

The effects of plant genome instability on breeding

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Credits

- Mitotic recombination in potato

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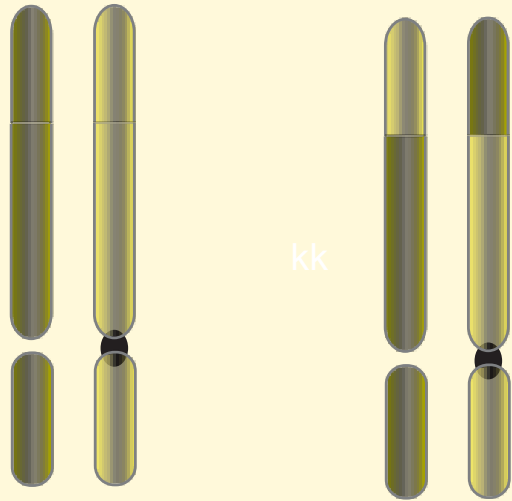
Isabelle Henry



Michelle Fossi

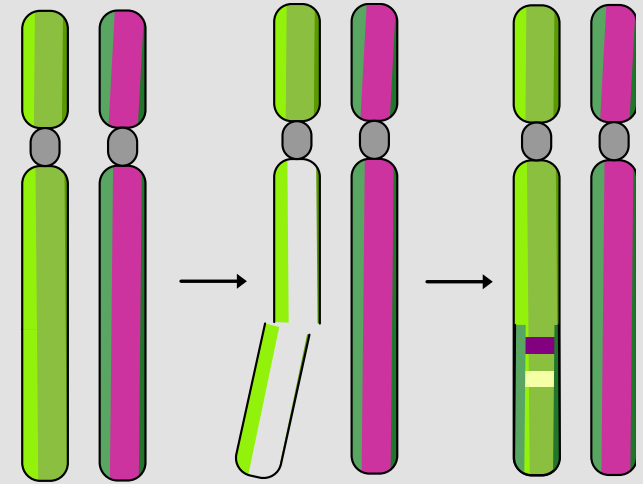
Funding: IGI, NSF PGRP, Fossi fellowship by H.M.Clause

Desirable mitotic recombination events



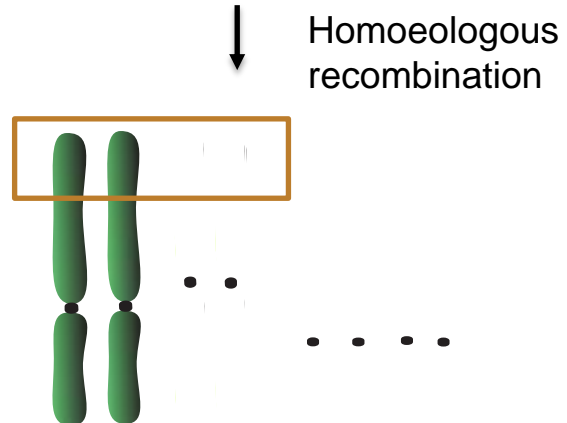
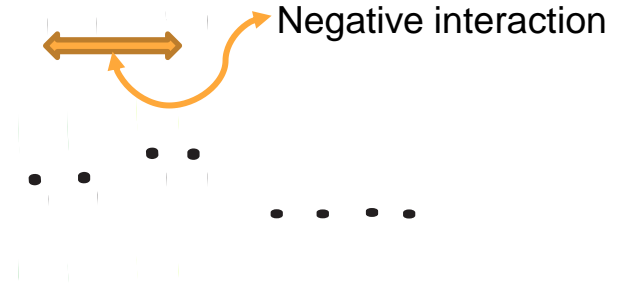
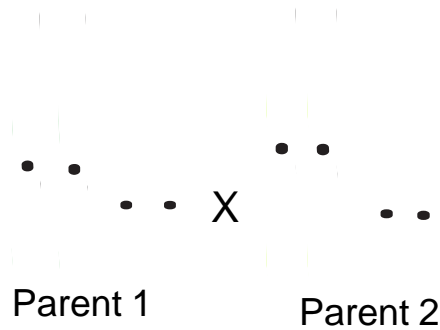
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Homologous translocation
(mimics meiotic recombination)



Large-scale conversion

Example of utility: homoeologous recombination



- Synthesized allopolyploids could revolutionize agriculture, but display deleterious interactions between genomes
- Solution: “convert” selected loci

Potato (*Solanum tuberosum*)

Bred sexually, propagated clonally

Clonal variants (sports) common (10^{-4})

Autotetraploid and diploid

Outbreeding and heterozygous

Haploid inducers available



Early Rose

Sexual
breeding



1867 - Selfing or unknown
cross-pollinator

Burbank (no russetting)



