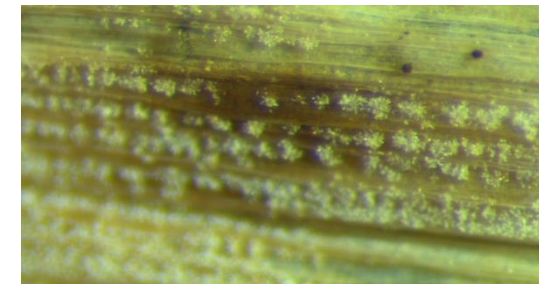
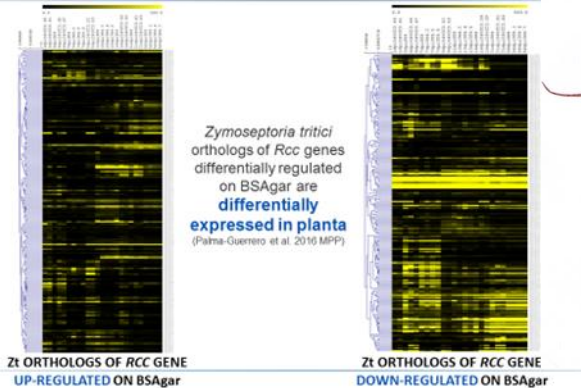


A population genetic approach gives new insights into the epidemiology and control of *Ramularia collo-cygni*

Michael Hess
m.hess@tum.de

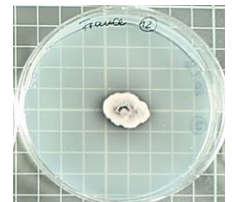
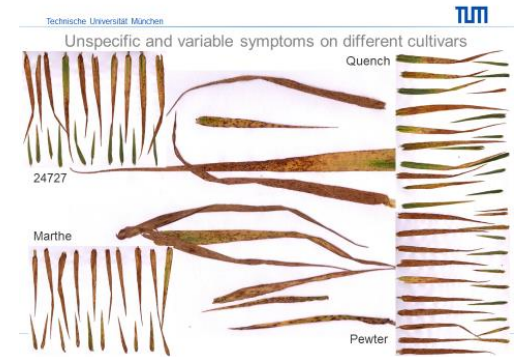


rubellin A B C D Dehydro-D E

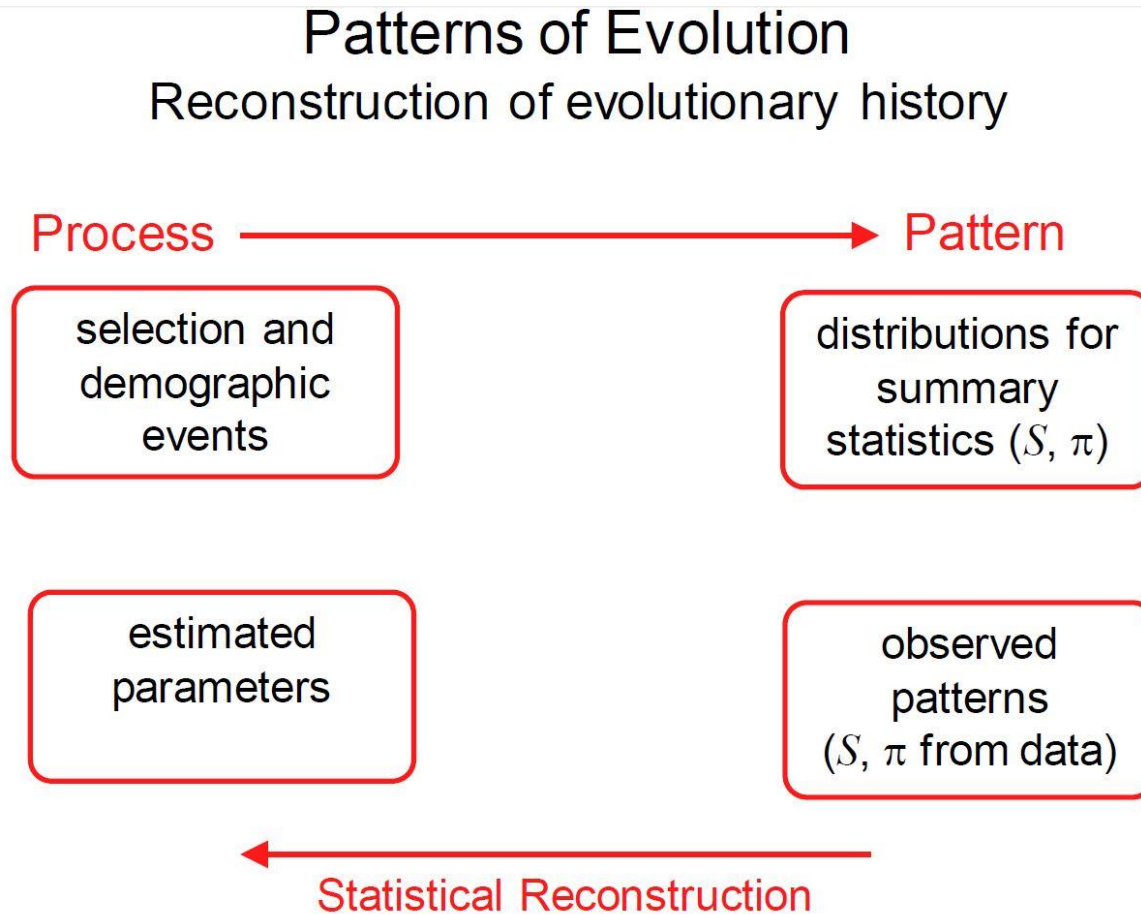


Limitations and open questions

- Unspecific symptoms
- High latency
- Quick adaptation to fungicides or resistance genes
 - Multiple resistances described, high risk FRAC
 - No sufficient host resistance
- **Limited knowledge** on infection biology, variable life style
 - Seed transfer versus airborne spores
 - Endophytic versus pathogenic
 - Host specificity
 - Sexual reproduction
- **Limited accessibility** with traditional methods (growth in vitro, spore production,...)
- **New approach** for understanding pathogen biology:
 - Sequencing (reference+18 isolates) and population genetic study



Evolutionary pattern (Aurélien Tellier)



Based on polymorphism data, we can find out the history of a species! And even know when this happens.

