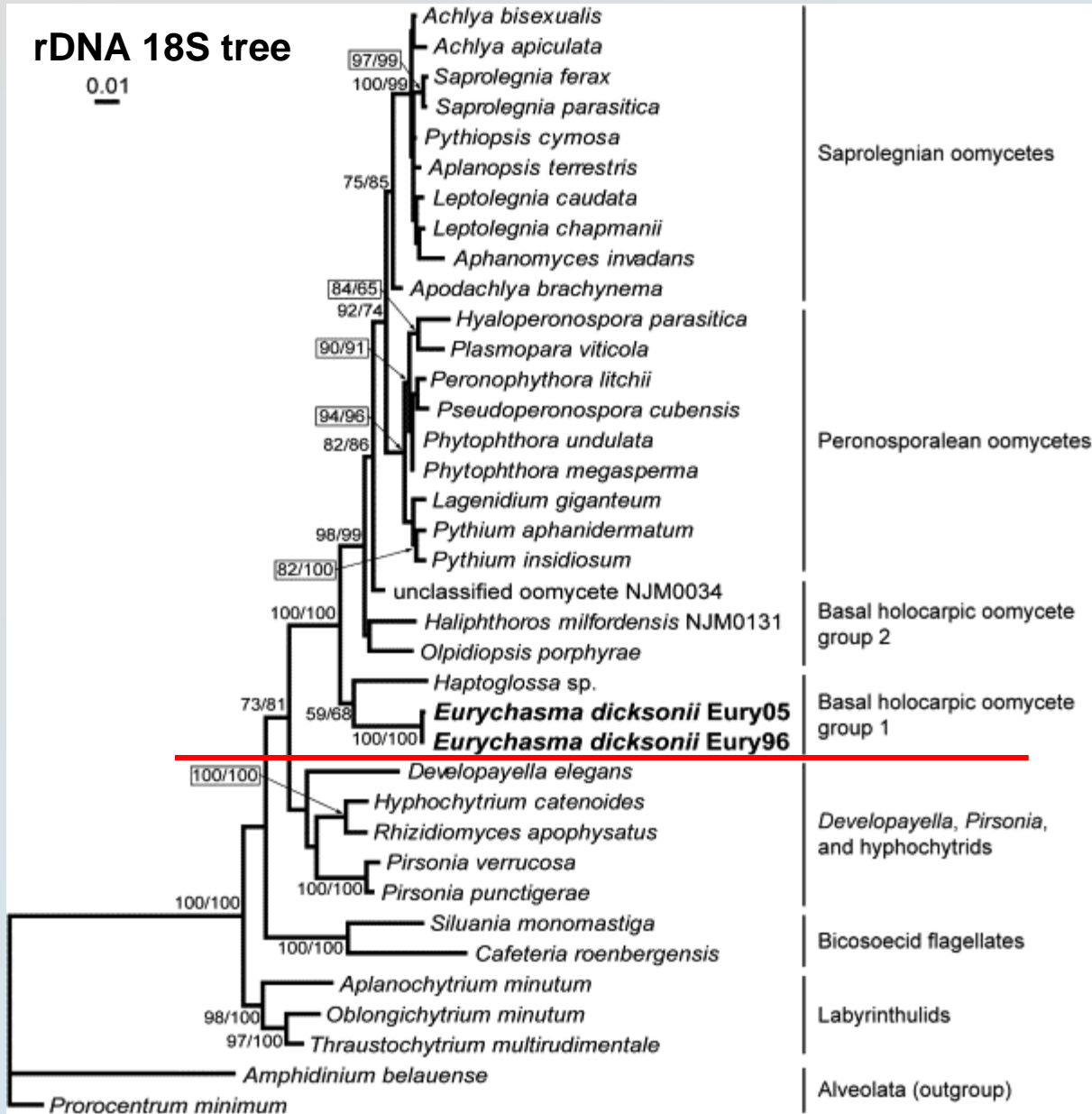
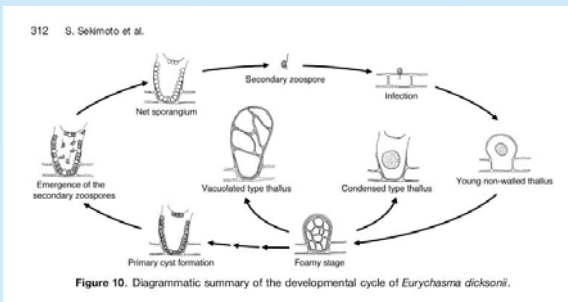


Basal oomycetes

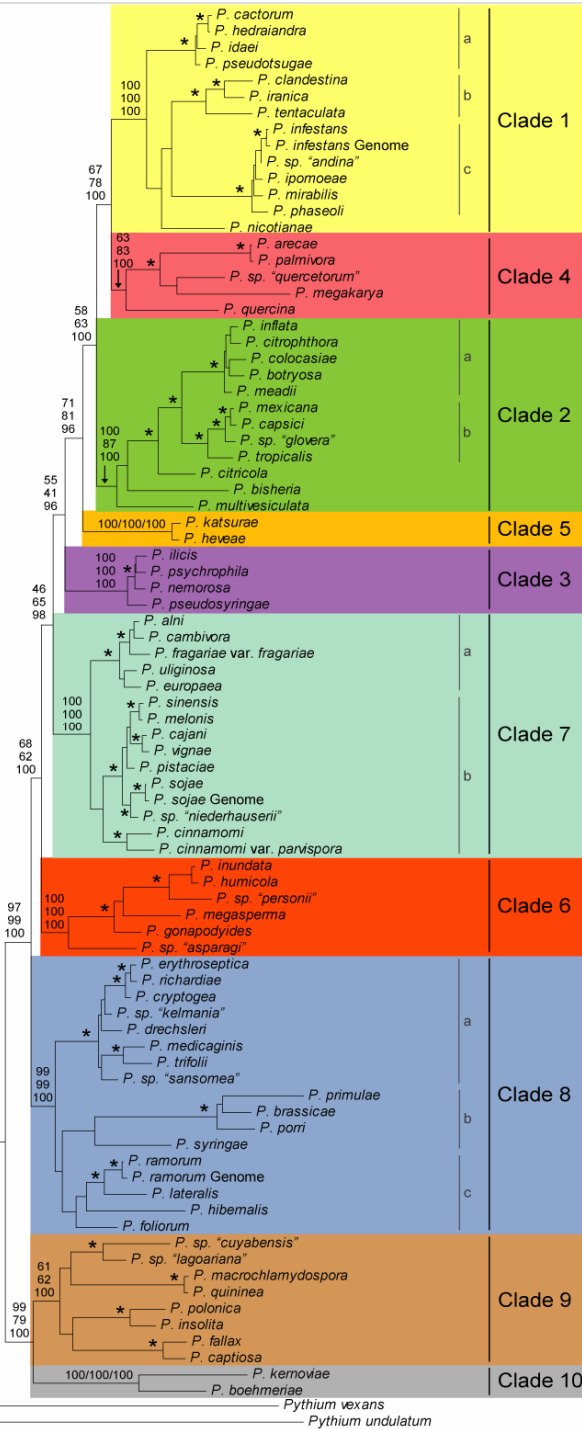
The Development, Ultrastructural Cytology, and Molecular Phylogeny of the Basal Oomycete *Erychasma dicksonii* ...

