

Are simple *Phytophthora infestans* races really that simple?

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1 Description of diversity of *P. infestans*

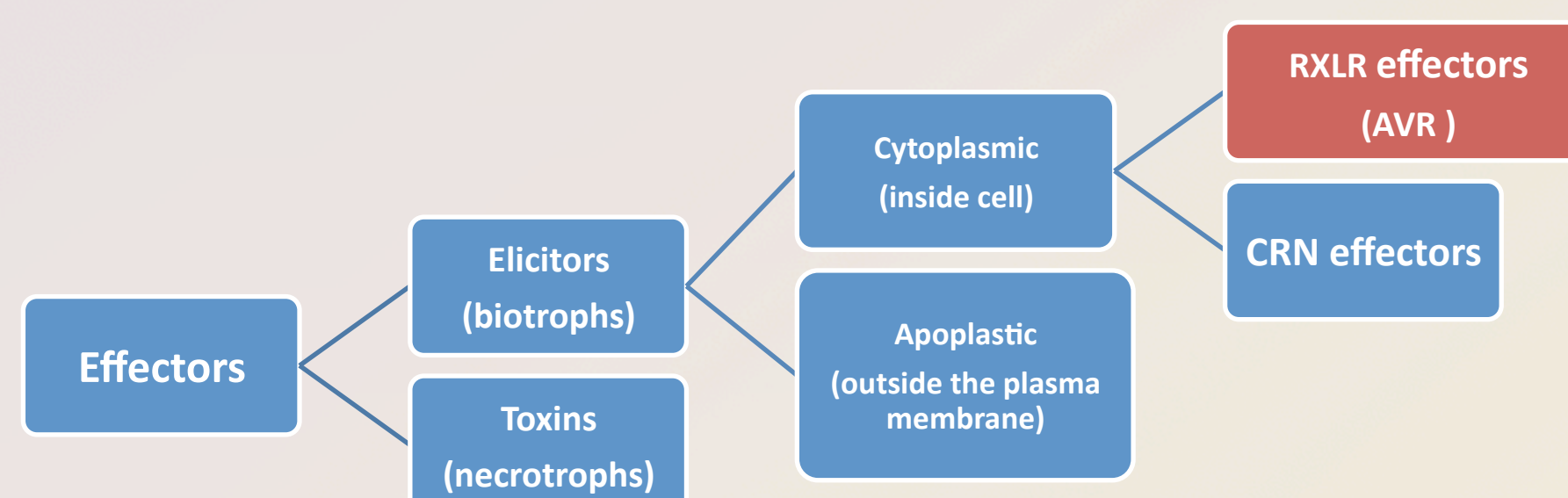
Various methods and protocols were developed to identify, distinguish and classify *P. infestans* isolates collected in the fields worldwide. Among these are:

- pairing test (mating type A1, A2);
- response to metalaxyl (Sozzi et al., 1992);
- isozymes (Goodwin et al., 1995);
- RFLP markers mtDNA and RG57 (Goodwin et al., 1991 and 1992);
- AFLP fingerprinting (Chen et al., 2008);
- microsatellite analysis (SSR; Lees et al., 2006);
- AVR profiling (Cardenas et al., 2011);
- virulence spectra on differential plants ("simple" and "complex" races).

In our study we critically analysed the concept of "simple" and "complex" races using current knowledge about the molecular basis of *P. infestans*-plant interactions.

2 What determines specificity in race-specific resistance

During attack on host plants, *P. infestans* secretes hundreds of molecules aimed at promoting infection by various mechanisms including reprogramming of plant immune system by interaction with proteins involved in the immune response. Such molecules are called effectors (Kamoun, 2006). The diversity of characterised effectors can be classified according to the scheme below.



Inside a cell, effectors can be directly or indirectly recognised by the sentinel receptors encoded by *R* genes - determinants of race-specific resistance according to Van der Plank (1963). All intracellular effectors characterised as triggering *R* gene-mediated immune response belong to the class of RXLR, also known as Avr (avirulence), effectors. The genome of *P. infestans* strain T30-4 contains 563 genes encoding proteins with unique RXLR motifs (Haas et al., 2009). Eight RXLR effectors were characterised as factors triggering immune response by their recognition by *Solanum* *R* proteins (reviewed in Vleeshouwers et al., 2011).

