Tracking *Phytophthora infestans* from field to continent via the Euroblight database

David Cooke

Jens Hansen, Poul Lassen, Sanmohan Baby, Alison Lees, Geert Kessel
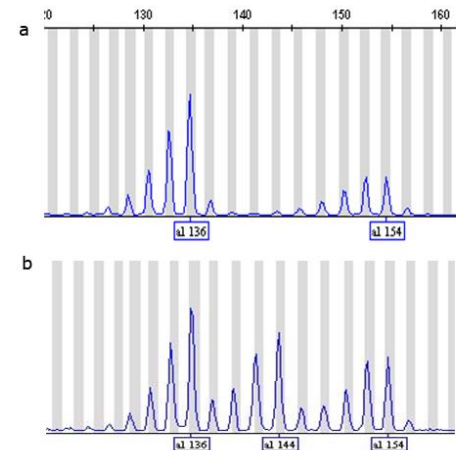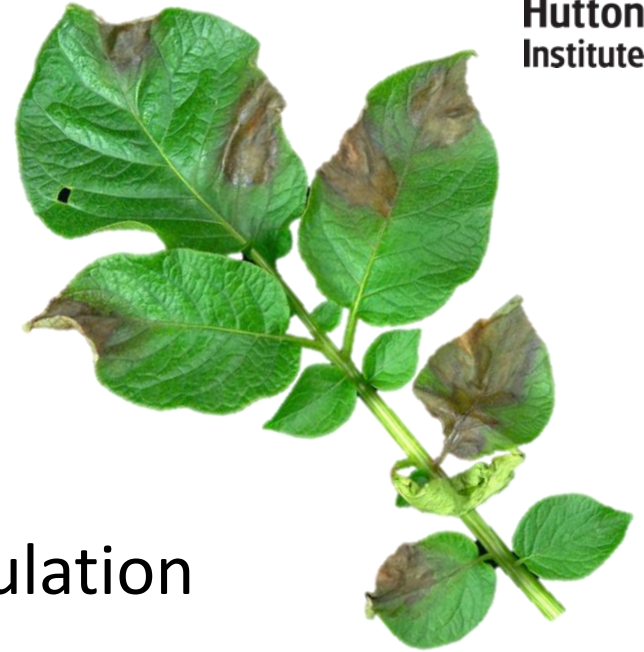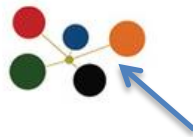
Thanks - scouts, industry and data contributors

Overview

- Context of research

- What is Euroblight?

- *P. infestans* genetics

- Why and how we monitor population

- Database structure and analysis tools

- Results

- Future directions

www.euroblight.net

5 continents

- Born from EU.ICP.NET and Eucablight projects
- Informal consortium of research, commercial and extension staff
- Meetings sponsored by industry but research funded by EU, national or commercial programmes

Huub Schepers
Jens G Hansen
Alison Lees

Why study populations?

Disease Management

- **Environ.** Temp & Humidity
- Fungicide resistance
- Fitness Aggress. Virulence
- Survival (asexual, Sexual)
- Rate of evolution (mating)

6_A1   8_A1   10_A2   13_A2   13_A2   13_A2
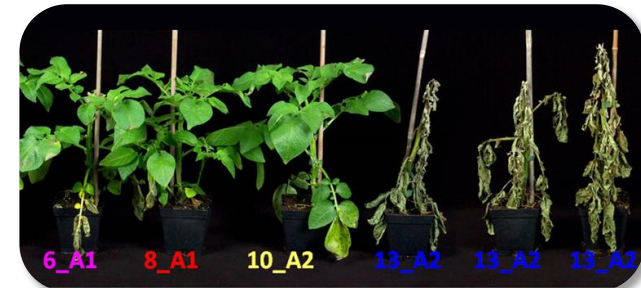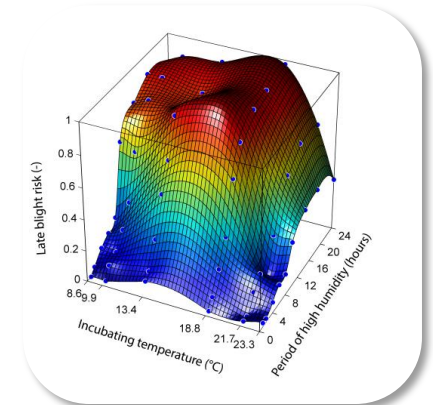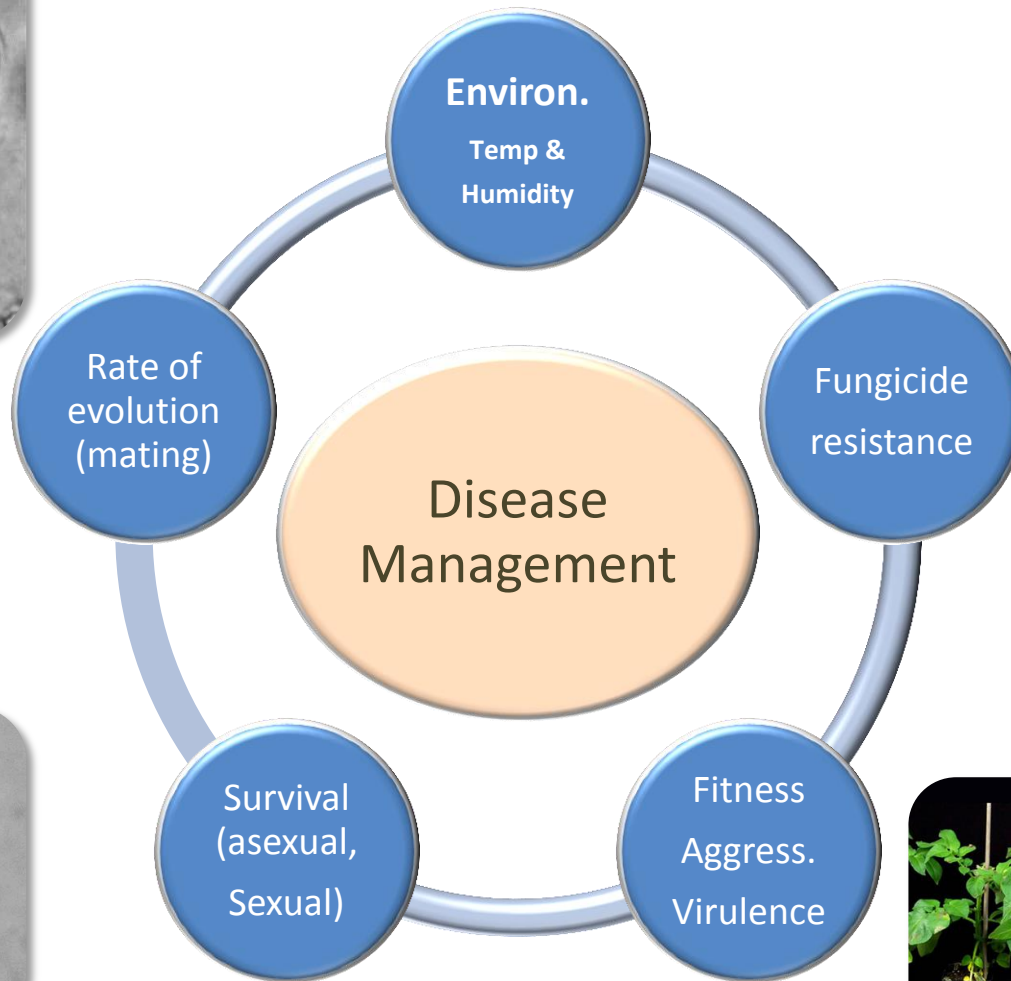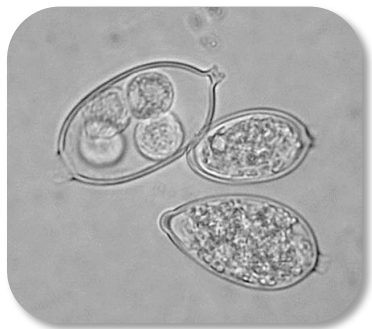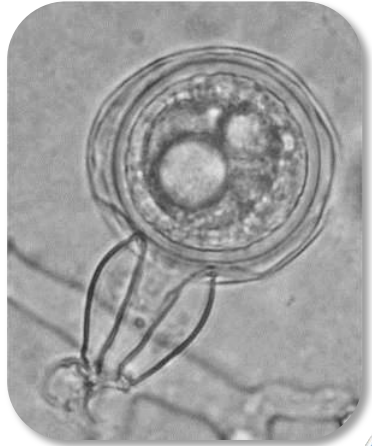
Monitoring methods