Satoshi Sekimoto, Gordon W. Beakes, Claire M.M. Gachon, Dieter G. Müller, Frithjof C. Küpper, Daisuke Honda *Protist*, 2008

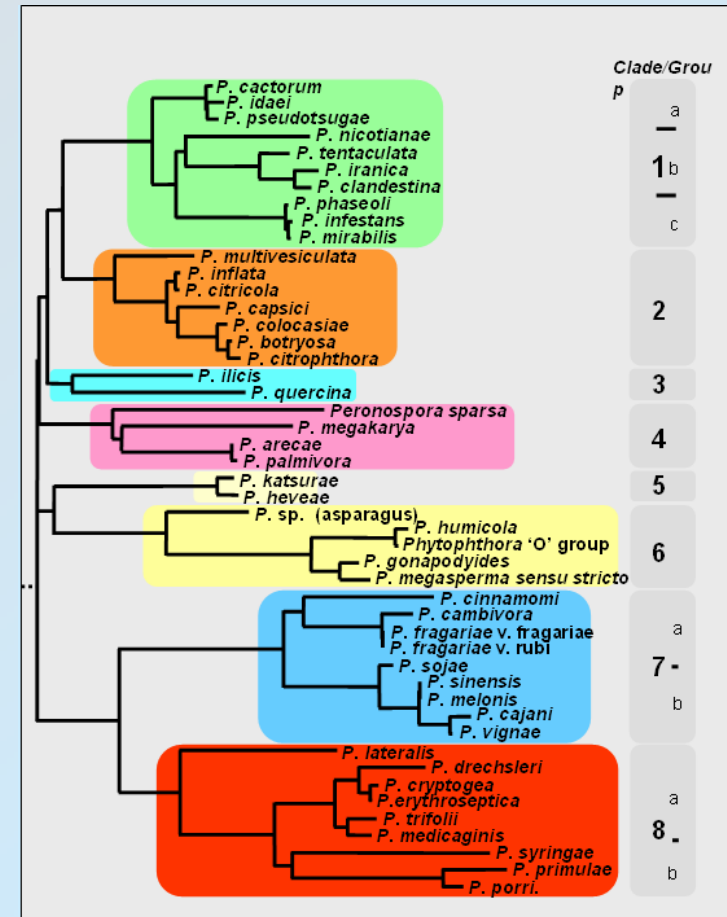
Parasites of plants across oomycete lineages



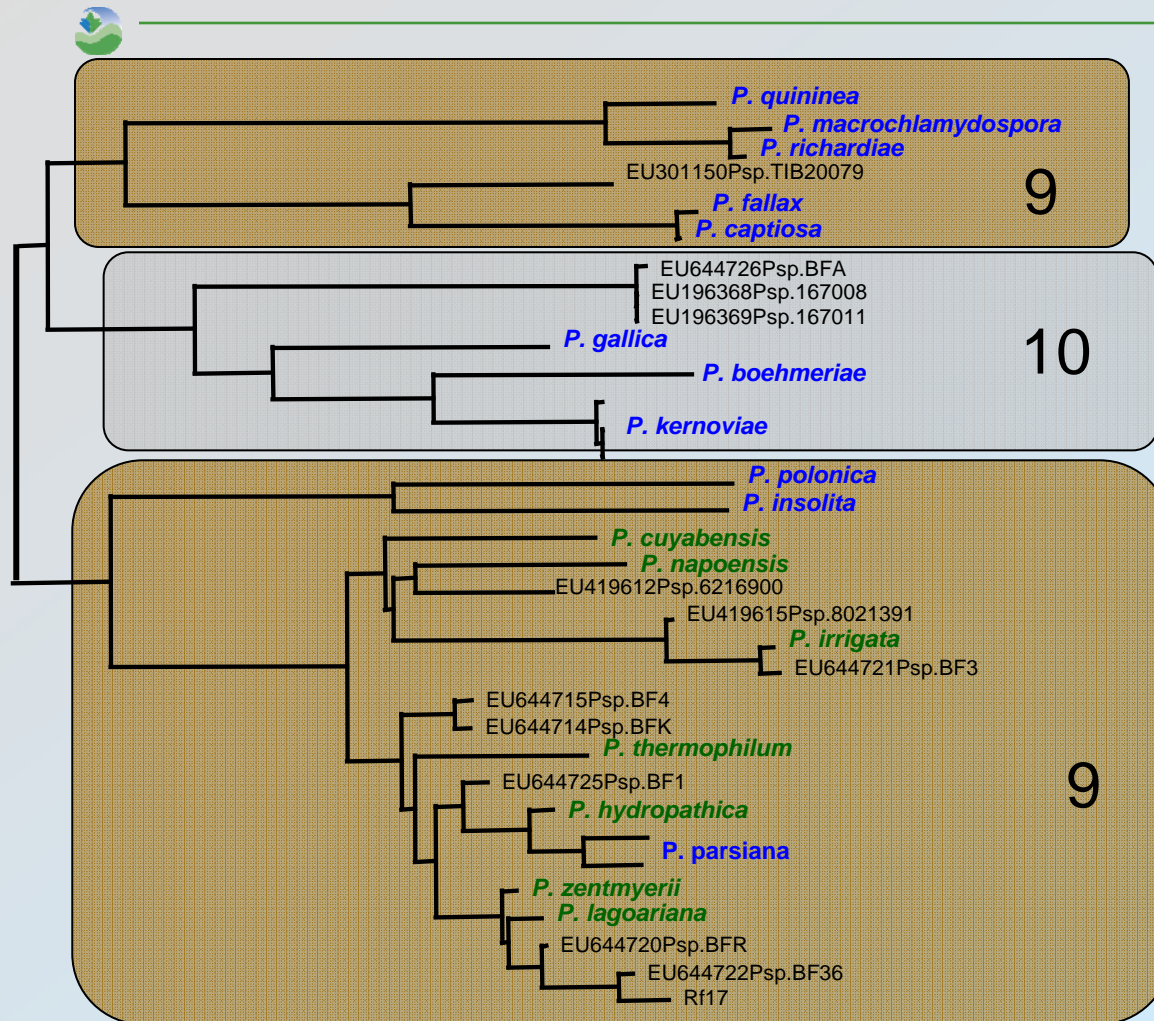
Cooke et al 2000 c.f. Blair et al 2007



- Multigene approach more robust
- Clades shared
- Basal branch structure similar



Expansion of clade 9 & 10



- Discrete lineage from majority of genus
- No obvious common morphological/behavioural traits
- Distinct geographical origin but blurred by migration?

Status of *Phytophthora* phylogenetics?



