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Hidden effector diversity in *Phytophthora infestans*: uncovering population structure beyond SSR genotyping

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21st Euroblight workshop
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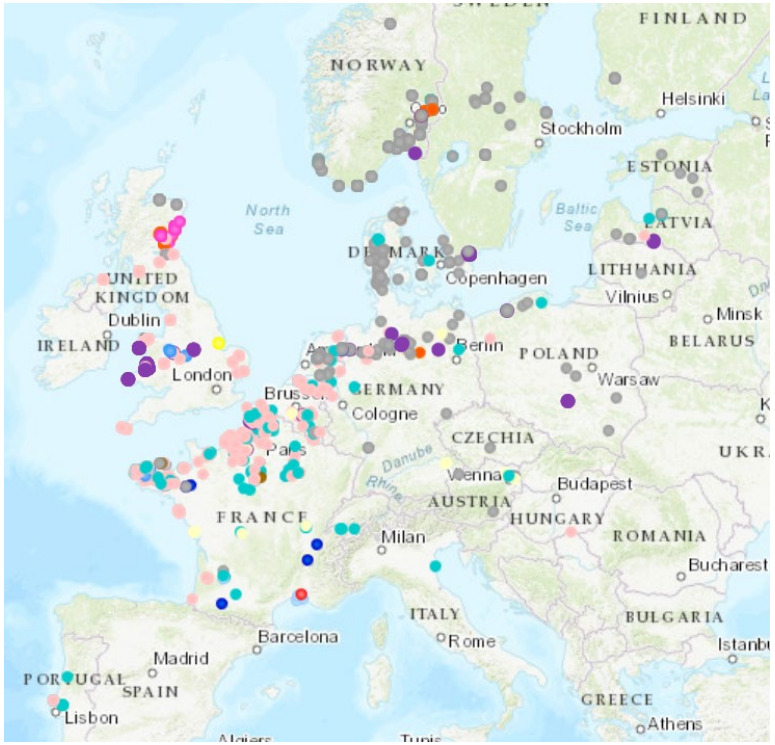
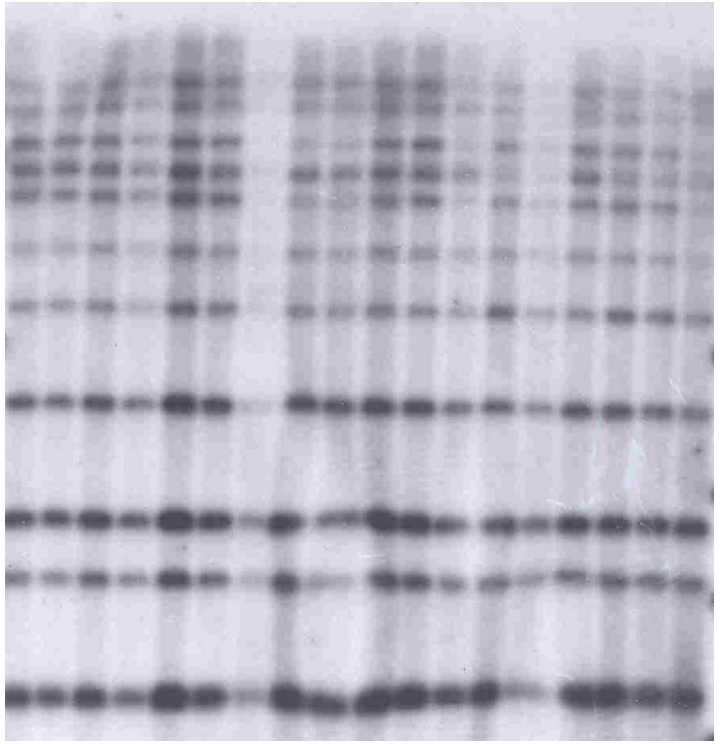
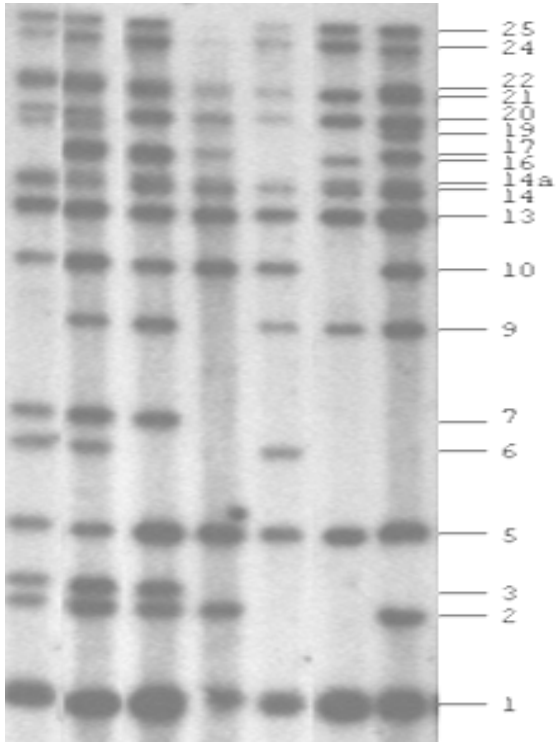
Universidade de Vigo (Ourense, Spain)

Monitoring *Phytophthora infestans* in Europe I

- Euroblight (www.euroblight.net)
- Clonal versus non-clonal







Brurberg et al 1999

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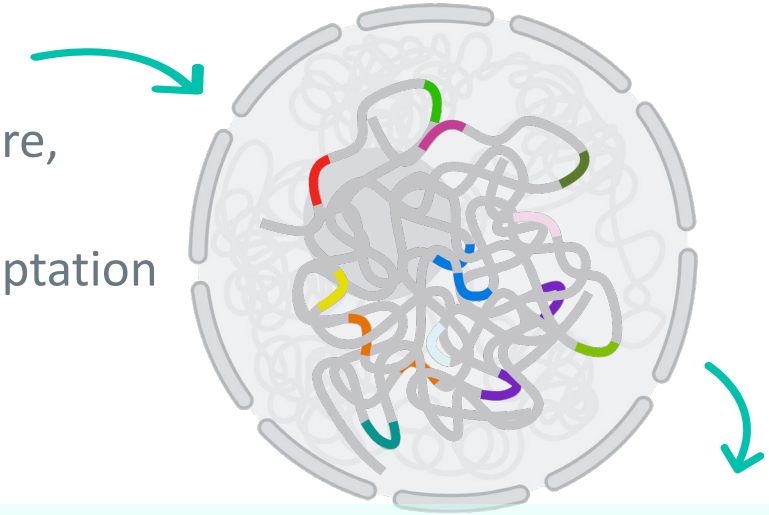
Monitoring *Phytophthora infestans* in Europe II