- Scouts (advisers, industry, scientists) given FTA cards & sample forms (live cultures - FAB)

- Outbreak data (e.g. location, crop type, cultivar) recorded

- Lesions pressed onto FTA cards to capture pathogen DNA

- DNA fingerprinted using 12-plex SSRs (Li *et al.* 2013)

- Genotypes defined by DNA fingerprint and data uploaded to database via web interface (Arhus University)

- Data mapped on www.euroblight.net

SSR loci (1-12) showing alelle peaks and size markers (orange)
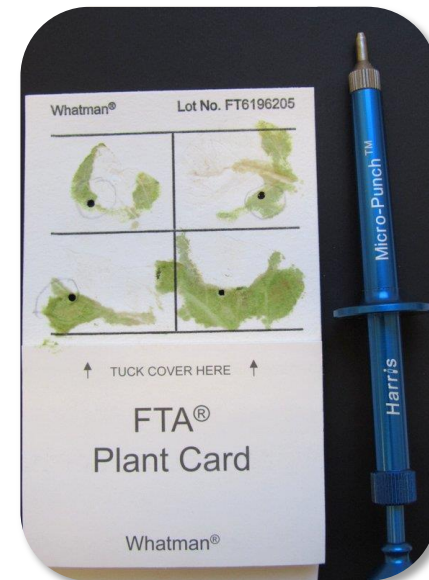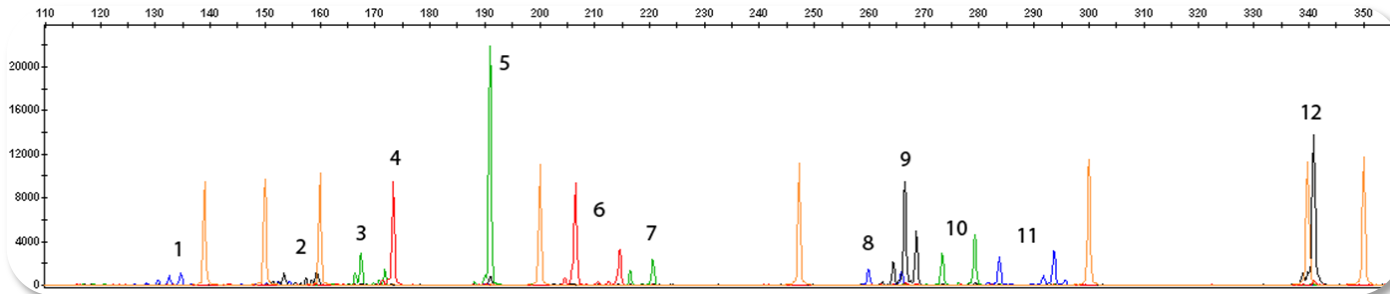
Sample statistics

- 2982 samples from 30 countries genotyped
- Most samples from north-western Europe
- Long 'tail' of countries with few samples
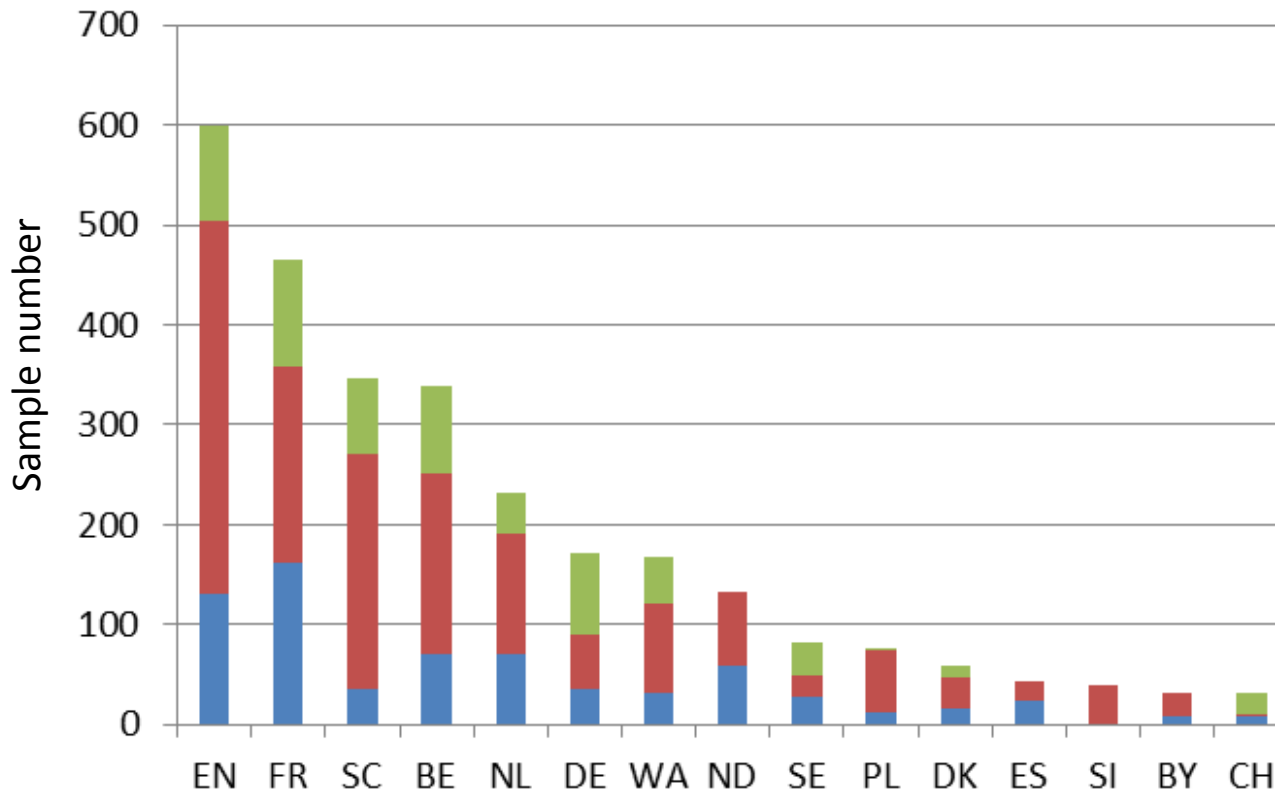
EN – England
FR – France
SC – Scotland
BE – Belgium
NL – the Netherlands
DE – Germany
WA – Wales
ND – Northern Ireland
SE – Sweden
PL – Poland
DK – Denmark
ES – Spain
SI – Slovenia
BY – Belarus
CH - Switzerland

