Genotypic diversity of European *P. ramorum* isolates based on SSR analysis

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Objectives

Determine genotypic diversity using SSR:

→ pathways of spread at country / continent scale?

→ host specificity?

→ signs of sexual recombination?

→ presence of other lineages?

Origin and number of samples genotyped

1394 samples genotyped

Country	# samples	% samples
Belgium	441	31.6
UK	274	19.7
Germany	146	10.5
Spain	111	8.0
France	101	7.2
Norway	81	5.8
Switzerland	74	5.3
Netherlands	61	4.4
USA	44	3.2
Denmark	20	1.4
Ireland	16	1.1
Poland	9	0.6
Finland	6	0.4
Canada	3	0.2
Greece	3	0.2
Portugal	2	0.1
Serbia	1	0.1
Slovenia	1	0.1

- cultures (via JKI or directly)
- DNA of pure cultures
- DNA of infected plant samples

Isolation year of samples genotyped for each country

country	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	no year
Belgium										30	49	111	55	37	39	92	27			1
Canada											2		1							
Denmark													20							
Finland												1	2	1			2			
France										15				25	17	23	13			8
Germany		2	4			2	4		35	6	20	9	28	10	20	5	1			
Greece																		1	2	
Ireland																	6	10		
Netherlands	2	1				5			3	5	6			16	21					2
Norway										1	3	7	9	4	12	42	3			
Poland						1			5											3
Portugal															2					
Serbia																1				
Slovenia											1									
Spain										9	6	18	2	3	42	30				1
Switzerland											2									72
UK										65	4	5		3	31	12	65	77	4	8
USA											5		11	5	14	9				
Total	2	3	4	0	0	8	4	0	43	131	98	151	128	104	198	214	117	88	6	95

SSR markers used

Name	repeat	Authors
18	(AC)39	Ivors et al. 2006
64	(CT)16	Ivors et al. 2006
82a	(GT)14	Ivors et al. 2006
82b	(GT)14	Vercauteren et al. 2010
ILVOPrMS133	(GACA)77	(= PrMS43) Prospero et al. 2007
ILVOPrMS145a	(AGCGAC)15	Vercauteren et al. 2010
ILVOPrMS145c	(AGCGAC)15	Vercauteren et al. 2010

Genotypes

Currently 66 genotypes identified

- EU1MG1: most observed genotype (63.6%)
- Other genotypes: 1 2 3 mutations

	1	2	3	
	51%	35%	14%	of non-MG1 genotypes
	66%	28%	6%	of non-MG1 isolates

	18		64		82a		82b		133		145a		145c	
EU1MG1	220	264	350	394	127	137	111	235	-	165	187	223	205	277
EU1MG2	220	264	350	396	127	137	111	235	-	165	187	223	205	277
EU1MG3	220	264	350	394	127	137	111	241	-	165	187	223	205	277
EU1MG4	220	264	350	394	127	137	111	235	-	165	187	223	205	259
EU1MG5	220	264	350	394	127	137	111	235	-	165	187	223	205	271
EU1MG6	220	264	350	394	127	137	111	231	-	165	187	223	205	277
EU1MG7	220	264	350	394	127	137	111	237	-	165	187	223	205	271
EU1MG8	220	264	350	396	127	137	111	235	-	165	187	217	205	259
EU1MG9	220	264	350	394	127	137	111	235	-	165	187	217	205	259
EU1MG10	220	264	350	396	127	137	111	235	-	165	187	223	205	271

⋮

- No sexual recombinants but a few isolates still need to be checked.
- 3 isolates belong to a different lineage (EU2 - see separate talk)
- Host specificity?: EU1MG5 &13 more on *Rhododendron*, EU1MG22 more on *Viburnum* but…

