

The use of microsatellite markers to examine European *P. infestans* populations using the EUCABLIGHT database.

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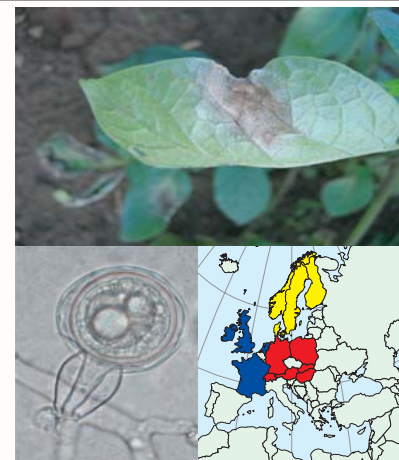
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Summary

The use of SSR markers is allowing a detailed and objective examination of *P. infestans* populations at a range of scales from the leaf to the continent. A set of 12 SSR loci have been optimised into 3 multiplex PCR assays for running on the ABI 3730 instrument. This assay is suited for high throughput analysis on either DNA from pure cultures or plant lesions. Comprehensive testing against isolates of worldwide origin has demonstrated a range from 2 to 26 alleles per locus and revealed tremendous genetic diversity.

The EU-funded project EUCABLIGHT was set up to examine the structure of European *P. infestans* populations on the basis of SSR marker and other data. A major goal of the project was to develop a database and populate it with data on *P. infestans* isolates from across Europe. This database contains over 50 fields and currently holds data on almost 14,000 isolates from 20 EU countries. Data is presented using a series of web interfaces that allow key parameters of the population to be examined on a range of spatial and temporal scales. Interesting findings are already emerging on the spatial distribution of *P. infestans* mating types across Europe. Interpretation sections for knowledge transfer to industry are planned as are detailed publications on the population structure.



SSR development and their use in the Eucablight project:

SSR development and optimisation

A range of di and tri-nucleotide markers were selected from ESTs or non-coding regions of BAC or short random clones. Multiplex assays of up to five loci in a single PCR have proved successful. The protocols will be published on the Eucablight web site.

Testing on over 1500 isolates has revealed allele diversity broadly reflecting the source of each loci; non-coding regions with longer repeats showing greatest variation.

Genotyping of Scottish *P. infestans* populations using SSRs has confirmed their power to discriminate clonal from sexually recombining populations at a range of scales.

Table 1 Overview of the origins, category and diversity of the 12 SSR loci. *Knapova & Gisi 2002 (Plant Path. 51, 641-53)

Marker	Origin	Repeat	Number alleles
P102	EST (PGI)	(TG) ₁₁	10
P104	BAC (SCR)	(GT) ₆	6
P116	EST (PGI)	(GA) ₁₁	11
P133	EST (PGI)	(CAG) ₁₀	3
P166	EST (Syn.)	(AT) ₁₀	2
P163	EST (Syn.)	(GAG) ₁₀	3
P166	EST (Syn.)	(GT) ₁₁	7
P170	EST (Syn.)	(AAG) ₁₀	3
P189	EST (Syn.)	(AT) ₁₁	5
4B*	Random (Knap.)	(TC) ₁₀	8
G11*	Random (Knap.)	(TC) ₁₀	19
D13	Random (Wall.)	(CT) ₁₂	26

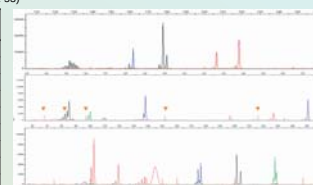


Figure 1. Composition of the three SSR panels.

The Eucablight database: examining the population structure of *P. infestans* in the EU.

Teams examining the population biology of *P. infestans* across Europe agreed a database structure and standard protocols.

The database was constructed by DIAS and is carefully designed to remain functional and expandable with minimum maintenance beyond the project end-date (January 2006). Expansion beyond Europe has also been built in to the database design. A PC-program – Phytophthora.exe – has been developed which enables the entry, storage and transfer of data to the EUCABLIGHT database.

An overview is presented as a table (Figure 2) in which the data for a range of key traits is shown by country and date. A range of additional tools can be selected and results for either the entire range or specific country/year combinations presented. The graphics are generated in real-time from the database and are thus updated as new data is entered.

The overall frequency of the A2 mating type amongst 12,000 isolates is 20%. The Graphic Analysis Tool allows the relationship between two factors such as mating type and fungicide resistance to be examined. The association between the A2 and metalaxyl sensitivity, for example, can be seen (Figure 3) indicating that EU A2 lineages were predominantly sensitive.

The frequency of virulence against the 11 R-gene differentials is shown using the Virulence Analysis Tool (Figure 4). The frequency of specific combinations of virulences can also be presented.

Summaries of the genotypic diversity are presented using the SSR Analysis Tool that, in a simple plot of allele frequencies, illustrates any major differences between the populations in different countries (Figure 5).

The Eucablight team are already planning the use of the database format and protocols beyond Europe and will be happy to discuss this further with any research groups.

Further detailed analysis of the populations will come from joint data analyses by Eucablight project and other partners later in 2006.

Figure 2 Eucablight *P. infestans* database overview.

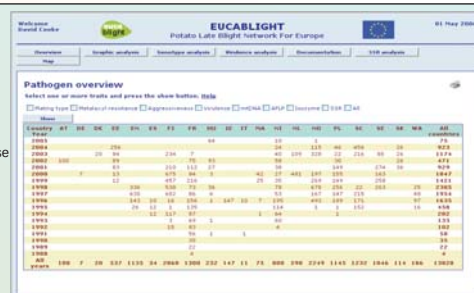


Figure 3 Sample of the output from the Eucablight Graphic Analysis Tool showing the relationship between mating type and metalaxyl resistance for 6430 isolates from 15 countries.

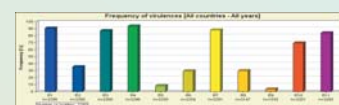


Figure 4 Sample of the output from the Eucablight Virulence Analysis tool showing the frequency of each virulence in 3290 *P. infestans* isolates from 16 countries.



Figure 5 An example of the output from the Eucablight SSR Analysis Tool showing the frequencies of the alleles of SSR marker P102 across 5 countries.