Population genetics
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Consequences on early blight disease

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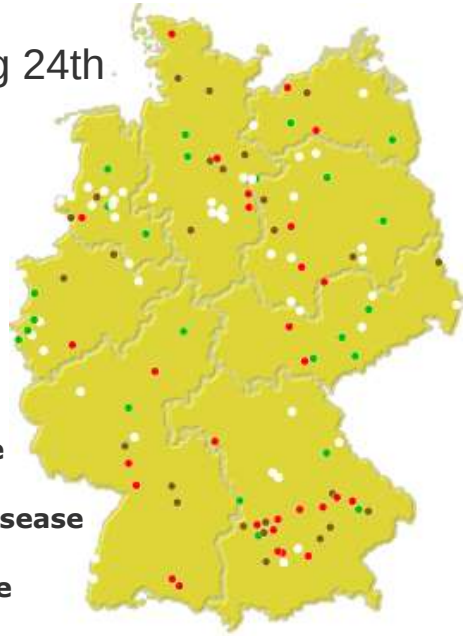
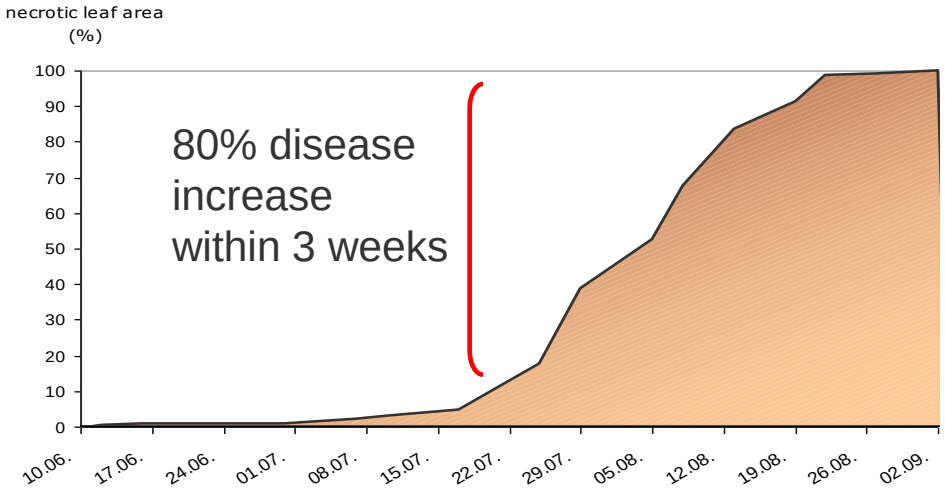
Euroblight Workshop
4.-7. May 2010

Diagnosis and disease quantification

2009, Aug 24th



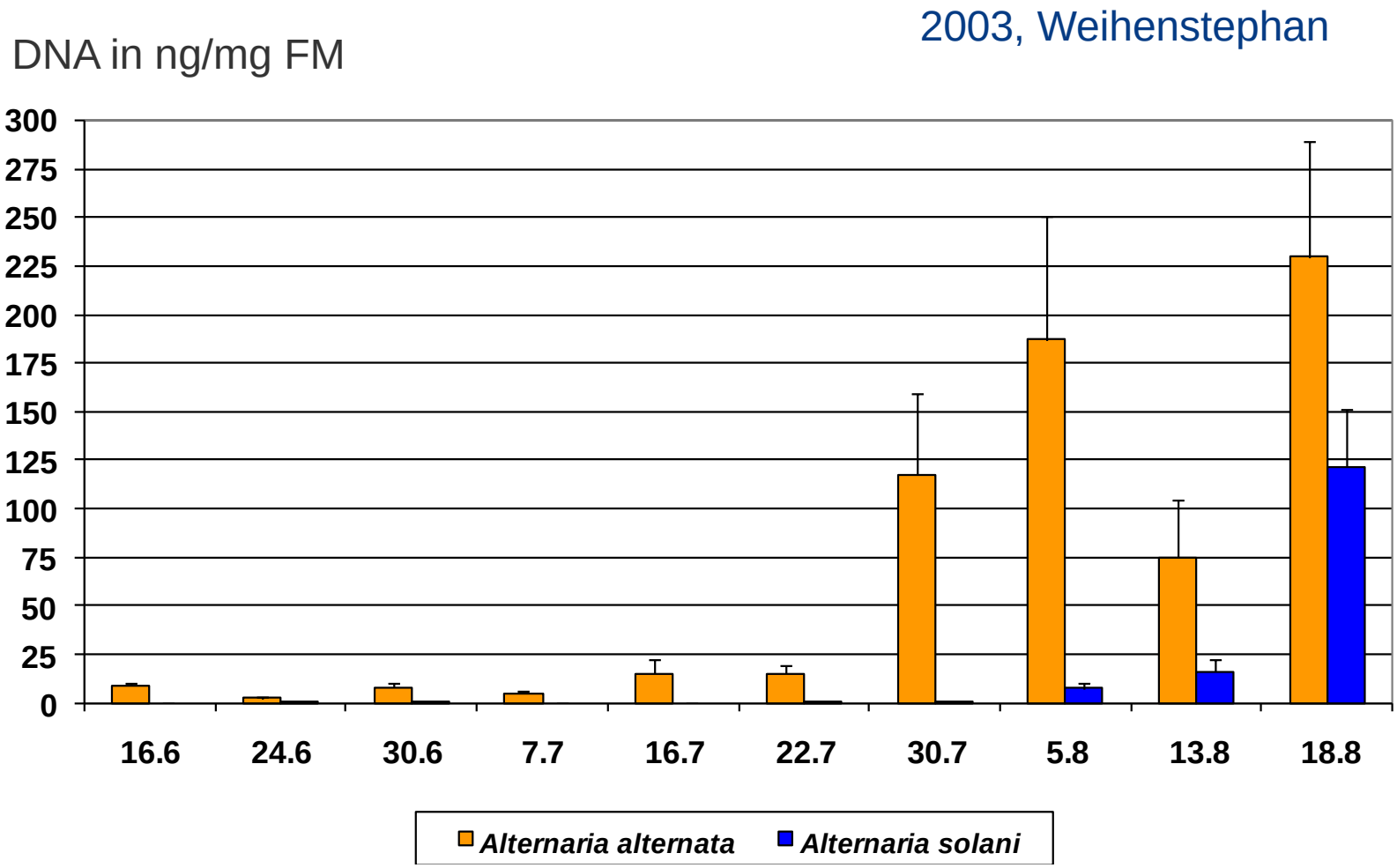
- No disease
- Low disease
- Moderate disease
- High disease



