Legume
Generation

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WP6.3 Self-incompatibility in White Clover

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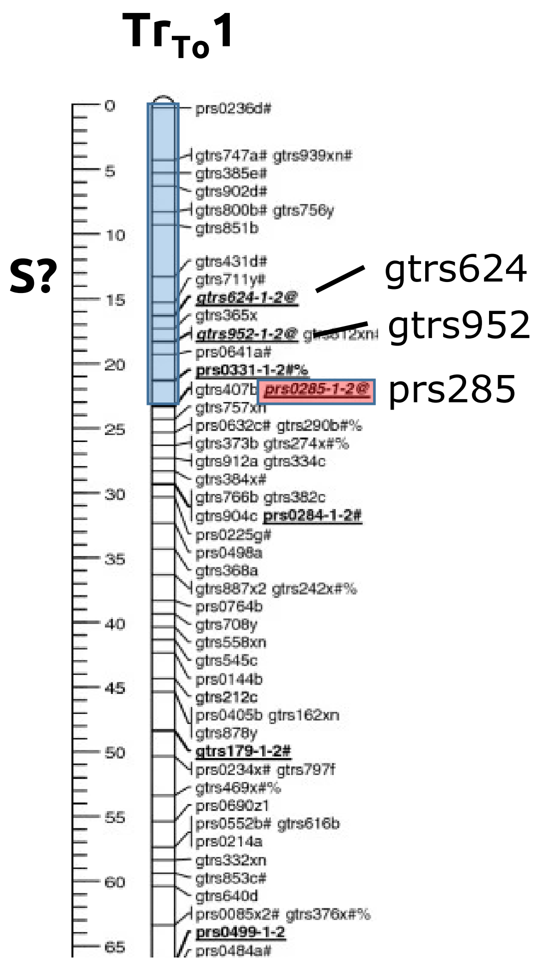
What we know about gemetophytic SI in white clover

- Mapped to a single locus[1]
- $Tr_{To}1$ (derived from *T. occidentale*) ≈ 12 Mbp region[2]
- Adjacent to a single-locus SSR (prs285)
- Potentially many alleles (74-139)
- Impacts seed number and segregation distortion
- Genotype S-alleles
  - Maintain heterozygosity in breeding pools
  - Semi-hybrid system to utilise heterosis