1876 - Clonal variant in
garden

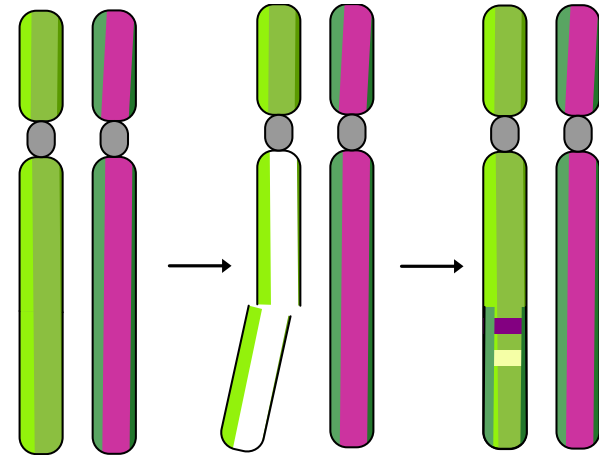
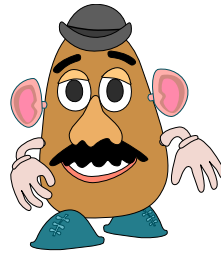
Russet Burbank

Clonal
breeding

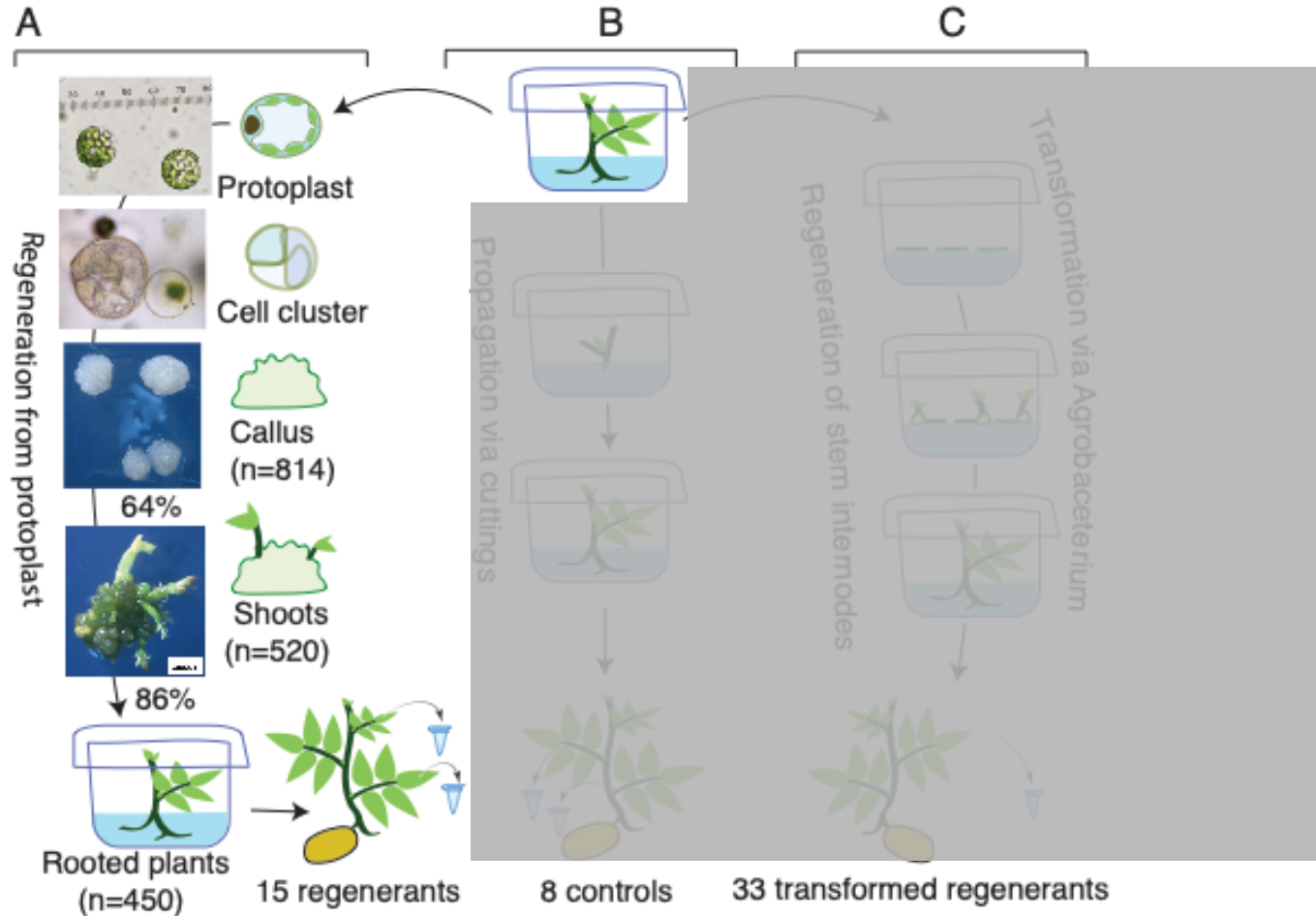


Large scale chromosome conversion in potato

