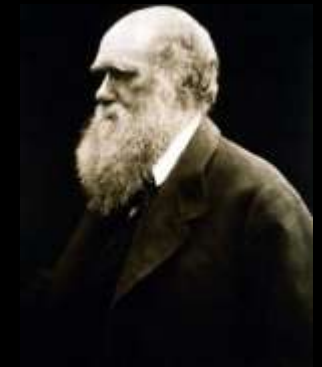
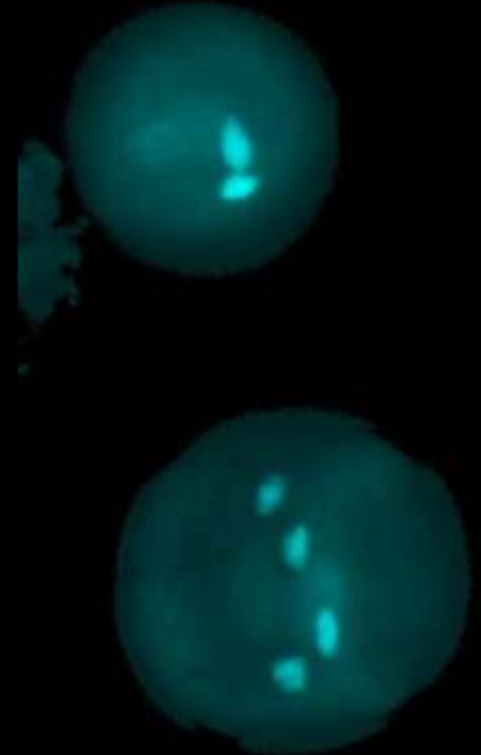


Apomixis in *Boechnera* (Brassicaceae): From the population, to the ovule, and back