- Population monitoring using SSR genotyping in EuroBlight
  - 12 SSR markers (Simple Sequence Repeats)
  - Neutral markers distributed across the genome
  - Well suited for understanding population structure, tracking epidemics
  - Less suited for understanding environmental adaptation

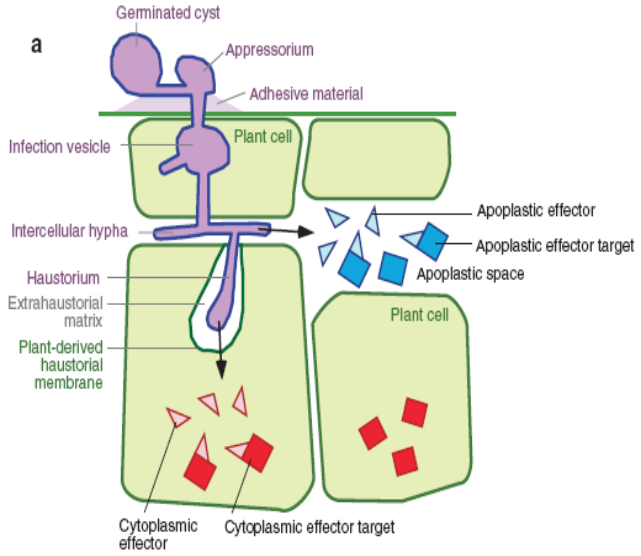


```
Markør Pi02
Isolate 1: CACACACACACACACACACACACA
Isolate 2: CACACACACACACACACACACACACA
Isolate 3: CACACACACACACACACACACACACA
Isolate 4: CACACACACACACACACACACACACACA
```

Variation in functional genes?

Effectors

- Secreted proteins that alter host physiology and facilitate colonization and/or trigger defense responses
    1. Apoplastic effectors - accumulate in the plant intercellular space
    2. Cytoplasmic effectors - are translocated directly into the plant cell
- The genome contains large complex families of hundreds of effector genes
- The effector genes are localized to highly dynamic and expanded regions of the genome thus evolving fast