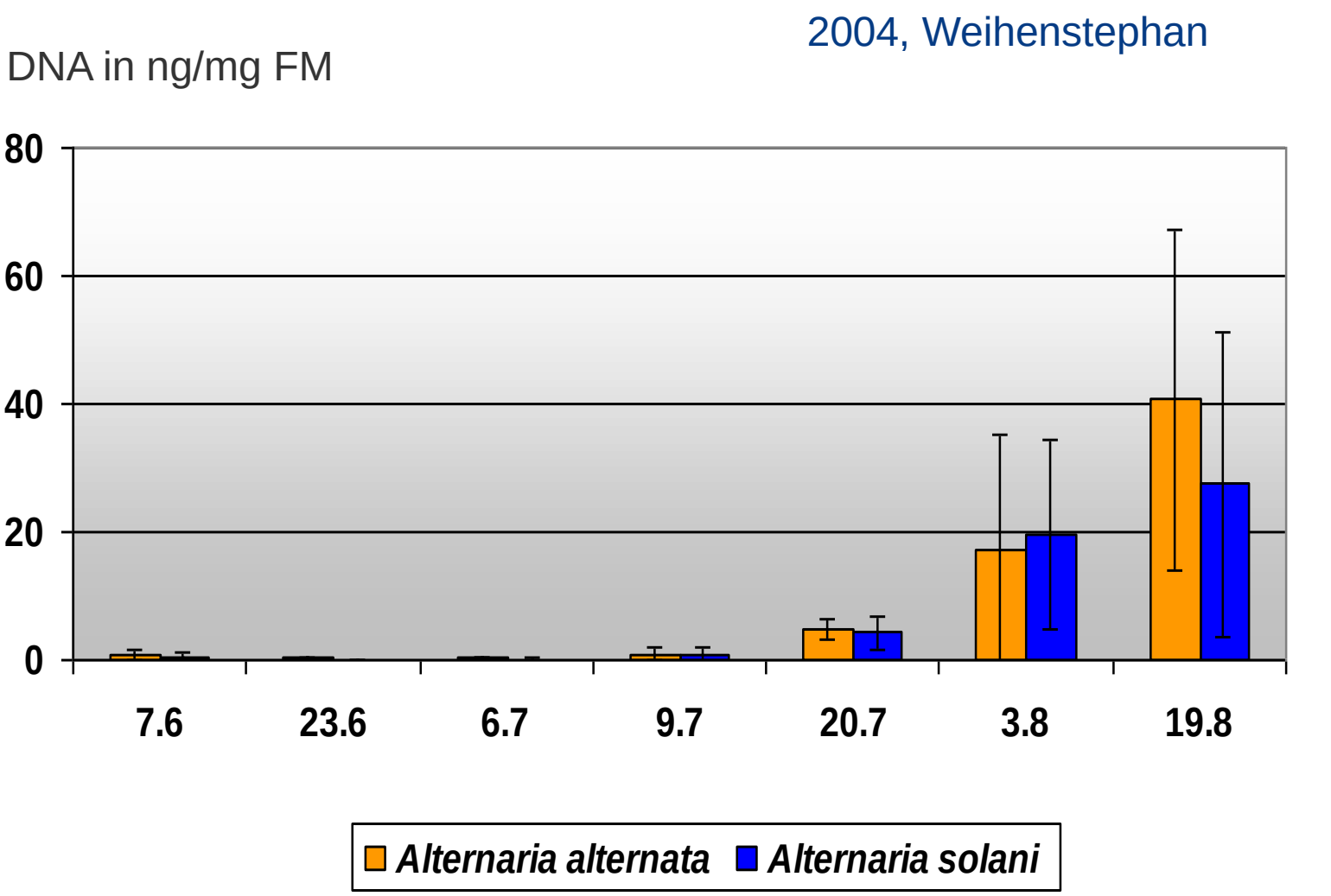
Early blight diagnosis 2009

sampling	location	A.a	A.s.
09. Jun	Gundhöring	✓	✓
14. Jun	Laberweinting	/	✓
13. Jun	Aiterhofen	✓	/
17. Jun	Worms	✓	/
12. Jul	Cappel-Cloppenburg	✓	/
14. Jul	Dessau	✓	✓
19. Jul	Salching	✓	✓
21. Aug	Meppen	✓	✓
21. Aug	Meppen	✓	✓
21. Aug	Rupenest	✓	✓
21. Aug	Niewe-Schanz	✓	✓
25. Aug	Lichtenau	✓	✓
05. Sep	Pönning	✓	✓
05. Sep	Landshut	✓	✓
06. Sep	Aiterhofen	✓	✓
09. Sep	Pulling	✓	✓