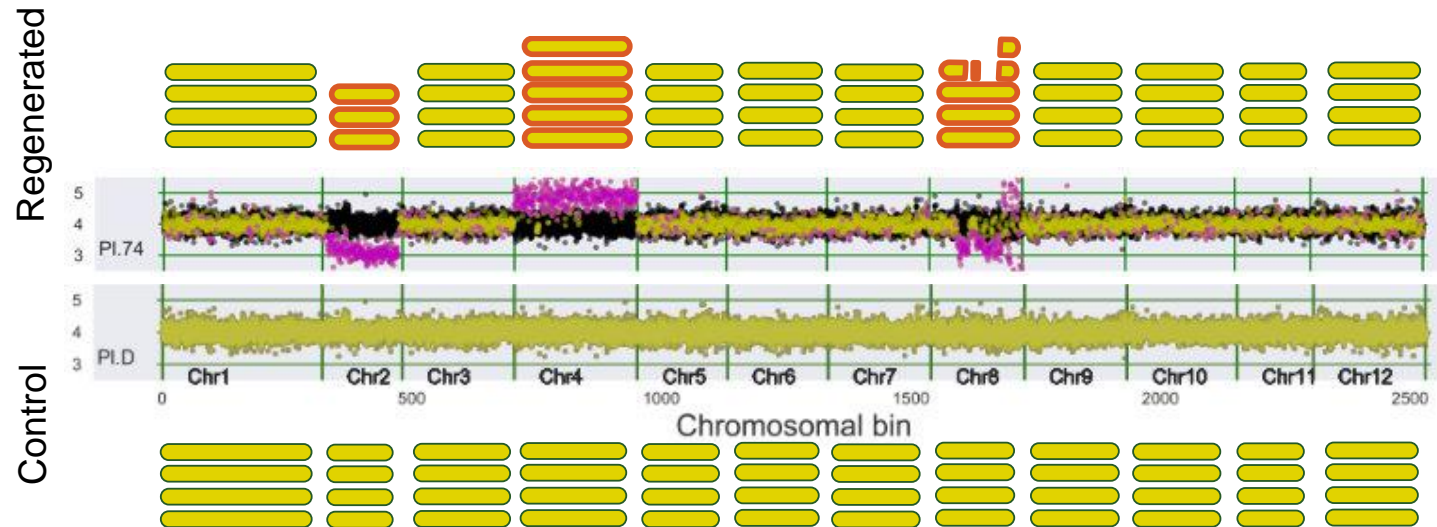
- Selective instability of chr.8 in Desiree
- Consistent with fragile site
- Homologous Recombination → chromosome conversion (BIR?)



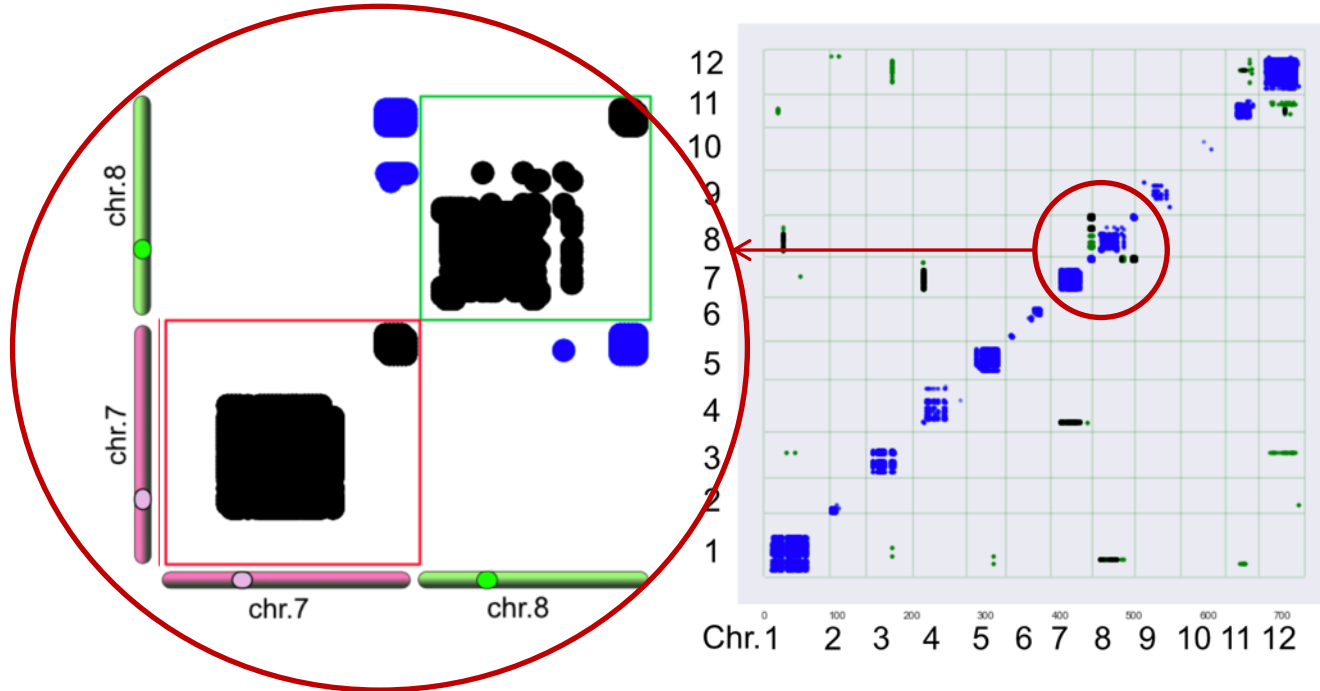
Potato genome display instability during regeneration