From: Kamoun 2006

Cytoplasmic effectors - molecular keys to infection

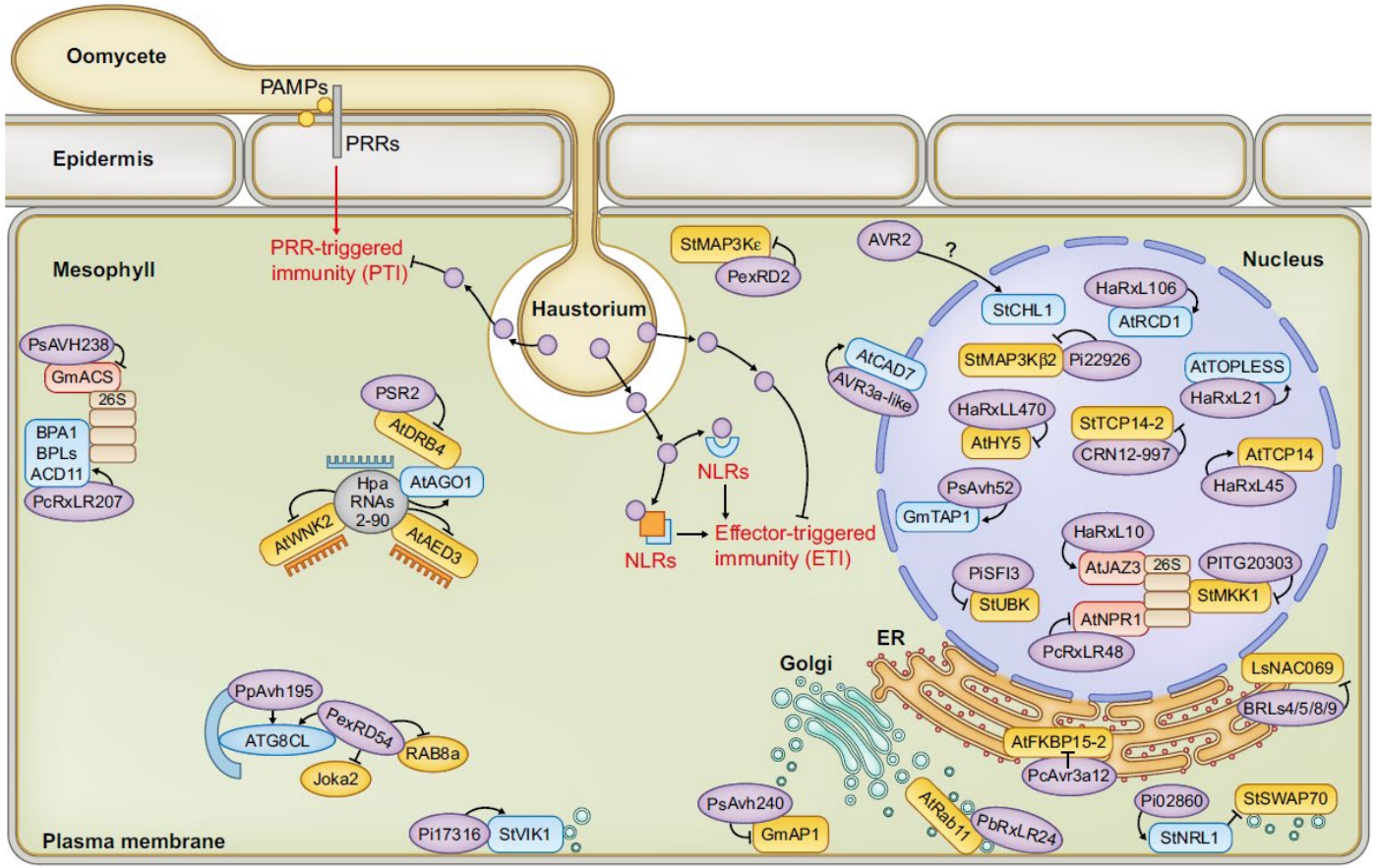


Figure: Fabro, New Phyologist 2021

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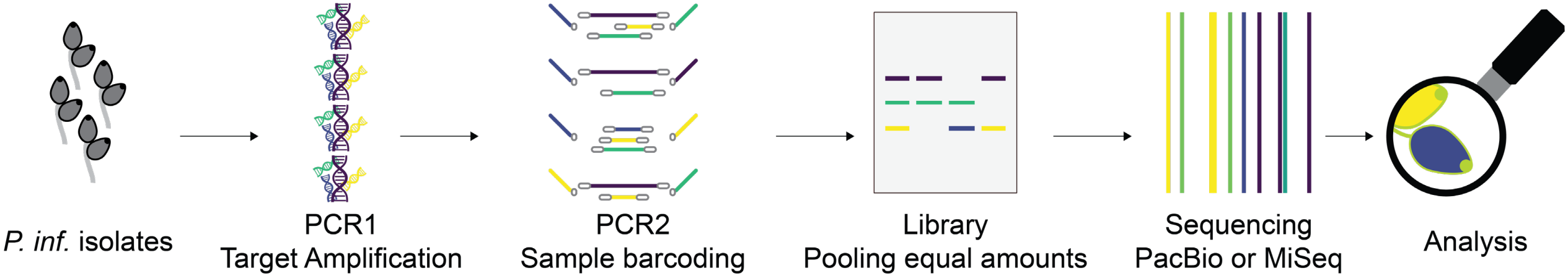
Amplicon sequencing (AmpSeq) of effector genes

Initial targets: 70 effector genes; ~~8~~ house keeping genes, ~~12 SSR markers~~



Rossmann et al., unpublished

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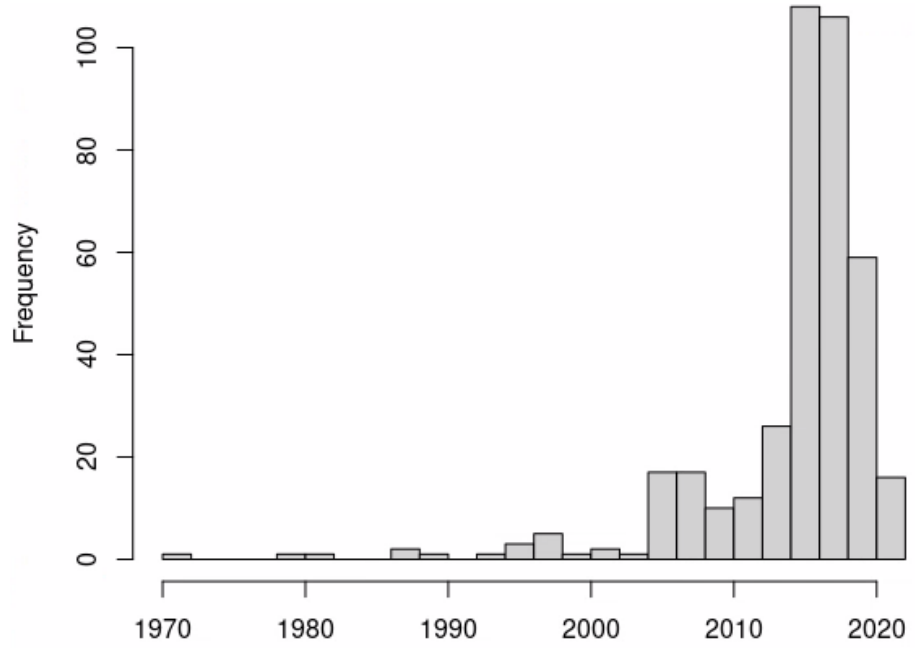
P. infestans strains

- 394 isolates from Polish and Norwegian collections
  - 389 from potato (86 varieties)



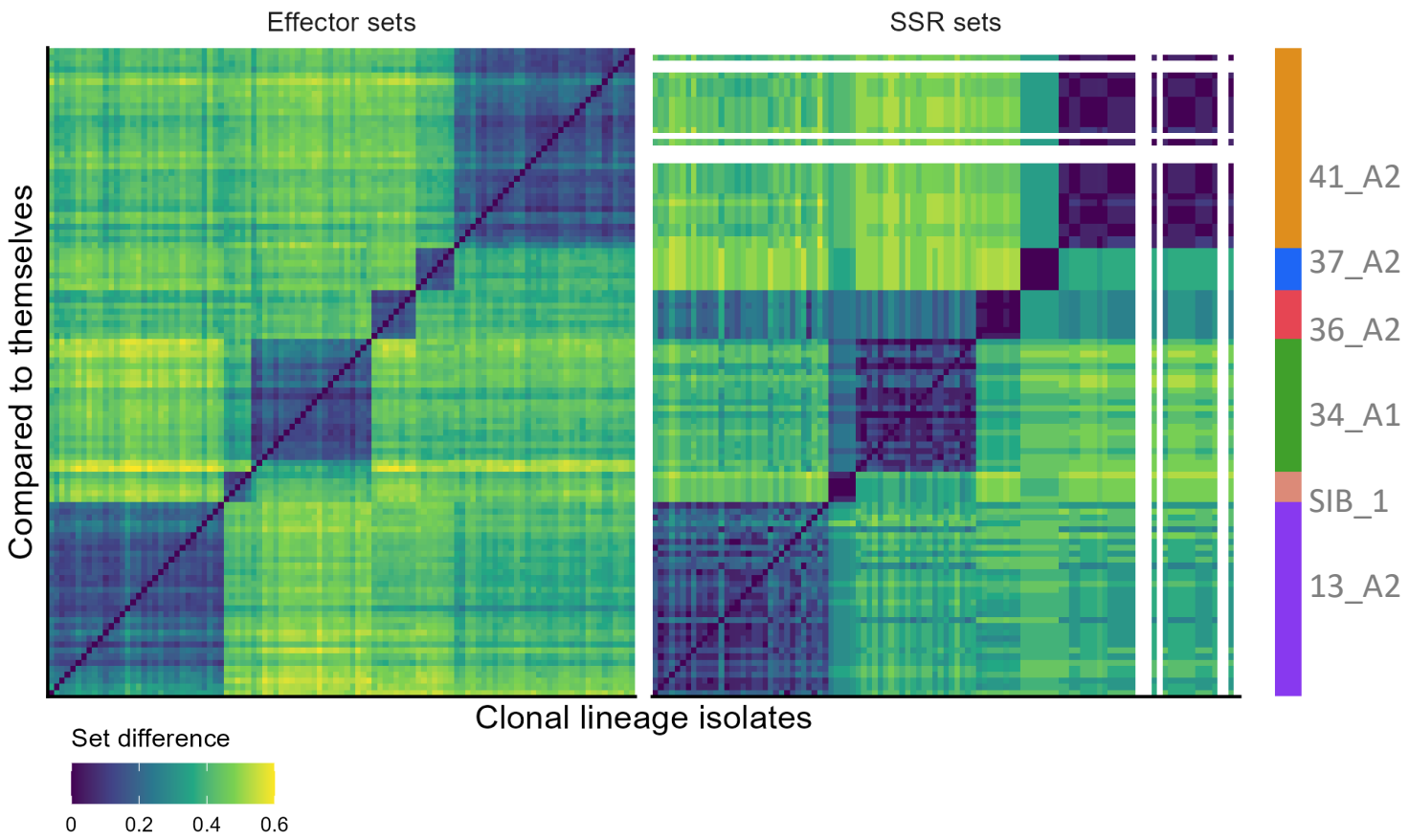
	P	N
13_A2	27	2
34_A1	19	
36_A2	8	
37_A2	7	
41_A2	9	27
EC1	1	
P. andina	1	
US-1	5	
US-8	1	
Others	96	160
Unknown	26	5