Legend:
- 2015
- 2014
- 2013

Countries with >30 samples shown

The James Hutton Institute

EuroBlight
A potato late blight network for Europe

Great Britain population change

Isolate no. 74 138 73 899 1452 1118 522 247 426 716 196 705 222
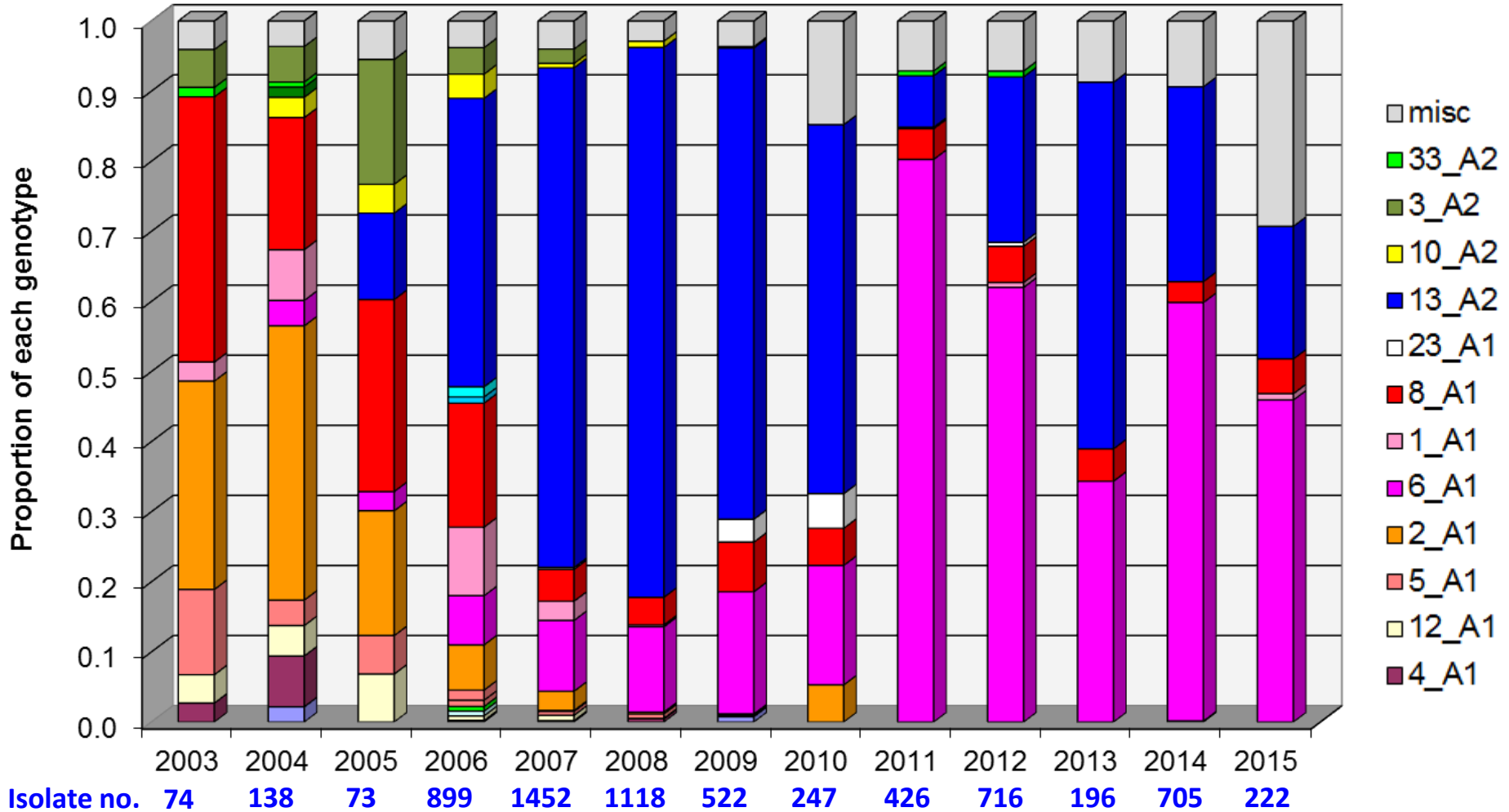
EU *P. infestans* genotype change
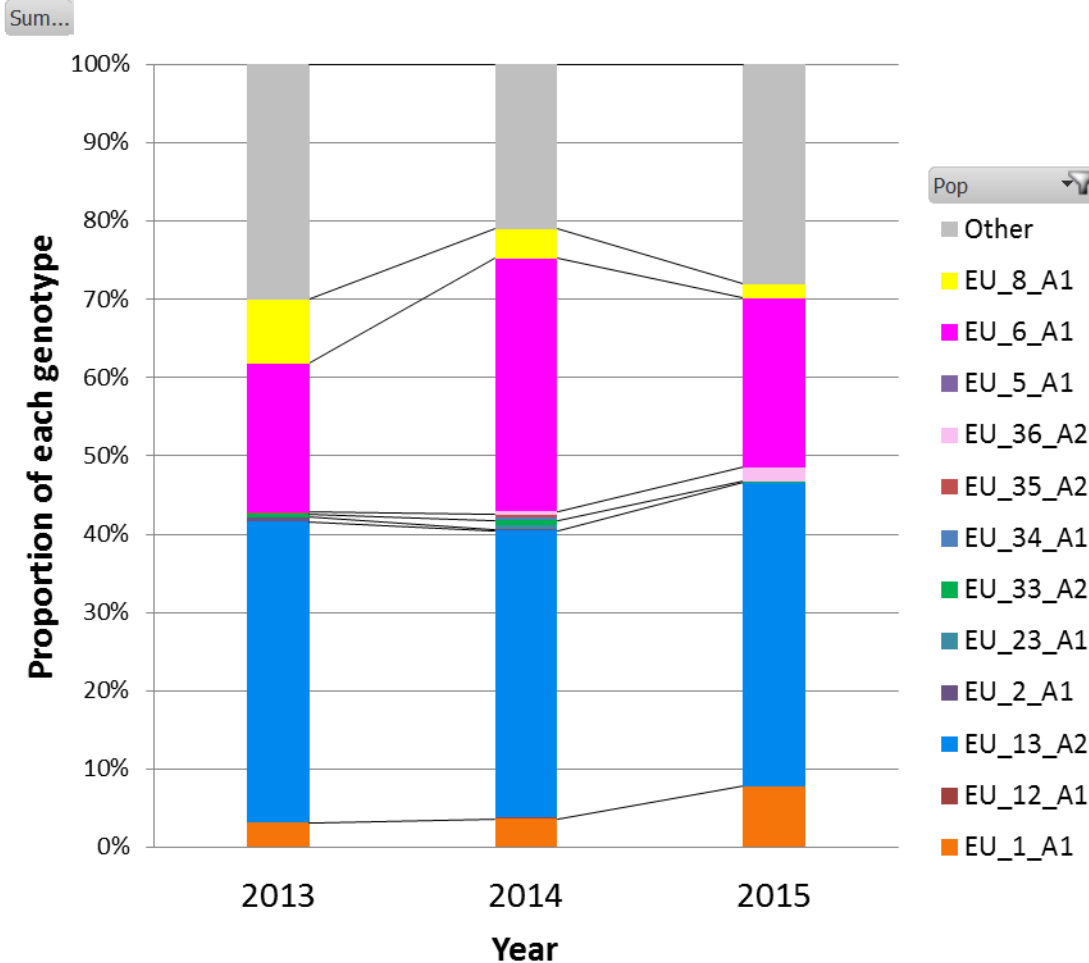
- ~ 70% of population clonal
- EU_13_A2 dominant followed by EU_6_A1, EU_8_A1 and EU_1_A1
- EU_33_A2 'green 33' very low frequency
- EU_36_A2 novel clone in 2014 and 2015
- Most inoculum survives overwinter in tubers

- 20-30% of population each year genetically diverse 'Other' group probably from oospores

The James Hutton Institute

2013 samples

The James Hutton Institute

EuroBlight
A potato late blight network for Europe
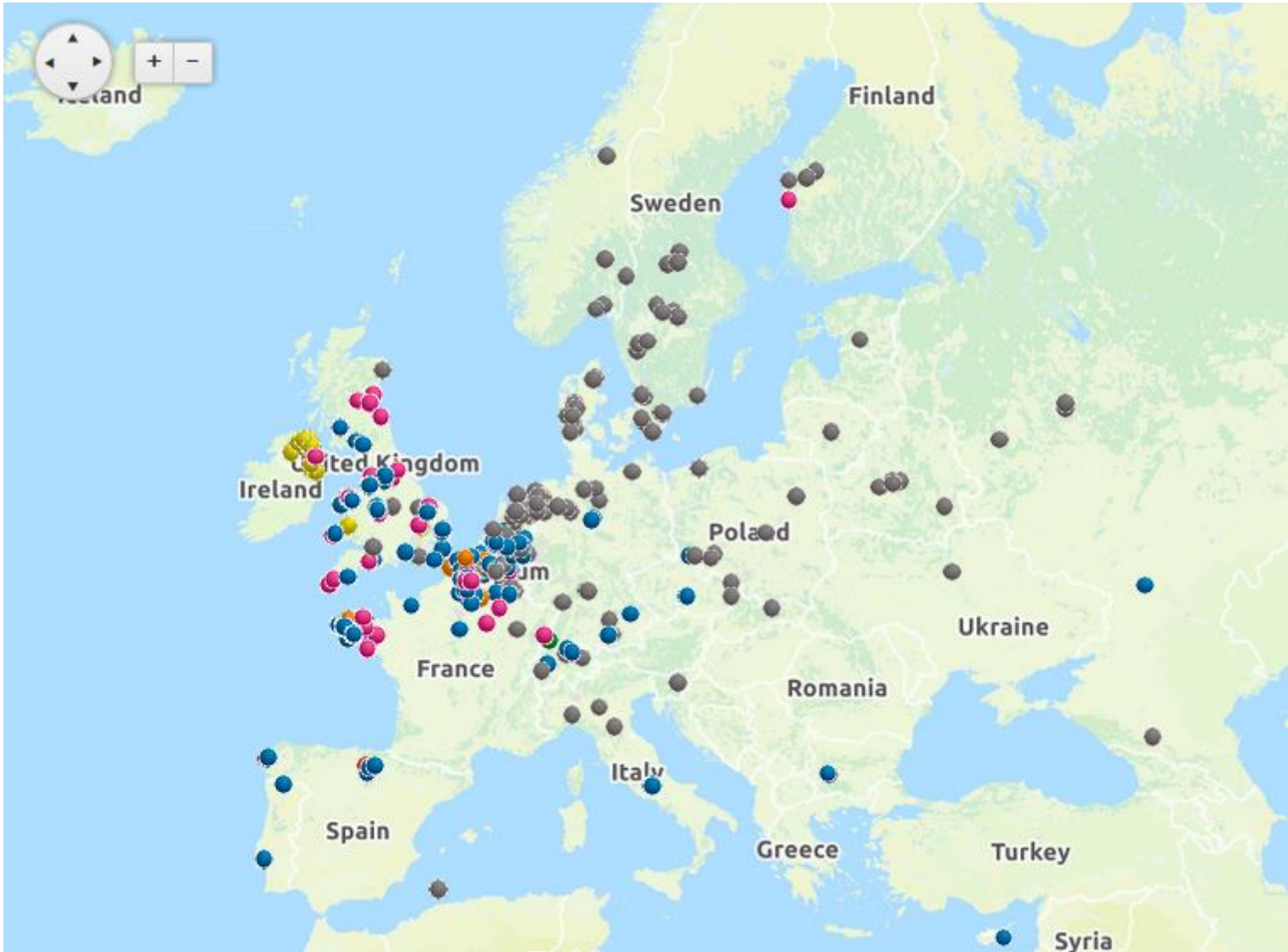
733 samples from 27 countries