 An Institute of the
Leibniz Society

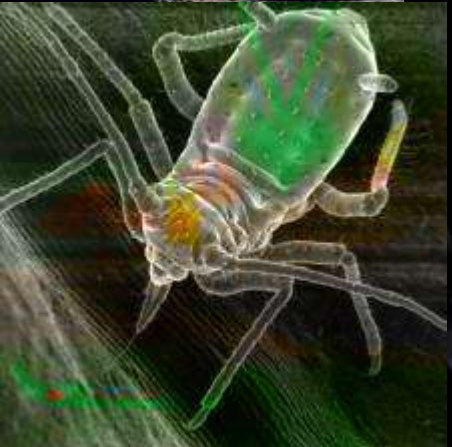
Tim Sharbel – Apomixis Research Group

Apomixis is asexual reproduction

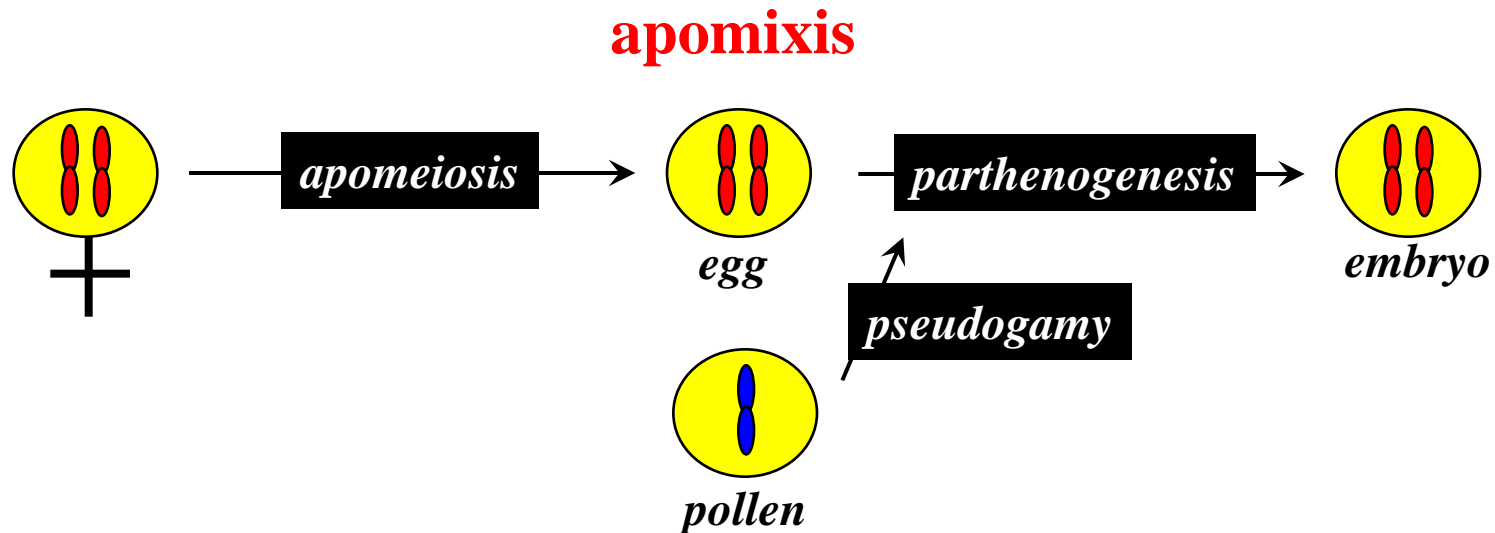
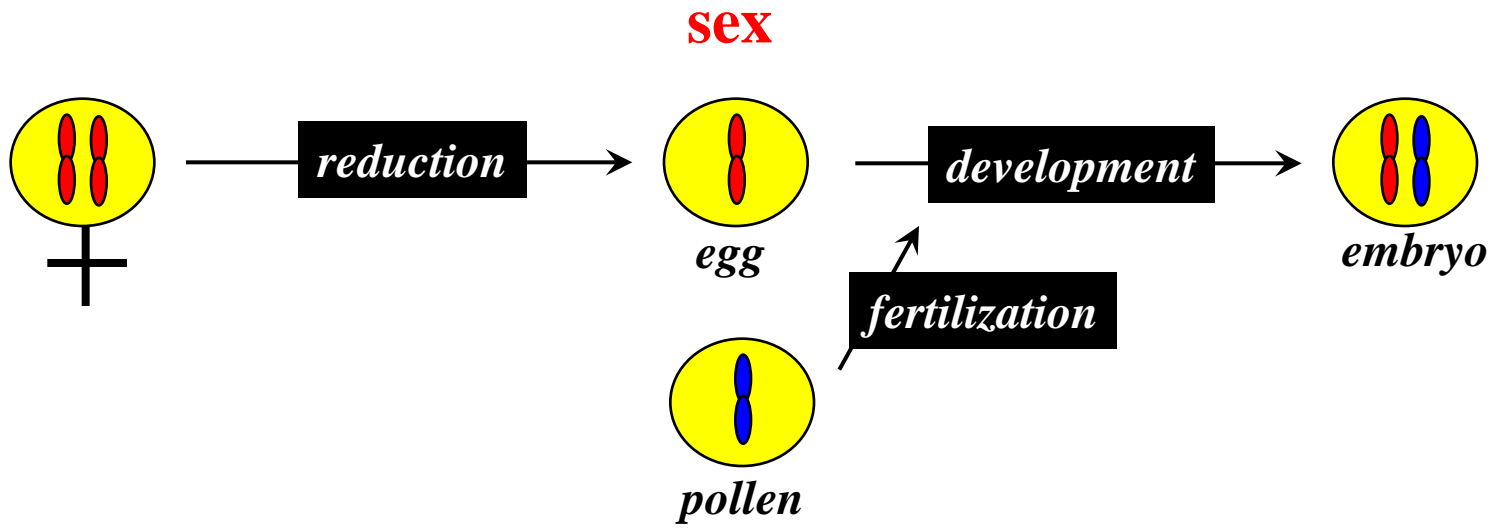