Rossmann et al., unpublished

AmpSeq analysis

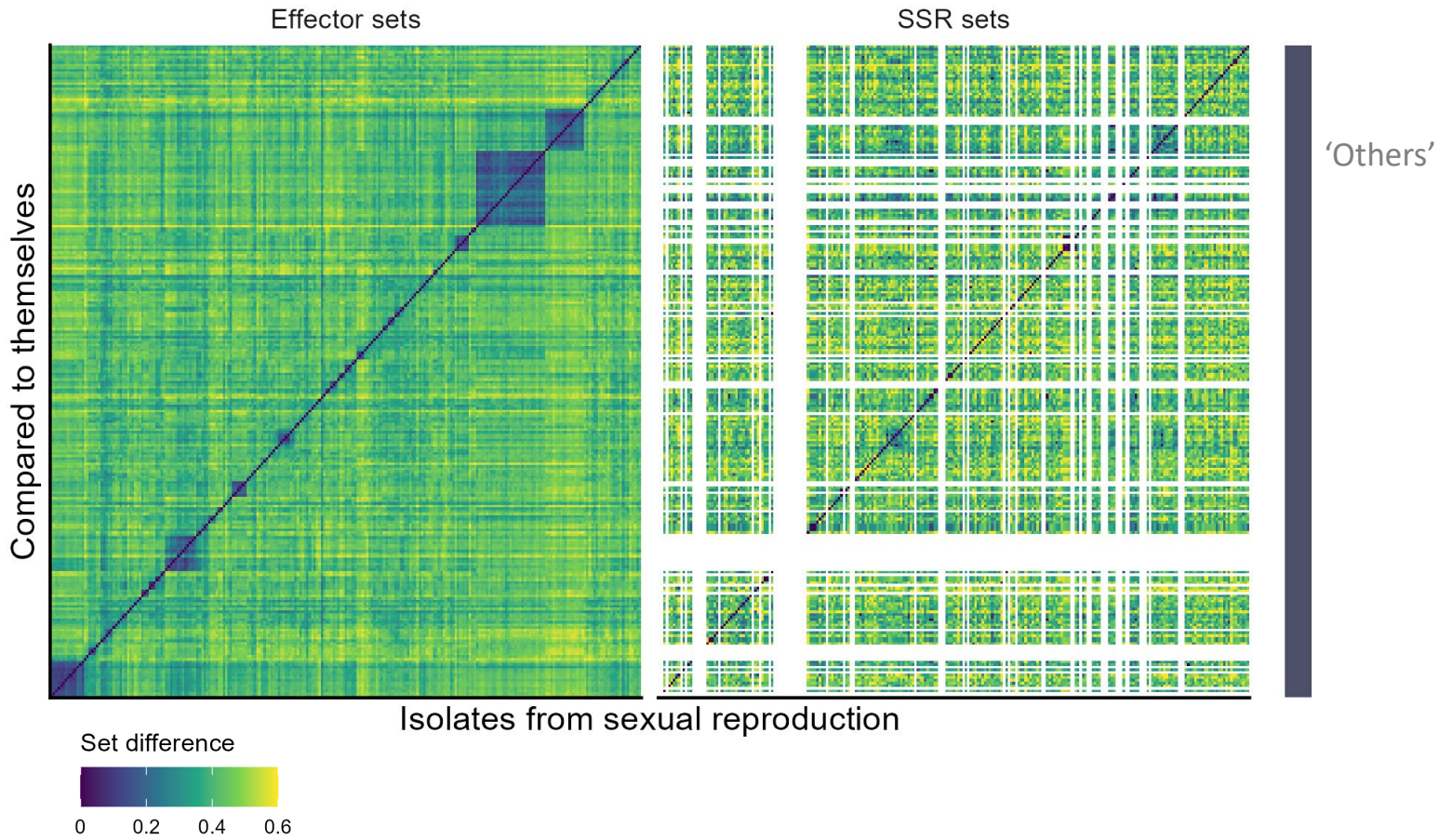
- Generating effector sets for each isolate:
  - 3 most frequent alleles for each gene for each isolate
- Pairwise comparison of isolates
  - An effector set difference of e.g. 0.2 means that 20% of the effector variants differ between two isolates