Genotype frequencies

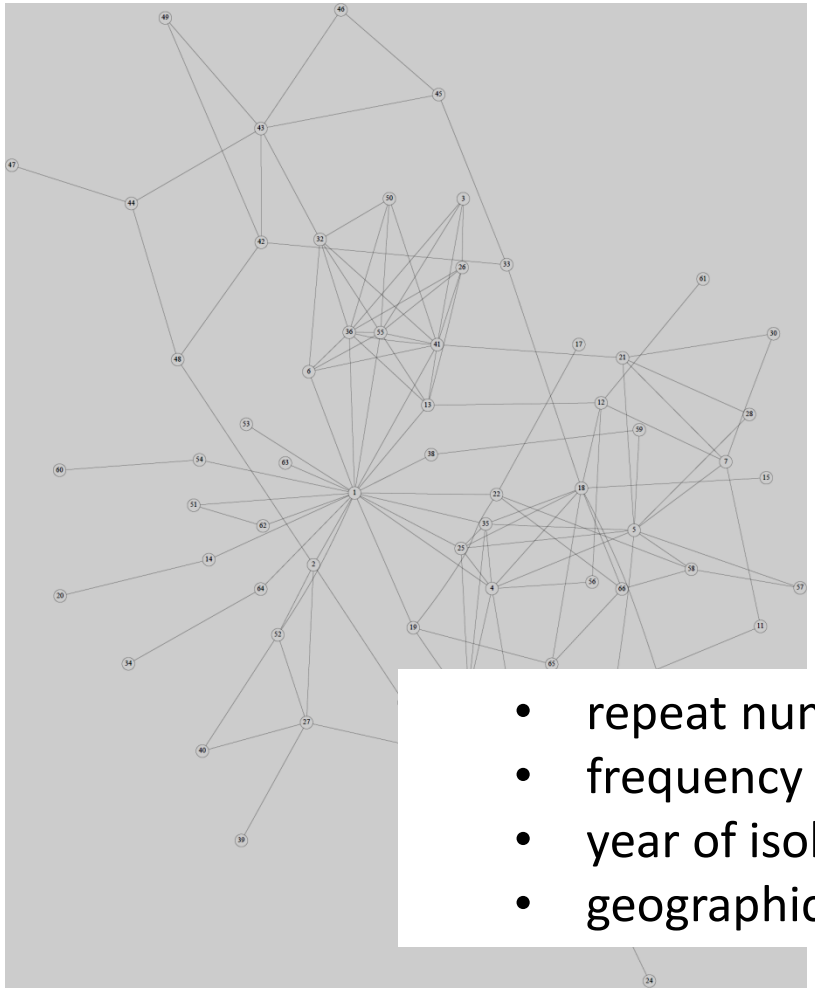
EU1MG	#isolates	%
1	886	63.6
18	75	5.38
5	74	5.31
43	67	4.81
22	35	2.51
42	25	1.79
4	22	1.58
13	21	1.51
2	18	1.29
7	14	1.00
29	13	0.93
9	12	0.86
46	10	0.72
19	9	0.65
14	7	0.50
31	7	0.50
6	6	0.43
12	6	0.43
26	6	0.43
45	6	0.43
3	5	0.36
24	5	0.36

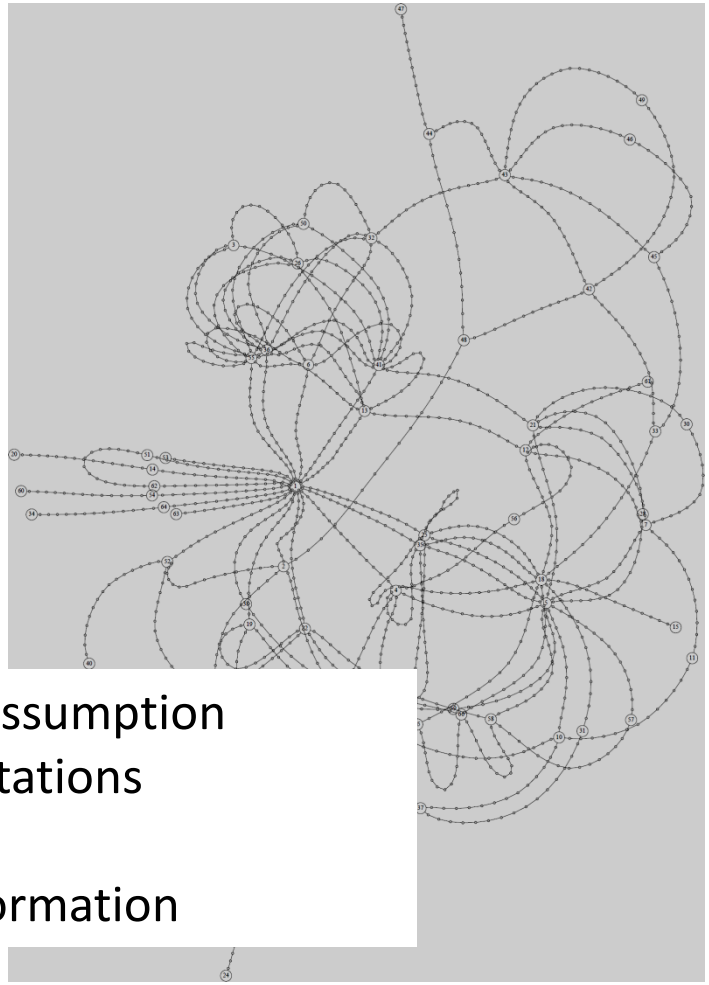
EU1MG	#isolates	%
10	4	0.29
38	4	0.29
58	4	0.29
17	3	0.22
32	3	0.22
35	3	0.22
41	3	0.22
23	2	0.14
25	2	0.14
44	2	0.14
54	2	0.14
8	1	0.07
11	1	0.07
15	1	0.07
16	1	0.07
20	1	0.07
21	1	0.07
27	1	0.07
28	1	0.07
30	1	0.07
33	1	0.07
34	1	0.07