- Move from single gene to multigene phylogenies
- ITS remains good starting point
- Number of species increasing but main structure unchanged
- Expansion of clades 9 & 10 most marked
- Ever expanding range of taxa in clade 6
- Monitoring methods by Silvia Scibetta – powerful tool
- Still issues with GenBank – reference collection better in independent source (Phytophthora database)

Oomycete pathogen population analysis



- **Neutral markers**

AFLPs

SSRs

P. infestans, *P. ramorum*, *Pl. viticola*

Accelerated discovery examined by Schena and Cooke (*BMC Genomics* submitted)

Sequencing

Coalescent analysis

- **Functional markers**

Host range

Virulence

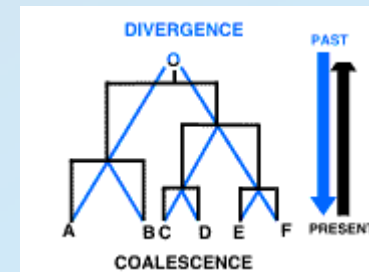
Aggressiveness & Fitness

Fungicide resistance

Sequencing genes under strong selection

effector genes e.g. RxLRs *P. infestans*, *Hy. parasitica*

- Mutation
- Recombination
- Natural selection
- Gene flow
- Random genetic drift
- Migration



Carbone web page

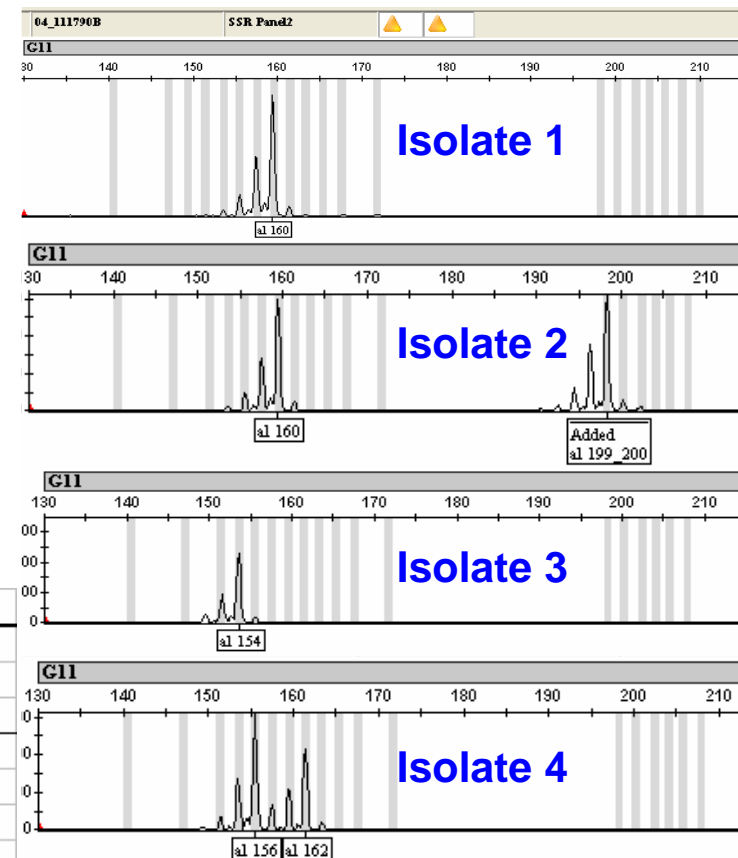
Simple Sequence Repeats – *P. infestans*



ABI 3730 capillary sequencer

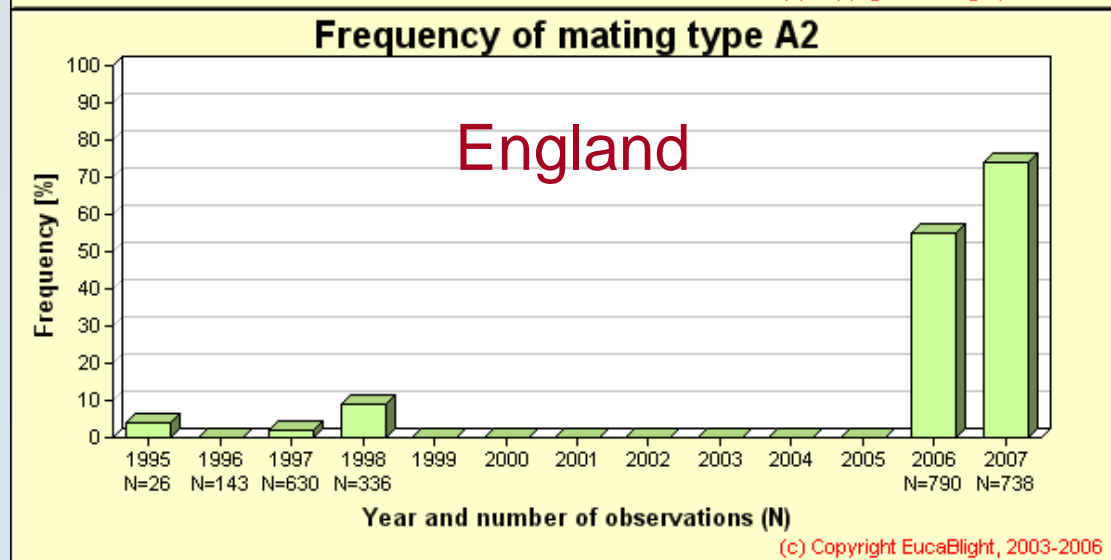
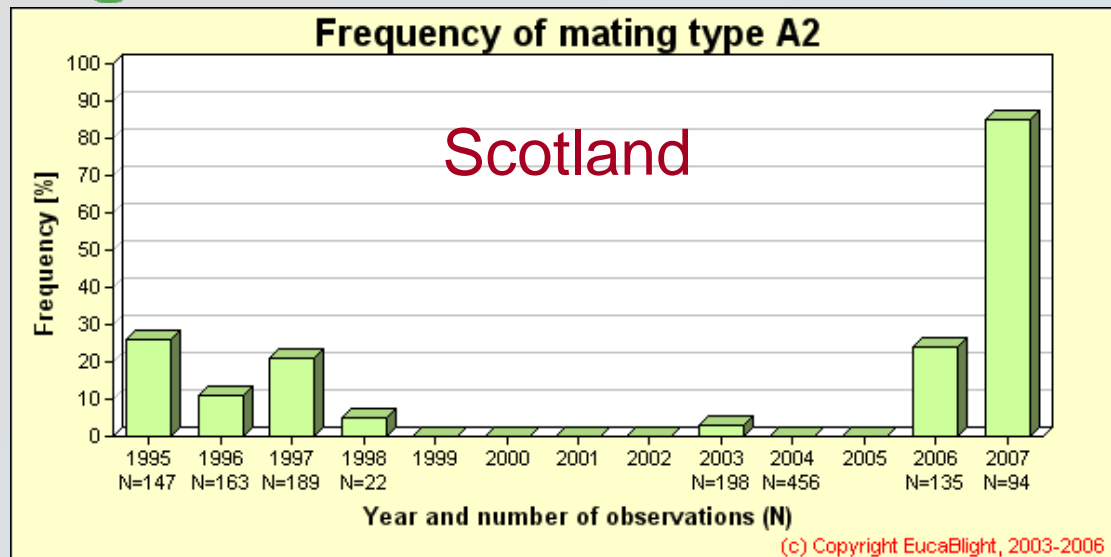
Marker	Panel	Source	Seq	Type	No of alleles
Pi02	Panel1	SCRI	(TG)11	EST	11
Pi89	Panel1	SCRI	(AT)9	EST	16
Pi4B	Panel1	Syngenta	(TC)34	?	8
PiG11	Panel2	Syngenta	(TC)26	?	30
Pi04	Panel2	SCRI	(GT)6	BAC	6
Pi70	Panel2	SCRI	(AAG)8	EST	4
Pi56	Panel2	SCRI	(AT)10	EST	2
Pi63	Panel2	SCRI	(GA)8	EST	3
D13	Panel3	Bangor	(CT)27	?	37
Pi16	Panel3	SCRI	(GA)7	EST	10
Pi33	Panel3	SCRI	(CAG)5	EST	4