NGS - Whole genome and RNA sequencing

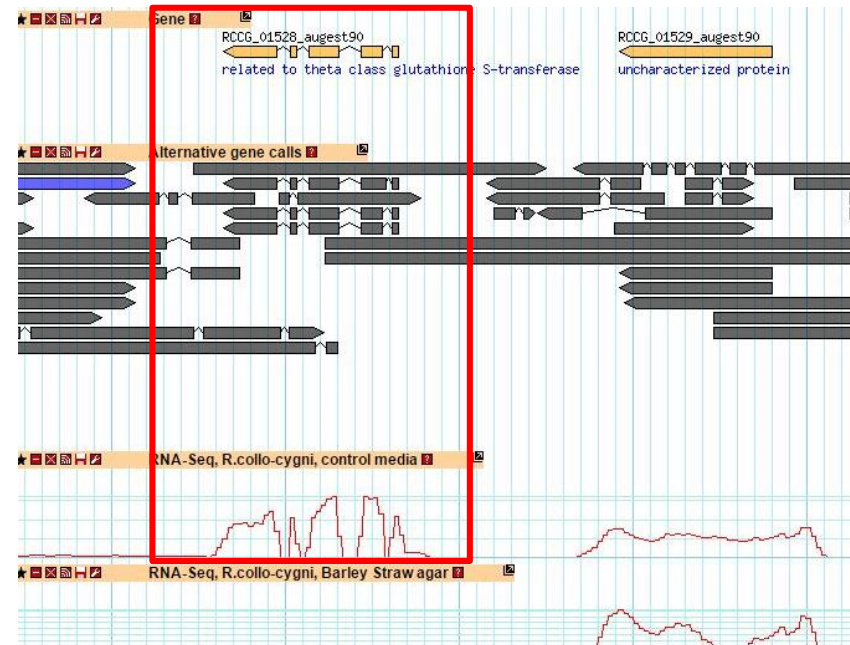
Genome annotation

Using different gene predictors
(Genmark, Fgenesh, Augustus)

Rcc RNA-seq of samples from 6 different conditions

- Control Media
- Old Mycelium
- No Glycerol media
- Barley Straw Agar
- pH 5
- pH 9

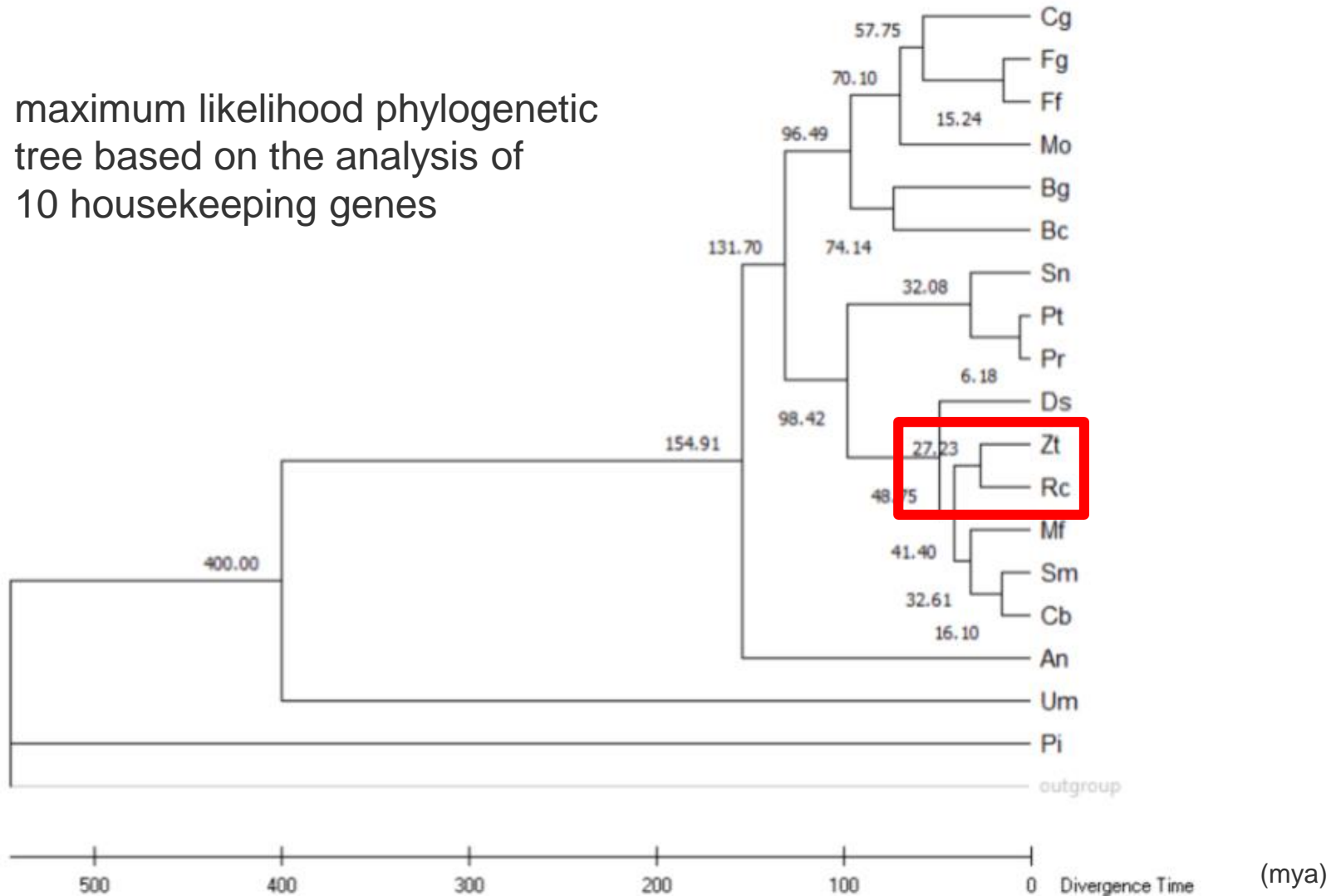
Mapped to the genome
using TopHat to help the
annotation