Understanding Self-Incompatibility – a powerful tool to harness heterosis



1-Casey et al 2010
2-Griffiths et al. 2013

Approach

Legume Generation

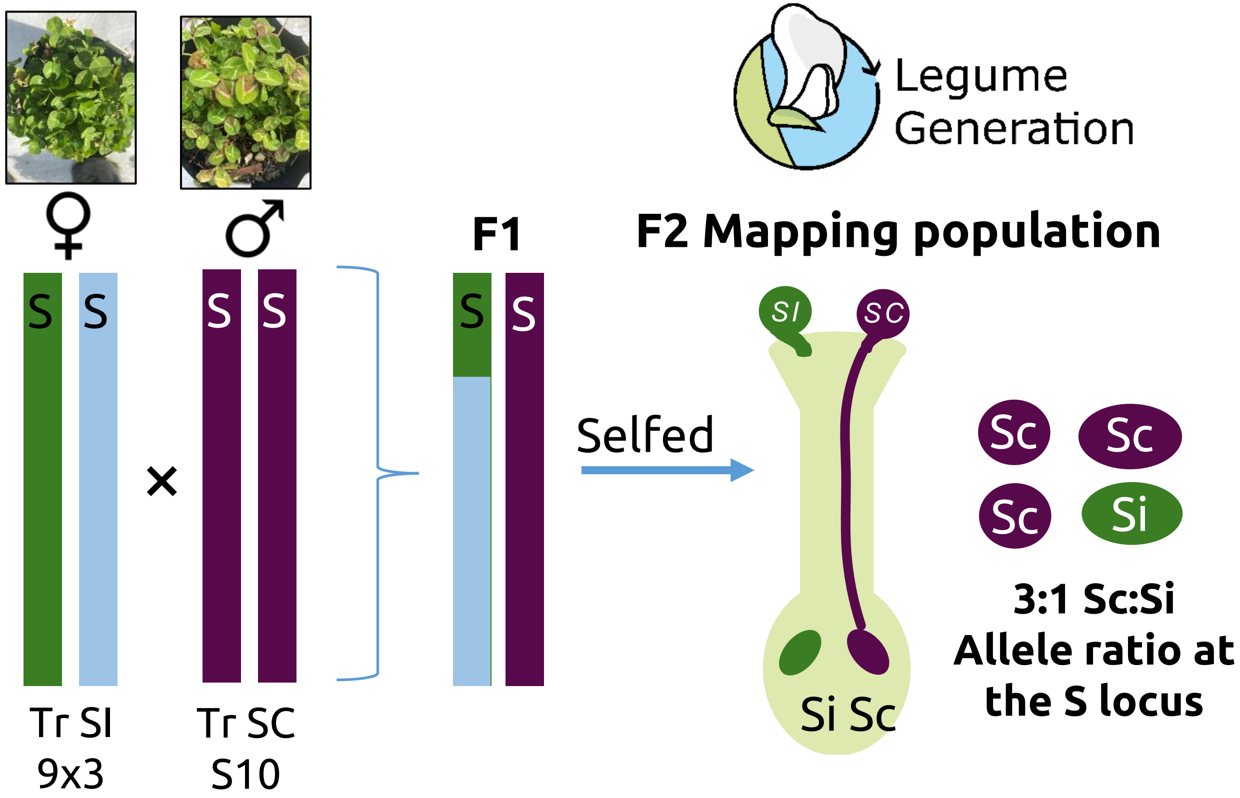
Fine map the SI locus

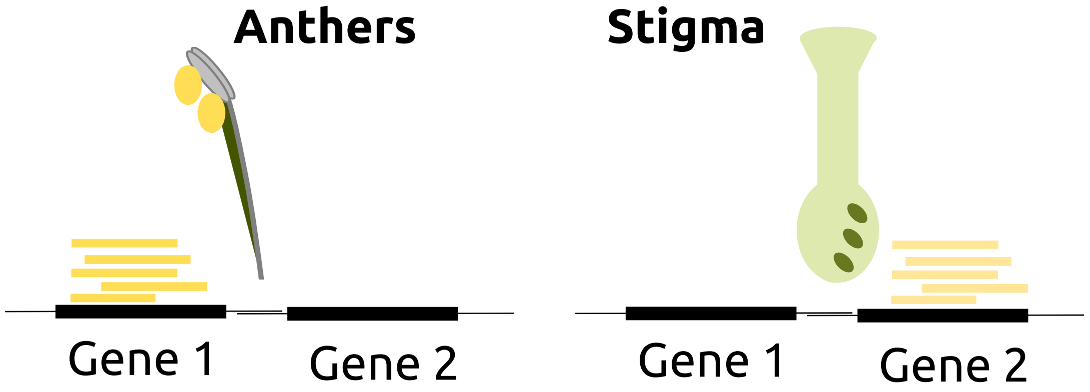
- Self-compatible (SC) plant (S10)
- Self-incompatible (SI) plant (9x3)
- Cross Sc plant onto SI plant
- Self F1
- Sequence populations of F2 (map allele bias)

Transcriptome analysis

- S incompatibility genes expressed in pollen and stigma
- RNA-seq from 700 florets
  - S10 (Sc), 9x3 (Si), Tribute
  - anthers, stigma/style, leaf
- Identified differentially expressed genes