During regeneration from protoplasts, chromosomes can missegregate and break resulting in large indel and other aberrations



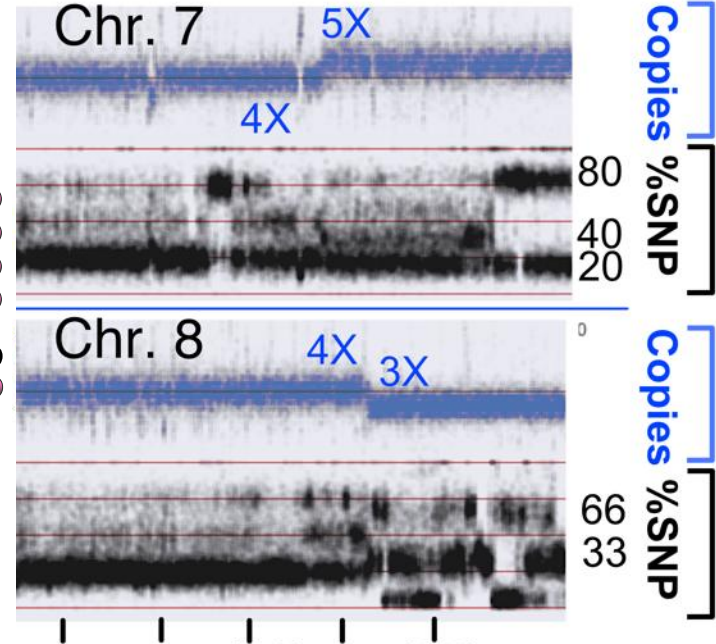
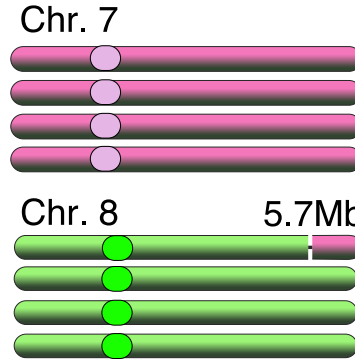
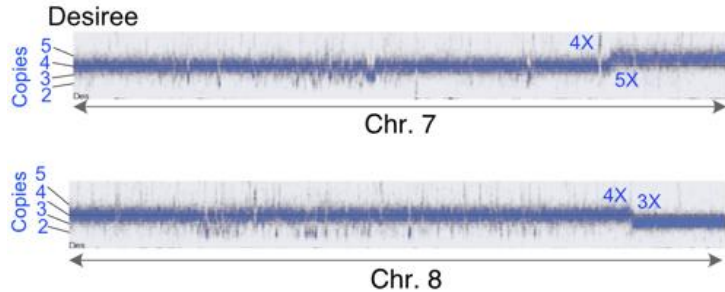
Var. Desiree: right tip of 7 is linked to the right tip of 8