**Manual curation for
high quality annotation**

Split from *Zymoseptoria tritici* 27 million years ago (mya)

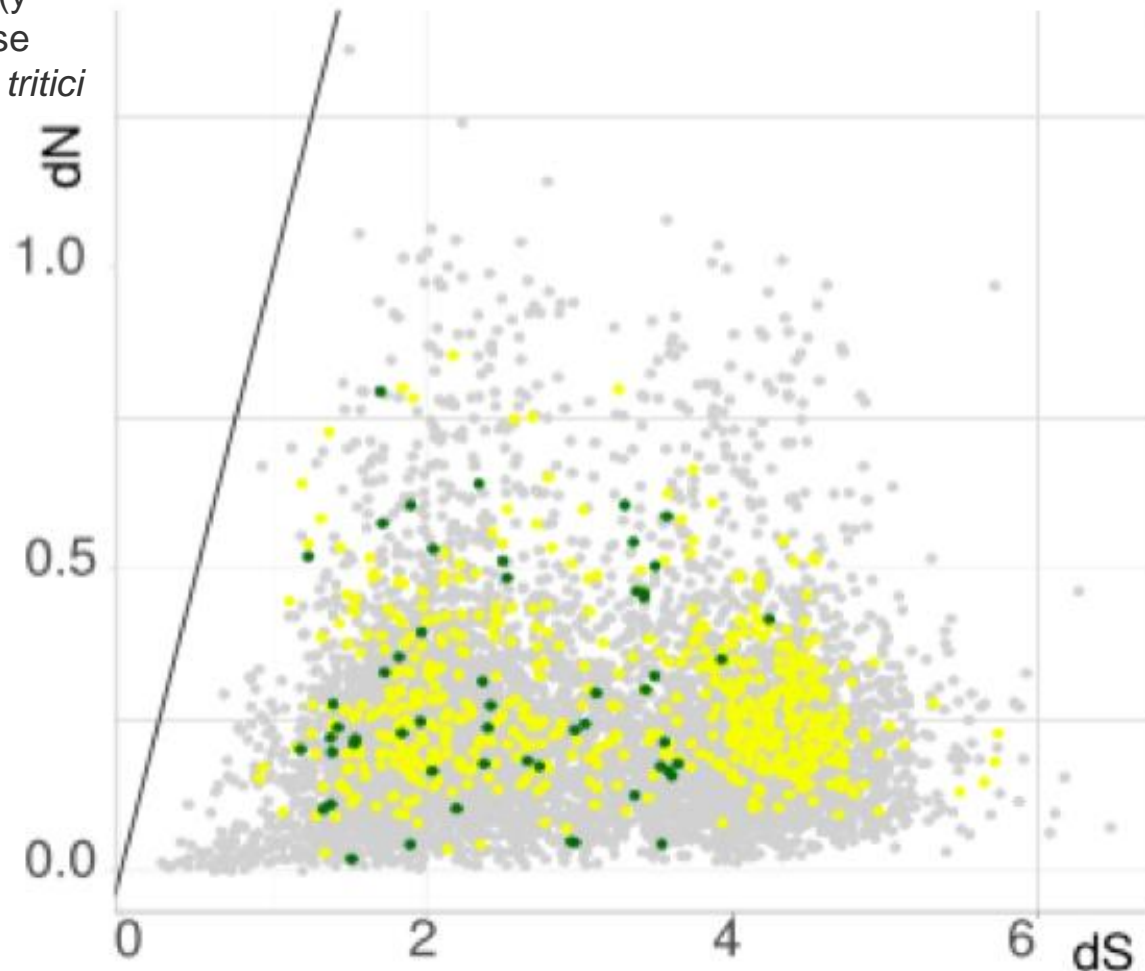
maximum likelihood phylogenetic tree based on the analysis of 10 housekeeping genes