2014 samples

1545 samples from 24 countries

2015 samples

614 samples from 16 countries

EuroBlight
A potato late blight network for Europe

The James Hutton Institute

2015 samples – EU_6_A1

Live map at
http://euroblight.net/pathogen-characteristics-and-host-resistance/sampling-sites-and-genotype-maps/

2015 samples – EU_13_A2

EuroBlight
A potato late blight network for Europe

The James Hutton Institute

2015 samples – EU_1_A1

EuroBlight
A potato late blight network for Europe
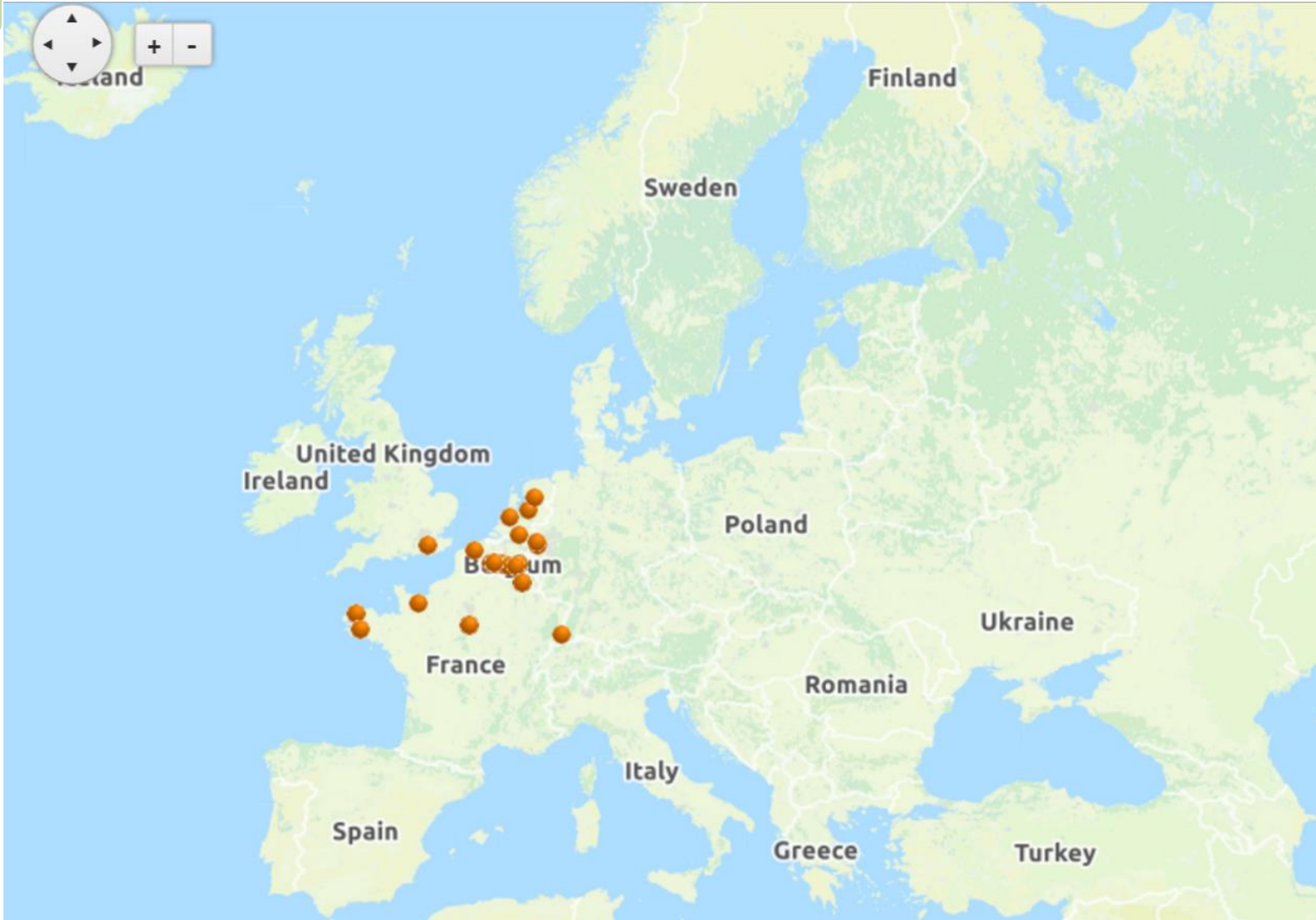
2013-2015 summary
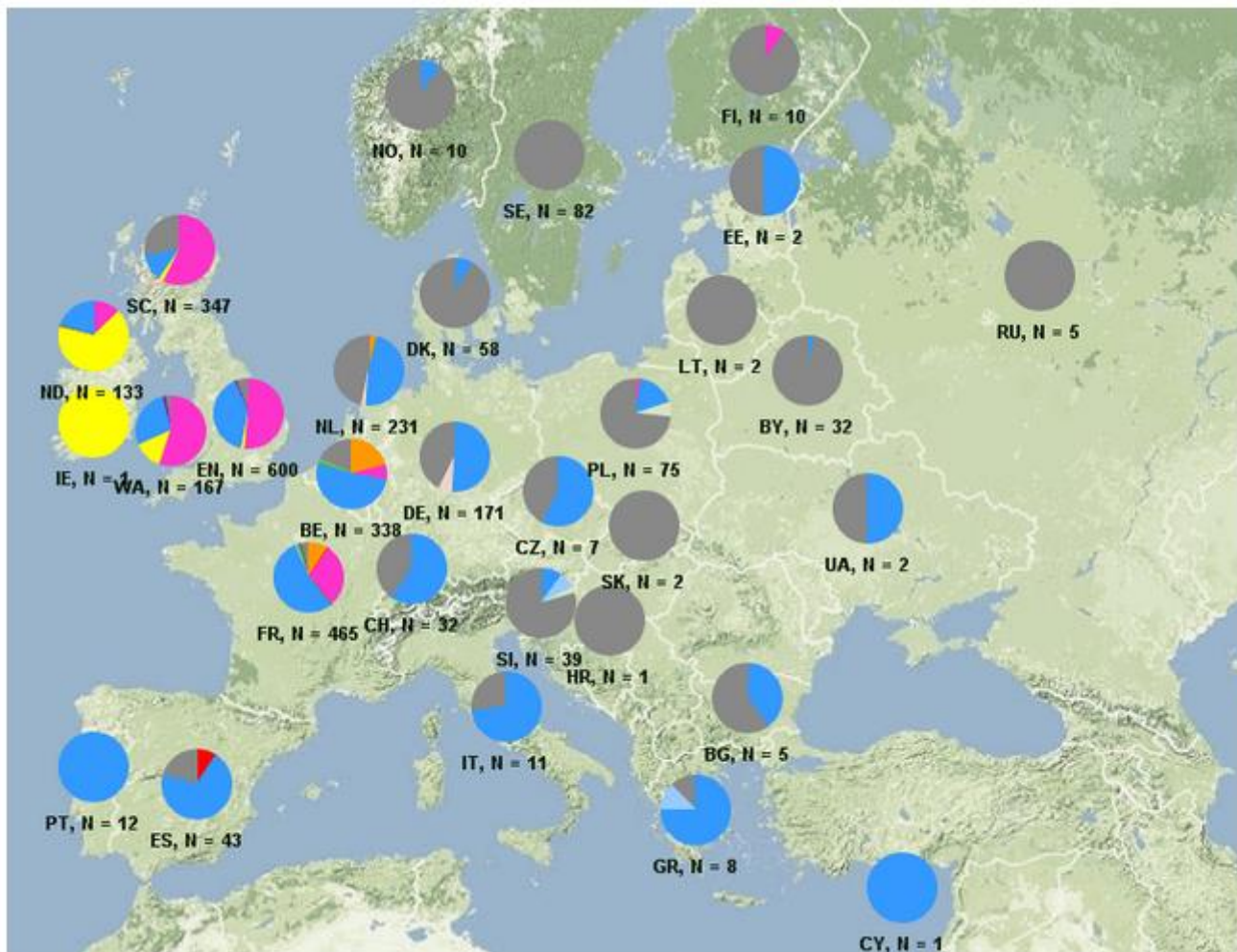
- EU_13_A2 clone most widespread
- Other clones clustered, e.g
  - EU_6_A1 in UK and France
  - EU_8_A1 in Northern Ireland
  - EU_1_A1 in France and Belgium
- Higher proportion of 'Other' types in northern and eastern Europe