Marker G11



Multiplex PCR – protocols
on www.eucablight.org

Changes in British *P. infestans* population



- Eucablight database - powerful
- Increase in A2 mating type seen in GB
- What about genotypes?

Derived data plotted on web

Welcome
David Cooke



EUCABLIGHT
Potato Late Blight Network For Europe

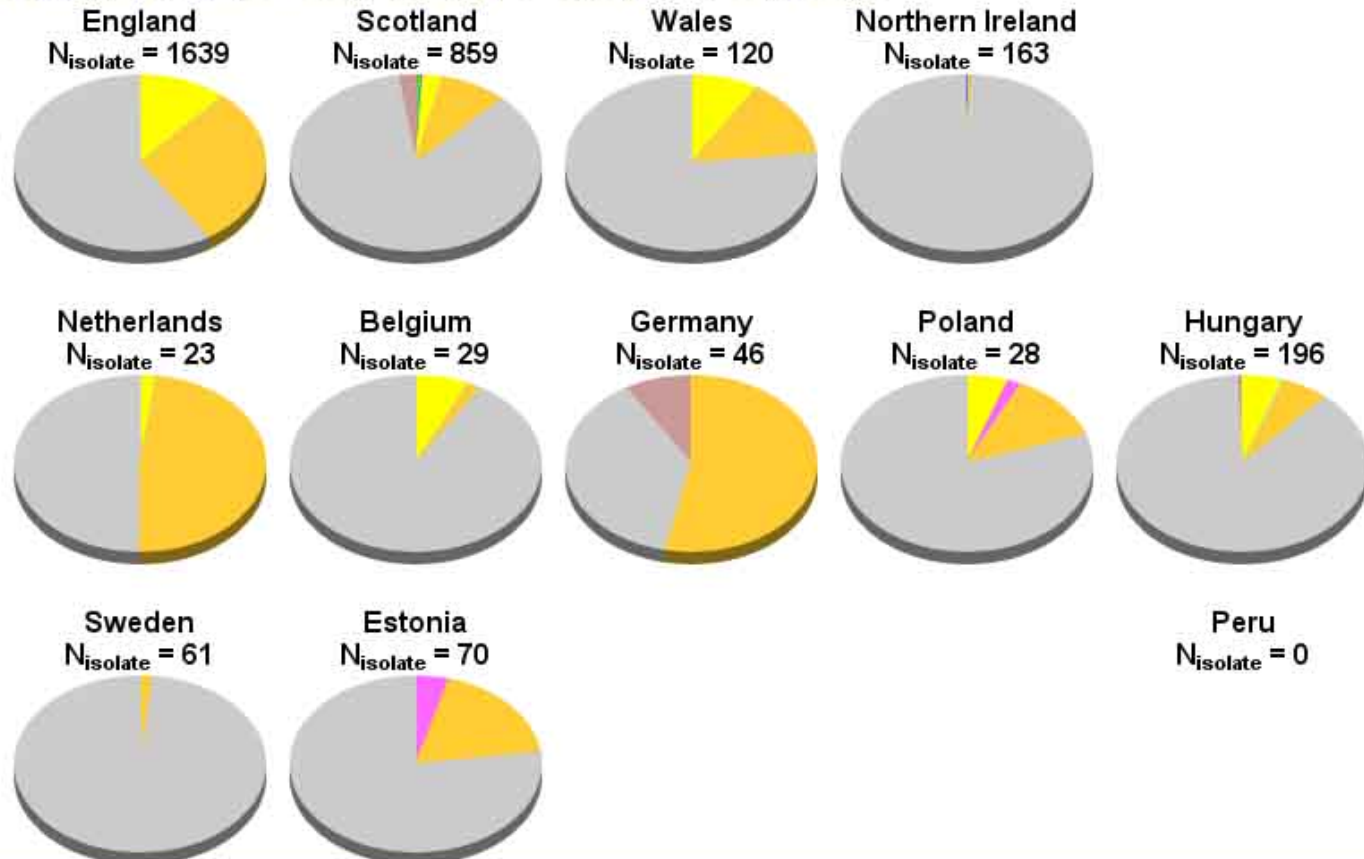


- Overview
 - Graphic analysis
 - Genotype analysis
 - Virulence analysis
 - Documentation
 - SSR analysis**
 - Map
- Pie
 - My isolates
 - My transfers
 - Gene names