EU1MG	#isolates	%
36	1	0.07
37	1	0.07
39	1	0.07
40	1	0.07
47	1	0.07
48	1	0.07
49	1	0.07
50	1	0.07
51	1	0.07
52	1	0.07
53	1	0.07
55	1	0.07
56	1	0.07
57	1	0.07
59	1	0.07
60	1	0.07
61	1	0.07
62	1	0.07
63	1	0.07
64	1	0.07
65	1	0.07
66	1	0.07

Networks

Genotype: Bruvo genotype distances, repeat number / stepwise mutation model
Minspnet: minimum spanning network based on distance matrix
Hapstar/Graphviz: visualise network

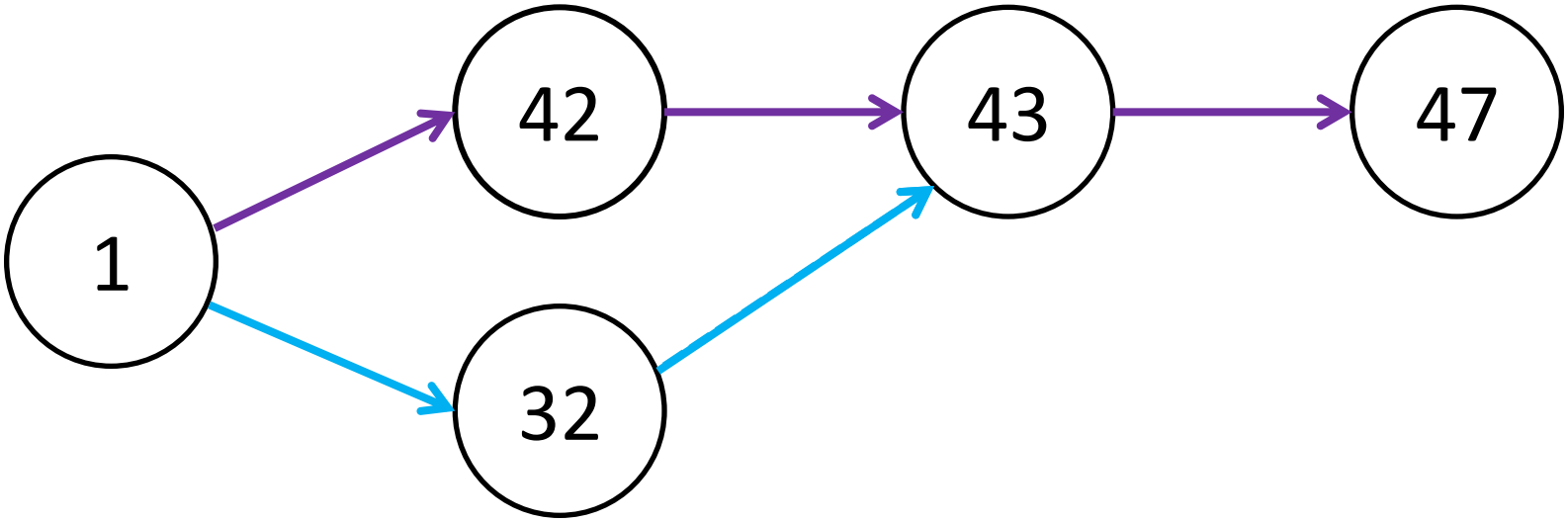




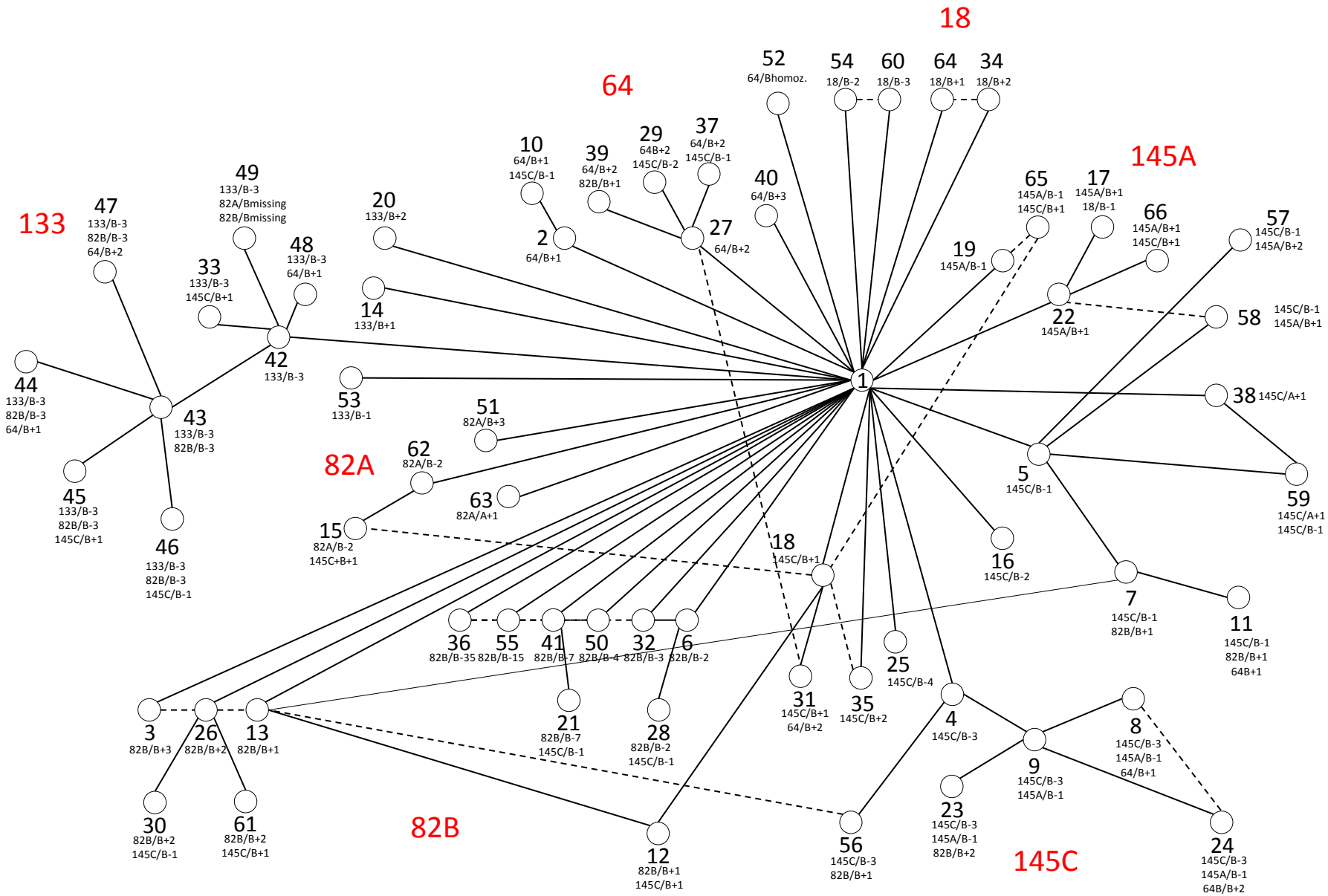
- repeat number assumption
- frequency of mutations
- year of isolation
- geographical information