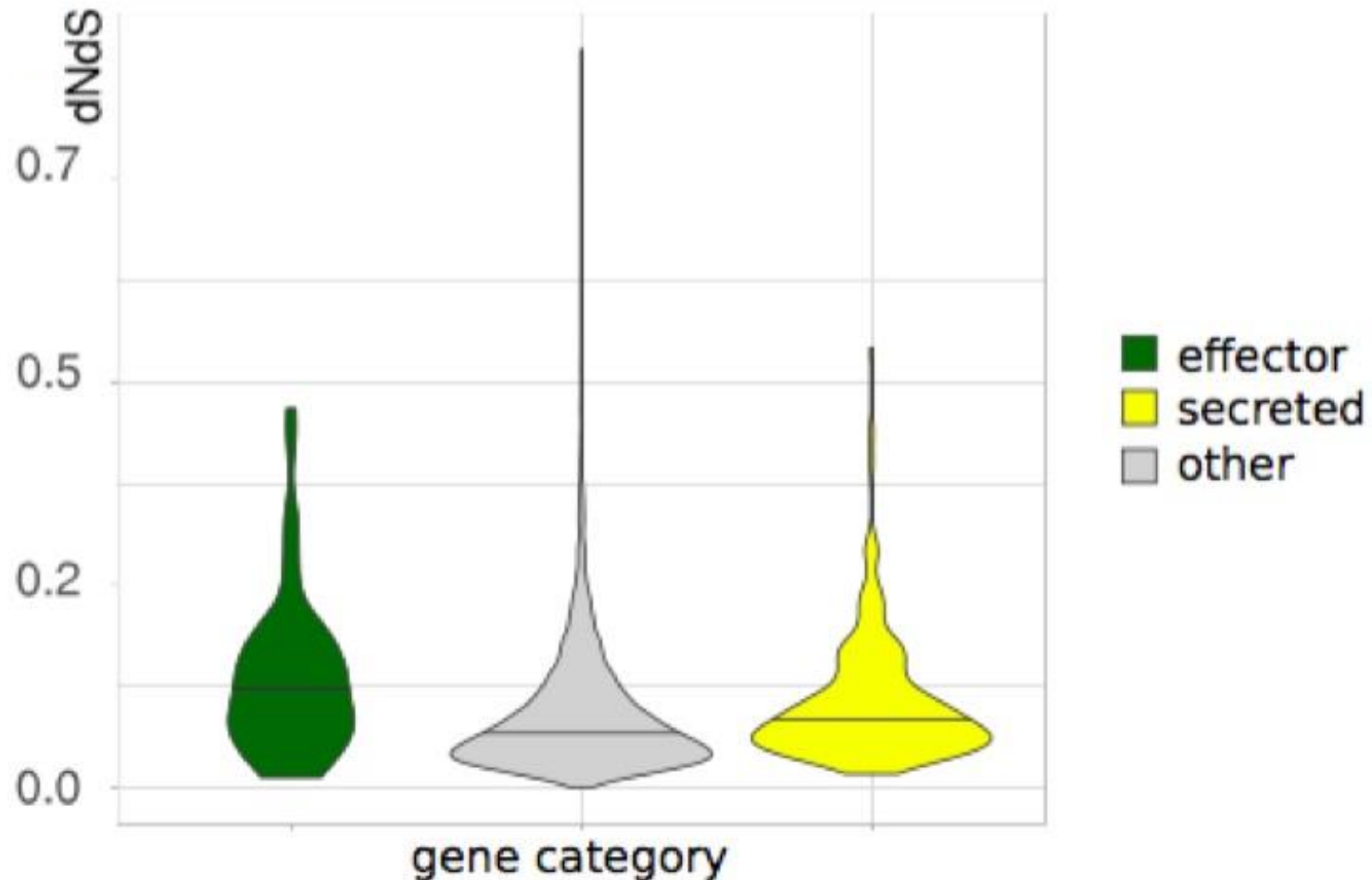
Very high divergence of *Z. tritici* und *R. collo-cygni* on the sequence level

Scatter plots of the dS (x axis) against dN (y axis) for each predicted protein in a pairwise comparison between *R. collo-cygni* and *Z. tritici*