Allele frequency for marker Pi02 by country for all years

Legend

- 0
- 142
- 150
- 152
- 156
- 158
- 160
- 162
- 164
- 166
- 168

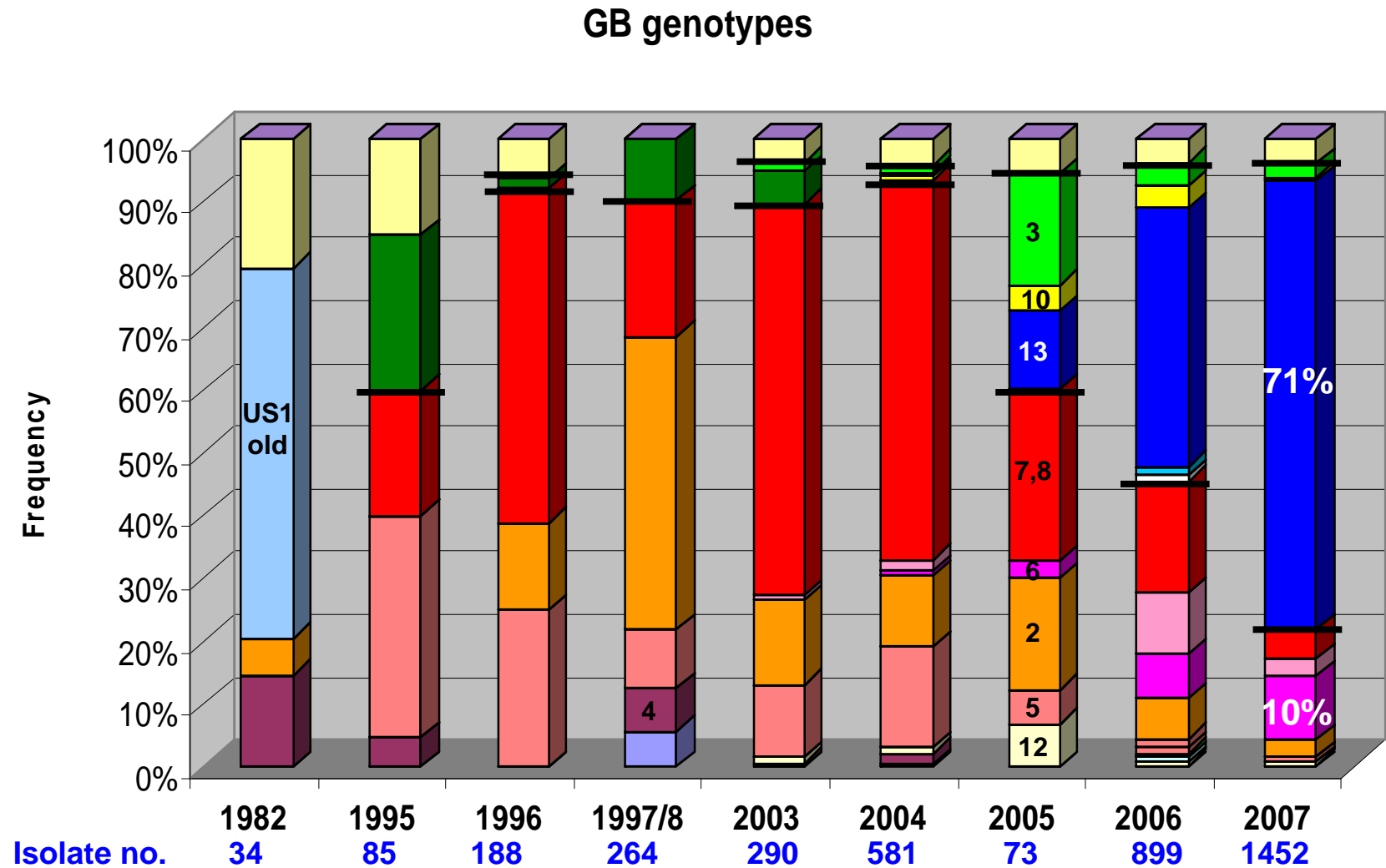


Differences in allele frequency between countries

Expansion to South and Central America complete