They mapped to candidate SI locus

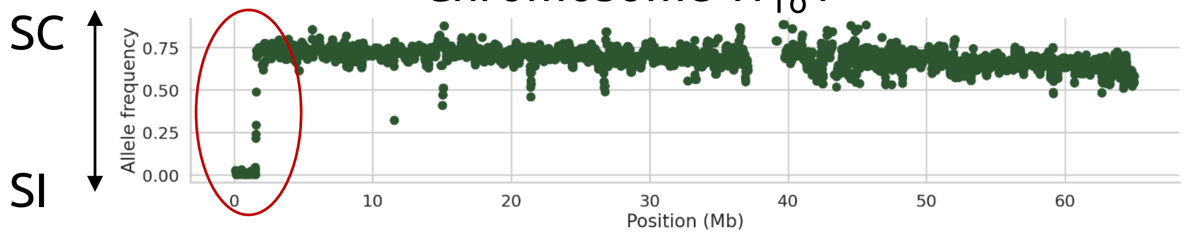




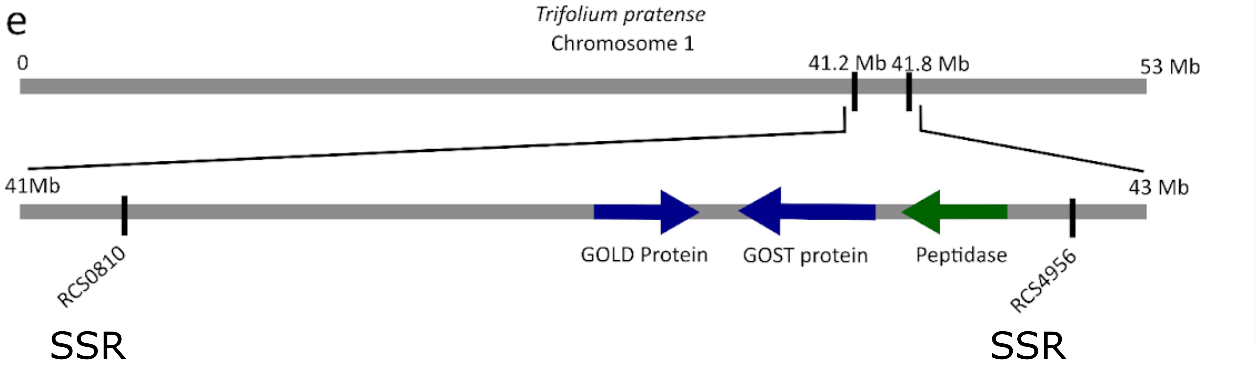
Found likely Self-incompatibility genes

- Identified 1.5 Mb deletion in $Tr_{To}1$ of S10 self-compatible line
- Annotation combined with transcriptome analysis identified three candidate genes in the deleted region – and in SI line 9x3
  - **Cysteine peptidase (in stigma/style)**
    - Involved in cell degradation when activated
  - **GOST (GOLD domain seven-transmembrane; in pollen)**
    - transport proteins across membranes
  - **GOLD domain (pollen)**
- High sequence divergence among haplotypes (important for allele discrimination)
- These genes are in a 2 Mb region of the S locus of Red Clover flanked by SSRs

Chromosome $Tr_{To}1$



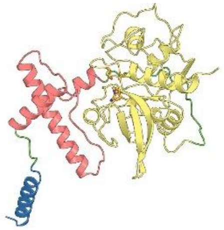
Red Clover Chromosome 1



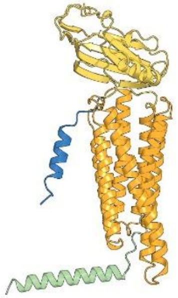
Validation – Protein models

- **Predicted protein structures**
  - **Peptidase** (stigma) degrades cells
  - **GOST (pollen)** traffics proteins across membranes
  - **GOLD (pollen)** protein stabilises GOST/ Peptidase complex
  - **GOST+GOLD** predicted to traffic peptidase into pollen tube to start tube destruction
- **Proteins from same haplotype form stable structure** – likely to interact
  - Peptidase pulled into pollen tube which is degraded
- **Proteins from different haplotypes form unstable structures** – unlikely to interact.
  - Therefore pollen tube grows

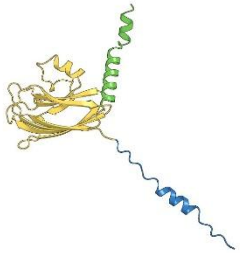
♀ **Peptidase** (Extracellular)



GOST ♂ **protein**



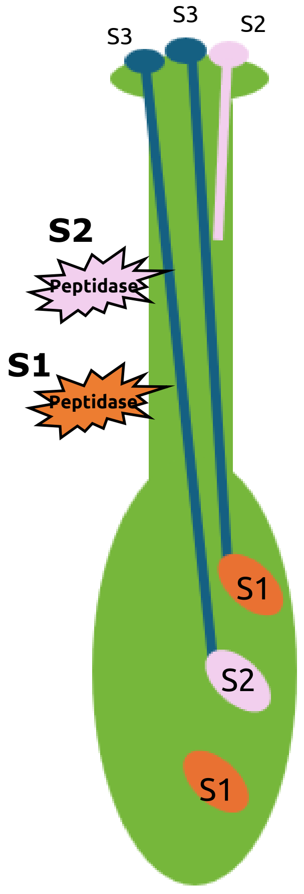
GOLD-Domain ♂ **protein**



Same haplotype: Stable (Incompatible)

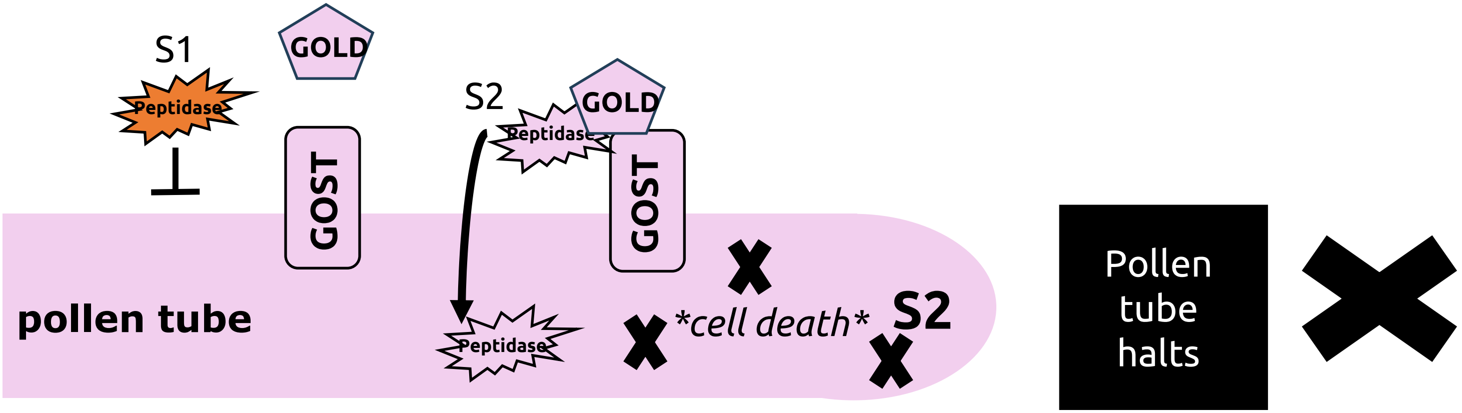
Different haplotypes: Unstable (Compatible)