Effector profiles versus SSR-profiles for clonal lineages



Rossmann et al., unpublished

Effector profiles versus SSR-profiles for ‘others’



Rossmann et al., unpublished

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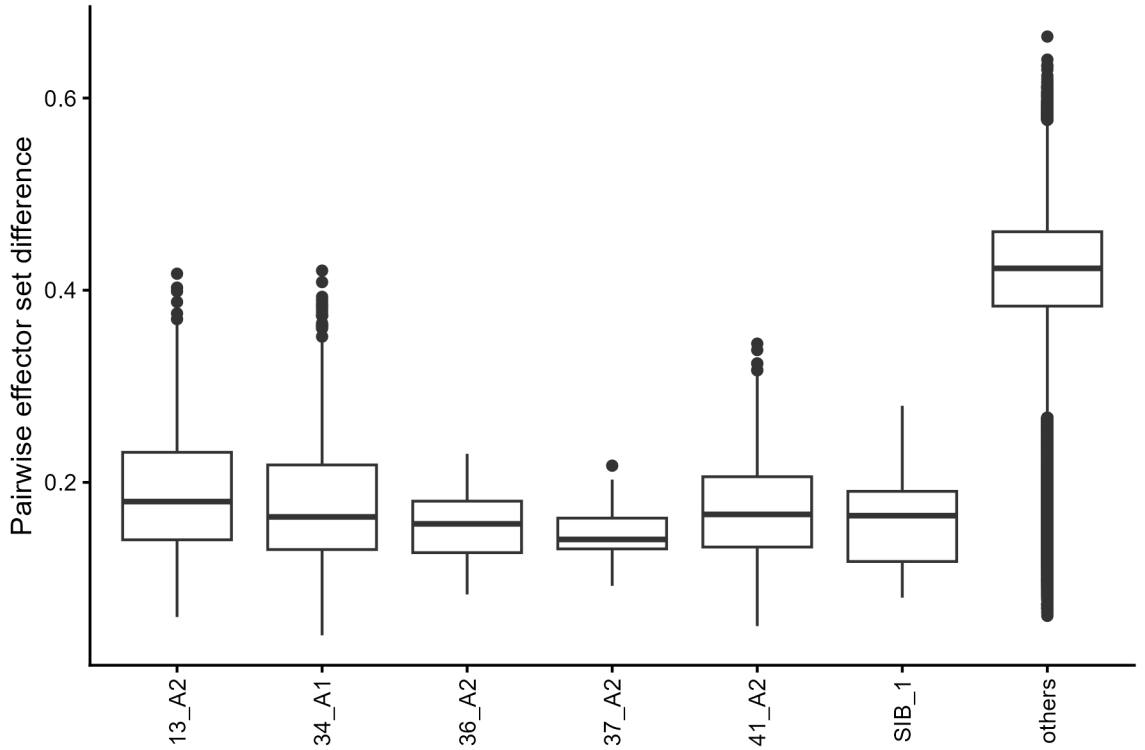
Distribution of pairwise effector set differences



Rossmann et al., unpublished

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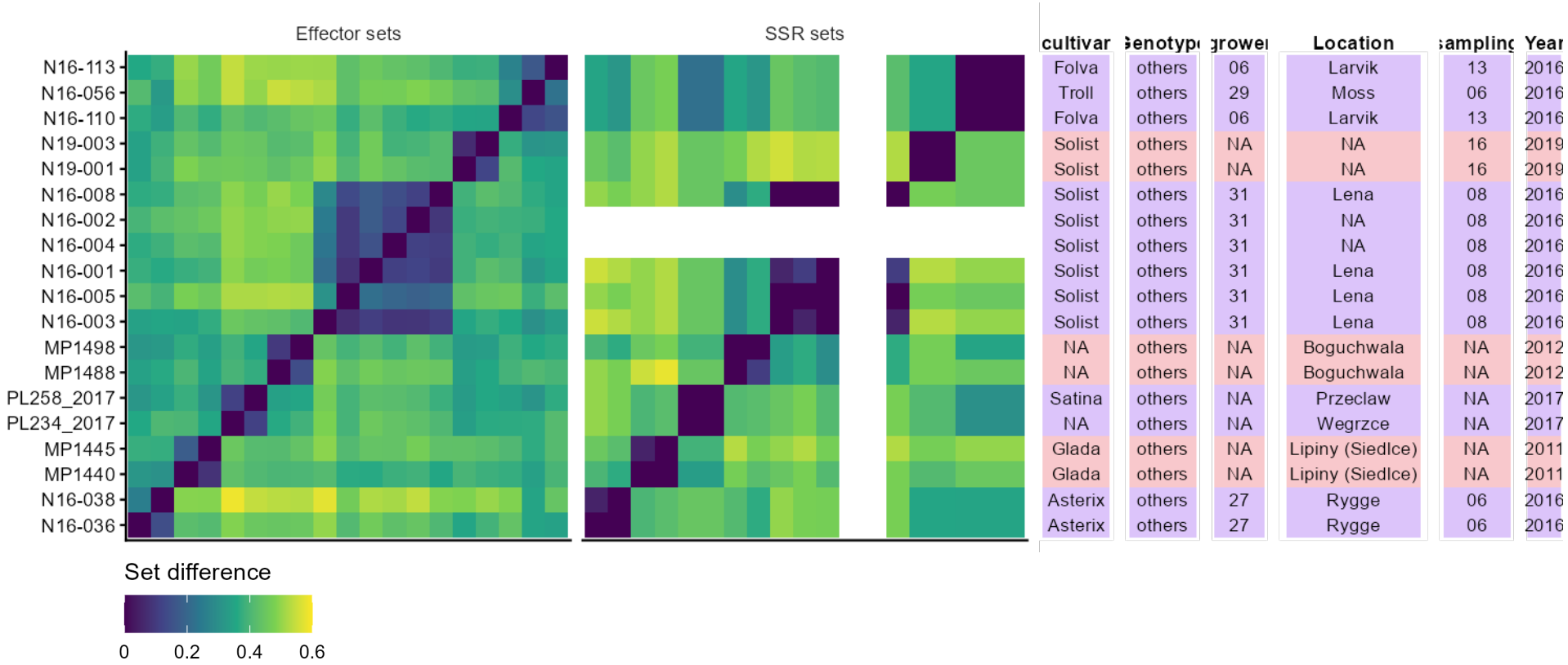
Examples of clusters I
Mini lines