- Evidence: Linkage Disequilibrium between chr.7 and chr.8

DNA sequencing evidence for Tr8-7 translocation model

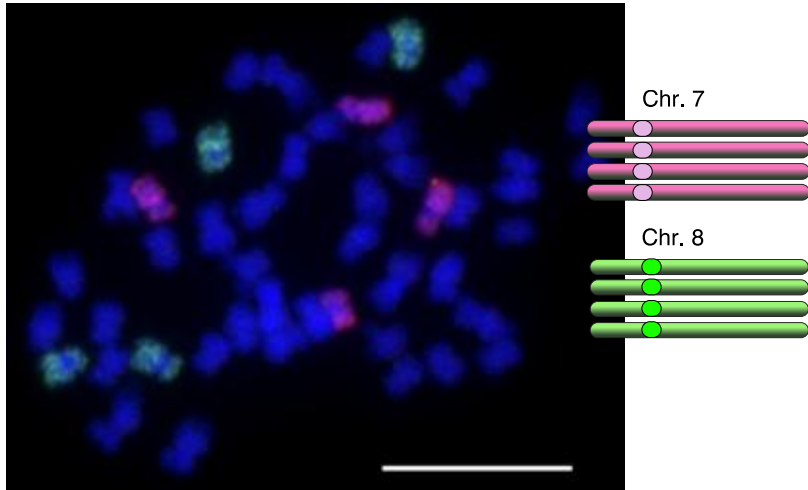
- DNA dosage -> **5 copies** of chr 7 tip, **3 copies** of 8 tip
- Heterozygous SNP ratio confirms it