Current hypothesis



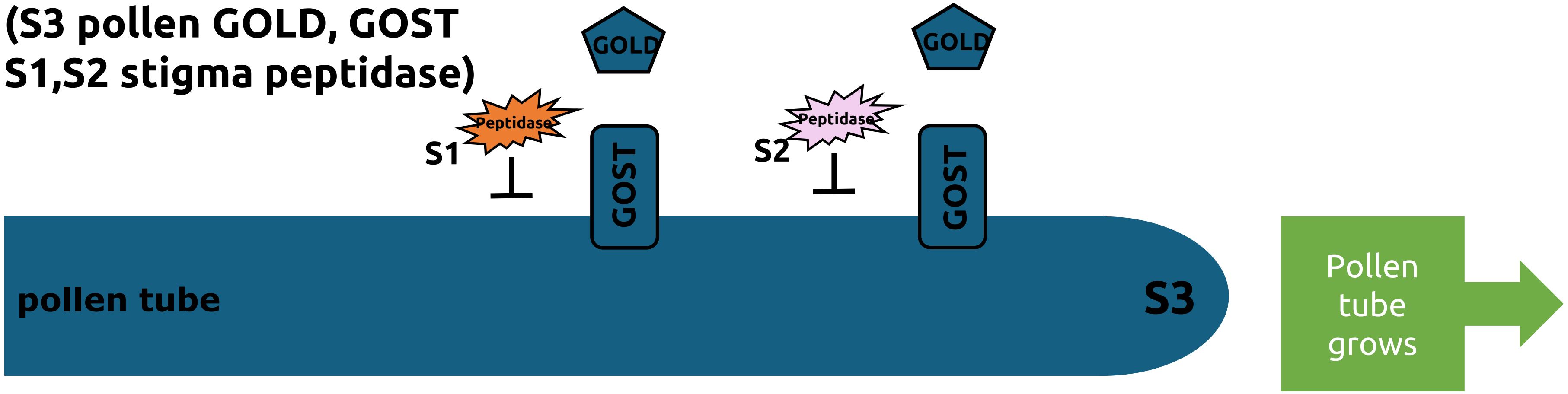
S1S2 x S2S3

Same haplotype (S2 pollen GOLD, GOST, S2 stigma peptidase)



Pollen tube halts

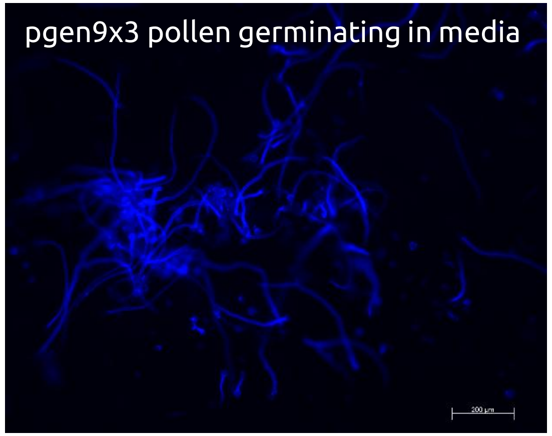
Different haplotype: (S3 pollen GOLD, GOST S1,S2 stigma peptidase)



Pollen tube grows

Validation

- *In vitro* pollen germination assay
  - Test effect of the GOST/GOLD/Peptidase



pgen9x3 pollen germinating in media

- Tobacco leaf infiltration to test combinations of haplotype proteins on cell death
- RNAi to silence candidate genes in both pollen and florets
- CRISPR-Cas9 knock-out lines

Further Analysis

- Access the sequenced clover diversity panel (WP6) to assess S-allele diversity and understand molecular diversity
- Or amplicon sequence the BSI-AgR diversity panel
- Design amplicon method to genotype S alleles as a breeding tool
  - May overcome issues with biased parental contribution in some polycrosses
  - Semi-hybrid development

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