- effector
- secreted
- other



No difference between effectors and other predicted proteins

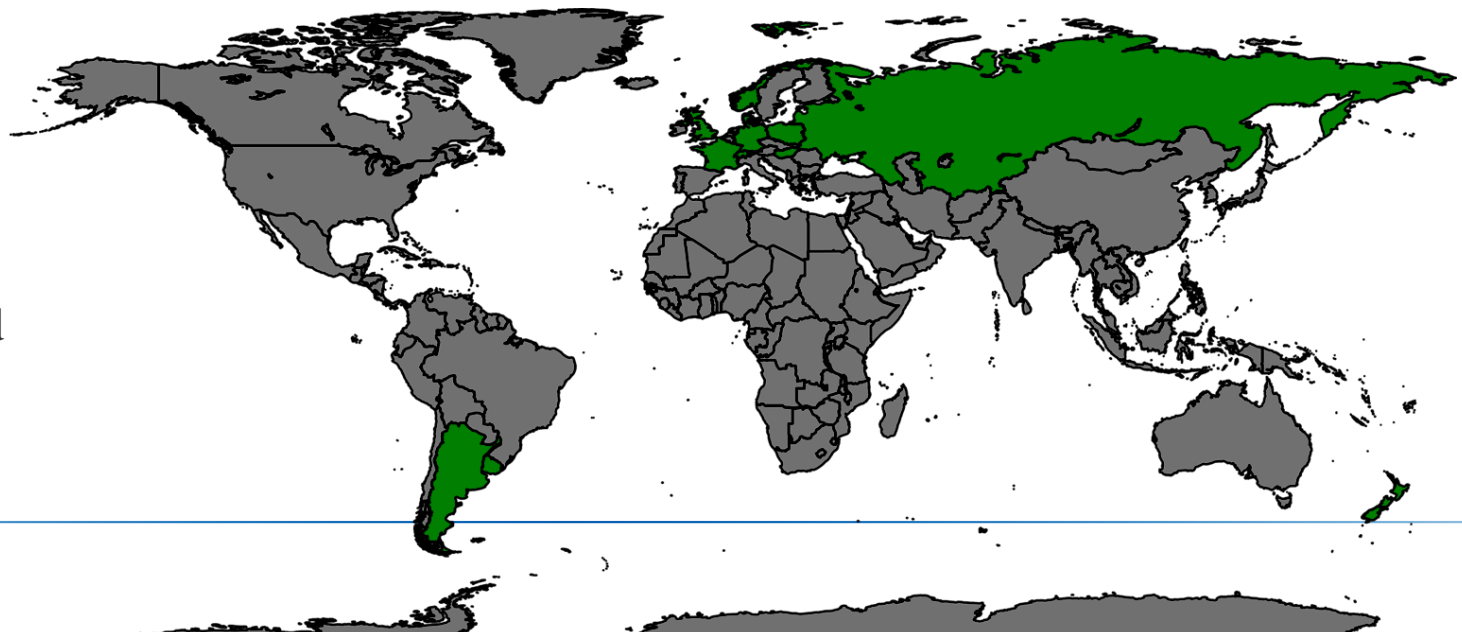


Population genetic study: Global strains & different hosts

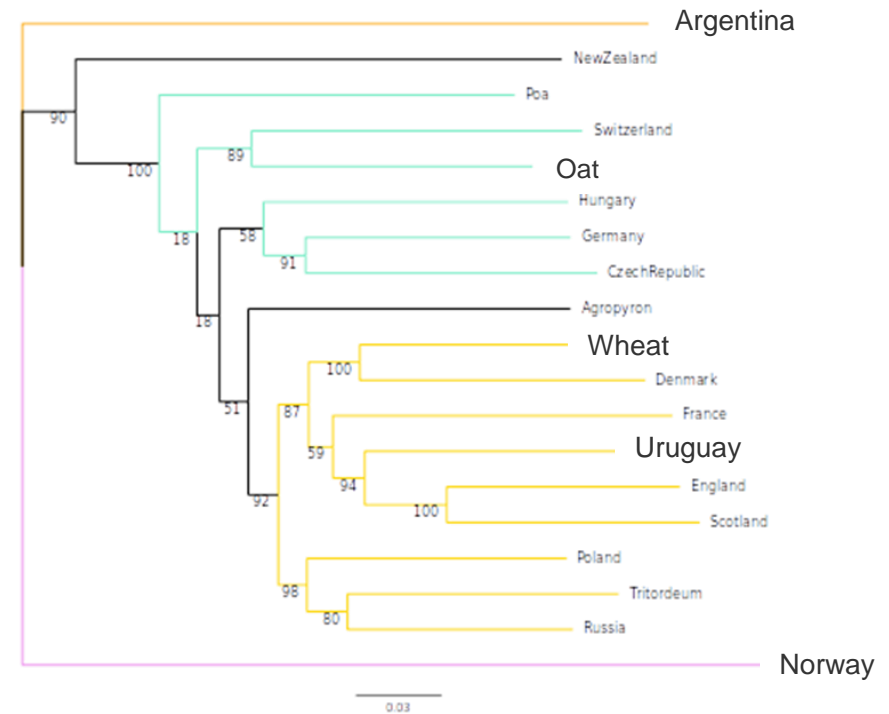
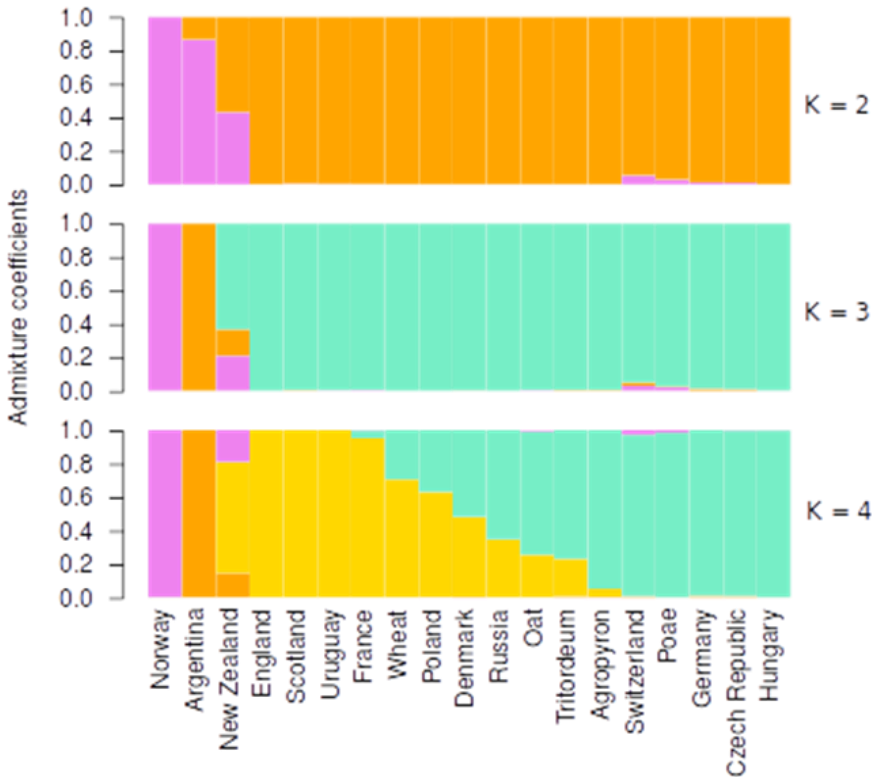
Norway	Oat
Denmark	Wheat
Scotland	Tritordeum
England	
	Agropyron
Russia	Poa
Poland	
Czechia	
Hungary	
Germany	
Switzerland	
France	
New Zealand	
Argentina	
Uruguay	



(source URL: <http://www.flickr.com/photos/fjbn/3721596727/>)