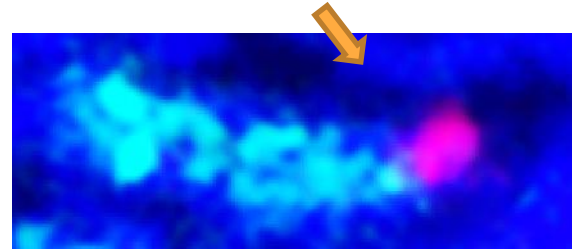
Chromosome painting

- Jiang lab (MSU) painted 7 and 8

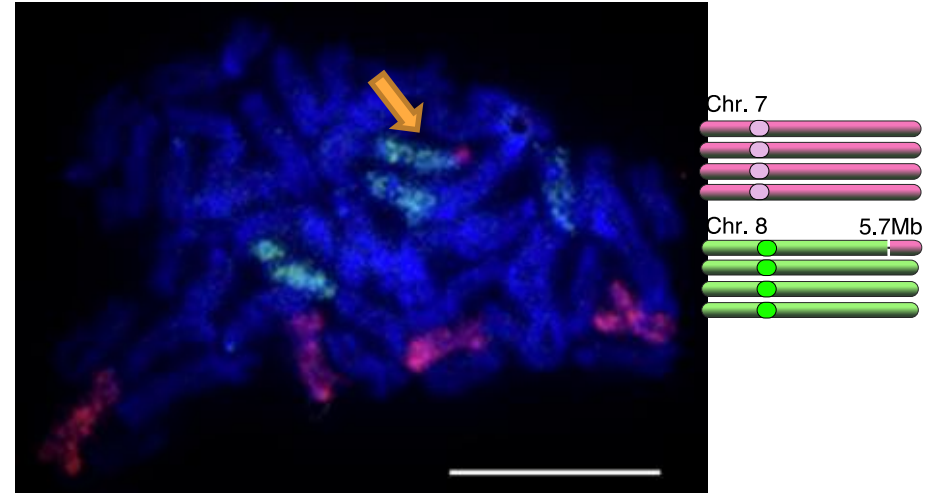
Normal karyotype



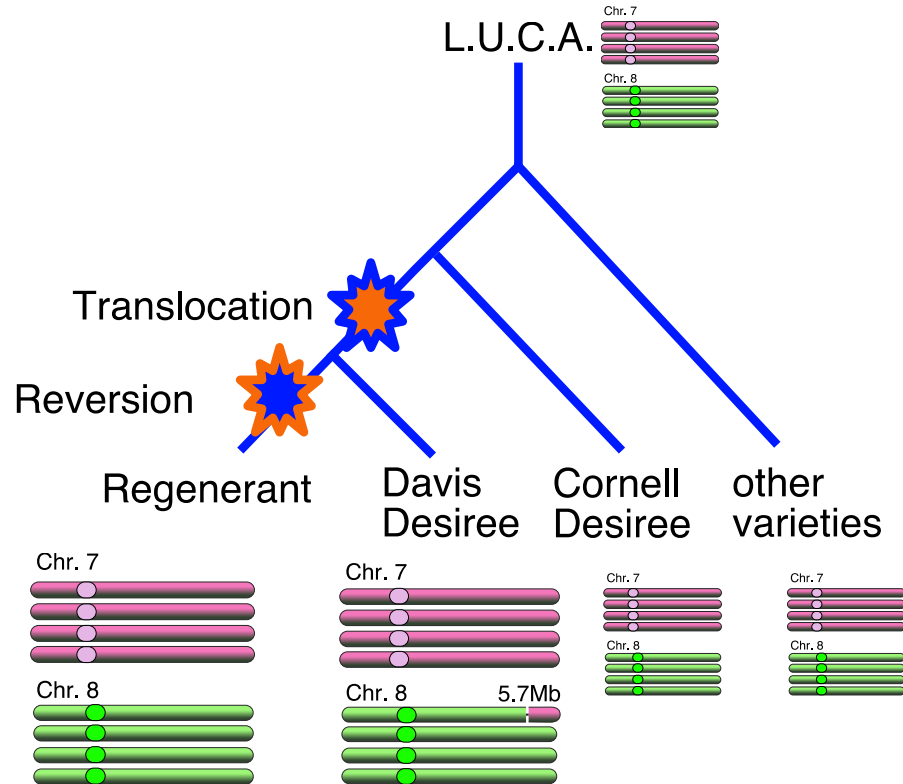
- Translocation evident



Desiree

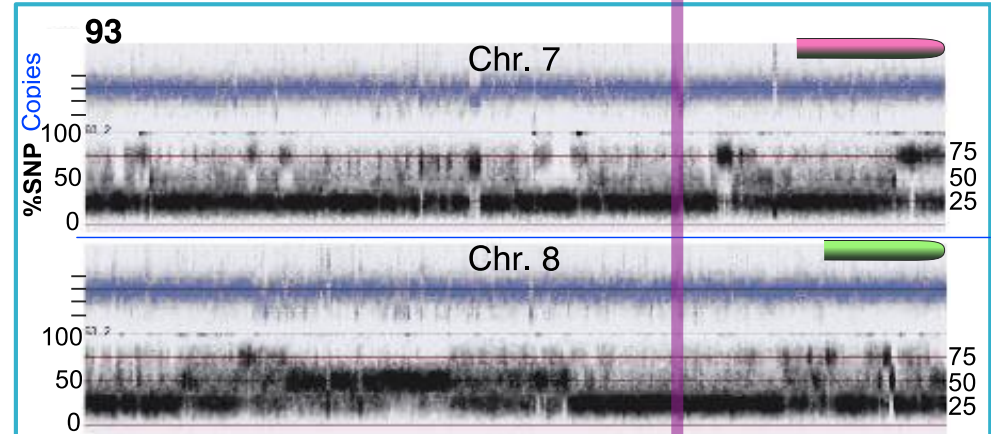
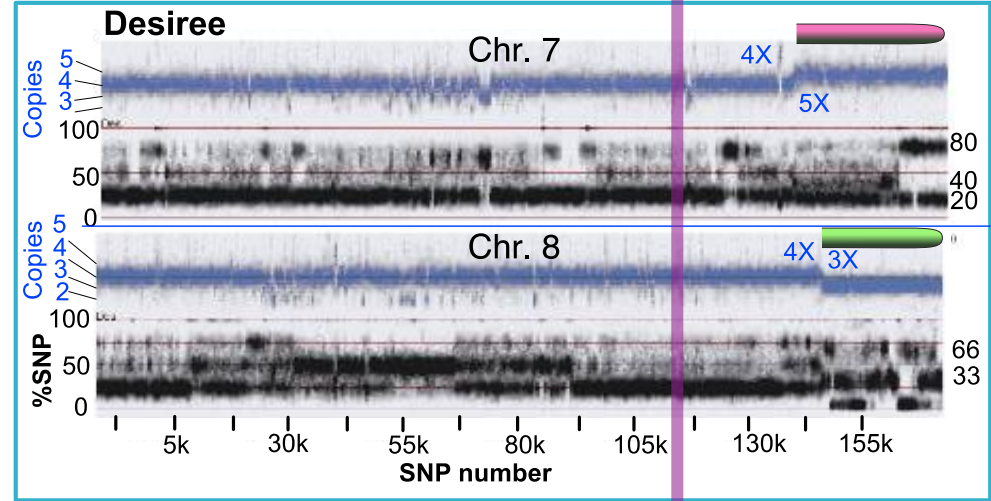
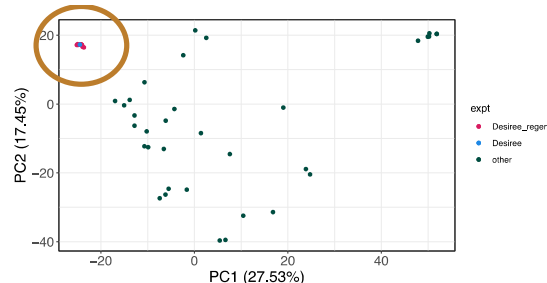


A short history of potato



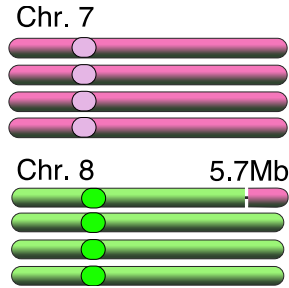
Sequence evidence for reversion of Tr8-7 translocation

- Several protoplast regenerants display four normal copies of chr.7 and chr.8
- Evidence
 - Sequence dosage
 - Heterozygous SNP ratio
 - Identity confirmed by SNP fingerprinting