1. All are hybrid and/or polyploid

2. Apomixis has evolved from sex

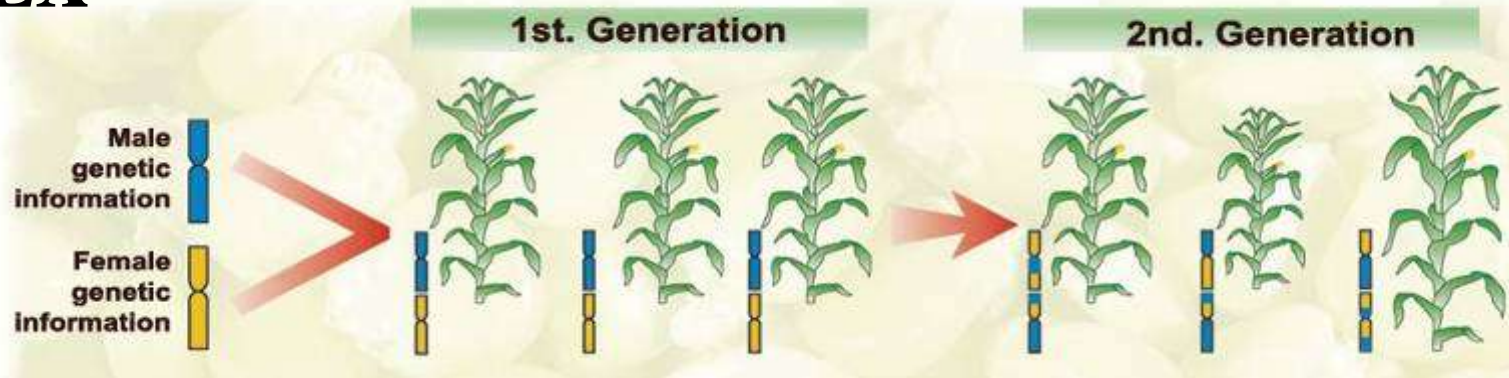


Apomixis in plants: asexual reproduction via seeds

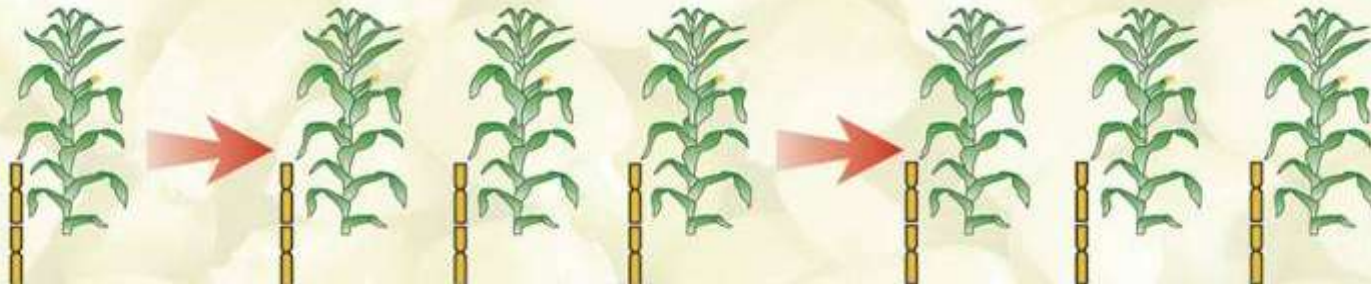


Why study asexual reproduction (apomixis) in plants?

SEX



APOMIXIS



(picture from CIMMYT homepage <http://www.cimmyt.org/index.htm>)

Apomixis can fix hybrid genotypes in crop plants

A wide-angle photograph of a vast field of yellow wildflowers, likely Drumheller wildflowers, under a bright sun in a clear blue sky. The sun is positioned in the upper left quadrant, creating a lens flare effect. The field stretches to the horizon, with a person visible in the distance for scale. The overall scene is bright and sunny.