Quantitative analysis of early blight by qPCR, 2003

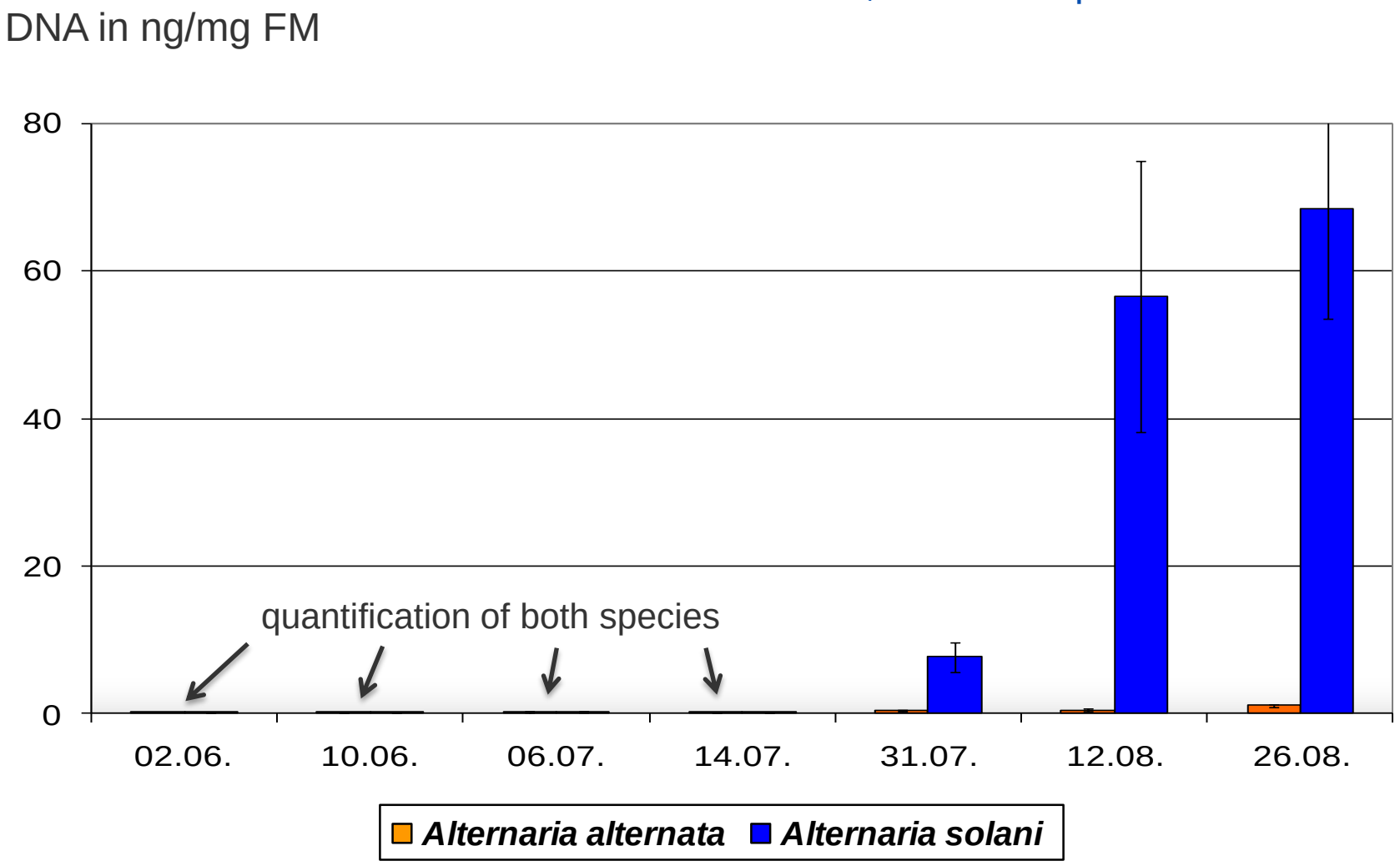


Quantitative analysis of early blight by qPCR, 2004



Quantitative analysis of early blight by qPCR, 2005

2005 , Weihenstephan



Early blight epidemics and differentitation

- Heavy early blight infections and epidemics throughout the past years
- Better insight into pathogen distribution through quantitative analysis (qPCR)
- Years with either more *A. solani* or *A. alternata*
- Earlier occurrence of *A. alternata* in 2003, detection of *A. solani* not before August
- Equal infestation by *A.solani* and *A. alternata* in 2004
- more severe and earlier appearance of *A. solani* in 2005

Investigation of population structure

The differential success of a population or species over time is proportional to its genetic variation = genetic diversity

- better insight into early blight distribution and population structure
- investigation of *A. solani* population in the field
- better understanding of disease progression and improvement of disease control

Random Amplified Polymorphic DNA (RAPD) analysis as a tool to study genetic diversity

- the RAPD technique uses a single arbitrary 10mer as a primer for PCR to amplify genomic DNA fragments. No sequence information is required
- The primer anneals to the template DNA at several positions, which generates a specific DNA profile that can be used for genotype differentiation
- different individuals gives different PCR products,