Species	R protein	Effector
dms	R1	Avr1
blb/dms	Rpi-blb3/R2	Avr2
dms	R3a	Avr3a
dms	R3b	Avr3b
dms	R4	Avr4
vnt	Rpi-vnt1.1	Avr-vnt1
blb/sto	RB/Rpi-blb1/Rpi-sto1	IpiO1
blb	Rpi-blb2	Avr-blb2

dms – *S. demissum*, blb – *S. bulbocastanum*, sto – *S. stoloniferum*, vnt – *S. venturii*

From the pathogen side, allelic diversity of Avr effectors apparently determines the specificity of their recognition by *R* proteins. Interestingly, a single amino acid change in Avr3a could inhibit its recognition by R3a (Bos et al., 2009).

3 What makes "simple" race simple

The concept of "simple" and "complex" races was introduced by Black and associates who bred the set of 11 so-called potato differentials each presumably containing a single *S. demissum* *R* gene (Black et al., 1953). *P. infestans* isolates virulent only on one or two of these differentials were considered as "simple", isolates with wider virulence spectra as "complex". "Simple" races were widely used to map *S. demissum* *R* genes (Leonard-Schippers et al., 1992) and to detect *R* genes in potato cultivars (Sokolova et al., 2011). "Simple" races are frequently referred to as carrying one or two virulence factors (e.g. Ghimre et al., 2001); but how does it fit the Avr-*R* gene model? We suggest that virulence factors as defined by classic phytopathology are products of mutated Avr genes evading recognition by *R* proteins. Therefore, we propose that "simple" races contain a single "broken" Avr gene, whereas other Avr effectors corresponding to *S. demissum* *R* genes are intact.

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See the Supplemental materials envelope for the list of the cited literature.

4 The concept of "simple" race may need to be revised

We suggest revising the use of the term "simple" and "complex" races based on the following arguments.

According to the so-called "arms race" model, pathogens and plants are constantly co-evolving by the re-assortment of the virulence and defence factors (e.g. alleles of Avr and *R* genes; Bergelson et al., 2001). Such co-evolution creates strong evolutionary forces that shape distribution of active and inactive alleles on both sides of the frontier. Recurrent selection of "simple" races on Black's differentials imposes constant selective constraint only on Avr effectors cognate to *S. demissum* *R* receptors, whereas allele frequencies of other effector genes (>500) apparently serve the best interest of the pathogen. Recently, wild *Solanum* species other than *S. demissum* attracted close attention from potato breeders and the corresponding *R* genes were isolated and successfully introduced into potato cultivars (Vleeshouwers et al., 2011). Virulence spectra of "simple" races on these cultivars will not be affected by selection on differentials and therefore will not be characteristic of "simple" race. In other words, "simple" races are simple only in relation to potato plants comprising *S. demissum* *R1-R11* genes. While in potato cultivars *R* genotyping with "simple" races is in line with the presence of particular *R* genes, the evidence for wild *Solanum* species is apparently misleading.

The second argument is more philosophical. The term "simple" race may carry connotations that pathogen isolate characterised as "simple" may have less ability to infect or be less aggressive and *vice versa* for "complex" race isolates. Montarry et al. (2010) showed that complexity of *P. infestans* race negatively correlates with pathogen fitness and selection may favor less complex isolates, which have less virulent factors (more intact Avr effectors). Such selection may be driven by the trade-offs between having more functional Avr effectors essential for fitness and the chance to be recognised by *R* protein. The evidence that mutation of Avr3a "hiding" this effector from R3a is also detrimental for the primary function of the effector in promoting infection fits this model (Bos et al., 2006). To summarise, isolates with "simple" race appear to be more fit and aggressive than "complex" race isolates.

5 Allelic diversity of *IpiO* effector gene in "simple" races

To confirm our suggestions, we studied allelic diversity of the *IpiO* gene, which encodes effector recognised by *Rpi-blb1/RB* gene from *S. bulbocastanum* and *S. stoloniferum* (Vleeshouwers et al., 2008). To this end, we designed the forward primer specific for the *IpiO* locus and the reverse primer was as described in Champouret et al. (2009). Screening 15 races (1, 3, 4, 10, 11, 1.2, 1.3, 1.4, 2.4, 3.4, 1.11, 1.2.3, 1.2.4, 1.3.4, и 1.2.3.4) from collection of the Institute of Phytopathology showed that *IpiO* was present in all these isolates. These results are in line with previous evidence that the diversity of *IpiO* locus is formed by allelic variation in nucleotide sequences rather than presence-absence polymorphism (Halterman et al., 2010).

Champouret et al. (2009) showed that the diversity of *IpiO* genes can be grouped in three distinct classes. IpiO effectors of classes I and II were recognised by Rpi-blb1/RB, whereas IpiO class III did not trigger RB-mediated hypersensitive response. To classify IpiO alleles in "simple" races, we analysed 10 clones of *IpiO* gene from races 3 and 4.