Rapid production of novel varieties (1 generation)

Drumheller Heart of the Canadian Badlands

www.traveldrumheller.com

How does naturally-occurring apomixis arise and evolve?

Apomixis origins

Phenotypic variation

Transcriptomal differences

The North American *Boechera holboellii* complex



Arabidopsis, *Boechera* (formerly *Arabis*) and *Brassica*

Arabidopsis thaliana



Arabidopsis lyrata



Boechera holboellii



Brassica rapa



Arabis alpina



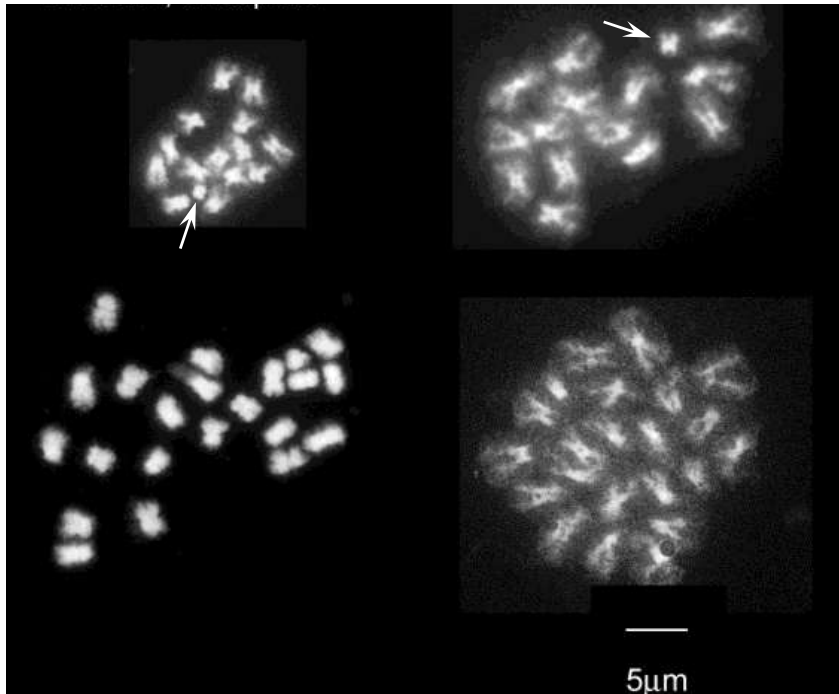
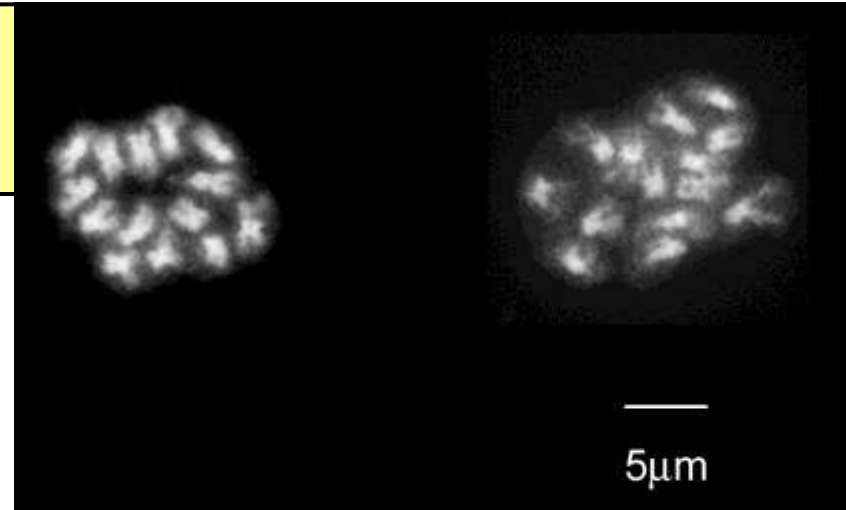
Cruciferae