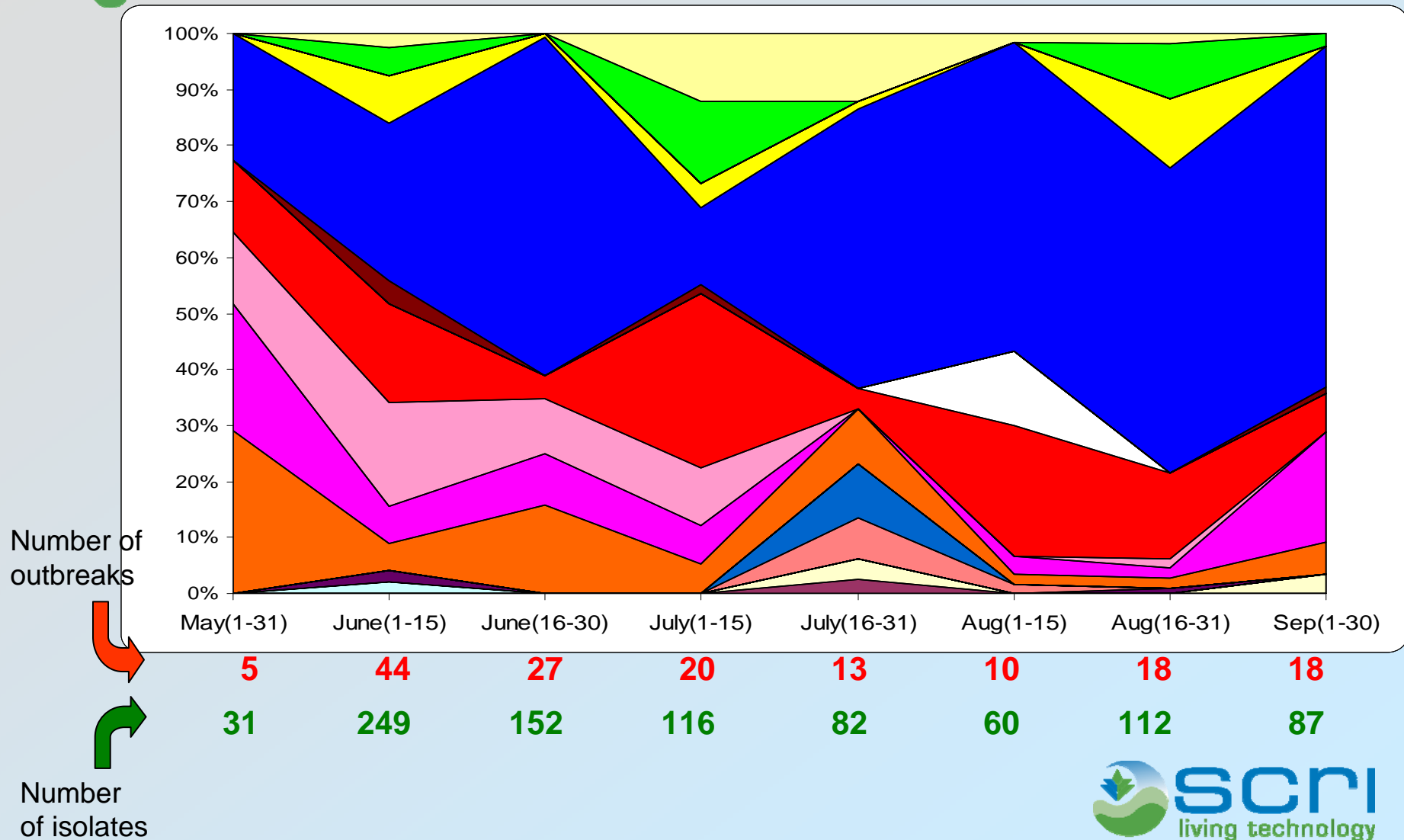
Further expansion under discussion

Great Britain SSR genotypes 1982-2007

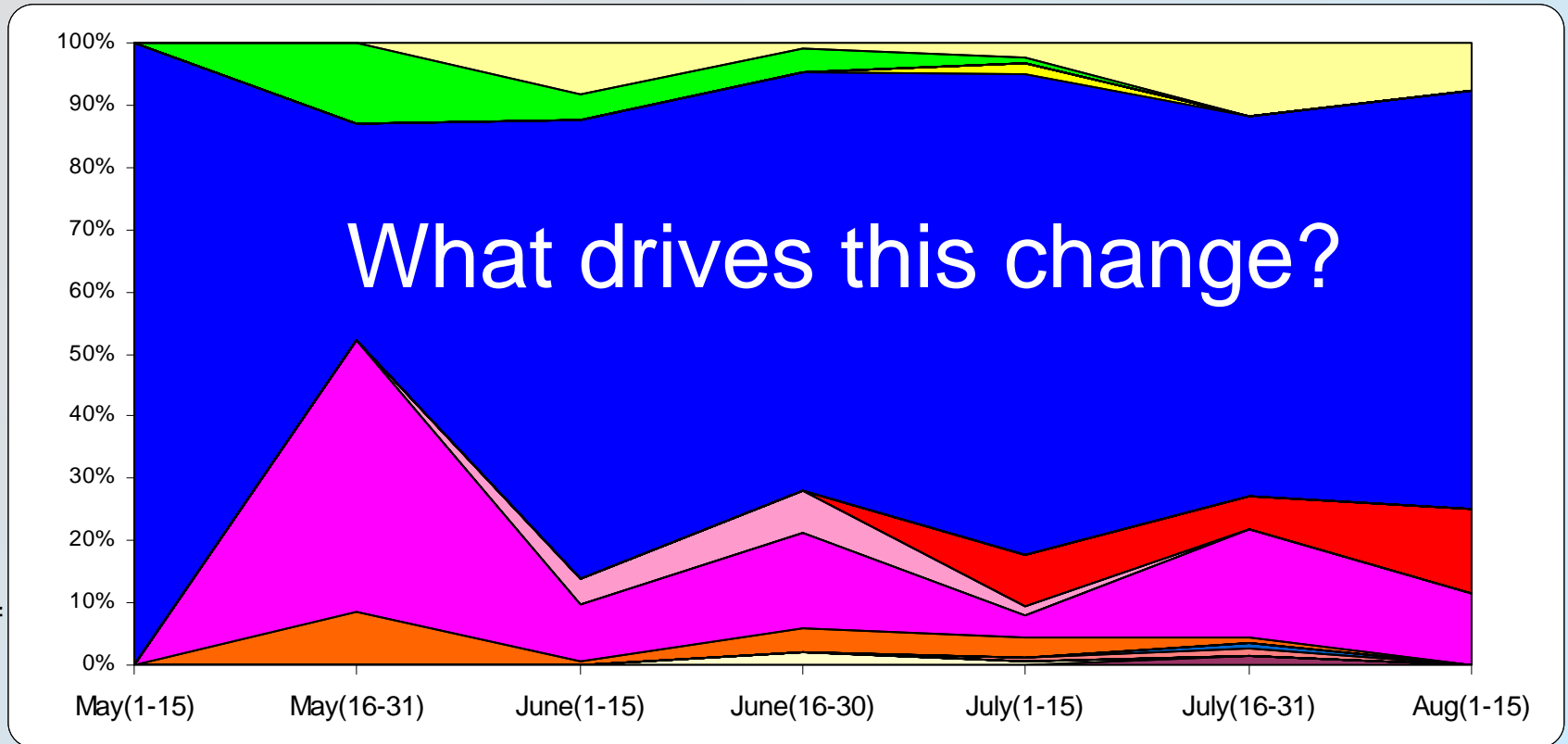


- Major change in 2006 and 2007
- Some genotypes very durable, others not. Why?

