

Population structure of *Phytophthora infestans* in the Netherlands during the first decade of the 21st century

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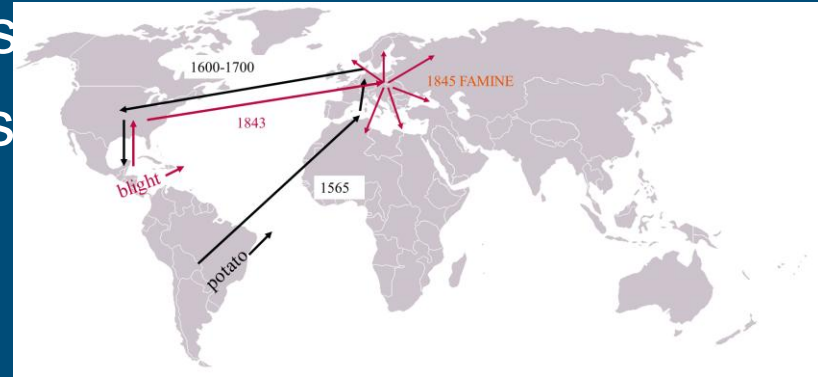
Outline

- Introduction
- Objectives
- Sampling
- Characterisation
- Data analysis
- Results
- Conclusion & discussion



Introduction

- Old population before the 1980's
- New population since the 1980's
 - Sexual cycle
 - Increased aggressiveness
 - High levels of genetic variation
 - Further adaptation to R-genes/fungicides
- Adaptations make potato late blight more difficult to control
- In this presentation we show the results of a 10 year monitoring of *P. infestans* in the NL (2000 – 2009)



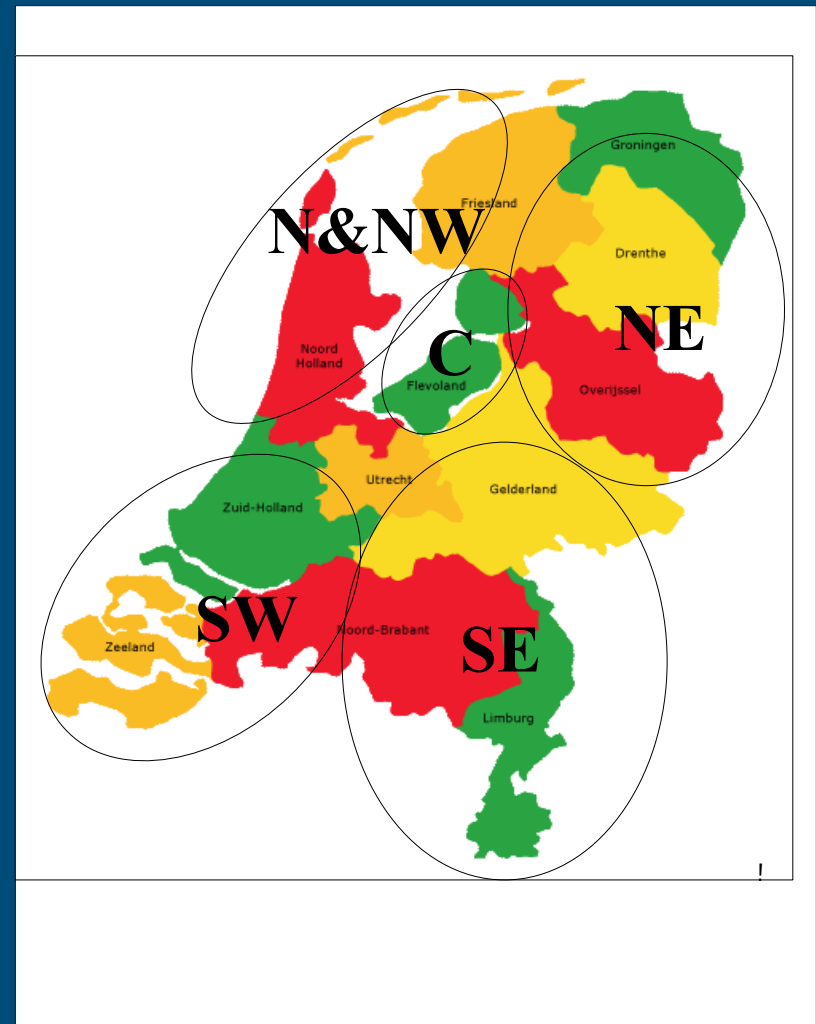
Objectives



- Describe 1 decade of *P. infestans* population dynamics in the Netherlands: 2000 – 2009
 - Spatial population dynamics:
Are we dealing with a national, regional or local population(s)?
 - Temporal population dynamics:
Population structure and dynamics of dominant clonal genotypes (clonal lineages)