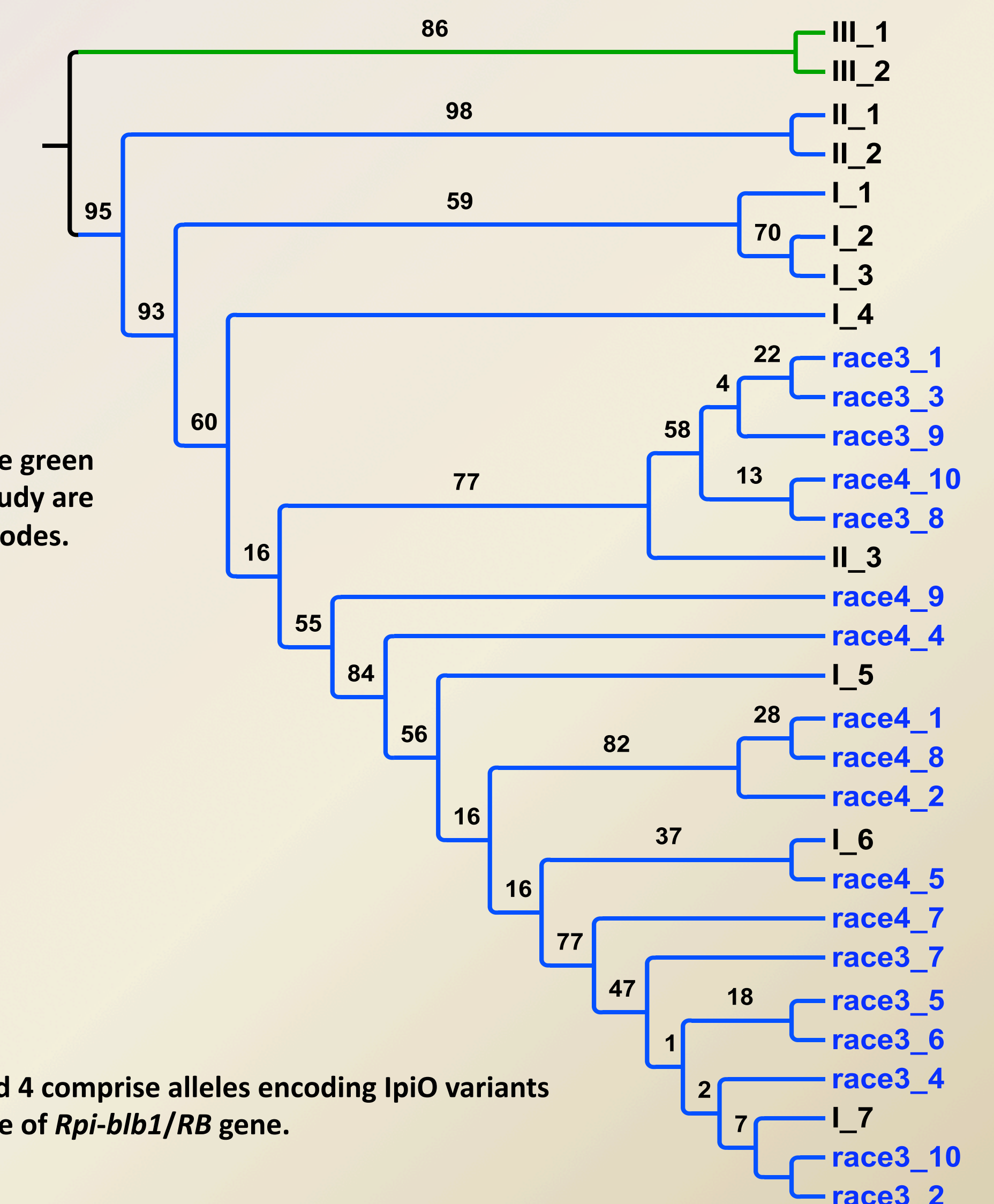


Fig. 1 Maximum likelihood tree of *IpiO* alleles.

Clusters of *IpiO* alleles of class III and classes I-II are green and blue, respectively. Alleles sequenced in this study are labelled blue. Bootstrap values are shown at the nodes.

Cluster analysis revealed that "simple" races 3 and 4 comprise alleles encoding IpiO variants avirulent on *Solanum* germplasm with active allele of *Rpi-blb1/RB* gene.

Conclusions

- We propose that virulence factors described by classic phytopathologists are "broken" Avr effector genes;
- We suggested and experimentally confirmed that "simple" races may comprise active alleles of effectors cognate to *R* genes other than *R1-R11* from *S. demissum*;
- The terms "simple" and "complex" race should be reconsidered and clearly defined to avoid ambiguities.