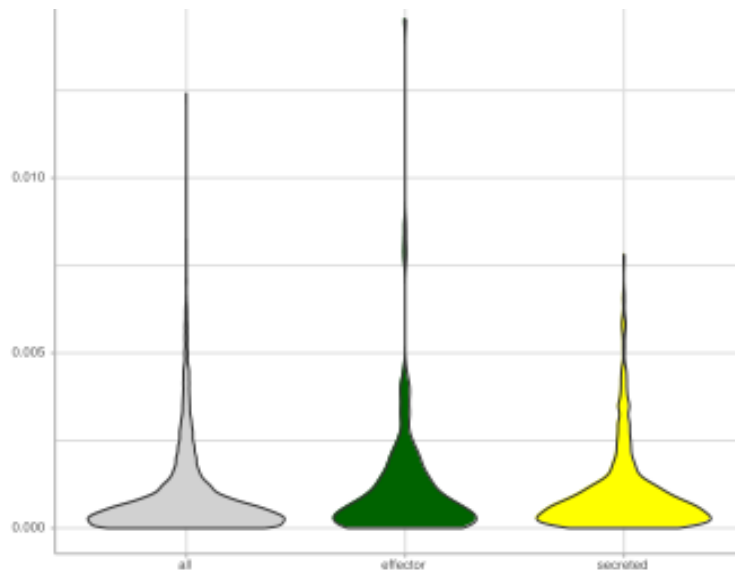


Population genetic study of the 19 sequenced isolates of *R. collo-cygni*

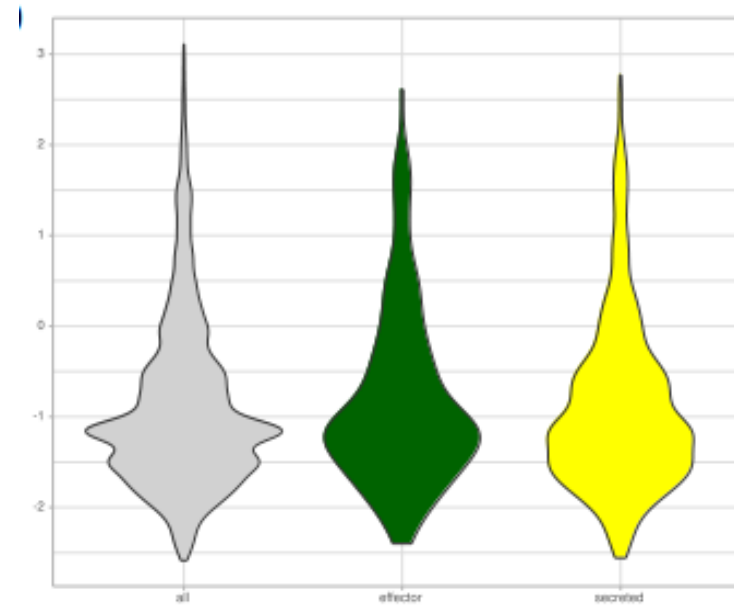


One group with 2 outliers (NO, AR), no host association, no geographic cluster

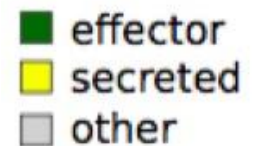
No accelerated sequence evolution for effectors



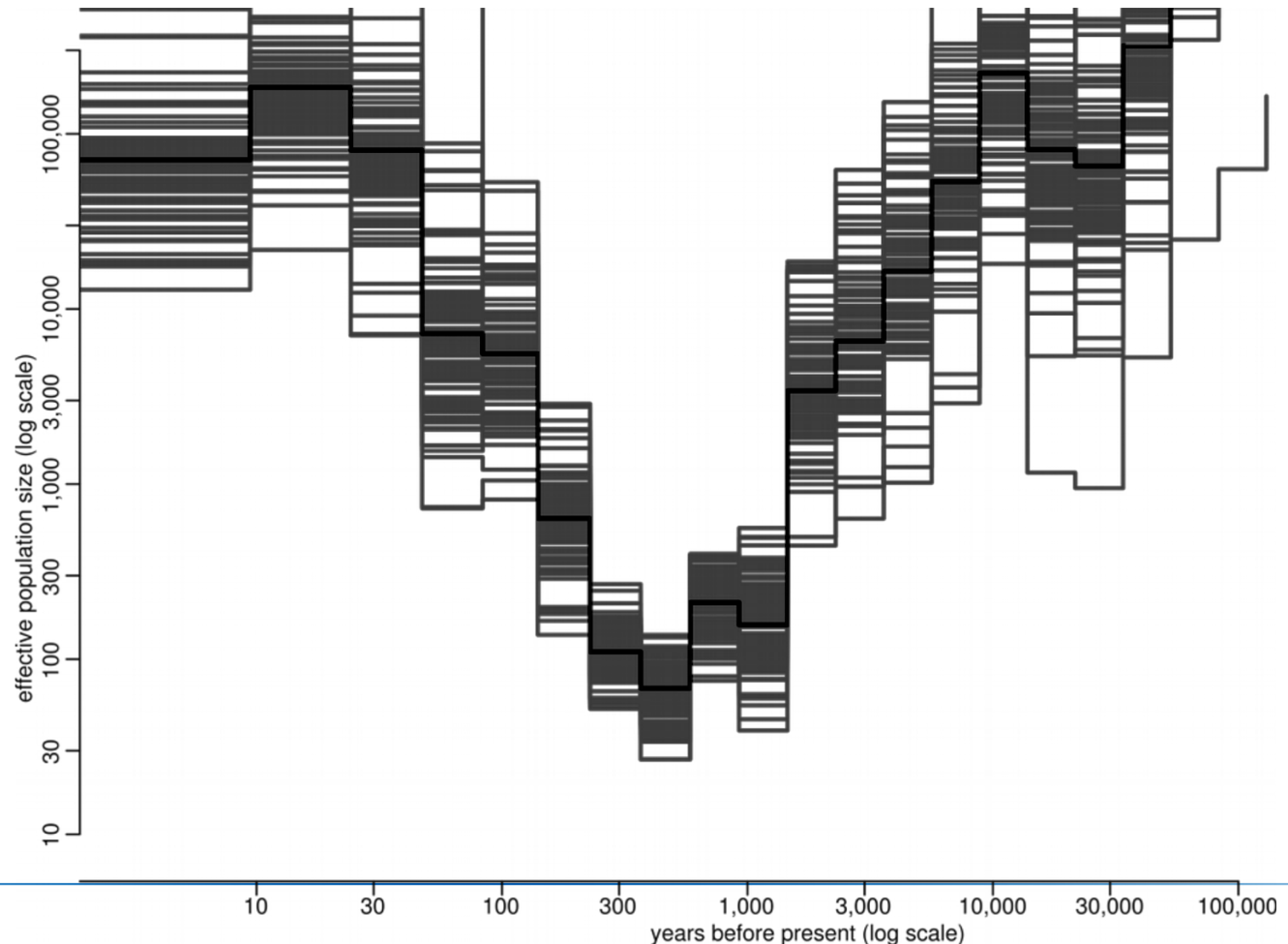
Nucleotide diversity (π)