Sampling of *P. infestans*

- 2000 – 2009 (10 years)
- 5 Dutch potato-growing regions
 - South West: Ware Potatoes
 - South East: Ware Potatoes
 - North & North West: Seed/Ware Potatoes
 - Central: Seed/Ware potatoes
 - North East: Starch Potatoes
- Infected leaflets from farmers fields
- 207 different sampling locations
- 652 isolates



Sampling of *P. infestans*



■ Sampling summary

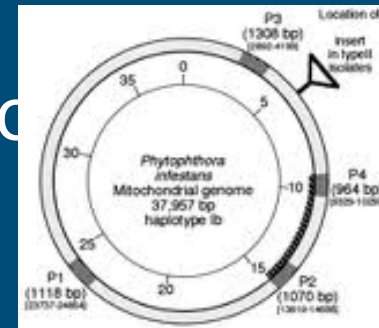
Region	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	SUM
C	8	0	4	10	6	34	28	16	10	34	150
N & NW	6	1	0	4	2	23	6	9	4	0	55
NE	18	22	20	21	28	30	18	23	9	20	209
SE	19	1	2	7	17	19	10	16	24	23	138
SW	4	0	3	10	3	46	10	16	2	6	100
SUM	55	24	29	52	56	152	72	80	49	83	652