primer: **OPD-1: 5-ACC GCG AAC C-3**

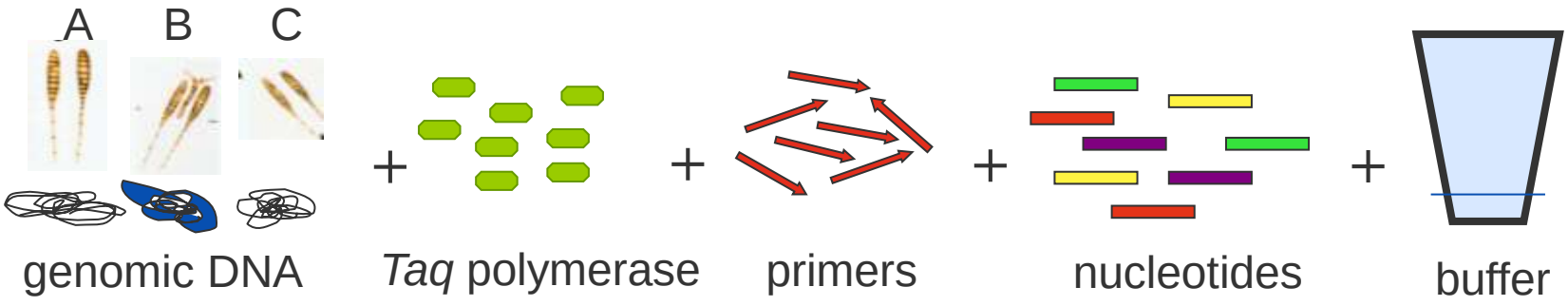
5-**ACC GCG AAC C**-3

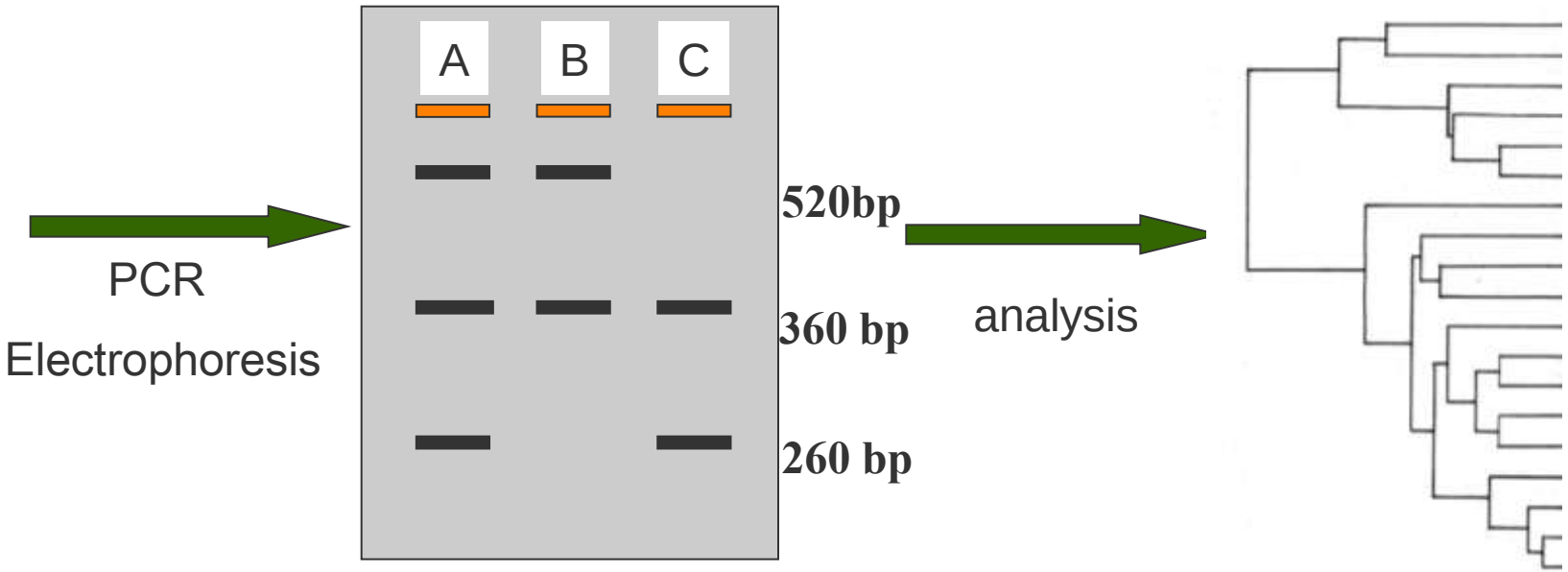
DNA A 3- TAC CTA TAA **TGG CGC TTG G**TA AGC GAT-5

5-**ACC** GC**G AAC C**-3

DNA B 3- TAC CTA TAA **TGG** TA**C TTG G**TA AGC GAT-5

Random Amplified Polymorphic DNA (RAPD)



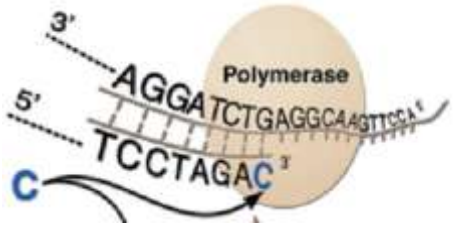


Random Amplified Polymorphic DNA (RAPD)

- isolation of 20 single spore isolates of *A. solani* (Weihenstephan, 2006, 2008)
- eight further isolates of *A. solani* from different German locations in 2008
- analysis with random primers (OP Operon Technologies, California)
  ~ test of 5 complete sets of OP random primers
- app. 120 different primers (OPA1-20, OPB 1-20, OPC-F...)
- 10 primers proved useful for differentiation of *Alternaria* genotypes

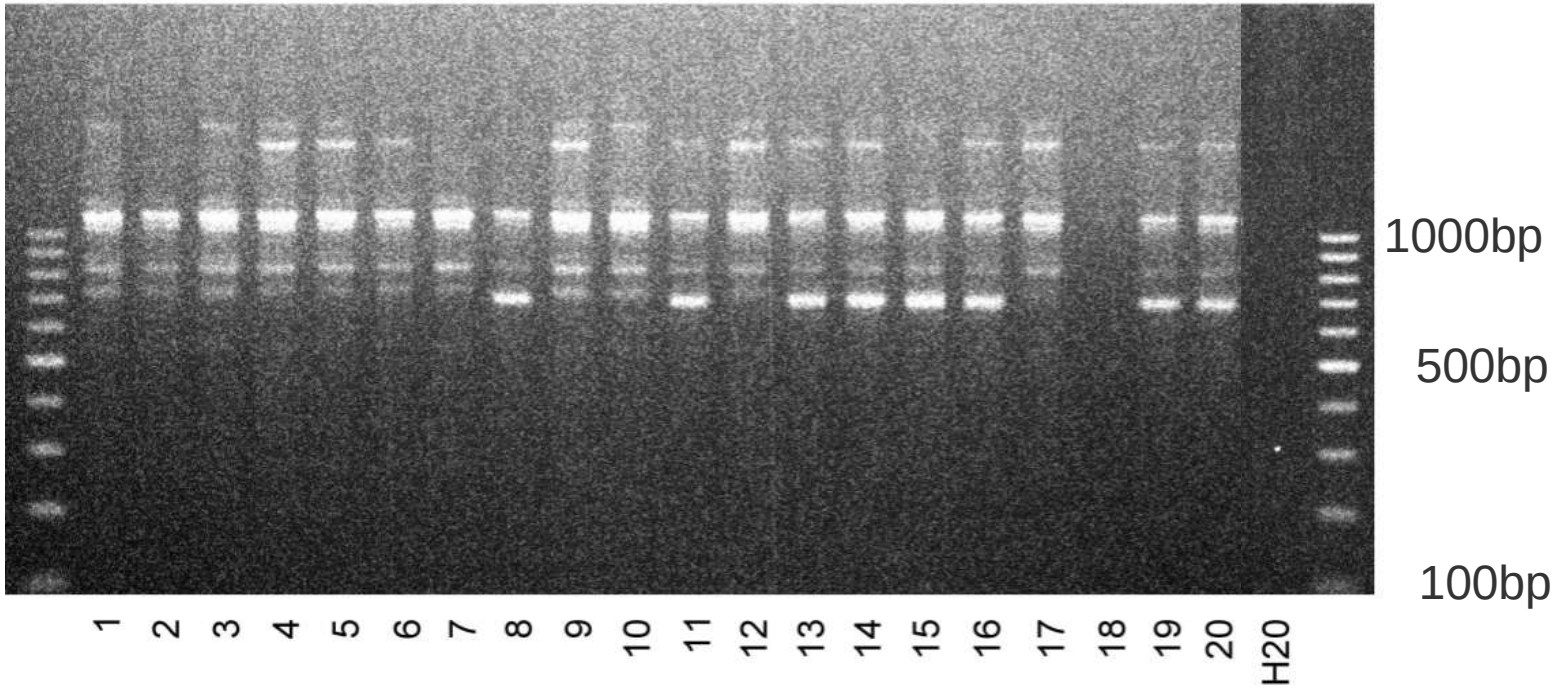
  OPC 5, OPC 6, OPC 9, OPC 10, OPC 11, OPC 14, OPC 19,