cultivar	Genotype	grower	Location	sampling	Year
Folva	others	06	Larvik	13	2016
Troll	others	29	Moss	06	2016
Folva	others	06	Larvik	13	2016
Solist	others	NA	NA	16	2019
Solist	others	NA	NA	16	2019
Solist	others	31	Lena	08	2016
Solist	others	31	NA	08	2016
Solist	others	31	NA	08	2016
Solist	others	31	Lena	08	2016
Solist	others	31	Lena	08	2016
Solist	others	31	Lena	08	2016
NA	others	NA	Boguchwala	NA	2012
NA	others	NA	Boguchwala	NA	2012
Satina	others	NA	Przeclaw	NA	2017
NA	others	NA	Wegrzce	NA	2017
Glada	others	NA	Lipiny (Siedlce)	NA	2011
Glada	others	NA	Lipiny (Siedlce)	NA	2011
Asterix	others	27	Rygge	06	2016
Asterix	others	27	Rygge	06	2016

Rossmann et al., unpublished

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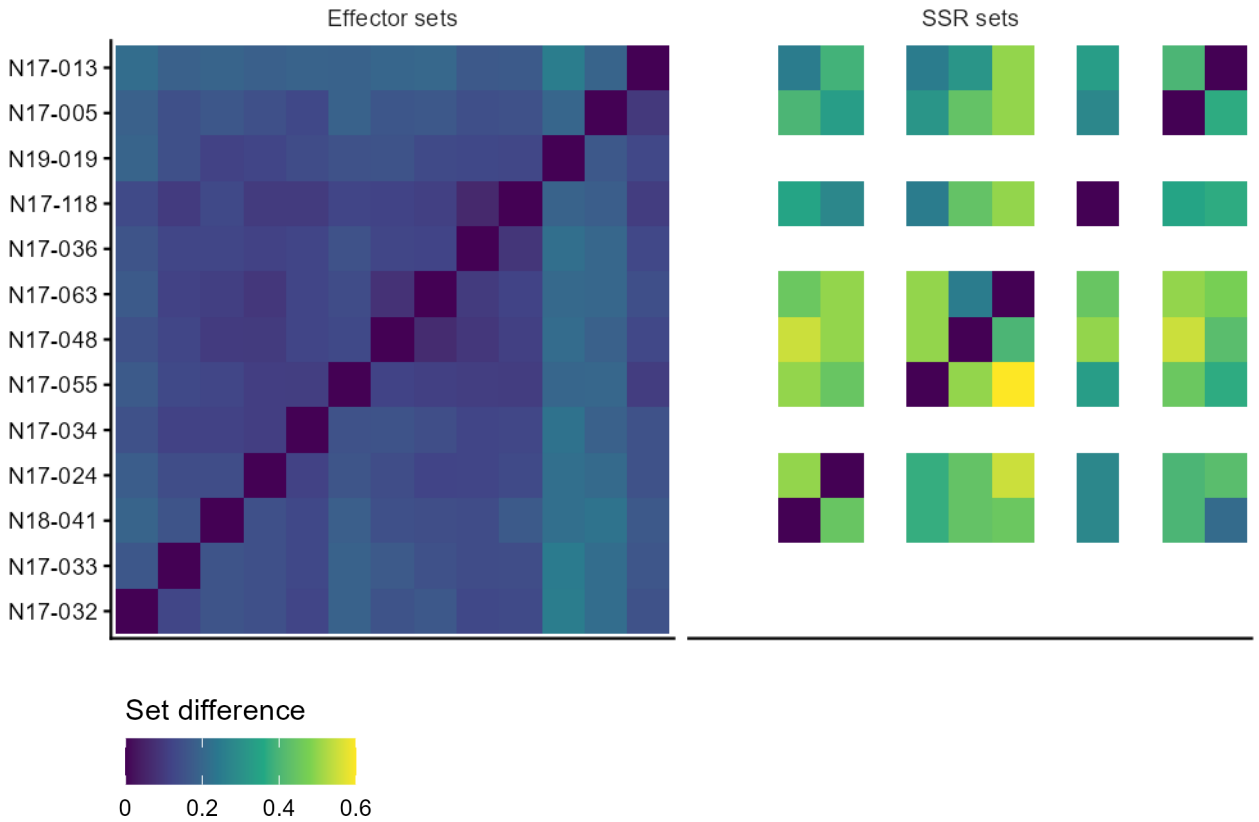
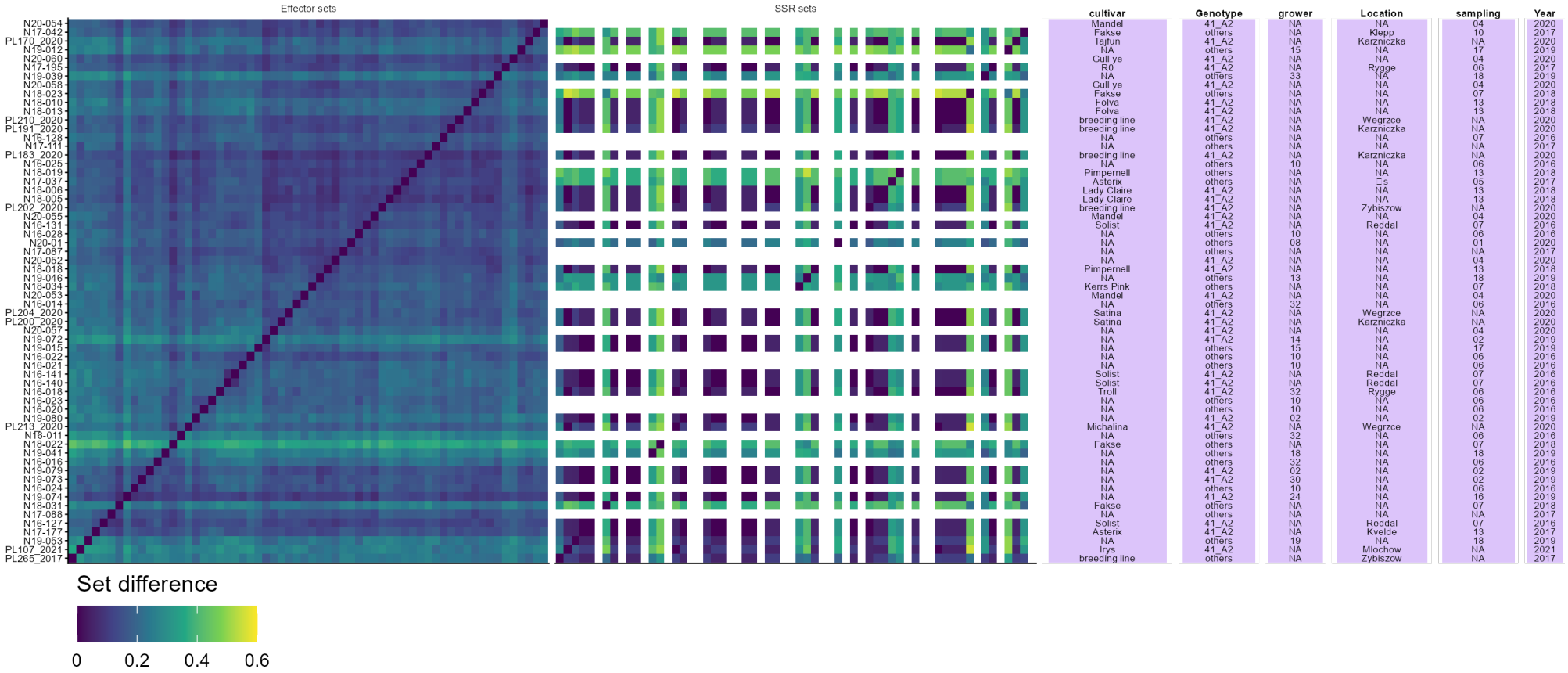
Examples of clusters II
‘Others’ with similar effector sets