Characterisation

- Mating type (A1 of A2) : test on agar

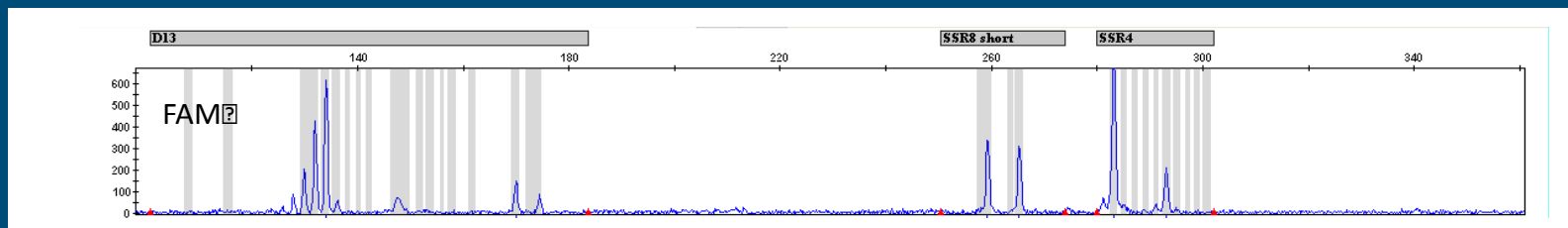


- Haplotype (Ia, IIa, Ib or IIb): pcr & digestion



- SSR: Single Sequence Repeats

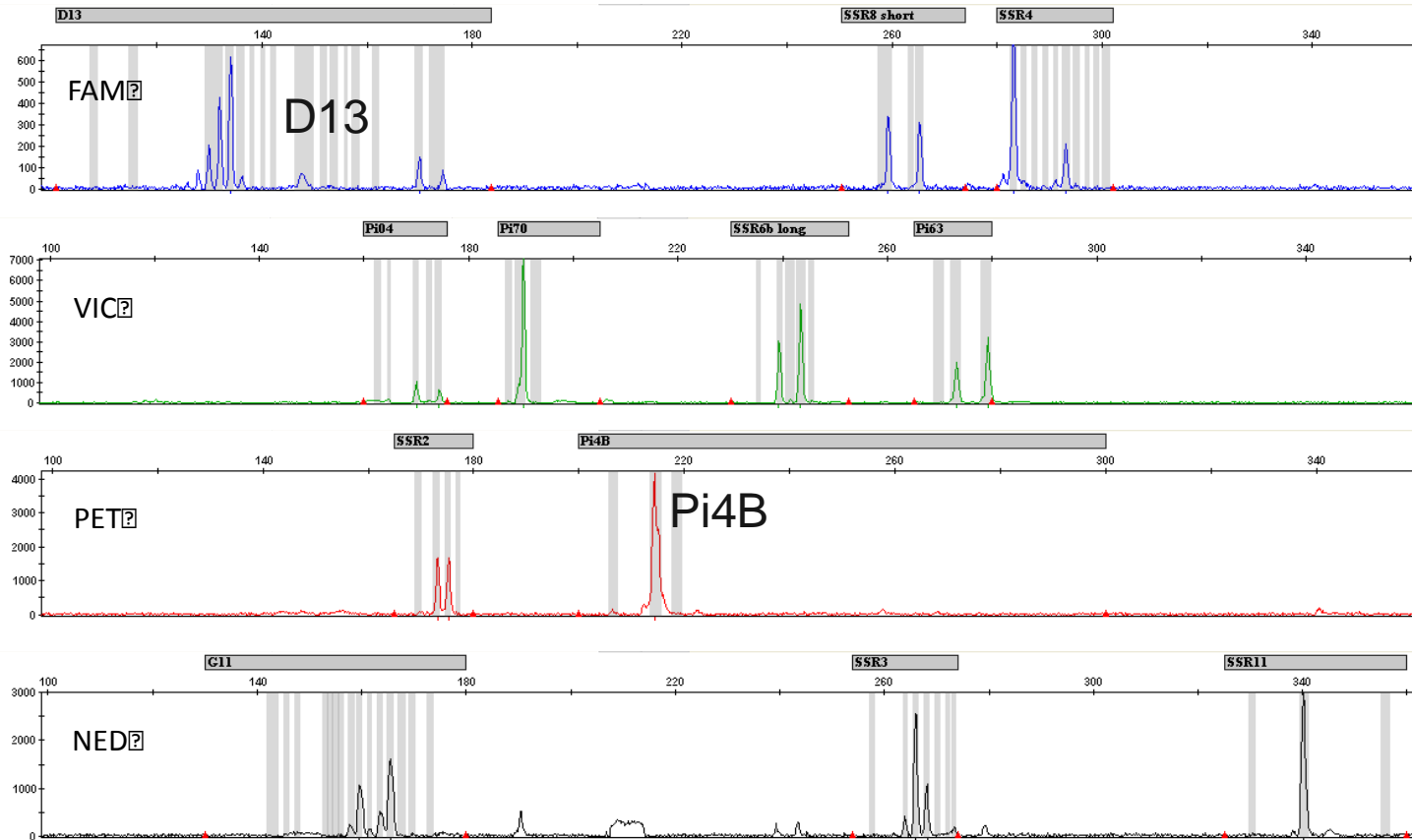
- multiplex PCR → DNA fingerprinting



Multiplex PCR with 12 SSR primers

- New 12-plex standard set:
 - Six SSRs from PRI
 - SSR2, SSR3, SSR4, SSR6, SSR8, SSR11
 - Six SSRs from James Hutton Institute (SCRI) Scotland
 - D13, Pi04, Pi70, Pi63, G11, Pi4B
- 12 SSRs integrated in one PCR reaction
- 4 fluorescent label: FAM, VIC, PET, NED detection on the ABI
- Each primer gives a specific PCR product size range (alleles)

Characterization



1-0 table: presence or absence alleles

		Pi04				SSR4							
		Allele 1 164 bp	Allele 2 170 bp	Allele 3 172 bp	Allele 4 174 bp	Allele 1 283 bp	Allele 2 287 bp	Allele 3 289 bp	Allele 4 291 bp	Allele 5 293 bp	Allele 6 295 bp	Allele 7 297 bp	Allele 8 301 bp
reference 80029		1	0	1	0	1	0	0	0	1	0	0	0
reference 88133		0	0	0	1	0	1	0	0	0	0	1	0
reference 90128		0	0	1	0	0	0	0	0	1	0	0	0
reference 98014		0	1	0	1	0	1	1	1	0	0	0	0
reference 428-2		0	1	0	1	0	0	0	0	1	0	0	0
reference ipo-complex		1	0	1	0	0	1	0	0	1	0	0	0
reference T30-4		0	0	0	1	1	1	0	0	0	0	0	0
reference VK1-4		0	1	0	1	0	1	1	0	0	0	0	1
NL10004		0	1	0	1	1	0	0	0	1	0	0	0
NL10087		0	1	0	1	1	0	0	0	1	1	0	0
NL10089		0	1	0	1	1	0	0	0	1	1	0	0
NL10092		0	1	0	1	1	0	0	0	1	0	0	0
NL10093		0	1	0	1	1	0	0	0	1	0	0	0
NL10105		0	1	0	1	1	0	0	0	1	0	0	0
NL10175		0	1	0	1	1	0	0	0	1	0	0	0
NL10180		0	1	0	1	0	0	0	0	1	0	0	0
NL10201		0	1	0	1	0	0	0	0	1	0	0	0
NL10252		0	1	0	1	0	0	0	0	1	1	0	0
NL10260		0	1	0	1	0	0	1	0	1	0	0	0
NL10289		0	1	0	1	0	1	0	0	1	0	0	0
NL10314		0	0	1	0	1	0	0	0	0	0	0	0
NL10317		0	1	0	1	1	0	0	0	1	0	0	0
NL10366		0	1	0	1	0	1	1	0	0	0	0	0
NL10384		0	1	0	1	1	0	0	1	0	0	0	0
NL10423		0	0	0	1	1	0	0	0	0	0	0	0
NL10425		0	1	0	1	0	1	1	1	0	0	0	0
NL10442		0	1	0	1	0	1	0	0	1	0	0	0
NL10453		0	1	0	1	1	0	0	0	1	0	0	0
NL10454		0	1	0	1	1	0	0	0	0	0	0	0
NL10473		0	1	0	1	1	0	0	0	1	0	0	0
NL10478		0	1	0	1	1	0	0	0	1	0	0	0
NL10480		0	0	1	0	1	1	0	0	0	0	0	0
NL10482		0	1	0	1	1	0	0	0	1	0	0	0
NL10485		0	0	0	0	0	0	0	0	0	0	0	0
NL10486		0	0	0	1	1	0	0	0	0	0	0	0