GB genotype frequency within 2006 season



GB genotype frequency within 2007 season



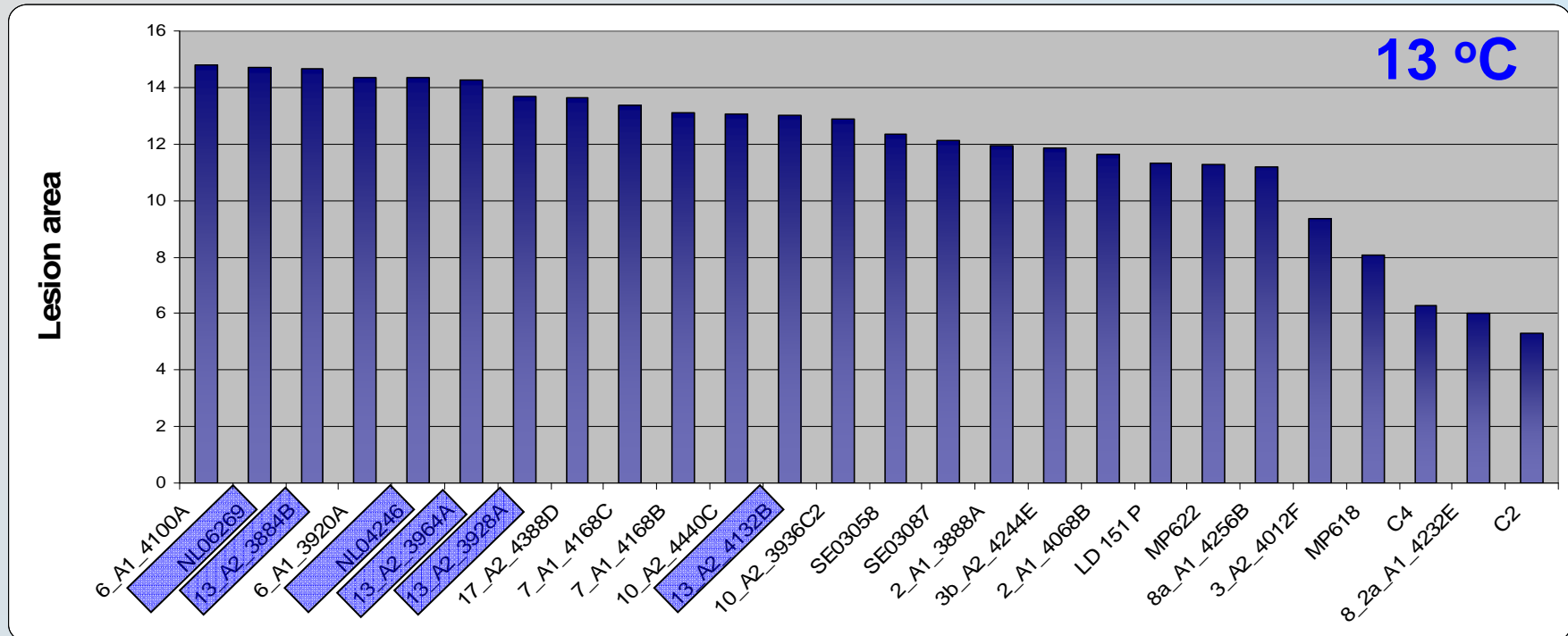
Number of outbreaks



Number of isolates

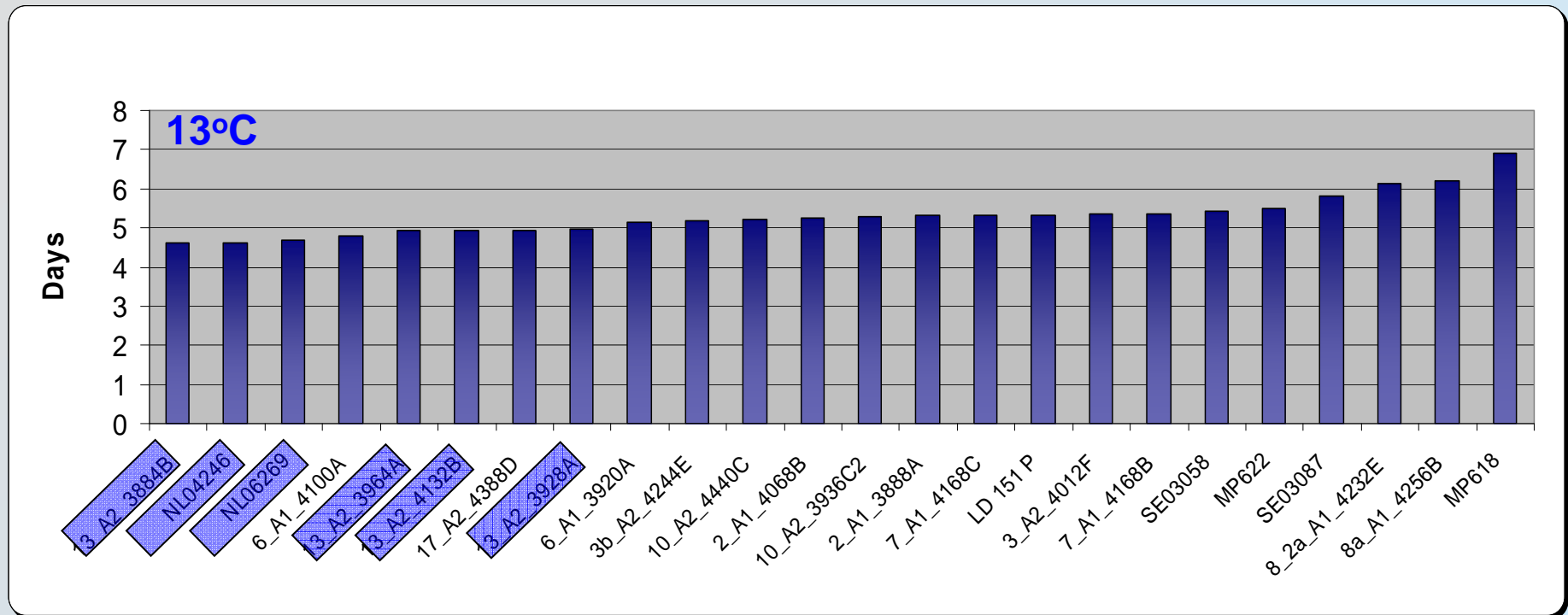
Period	Number of outbreaks	Number of isolates
May(1-15)	4	29
May(16-31)	4	23
June(1-15)	22	144
June(16-30)	80	387
July(1-15)	139	574
July(16-31)	45	220
Aug(1-15)	14	52

Lesion area (mean of all varieties)



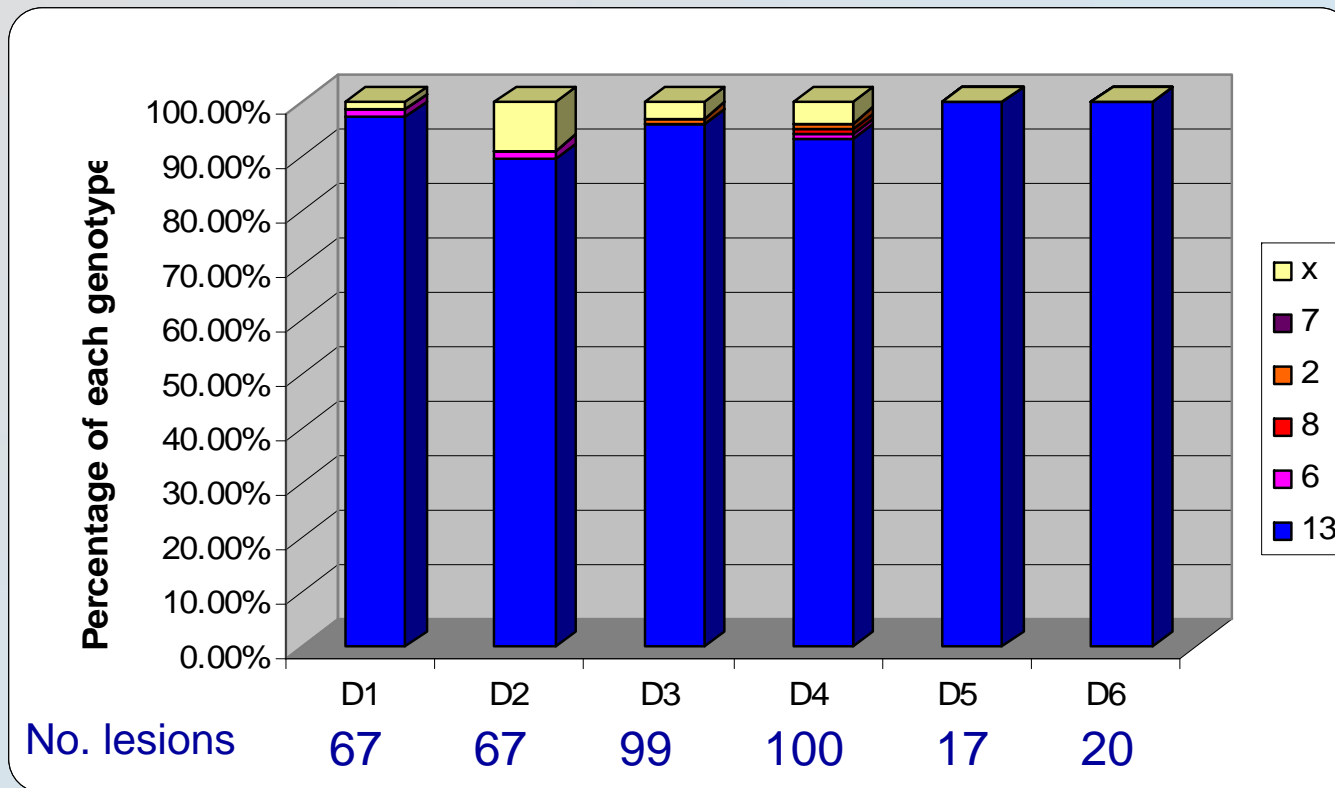
- On average, genotype 13 isolates result in larger lesions than other genotypes at 13°C
- Genotype 6 largest lesions of A1 genotypes