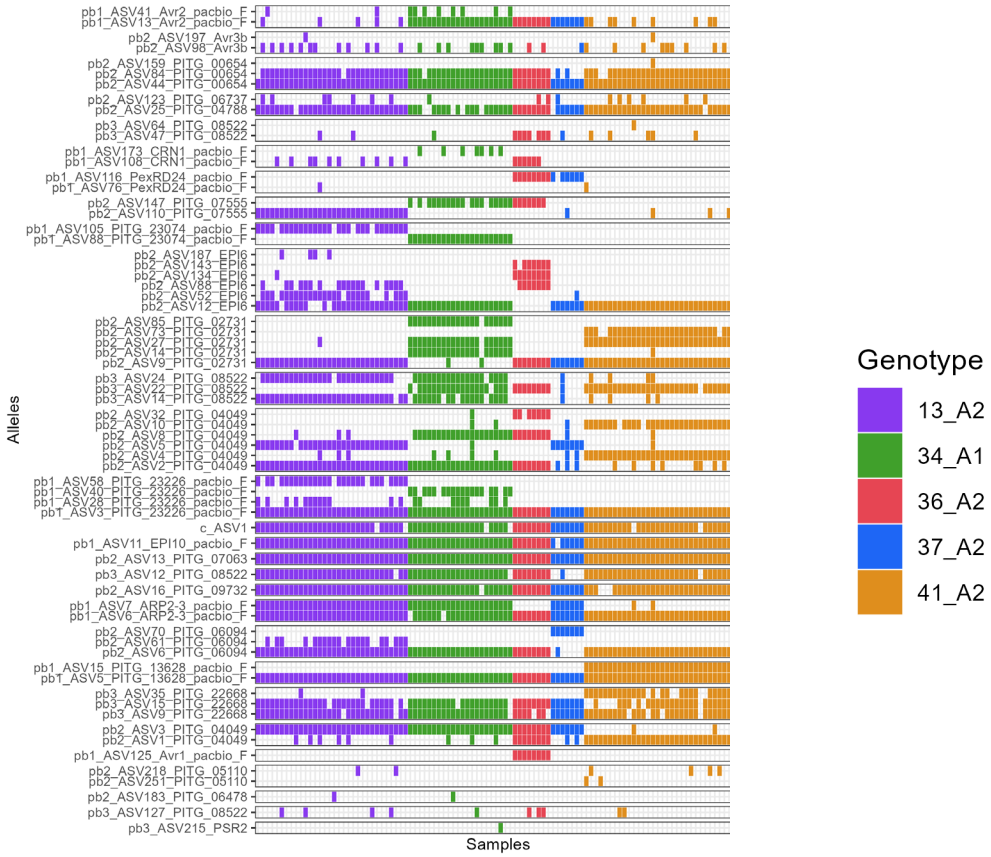
cultivar	Genotype	grower	Location	sampling	Year
Berber	others	NA	Sola	10	2017
Arielle	others	NA	Moss	06	2017
NA	others	16	NA	17	2019
R5	others	NA	Rygge	06	2017
NA	others	NA	NA	06	2017
Asterix	others	NA	Levanger	12	2017
Asterix	others	NA	Frosta	12	2017
Folva	others	NA	Sola	10	2017
NA	others	NA	NA	06	2017
Asterix	others	NA	Kvelde	13	2017
Rutt	others	NA	NA	07	2018
NA	others	NA	NA	06	2017
NA	others	NA	NA	06	2017