Isolates

Results of SSR → Population analysis

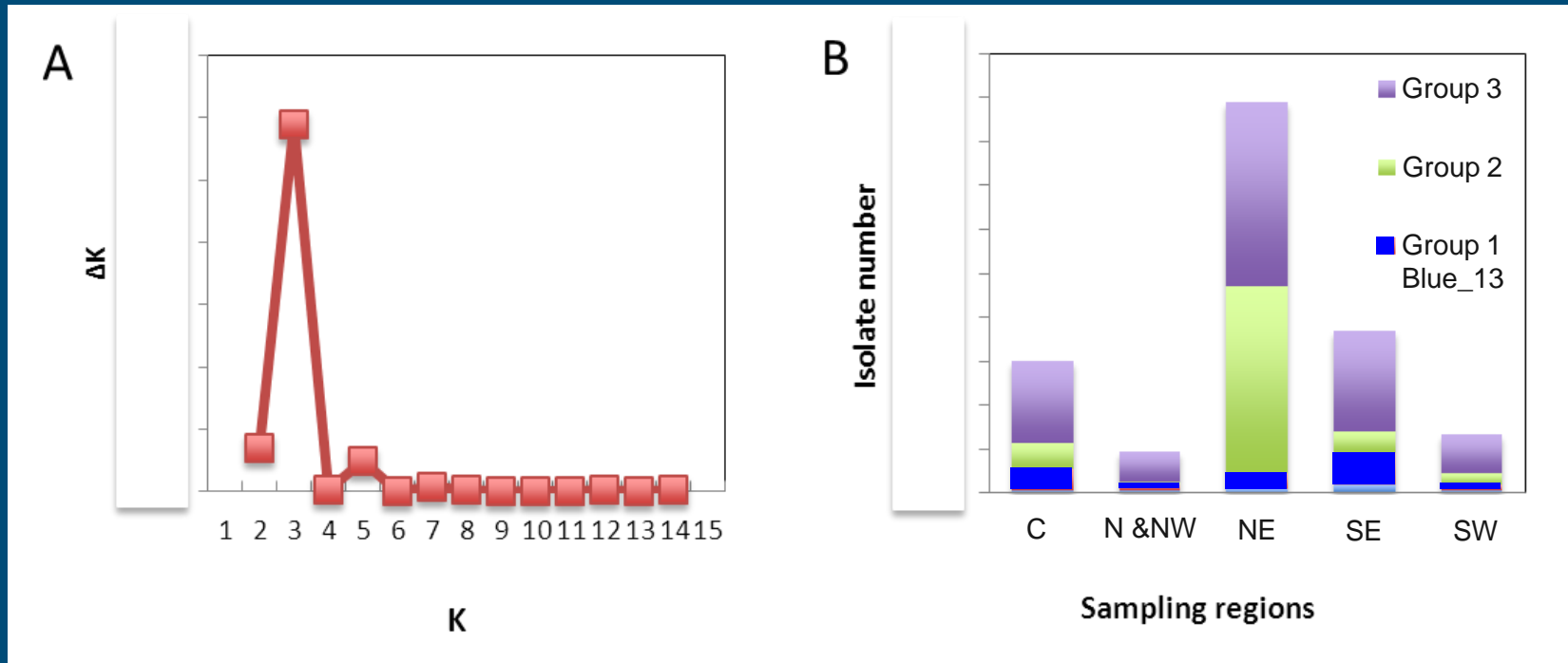
- Basic Genetic diversity (number of alleles etc.): POPGENE
- GENEPOP: Deviations from Hardy Weinberg equilibrium
- AMOVA: Genetic variation among and between populations
- Powermarker: Genetic distance matrix bases on Neighbor Joining
- Principle Component Analysis (PCA)
- STRUCTURE: clustering program
- MEGA: construct tree
-