Tajima's D



Recent expansion and historic large effective population size (Ne)



Overview of estimated historic population size back in time. Expected N_e was estimated by comparing observed summary statistics with 200.000 simulations (PopSizeABC; Boitard et al. 2016 PLOS Genetics)

Summary of genomic and populationgenetic study

- High quality reference genome
- Comparison with model species

Closely related to *Z tritici*, but split 27 mya and very high divergence on sequence level, no divergence for effectors

- Resequencing of 19 isolate from the global collection of *R collo-cygni*

No host association, no geographic cluster (**seed transfer**)

Recent expansion and historic **large effective population size** (**fungicide resitance**)

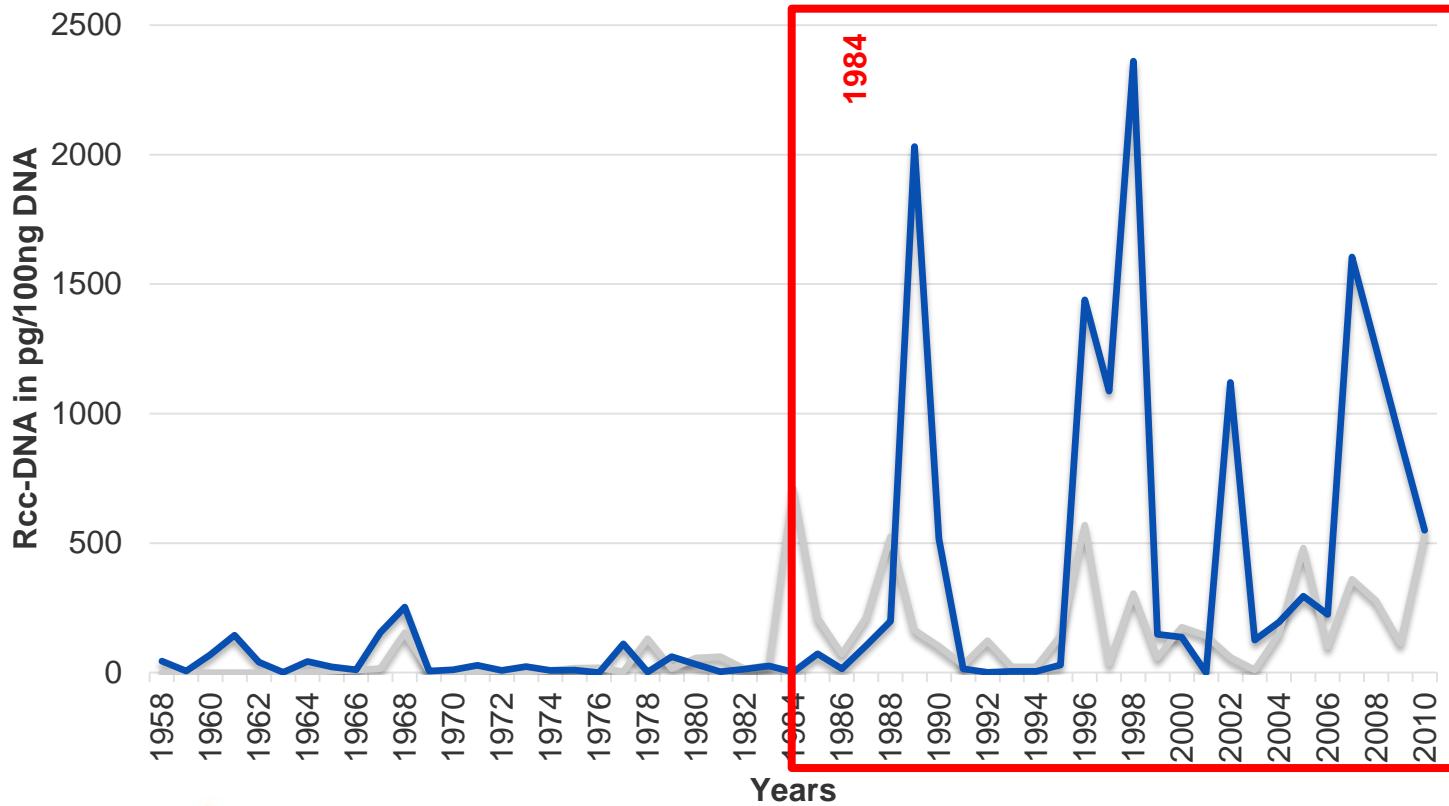
- **Outlook:** Further investigation of the reference genome showed unlike many other plant pathogens no evidence for higher selective pressure on putative effectors or other secreted proteins and repetitive sequences are spread evenly across the scaffolds (Remco Stam). We hypothesize that these findings could be associated to the predominantly endophytic life-style .



plant

environment (climate change)