- 2892 samples from 30 countries

EuroBlight
A potato late blight network for Europe

The James Hutton Institute

Legend
- EU_1_A1
- EU_2_A1
- EU_5_A1
- EU_6_A1
- EU_8_A1
- EU_12_A1
- EU_13_A2
- EU_23_A1
- EU_33_A2
- EU_34_A1
- EU_35_A2
- EU_36_A2
- Other

EuroBlight Population Genetics Platform

Potato Late Blight Toolbox

Population Statistics

Minimum Spanning Tree

Phylogenetic Tree

Principal Component Analysis

POPPR
Population Genetics in R

Zhian Kamvar & Nik Grünwald

Kamvar et al. 2015. Frontiers in Genetics 6:208

EuroBlight
A potato late blight network for Europe

Toolbox interface

2013-15 Genetic diversity - PCA
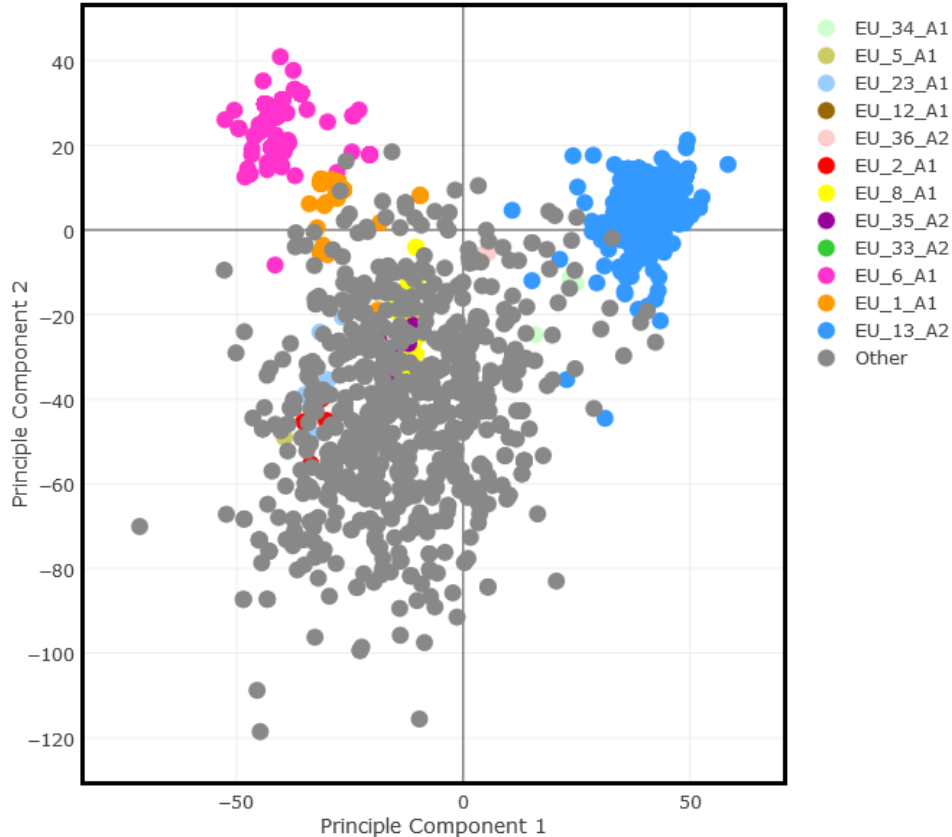
- Distinct clusters of major clones

- Minor variation within clones

- Greater genetic diversity of 'Other' genotypes apparent

- PCA an imperfect tool as the cloud of data obscures some points

EuroBlight
A potato late blight network for Europe

Genetic diversity - PCA
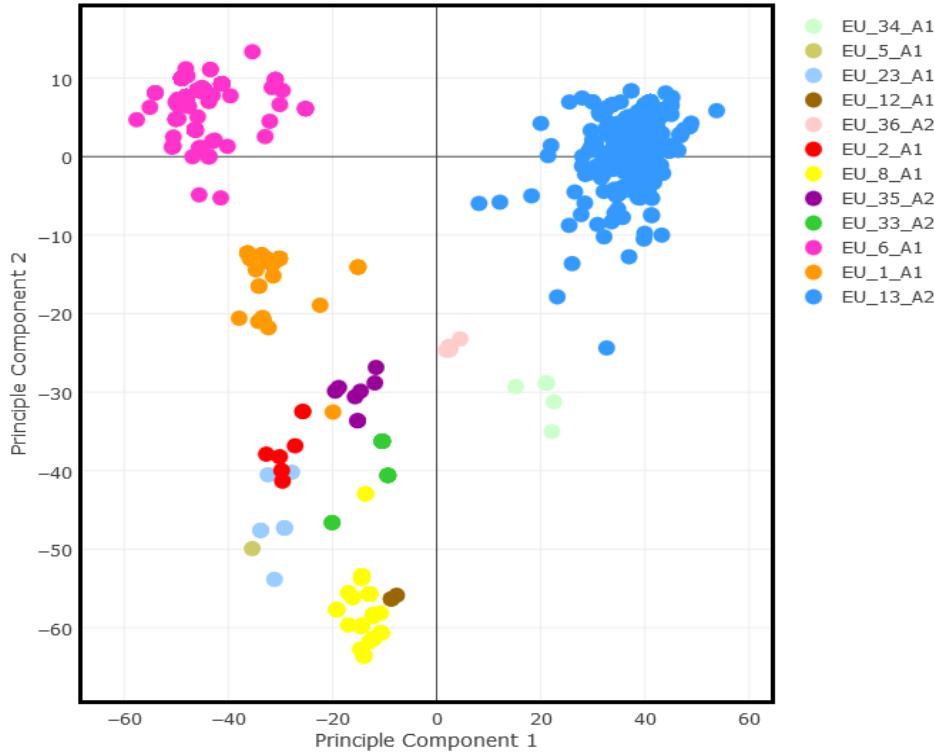
- 2013-2015 data after removing the 'Other' category

- Distinction between most clones is clear

- Minor genetic variation within clones apparent

- Example below shows minor differences in 1 to 3 SSR loci of genotype EU_13_A2

- Such variation useful to track variants of clonal lineages

Legend:
- EU_34_A1
- EU_5_A1
- EU_23_A1
- EU_12_A1
- EU_36_A2
- EU_2_A1
- EU_8_A1
- EU_35_A2
- EU_33_A2
- EU_6_A1
- EU_1_A1
- EU_13_A2

| SSR locus	Pi02		Pi4B		G11			Pi04		Pi63		Pi70		D13			SSR11		SSR2		SSR4			SSR6		SSR8	
13_A2_1	266	268	205	213	154	160	0	166	170	273	279	192	192	136	154	0	341	341	173	173	284	294	0	240	244	260	266
13_A2_2	266	268	205	213	154	160	0	166	170	273	279	192	192	136	140	154	341	341	173	173	284	294	0	240	244	260	266
13_A2_134	266	268	205	213	154	160	0	166	170	273	279	192	192	136	154	0	341	341	173	173	284	292	294	240	244	260	266
13_A2_42	266	268	205	213	154	160	164	166	170	273	279	192	192	136	158	0	341	341	173	173	284	292	294	240	244	260	266
EU_8_A1	268	268	205	217	166	166	0	166	170	273	279	192	192	118	136	0	331	341	173	175	288	294	0	242	244	260	266