General Results

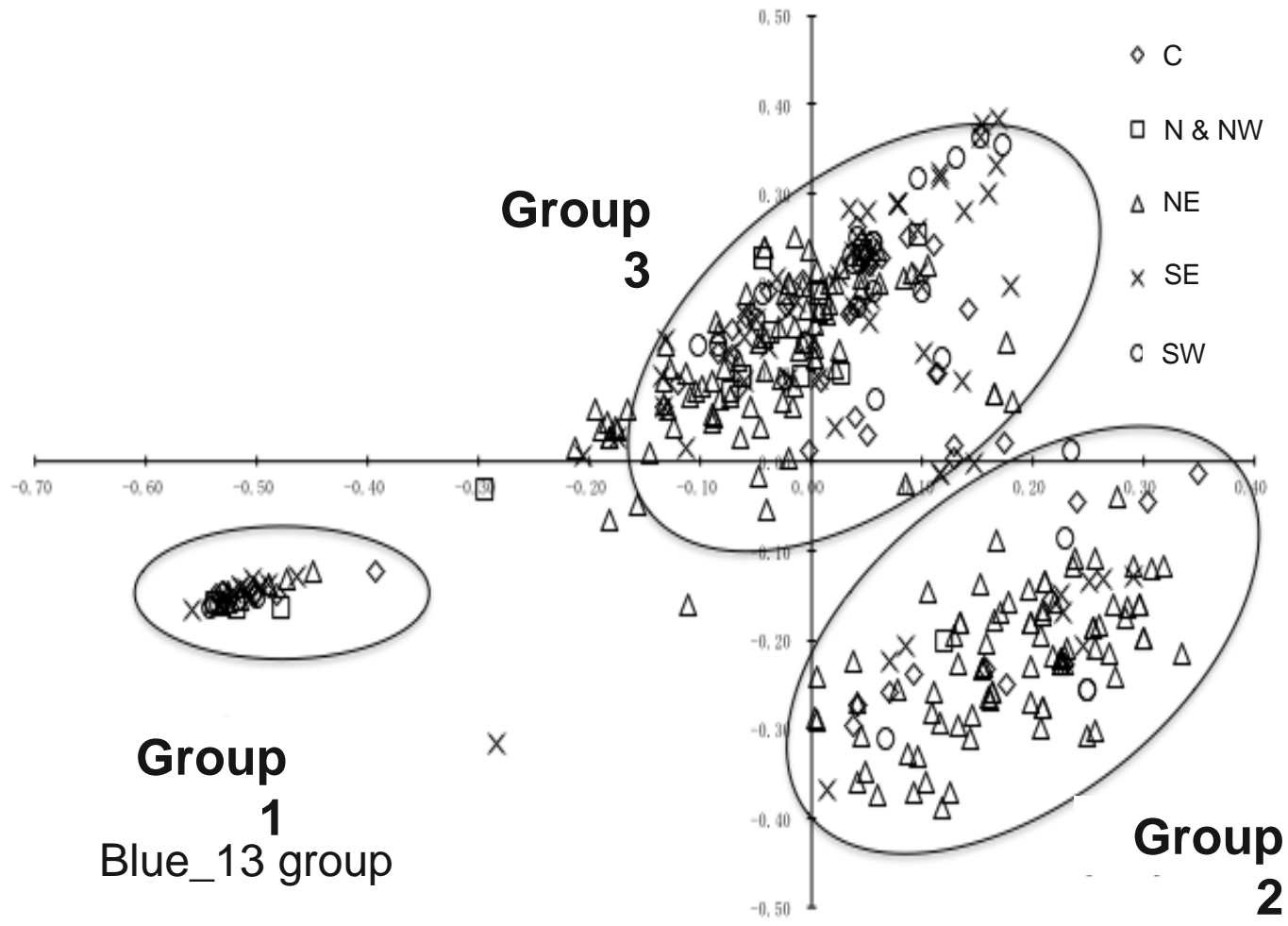
- 311 unique genotypes within the total of 652 isolates
- Both mating types found in all years and all regions
- Haplotype Ia, IIa and Ib found in all years and all regions
- SSR, general results:
 - 75 alleles detected over 12 loci
 - 3 – 17 alleles per locus (average of 6.25)

	D13	G11	Pi04	Pi4B	Pi63	Pi70	SSR2	SSR3	SSR4	SSR6	SSR8	SSR11	Mean
Freq of rare alleles	0.429	0.143	0.036	0	0.036	0	0.036	0.107	0.179	0	0.036	0	0.084
Nr of alleles	17	13	4	3	4	3	3	6	11	3	4	4	6.25