Emerging pathogen: Analysis of archive samples



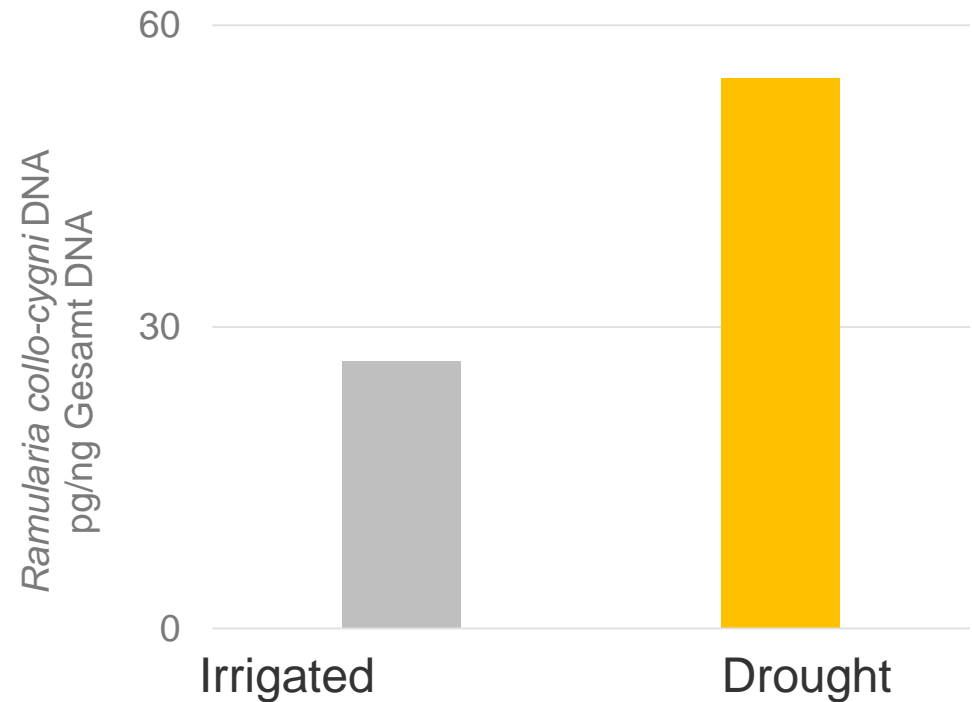
Drought stress experiments



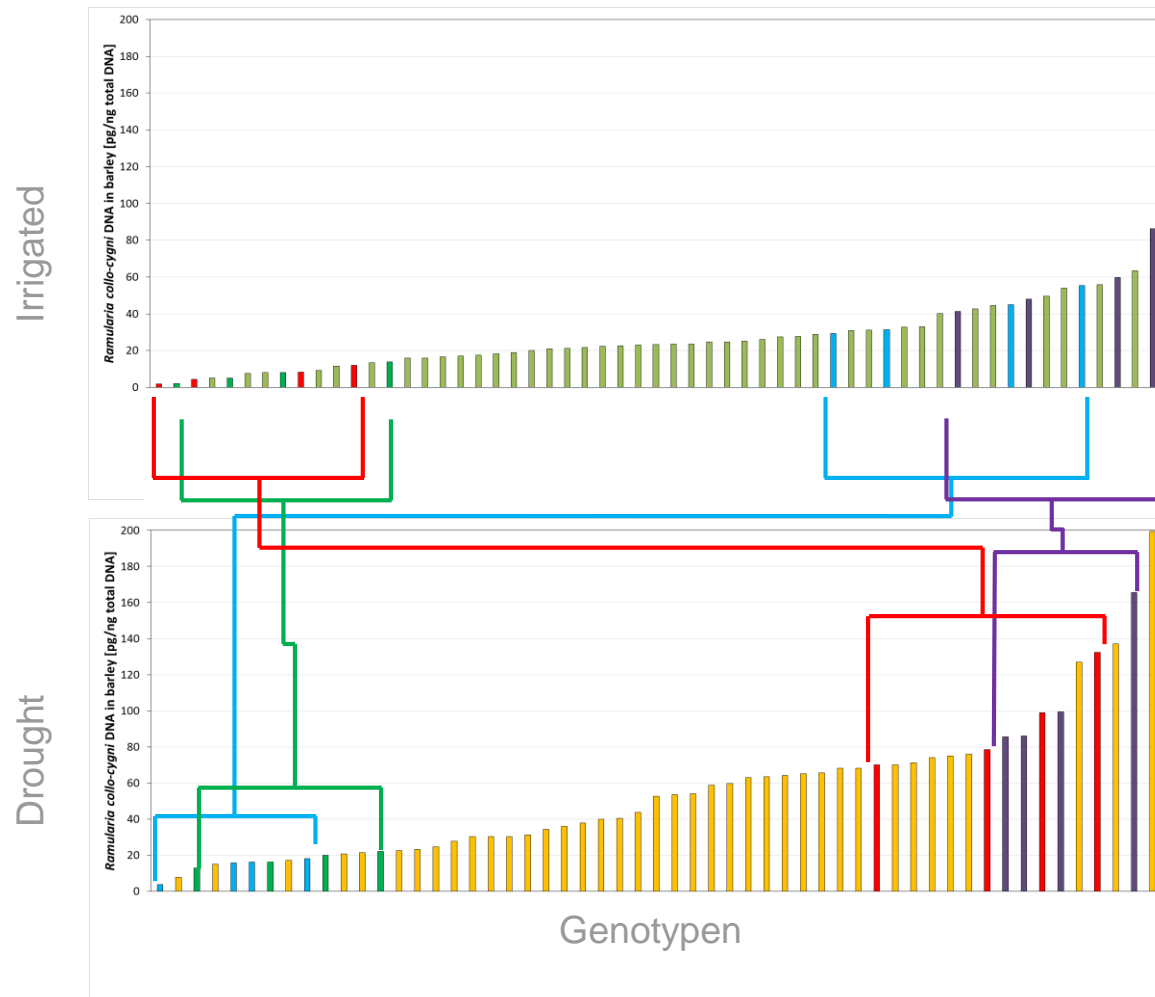
Disease under controlled drought stress in the rainout shelter of the Bavarian State
Research center

Effect of drought stress on *Ramularia collo-cygni* DNA

- Low disease with *Fusarium sp.* compared to field trials
- Similar level of *Ramularia collo-cygni*
- Higher DNA of *Ramularia collo-cygni* with drought stress, but lower symptoms



Pathophenotyping: *Ramularia collo-cygni* under drought stress



Four phenotypic extremes:

- Constant (**high/low**) disease level, independent of drought stress (neutral)
- **Higher** or **lower** leaf incidence after drought stress (**synergism**, **antagonis**)

Summary, Conclusions

- New insights into pathogen biology
- No geographic cluster, recent expansion and large effective population size
- Impact of environment

Ramularia collo-cygni remains a great challenge

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