Building a network: stepwise mutation model

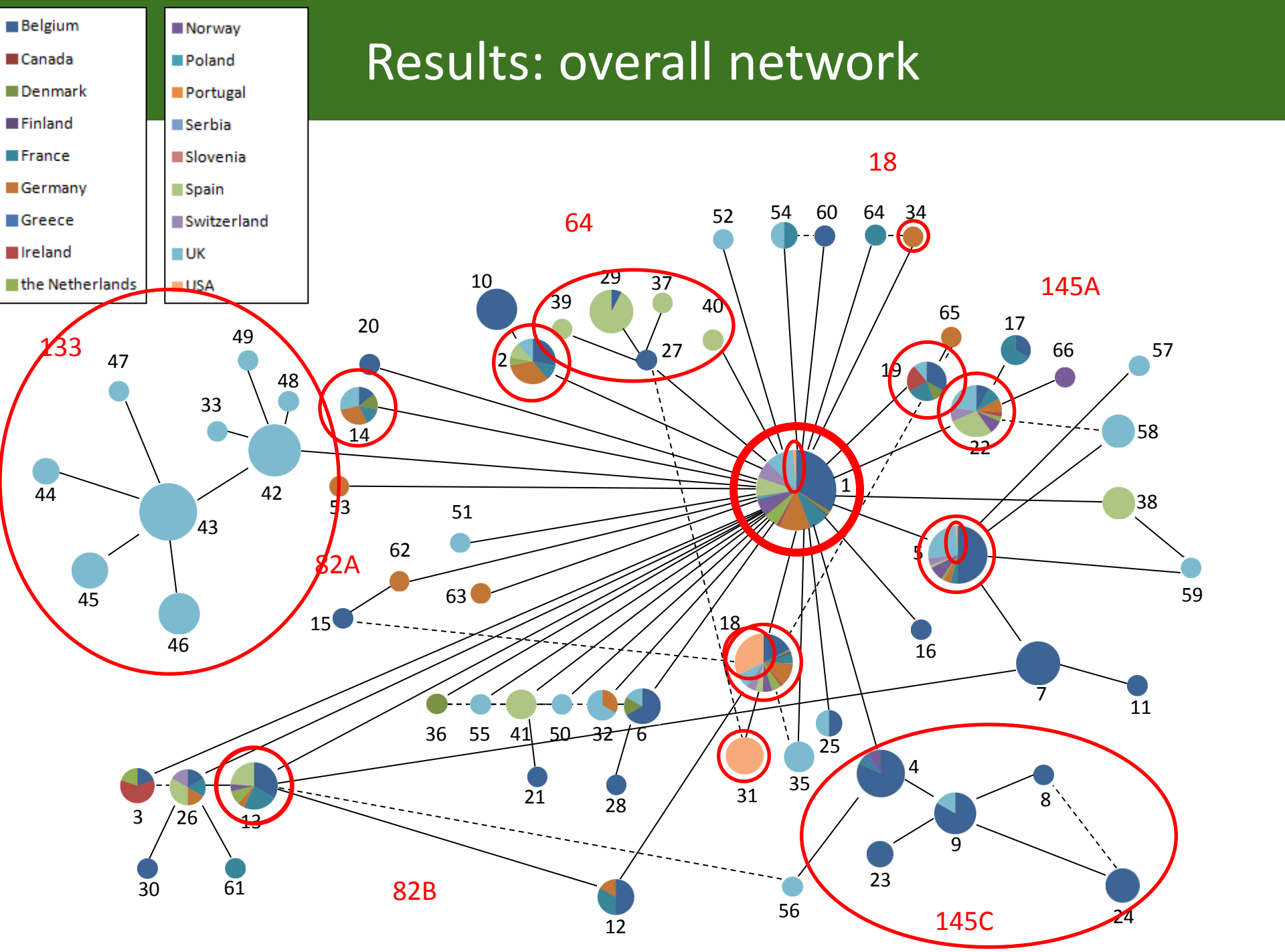
repeat size	2		2		2		4	
EU1MG	**64**		**82a**		**82b**		**133**	
1	350	394	127	137	111	235	-	165
42	350	394	127	137	111	235	-	153
32	350	394	127	137	111	229	-	165
43	350	394	127	137	111	229	-	153
47	350	398	127	137	111	229	-	153