EuroBlight
A potato late blight network for Europe

The James Hutton Institute

SSR diversity minimum spanning tree

- EU 2014 – 1552 isolates

EU_6_A1
EU_13_A2
EU_1_A1
EU_33_A2
Other
EU_8_A1
EU_35_A2
EU_2_A1
EU_12_A1
EU_23_A1
EU_5_A1
EU_34_A1
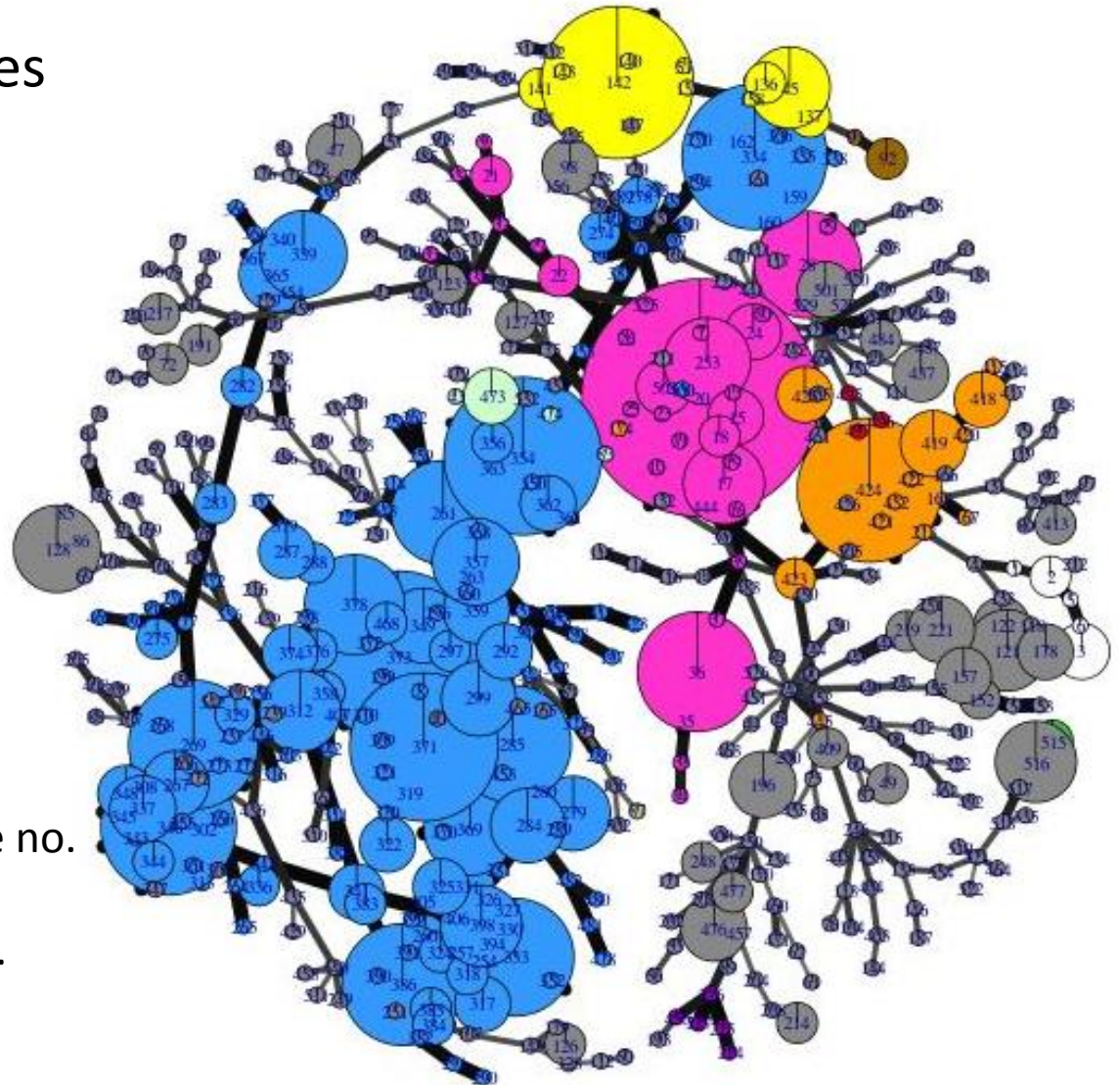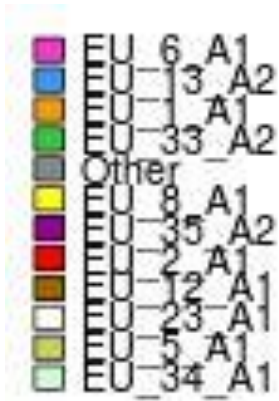
- Node size proportional to isolate no.
- Edge thickness and colour are proportional to Bruvo's distance. Edge lengths are arbitrary.
- Limited area so edges overlap

Genetic diversity - MSN
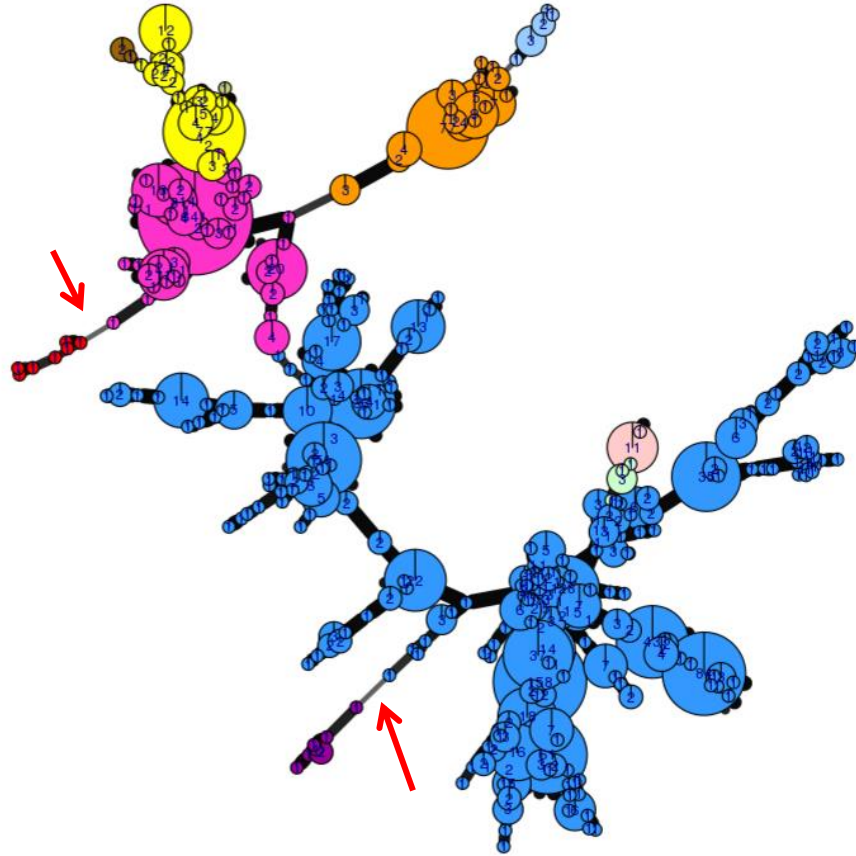
- Distances <u>within</u> variants of main clones short