Examples of clusters III
41_A2 mixed with ‘others’



cultivar	Genotype	grower	Location	sampling	Year
Mandel	41_A2	NA	NA	04	2020
Fakse	others	NA	Klepp	10	2017
Tajfun	41_A2	NA	Karzniczka	NA	2020
NA	others	15	NA	17	2019
Gull ye	41_A2	NA	NA	04	2020
R0	41_A2	NA	Rygge	06	2017
NA	others	33	NA	18	2019
Gull ye	41_A2	NA	NA	04	2020
Fakse	others	NA	NA	07	2018
Folva	41_A2	NA	NA	13	2018
Folva	41_A2	NA	NA	13	2018
breeding line	41_A2	NA	Wegrzce	NA	2020
breeding line	41_A2	NA	Karzniczka	NA	2020
NA	others	NA	NA	07	2016
NA	others	NA	NA	NA	2017
breeding line	41_A2	NA	Karzniczka	NA	2020
NA	others	10	NA	06	2016
Pimpernell	others	NA	NA	13	2018
Asterix	others	NA	□s	05	2017
Lady Claire	41_A2	NA	NA	13	2018
Lady Claire	41_A2	NA	NA	13	2018
breeding line	41_A2	NA	Zybiszow	NA	2020
Mandel	41_A2	NA	NA	04	2020
Solist	41_A2	NA	Reddal	07	2016
NA	others	10	NA	06	2016
NA	others	08	NA	01	2020
NA	others	NA	NA	NA	2017
NA	41_A2	NA	NA	04	2020
Pimpernell	41_A2	NA	NA	13	2018
NA	others	13	NA	18	2019
Kerrs Pink	others	NA	NA	07	2018
Mandel	41_A2	NA	NA	04	2020
NA	others	32	NA	06	2016
Satina	41_A2	NA	Wegrzce	NA	2020
Satina	41_A2	NA	Karzniczka	NA	2020
NA	41_A2	NA	NA	04	2020
NA	41_A2	14	NA	02	2019
NA	others	15	NA	17	2019
NA	others	10	NA	06	2016
NA	others	10	NA	06	2016
Solist	41_A2	NA	Reddal	07	2016
Solist	41_A2	NA	Reddal	07	2016
Troll	41_A2	32	Rygge	06	2016
NA	others	10	NA	06	2016
NA	others	10	NA	06	2016
NA	41_A2	02	NA	02	2019
Michalina	41_A2	NA	Wegrzce	NA	2020
NA	others	32	NA	06	2016
Fakse	others	NA	NA	07	2018
NA	others	18	NA	18	2019
NA	others	32	NA	06	2016
NA	41_A2	02	NA	02	2019
NA	41_A2	30	NA	02	2019
NA	others	10	NA	06	2016
NA	41_A2	24	NA	16	2019
Fakse	others	NA	NA	07	2018
NA	others	NA	NA	NA	2017
Solist	41_A2	NA	Reddal	07	2016
Asterix	41_A2	NA	Kvelde	13	2017
NA	others	19	NA	18	2019
Irys	41_A2	NA	Mlochow	NA	2021
breeding line	others	NA	Zybiszow	NA	2017

‘Allele’ distribution for selected ‘loci’



- High diversity of loci, some between genotypes and some within genotypes.
- Other effectors are highly conserved across genotypes and no alleles were found.
- Variability within genotypes seems the norm rather than exception

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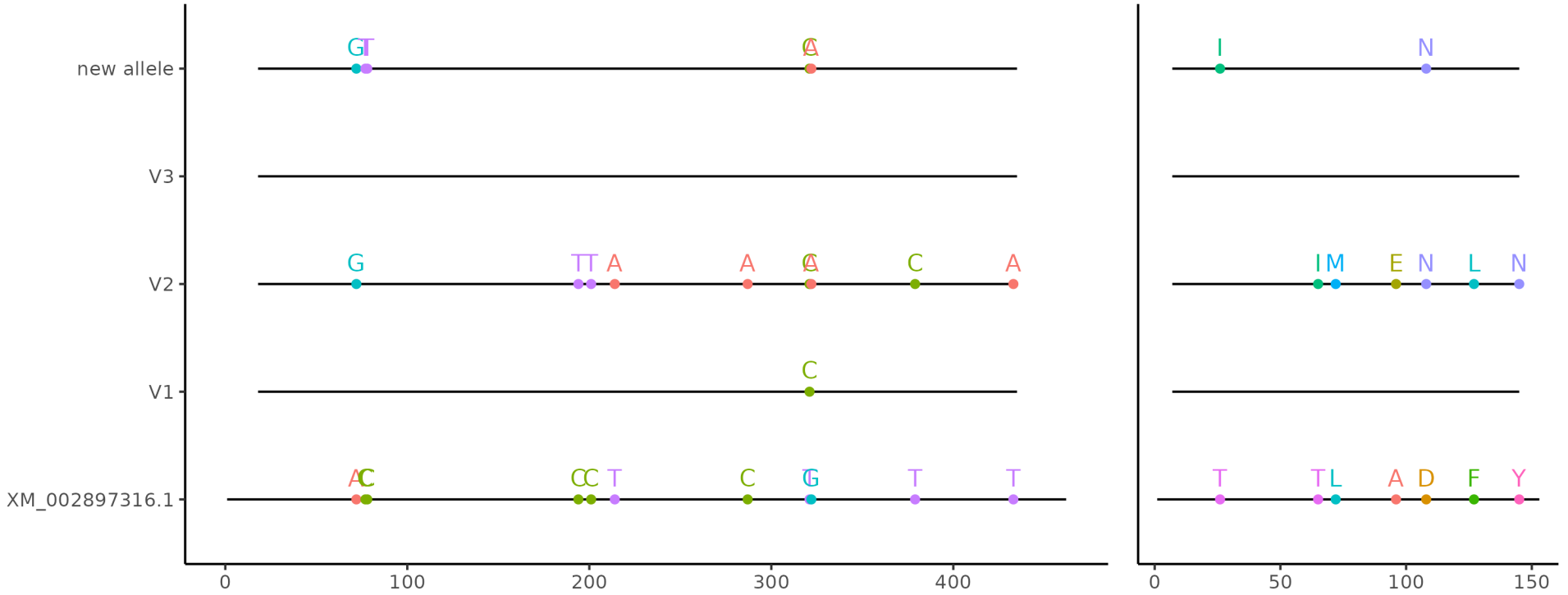
Example of sequence variation with potential functional effect; Avr-Vnt1 (targets host chloroplast metabolism; targeted by Rpi-vnt1.1)



Effector Ampseq

- A tool that allows us to see beyond the pure genetic variation that can be measured by SSR-genotyping
- Investigate effector repertoire and sequence variation that drive evolution of virulence and clones in *P. infestans* strains → future disease management
- Other genes than effectors

DivGene team

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Cross-platform validation of *Avr-vnt1* allele profiles