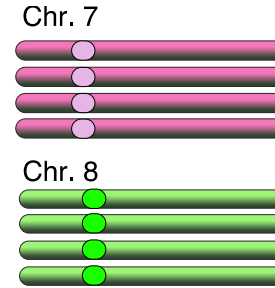
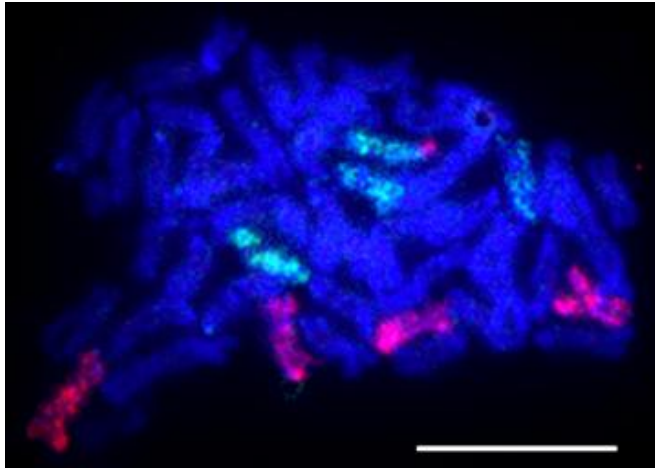


Reversion of the Tr8-7 translocation

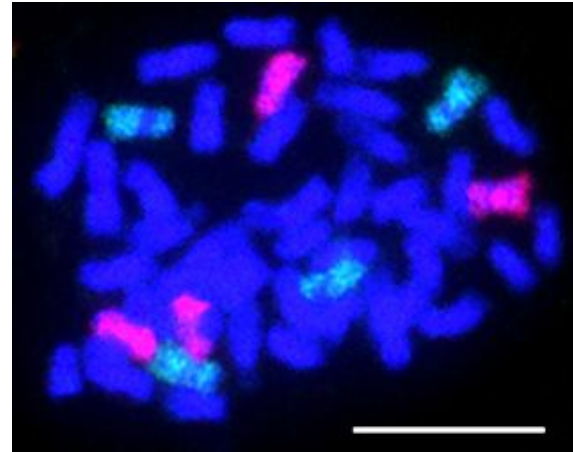
- Chromosome painting
- Cytology: X. Zhang,
- Painting: G.T. Braz, J. Jiang lab



Desiree

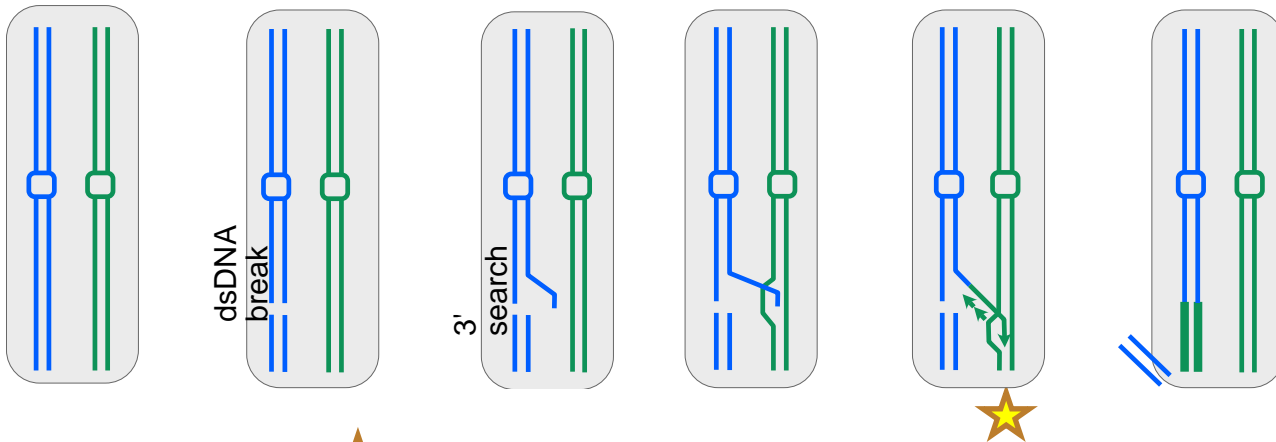


Regenerant 93



Candidate mechanisms for Tr8-7 reversion

- **Loss and Replacement** involving missegregation + nondisjunction (LR)
- **Mitotic crossover** (MiXo)
- **Break Induced Replication** (BIR)