- Distances <u>between</u> main clones long (arrows)
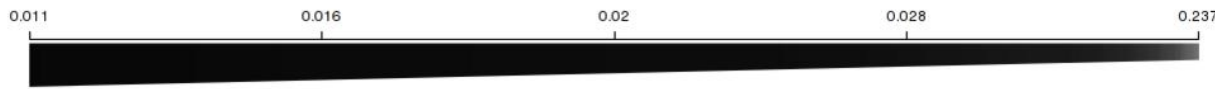
MSN – EU_13_A2 example
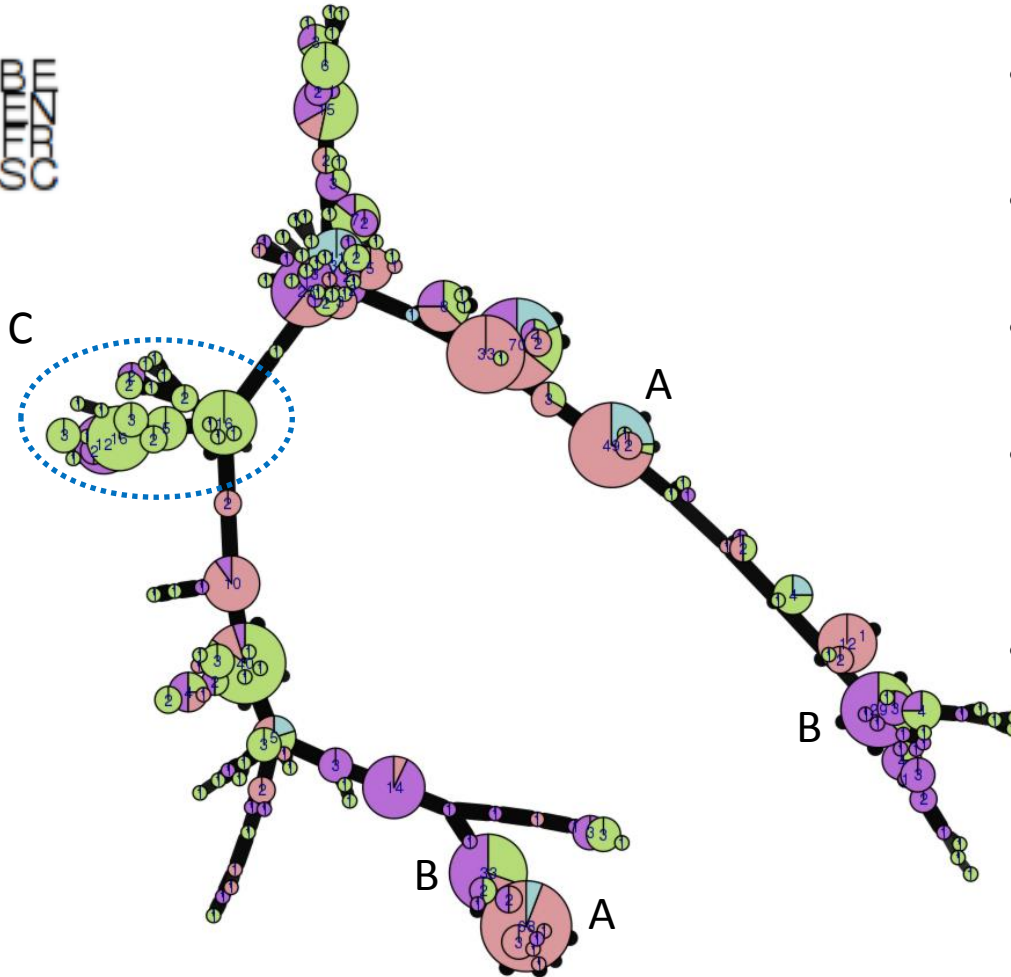
- EU_13_A2 only data from 4 countries
- 13_A2 variants shared between England and Scotland (A)
- 13_A2 variants shared between Belgium and France (B)
- Country (or region) specific clusters of related variants (C)

- Local spread of *P. infestans* populations can be tracked

Genetic diversity of *P. infestans* by country (2013-2015)
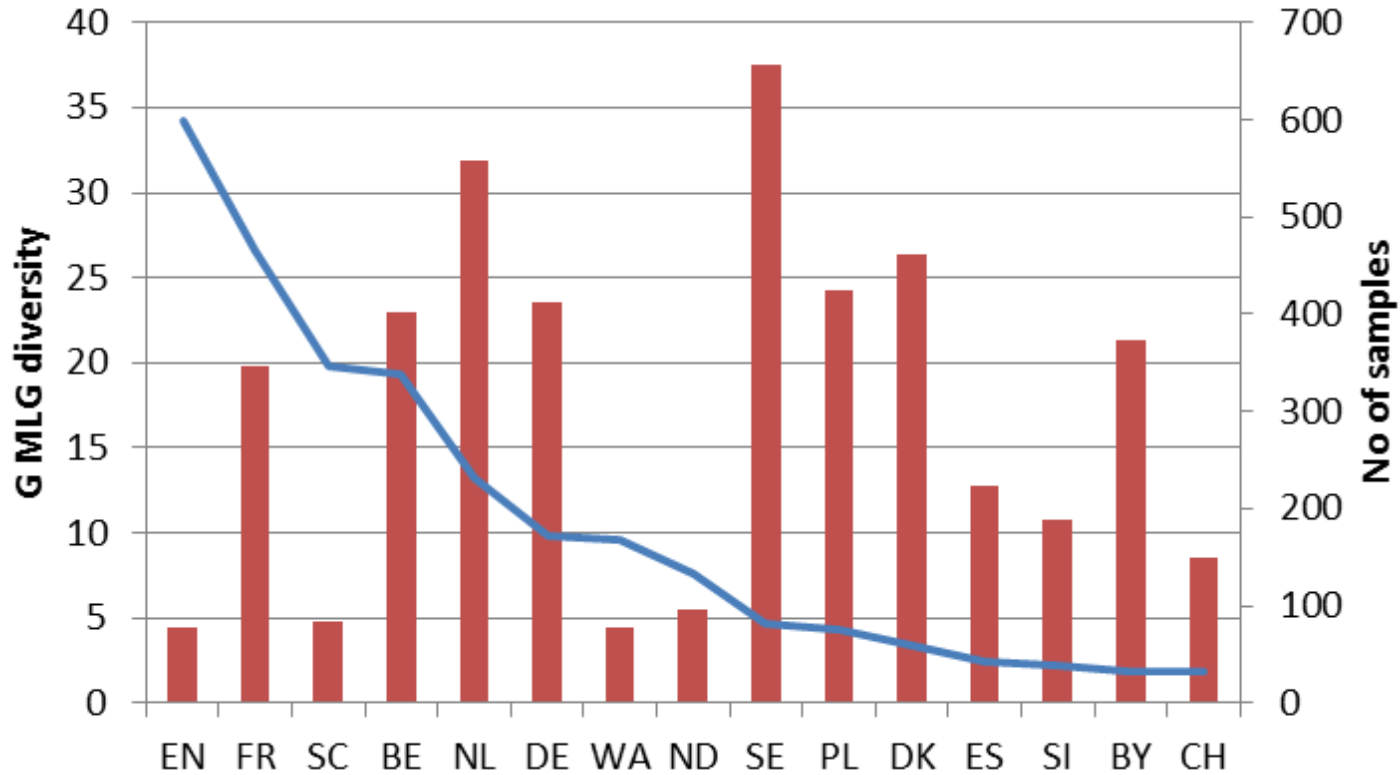
- Multi-locus genotype (MLG) diversity measure (Stoddart and Taylor's G) shows variation by country (red bars)
- High sample numbers in UK (EN, WA, SC, ND) but mostly clonal thus overall diversity is low
- Higher proportion of 'Other' genotypes in some countries results in greater diversity

Conclusions & future plans

- New insights on pathogen diversity

- Dominant clones across European crops - we share management challenges

- Much primary inoculum asexual and locally propagated. Better management of this needed

- Other populations highly diverse & ephemeral as a result of sexual oospore formation - competing evolutionary forces interesting

- High genetic diversity increases the risk of blight management problems

- New tools being developed (DAPC)

- Phenotypic traits of existing and novel genotypes examined in a 2016 new ERA-NET project (IPMBlight2.0)

- Discussions with other networks to apply the same model to study their populations and share data globally (AsiaBlight, USABlight, TizonBlight)

- Co-operation is critical

Thanks

ADAMA

Agrifirm

Agriphar

BASF

Bayer CropScience AG

Bayerische Landesanstalt für Landwirtschaft

Belchim Crop Protection

Centre Wallon de Recherches Agronomiques

Certis

Cheminova

CZAV

Dupont de Nemours

Emsland Group

Germicopa SAS

HZPC Holland B.V.