Latent period



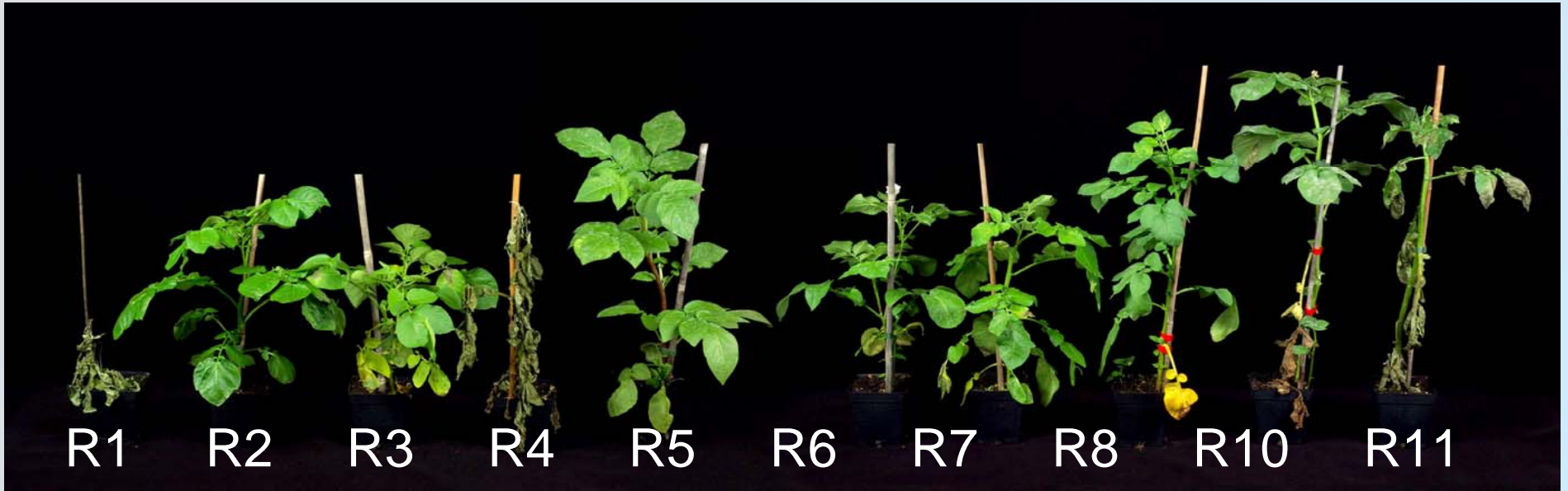
- On average, genotype 13 isolates sporulate sooner than other genotypes at 13°C
- Genotype 6 shortest LP of A1 genotypes

Field trial results



- All isolates were pathogenic in lab test at D0
- Domination of genotype 13 clear
- Four other genotypes (1 alien) rare

Virulence of genotype 8- A1



BPC_06_4256B = 8_A1 1,4,7,10,11

Less complex and less aggressive (note 7,10&11)

Virulence of genotype 13 - A2

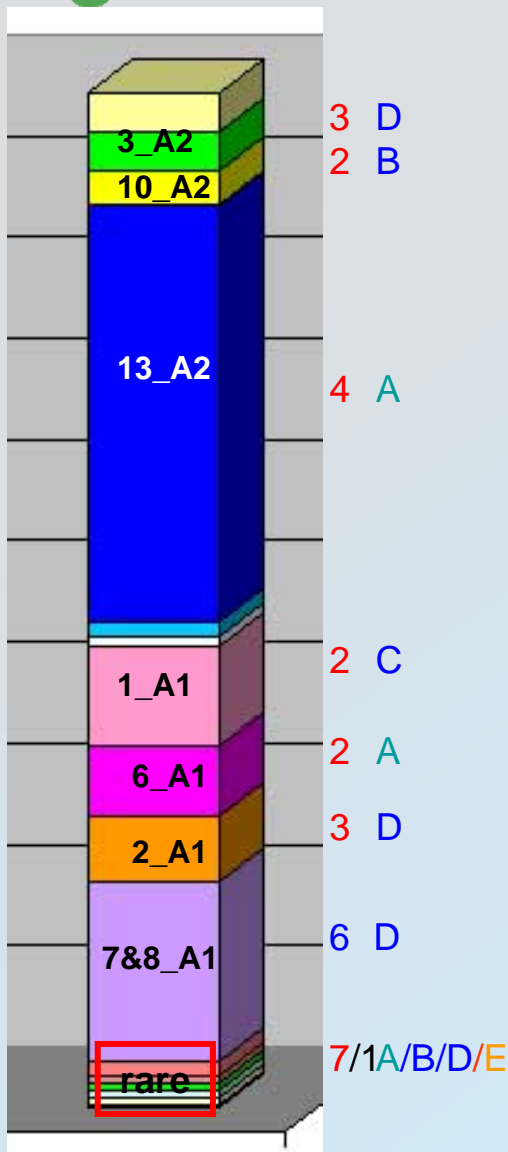


BPC_06_3928A = 13_A2 1,2,3,4,5,6,7,9,10,11

Complex virulence and aggressive

Resistant to metalaxyl

Structured screening of RxLR diversity



- Study of evolutionary forces on key pathogen traits
- Panel of 30 GB isolates

• Avr 3a

- 3 SNPs (3 replacement changes)

- 23 **EM** homozygous (virulent)
- 6 **EM/KI** heterozygous (avirulent)
- 1 **KI** homozygous (avirulent)

- avirulent strains confined to a specific lineage which explains decline of this avirulence

- R3a not deployed widely (3.5% area) – low selection pressure implies no ‘cost’ to virulence for avr3a

• PEX 161

- 9 SNPs (1 outside ORF, 2 silent, 6 replacement)

- 5 haplotypes

- 7 **TTGAATCGC** A
- 2 **YTGMMWYRY** B
- 2 **YWRMWWYRY** C
- 14 **YWRAAWYRY** D
- 1 **CAAAAATAT** E

- Haplotypes correspond to SSR lineages

Conclusions



- **Genomics**

High throughput, affordable (£5K per 100 Mb) DNA sequencing offering insights into mechanisms of pathogenicity, host range and ultimately host resistance responses. Post-genomics is going to be a 'long haul'.

- **Phylogenetics**

Exciting prospects for environmental monitoring of *Phytophthora* diversity in support of conventional approaches. Evaluating risks. Exploring what happens in 'balanced' natural ecosystems as an aid to understanding invasive species. Phylogeography to improve our understanding of origins of species. Phylogenomics to understand mechanisms conserved across genus.

- **Population analysis**

Tracking populations – co-ordinated responses needed – tracking using standardised tools – sharing 'intelligence' on local problems. Predicting risk? How does population structure influence management? Examining selection pressures on key genes.

Acknowledgments



- Eucabligh team Jens Hansen, Poul Lassen & Alison Lees
- SCRI Team: Alison Lees, Naomi Williams and Louise Sullivan,
- Italian team: Silvia Scibetta, Leonardo Schena, Santina Cacciola and others
- BPC team: David Shaw, Ruairidh Bain, Nick Bradshaw, Moray Taylor
- Many, many people who have contributed inspiration, friendship & cultures
- Potato Council & RERAD for funding