Results STRUCTURE Analysis



Principal Component Analysis (PCA)



Combine PCA & STRUCTURE in a tree

Blue = Group 1
Red = Group 2
Green = Group 3

Dutch population
(2000-2009)
311 genotypes 652 isolates

3 groups were distinguished
based on "PCA" & STRUCTURE results

Results: Haplotypes

GS-008
(2000)

Blue-13
(2004)

Dutch population
(2000-2009)
311 genotypes 652 isolates

Pink-6
(2002)

Mitochondrial
Haplotype

Ia = red

IIa = green

Ib = blue

Results: Mating types

GS-008
(2000)



0.02]

Blue-13
(2004)

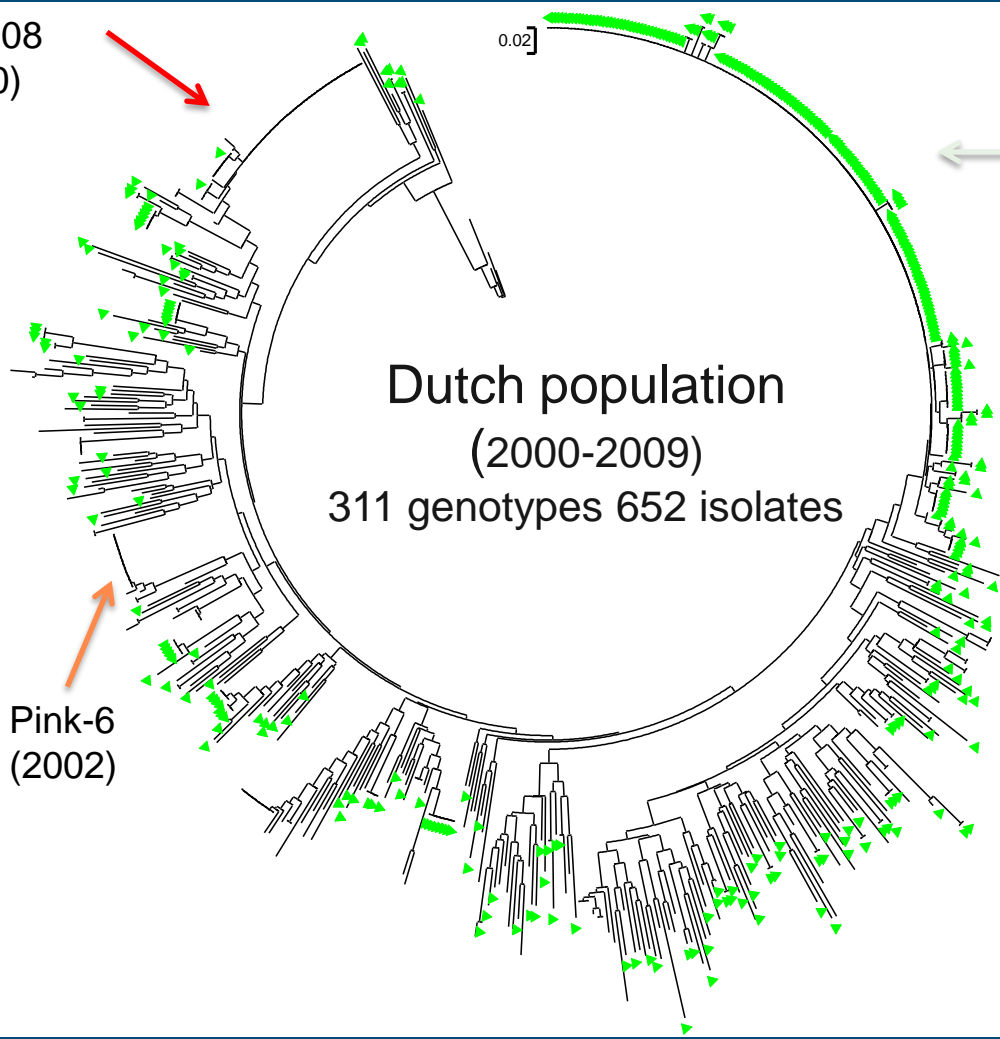


Mating type

Green = A2

Dutch population
(2000-2009)
311 genotypes 652 isolates

Pink-6
(2002)



Results: Regional populations?