  OPD1, OPD2, OPD 11
- similarity matrix



Results

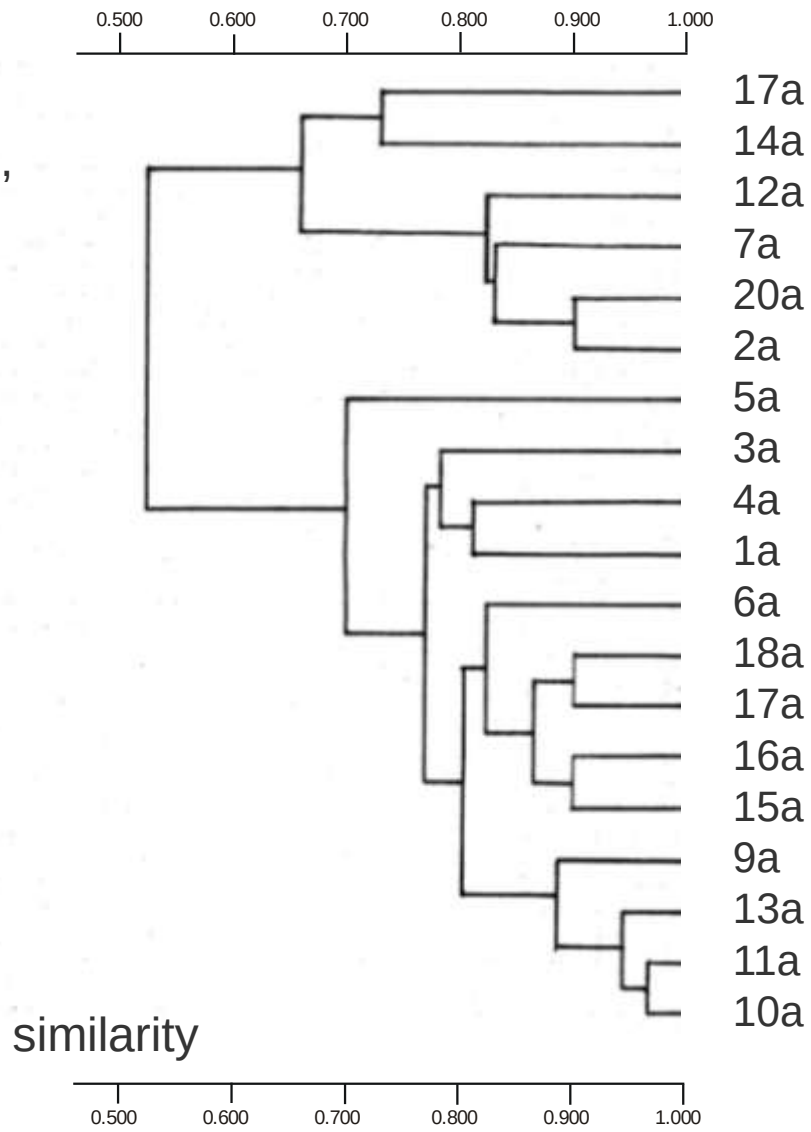
Weihenstephan, 2008, comparison of 20 isolates of *A. solani* with OPD-1



DNA of *Alternaria solani* isolates amplified with RAPD-primer OPD-1.
OPD-1: 5-ACCGCGAACC-3
Different band patterns are visible.