AHDB Potatoes

Neiker

Nordisk Alkali

PCA

Profytodsd

Swedish University of Agricultural Sciences

Syngenta Agro GmbH

Agricultural Institute of Slovenia

AFBI, Northern Ireland

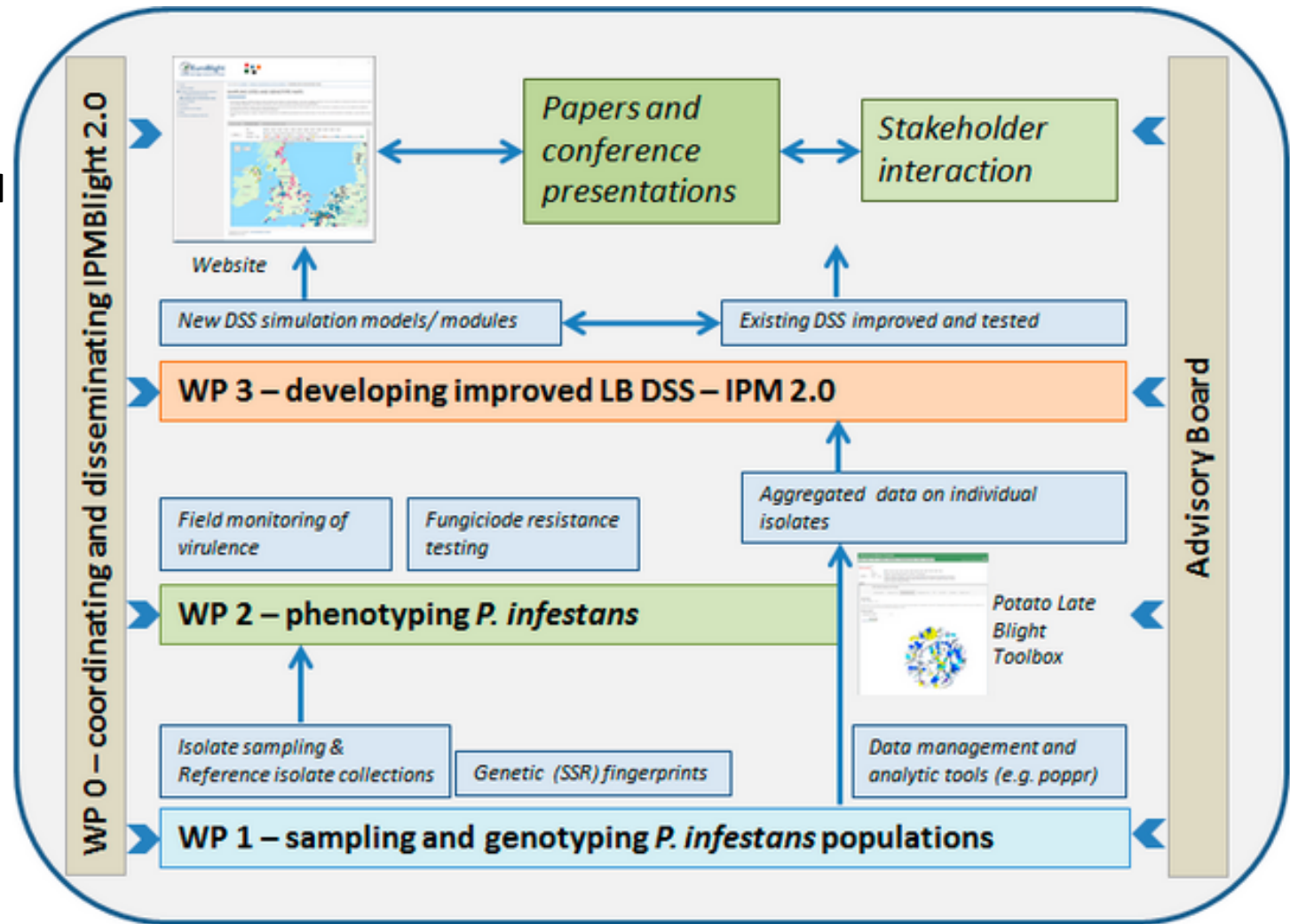Estonian University of Life Sciences

EuroBlight

A potato late blight network for Europe

IPM Blight 2.0 – EU project

ERA-NET IPM call Partners

- France
- Denmark
- Estonia
- Norway
- Scotland

- Starts Apr-16

Year ☐ All ☑ 2015 ☑ 2014 ☑ 2013 ☐ 2012 ☐ 2011 ☐ 2010 ☐ 2009 ☐ 2008 ☐ 2007 ☐ 2006
☐ 2005 ☐ 2004 ☐ 2002

Continent ⦿ Europe ◯ Africa & West/Central Asia ◯ East Asia ◯ South America

[Show] Country ☐ All ☐ Belarus ☐ Belgium ☐ Bulgaria ☐ Croatia ☐ Cypres ☐ Czech Republic ☐ Denmark ☑ England ☐ Estonia ☐ Finland
☐ France ☐ Germany ☐ Greece ☐ Ireland ☐ Italy ☐ Lithuania ☐ Netherlands ☐ Northern Ireland ☐ Norway ☐ Poland
☐ Portugal ☐ Russian Federation ☑ Scotland ☐ Slovakia ☐ Slovenia ☐ Spain ☐ Sweden ☐ Switzerland ☐ Ukraine ☐ Wales

Mode ◯ Default ⦿ DAPC

Input file

| DAPC | Subset Data | Find Clusters | Loading | Group Posterior |

Select no. of PCs to keep
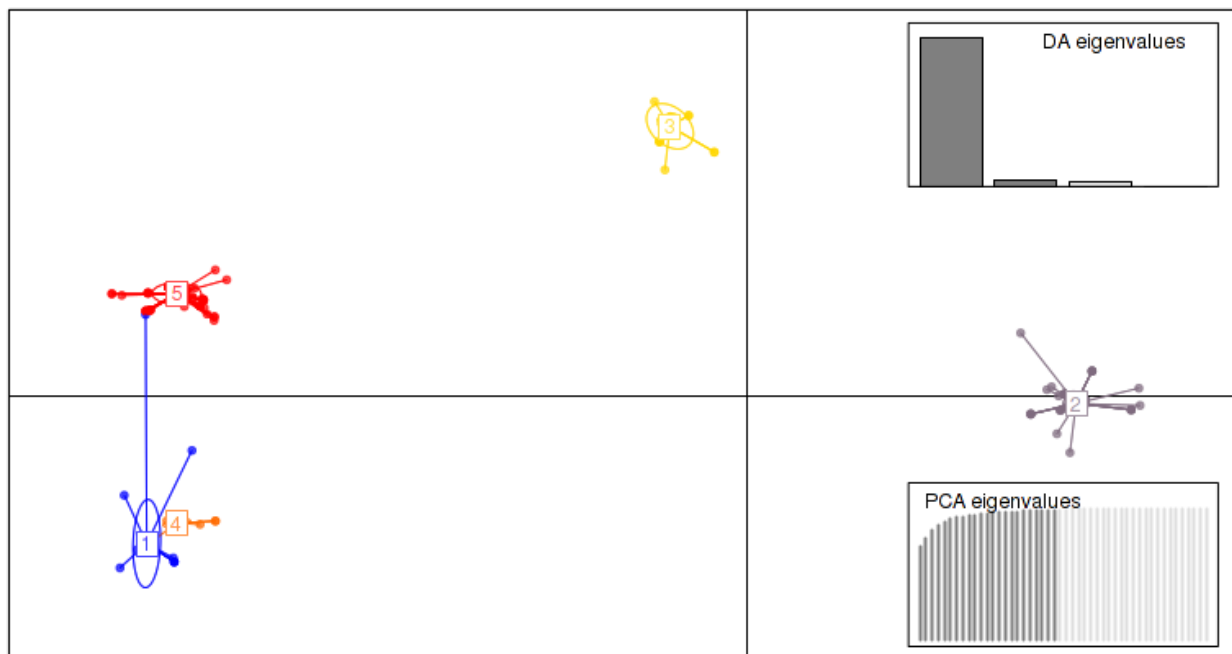
No. of PCs to keep

2 [23] 100

2 12 22 32 42 52 62 72 82 92 100

No. of clusters

2 [5] 20

2 4 6 8 10 12 14 16 18 20

No. of da fns

2 [4] 20

2 4 6 8 10 12 14 16 18 20
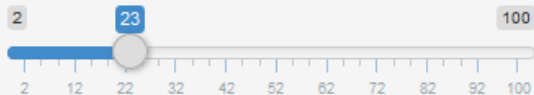
Statistics to be computed for each k

BIC ▾

DA eigenvalues

PCA eigenvalues