GS-008
(2000)



Blue-13
(2004)



Pink-6
(2002)



Sampling regions:

SW

SE

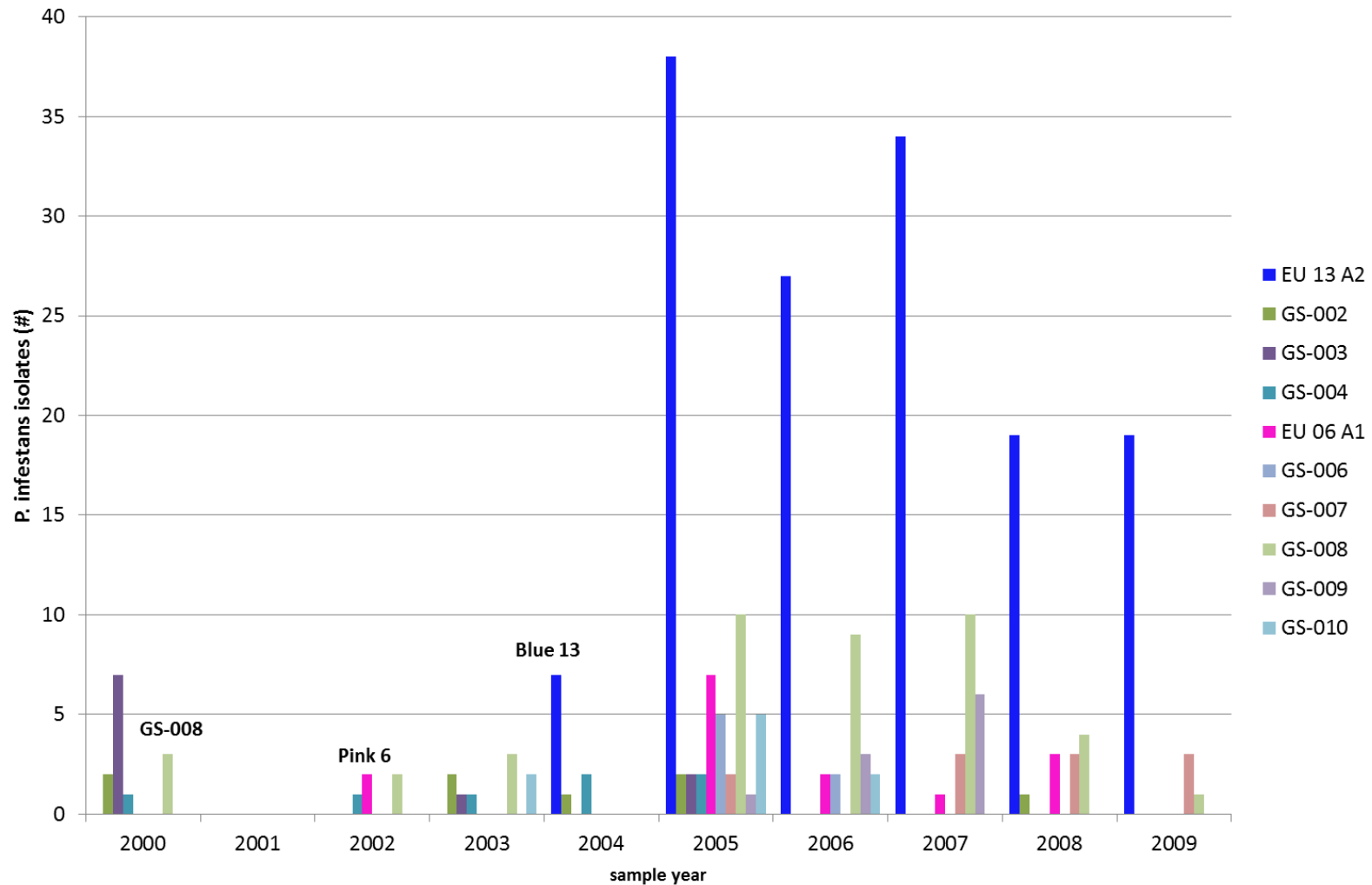
NE

N

C



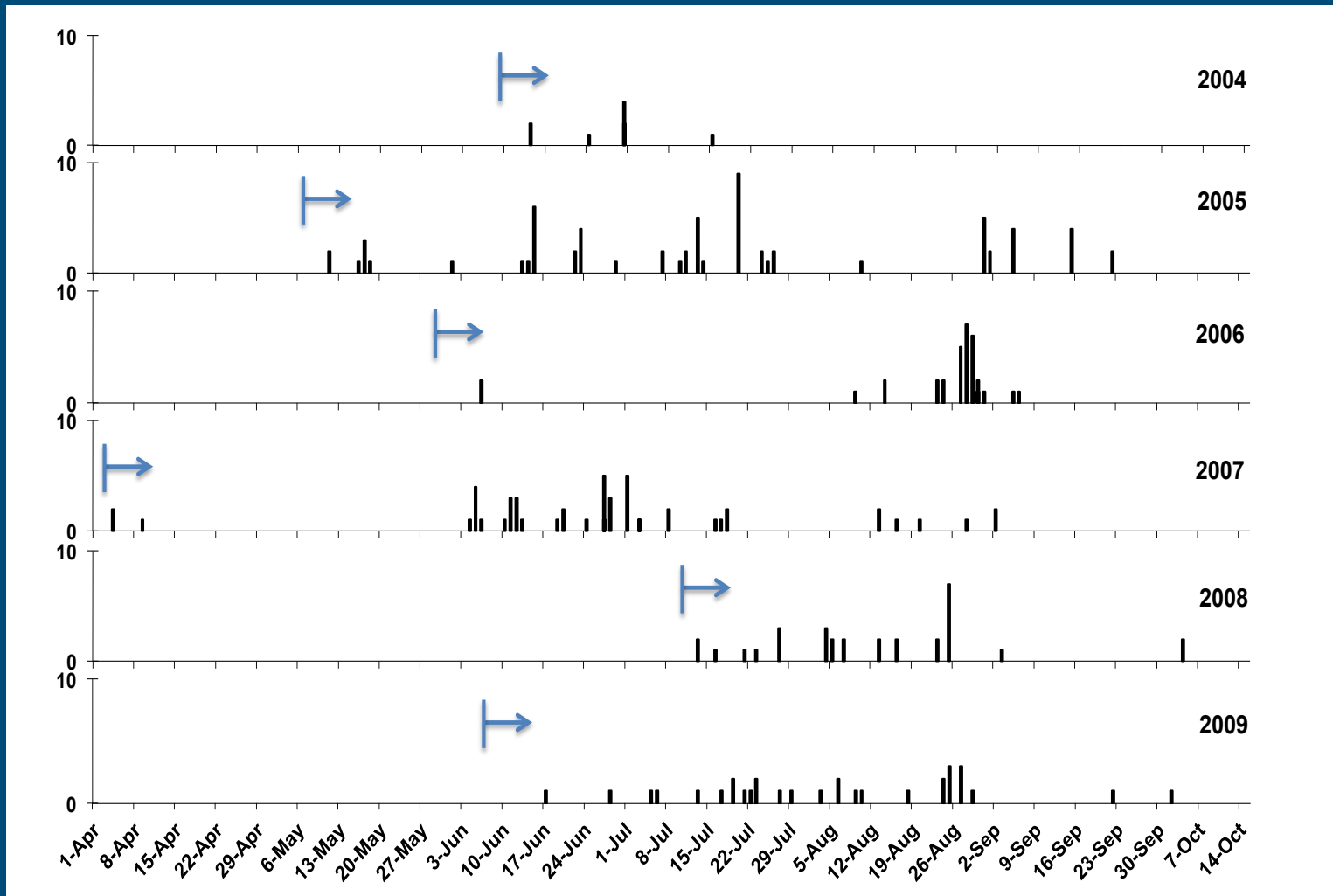
Dominant SSR genotypes 2000-2009



When is Blue-13 * showing up?

Region	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	SUM	% Blue 13
C	8	0	4	10	6	34/25*	28/16*	16/12*	10/5*	34/6*	150/64*	43
N&NW	6	1	0	4	2	23/14*	6/3*	9/5*	4/4*	0	55/26*	47
NE	18	22	20	21	28/4*	30/6*	18/4*	23/11*	9/7*	20/3*	209/35*	17
SE	19	1	2	7	17/5*	19/15*	10/6*	16/10*	24/13*	23/16*	138/65*	47
SW	4	0	3	10	3	46/5*	10/4*	16/12*	2/2*	6/6*	100/29*	29
SUM	55	24	29	52	56/9*	152/65*	72/33*	80/50*	49/31*	83/31*		
% Blue 13	0	0	0	0	16	43	46	63	63	37		34%

Findings of the Blue-13 clonal lineage



Conclusion and Discussion

- National, regional or local population?
 - National population: three groups
 - Significant variations among regions
 - Group 2 more dominant in NE region

- Population structure and dynamics of clonal lineages
 - Clonal lineages are formed and displaced
 - Three dominant clonal genotypes
 - GS-001 (Blue 13), since 2004, all regions
 - GS-008, since 2000, except in region North East (starch)
 - GS-005 (Pink 6), since 2002, except in region North East (starch)

- Blue 13
 - First find early 2004
 - Dominant since 2004
 - Found throughout the season
 - Seems to be slowly declining



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