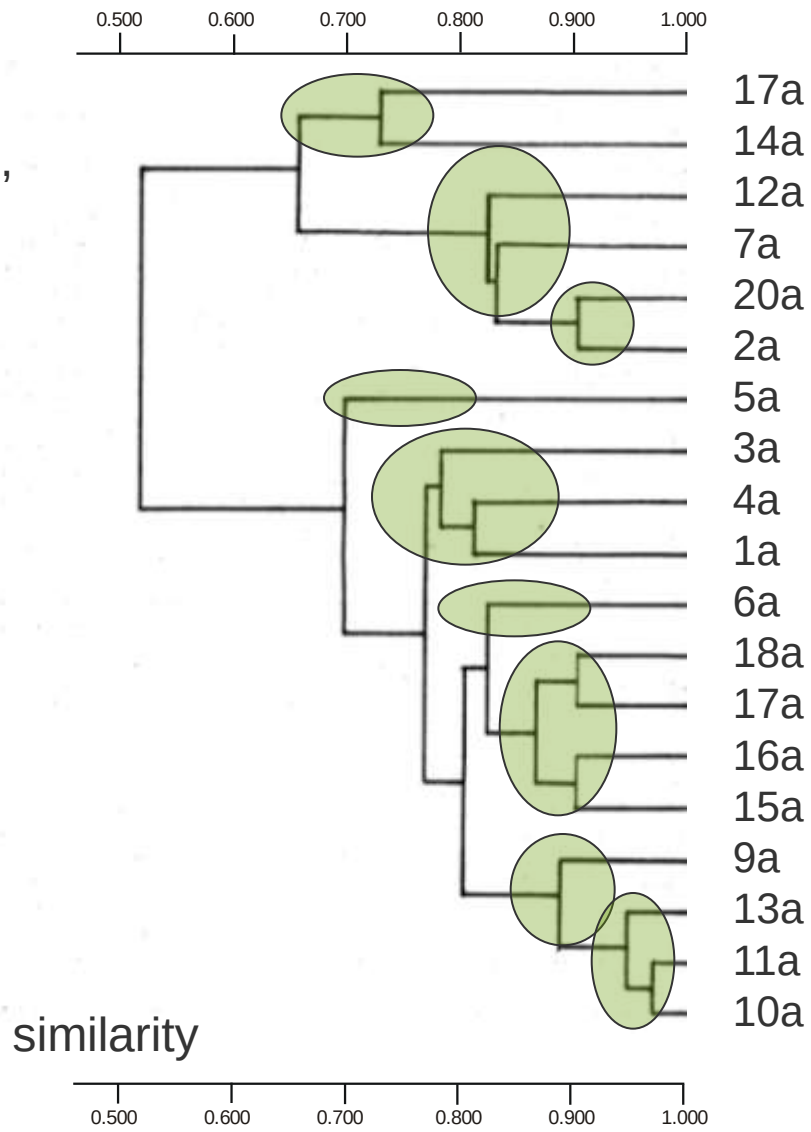
Results RAPD 2006

Weihenstephan, 2006

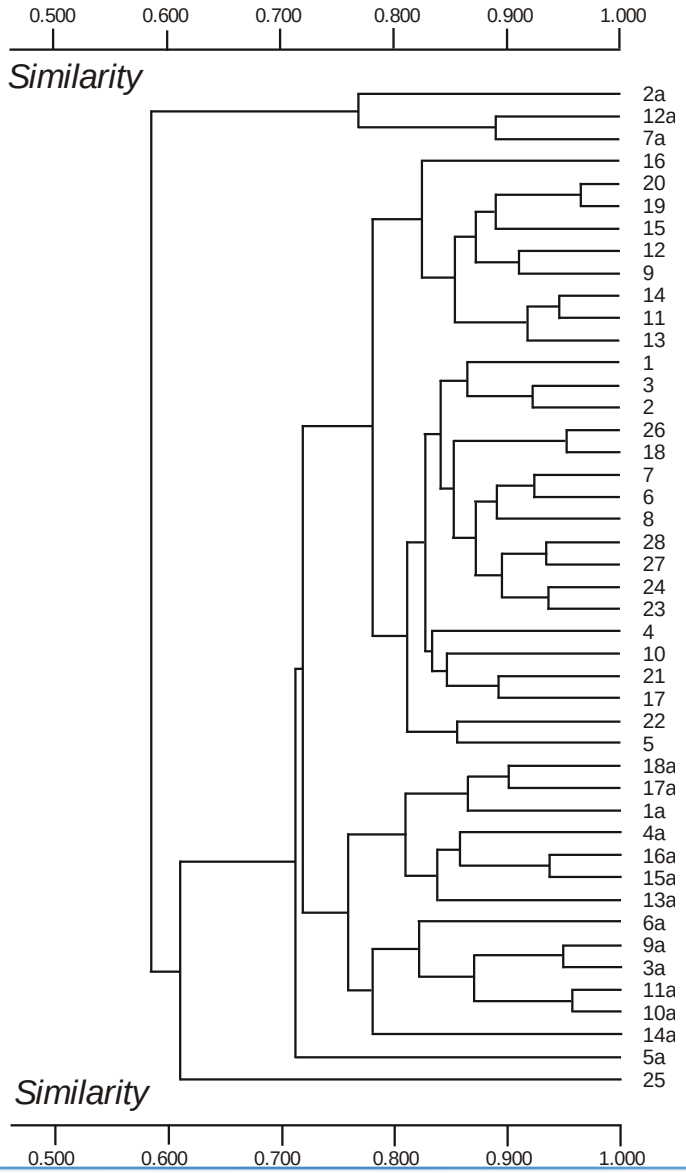


Results RAPD 2006

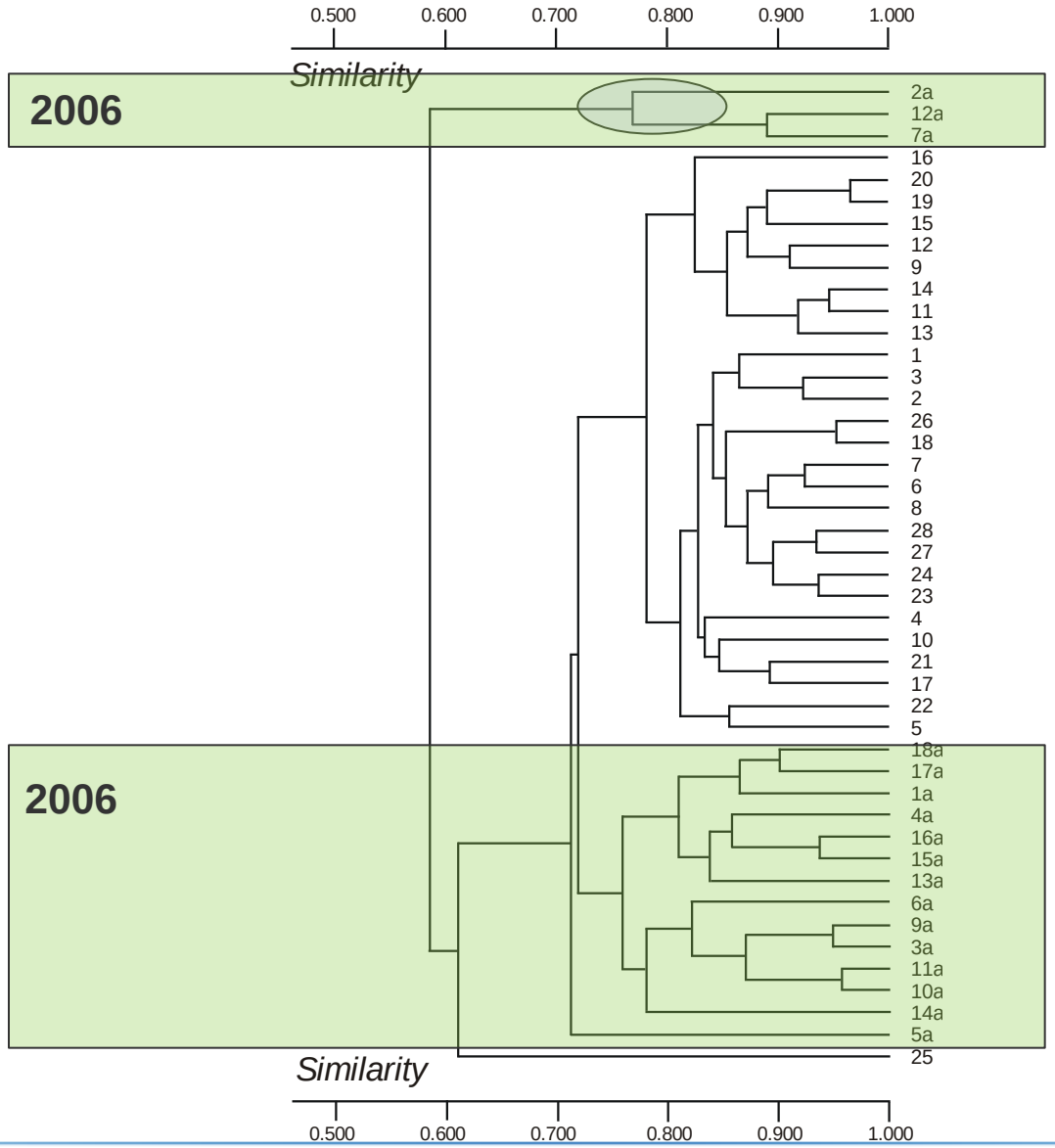
Weihenstephan, 2006



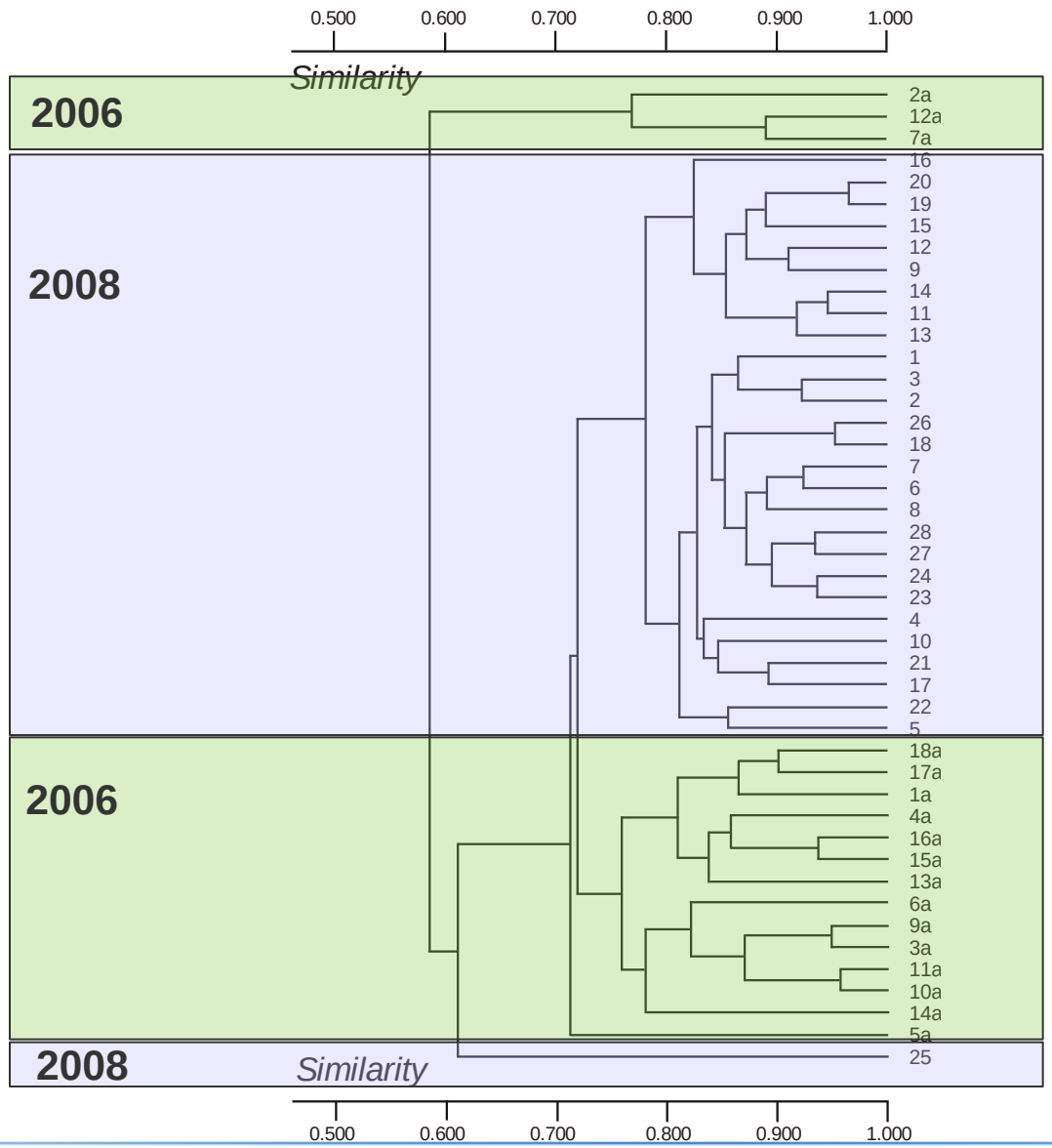
Results RAPD 2006 + 2008



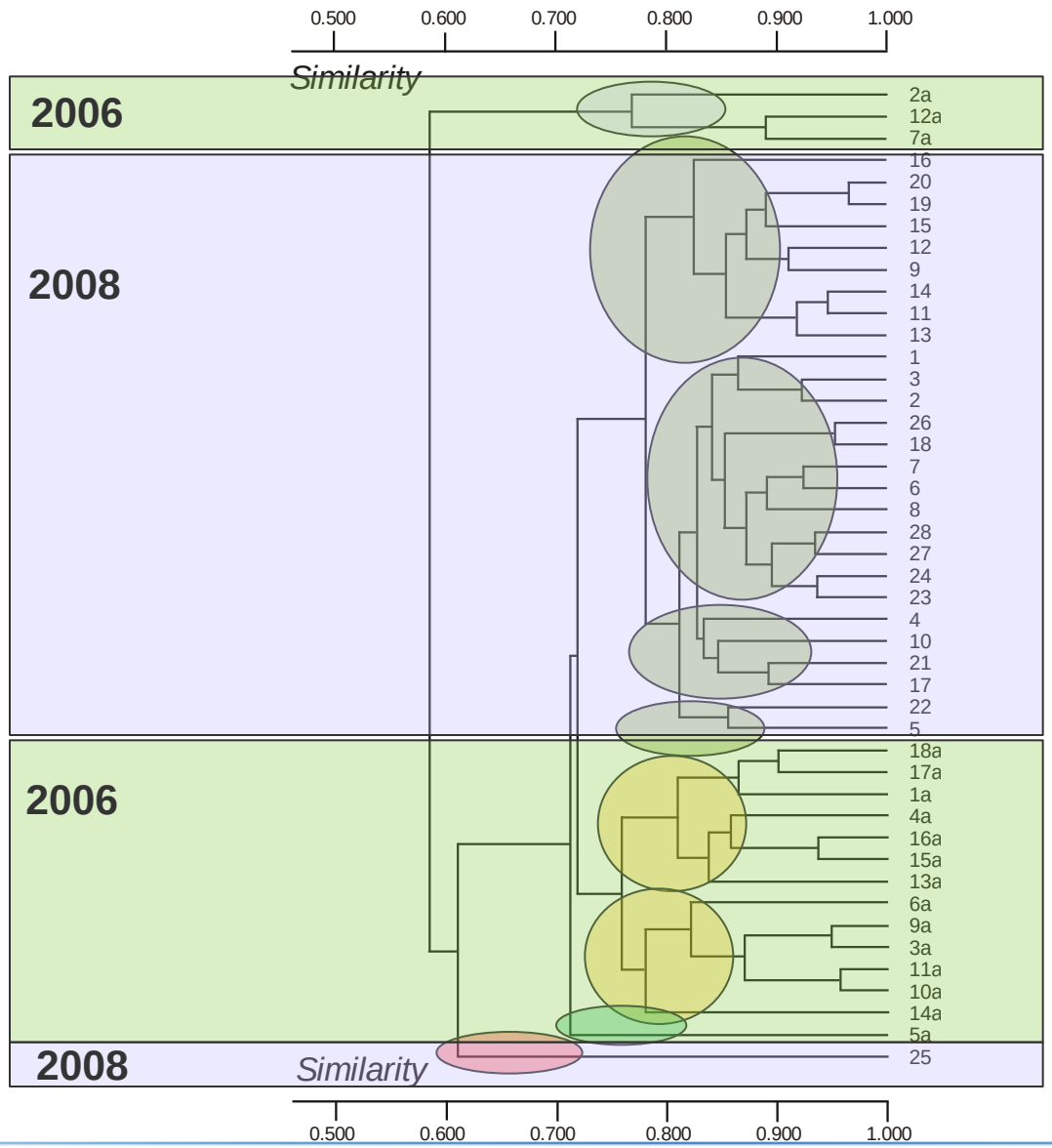
Results RAPD 2006 + 2008



Results RAPD 2006 + 2008



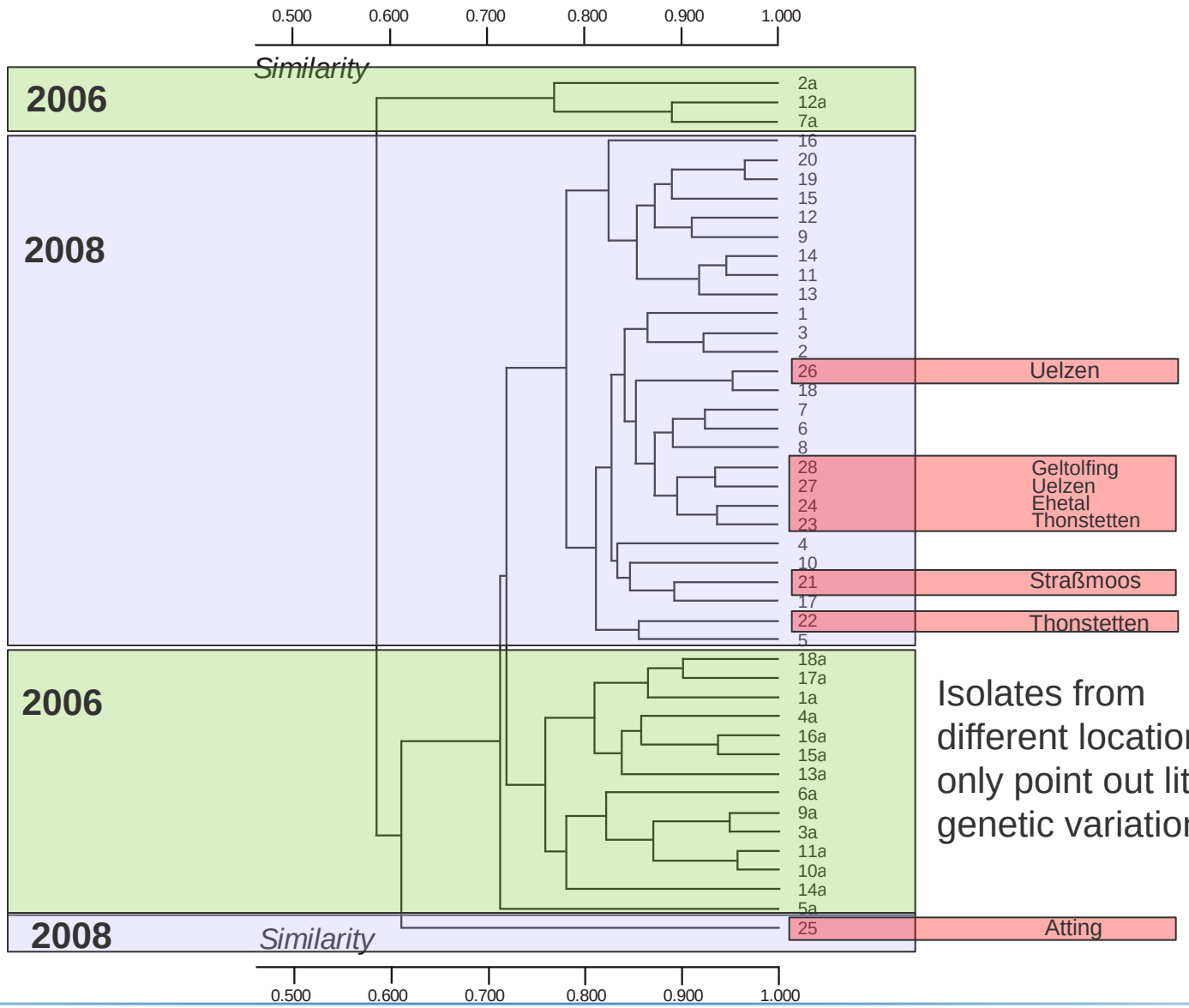
Results RAPD 2006 + 2008



Completely different clusters in 2006 and 2008

No clustering according to location

Results RAPD 2006 + 2008



Isolates from different locations only point out little genetic variation

Genome variability as selective advantage

- high levels of heterogeneity among isolates in different years
- significant differences among isolates of one particular geographic origin
- Various geographical subpopulations are not genetically isolated
- *A. solani* is a highly variable pathogen
- Diversity due to natural mutation or large population size (McDonald & Linde, 2002)
- Mutation is the driving force facilitating strain evolution, virulence factor variation (Lourenco et al., 2009)
- Causal agent of early blight disease is able to adapt to changing environments or agricultural practices which can vary from year to year

"Wild species must have available a pool of genetic diversity if they are to survive environmental pressures ….. If this is not the case, extinction would appear inevitable."

(Otto Frankel 1983)

Practical consequences on early blight epidemic

- high ability to adapt to changing environment facilitates build up of new populations, even within a small area
- preventive application of fungicide at an early disease stage (first application 7-8 weeks after crop emerge)
- monitor fields – note disease incidence & severity
- initial spray applications should not be carried out at disease progression (10-15% disease severity)
- prevent early blight by the use of few applications
- use full dose rates
- high genetic diversity supports selection of more virulent or fungicide resistant genotypes
- present potent fungicides (Strobilurines) with high resistance risk
- loss of sensitivity against QoI Inhibitors (F129L) Strobilurine within few years (USA)
- use combinations of single-site & multi-site fungicides

Thank you for your attention!