Results: overall network



Results: overall network

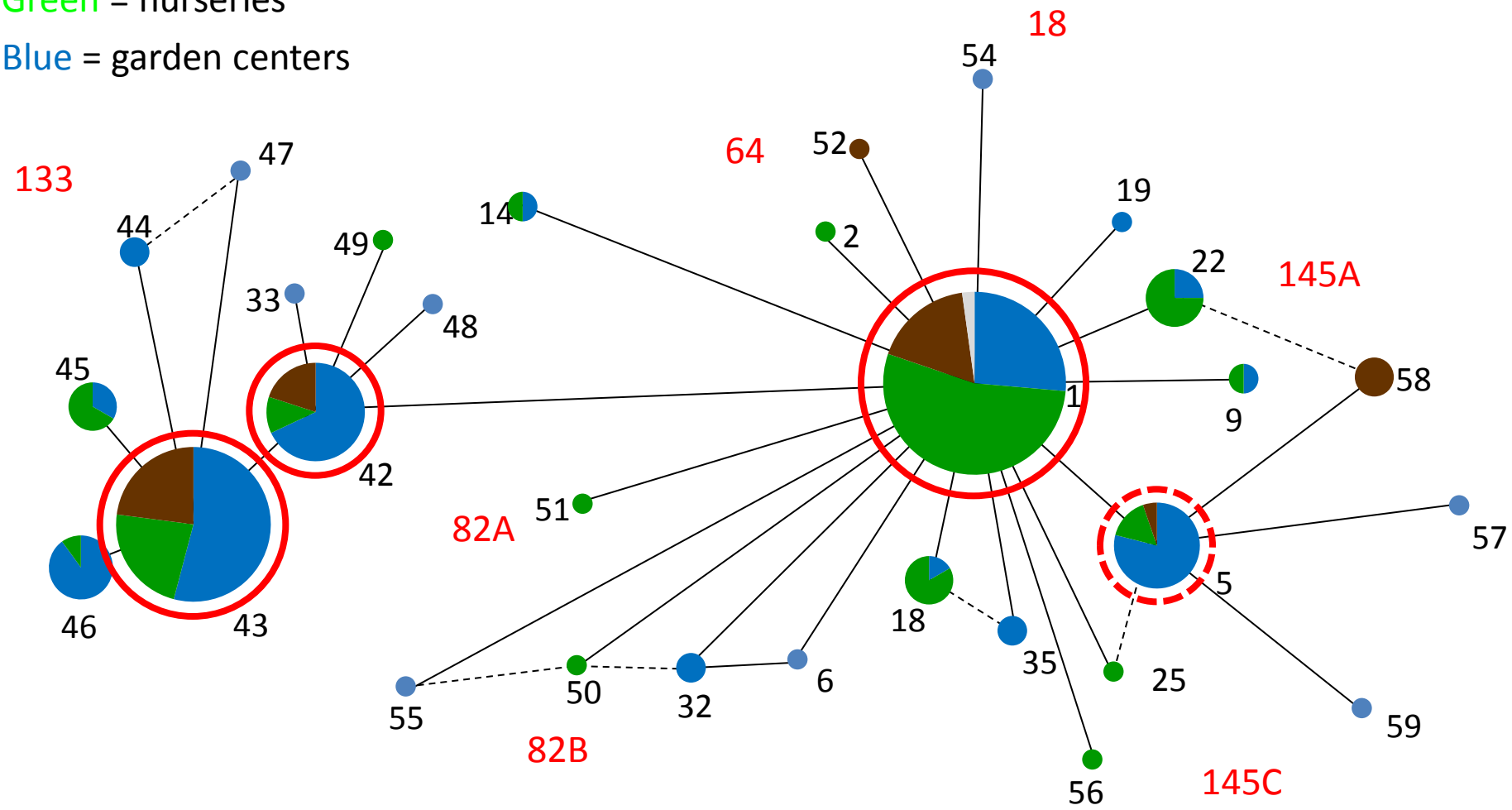


Nurseries/garden centers vs. woodlands (UK)

Brown = woodlands

Green = nurseries

Blue = garden centers



Conclusions

- 66 genotypes in 1394 EU1 isolates using 7 SSR markers
- Clonally expanding *P. ramorum* population, accumulating mutations
  - EU1MG1 dominant (64%) and widespread
  - genotypes with 1, 2 and especially 3 mutations increasingly rare
- When unique genotypes are found at different sites → pathway of spread
- UK and USA have large populations of non-EU1MG1 isolates
- No sexual recombinants (yet)
- No real indications of host specificity
- Discovery of a fourth *P. ramorum* lineage, EU2 (more recent UK introduction)

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