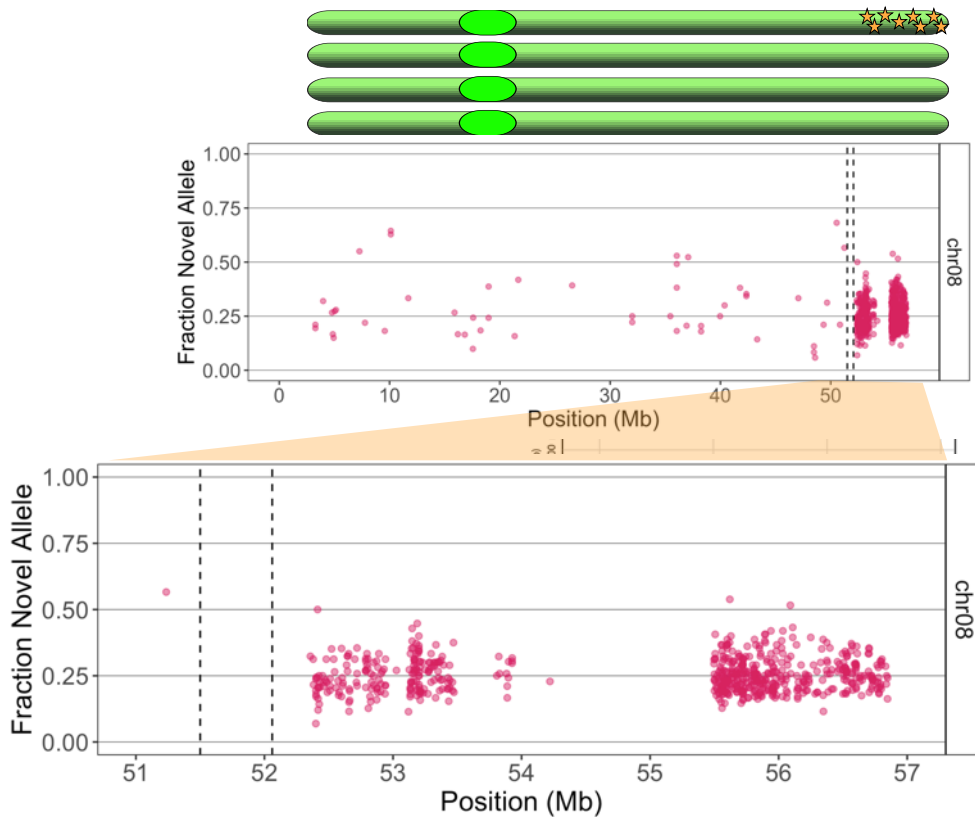
- Error prone replication ★
- Consequence: clustered, high-density mutation tracts (kataegis; Nik-Zainal, 2012)

Test hypothesis: is there kataegis (a dense mutation tract)?

- Expect clusters of novel mutations on
 - One homolog (25% freq)
 - Conversion tract
 - Novel

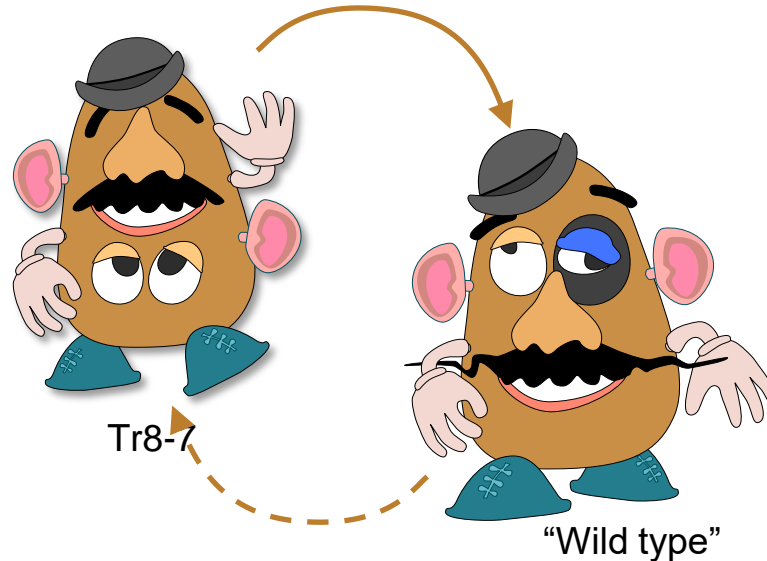
- Conclusions

- The observed mutation clusters are consistent with BIR (Nik-Zainal, 2012; Taylor, 2013)
- BIR is the favorite explanation for reversion



Summary

- Chr.8 in Desiree is unstable
 - A translocation between chr.7 and chr.8 is present as single copy
 - The translocation is reverted by a homologous recombination event
 - A mechanism called BIR is a strong candidate



Perspective

- Change of Heterozygosity following dsDNA breaks could contribute to somatic variation
- Unwanted outcome from traditional use
- Useful for targeted genome remodeling
- May be triggered by CRISPR-induced cuts