Arabidopsis genomics tools can be used in *Boechera*
(and we can apply them to *Brassica*)

The *Boechera holboellii* complex

Sexual

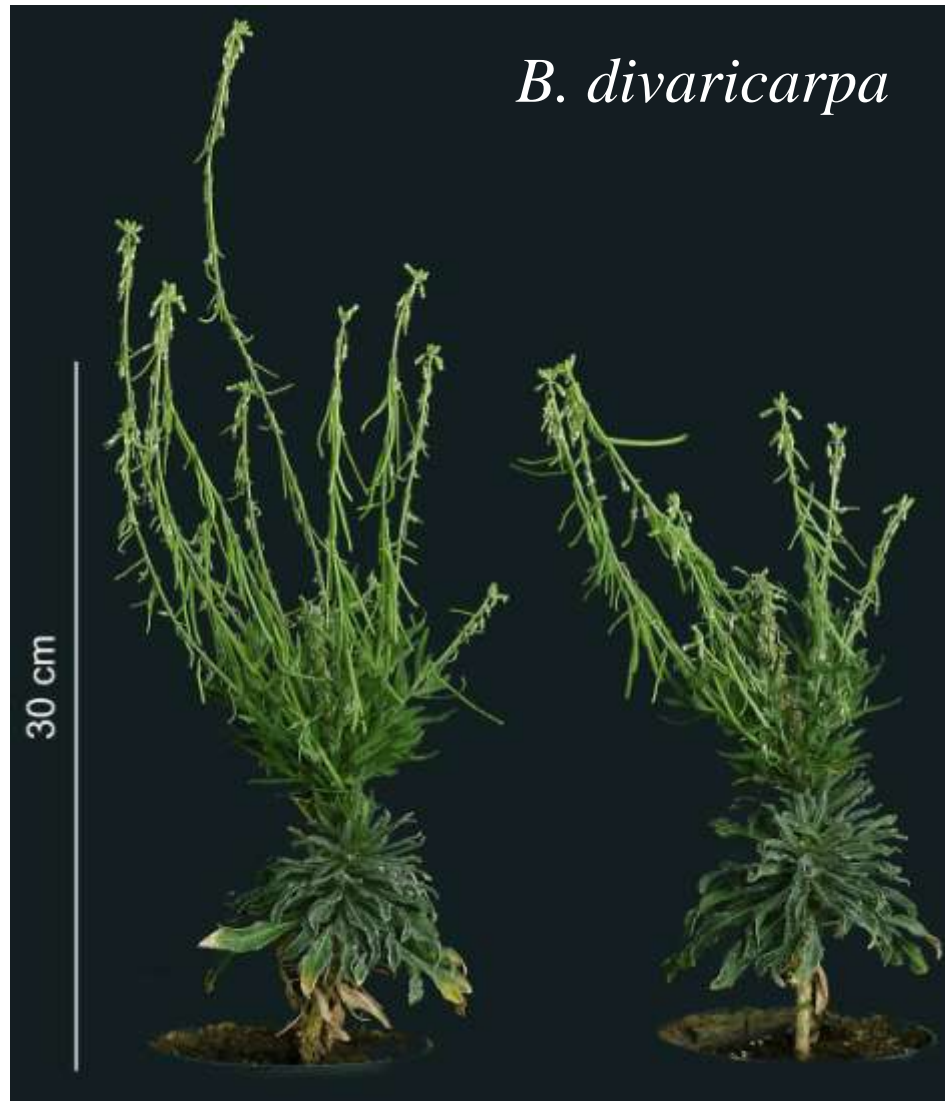
- $2n = 14$



Apomictic

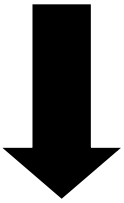
- Diploid and triploid

Pleistocene hybridization

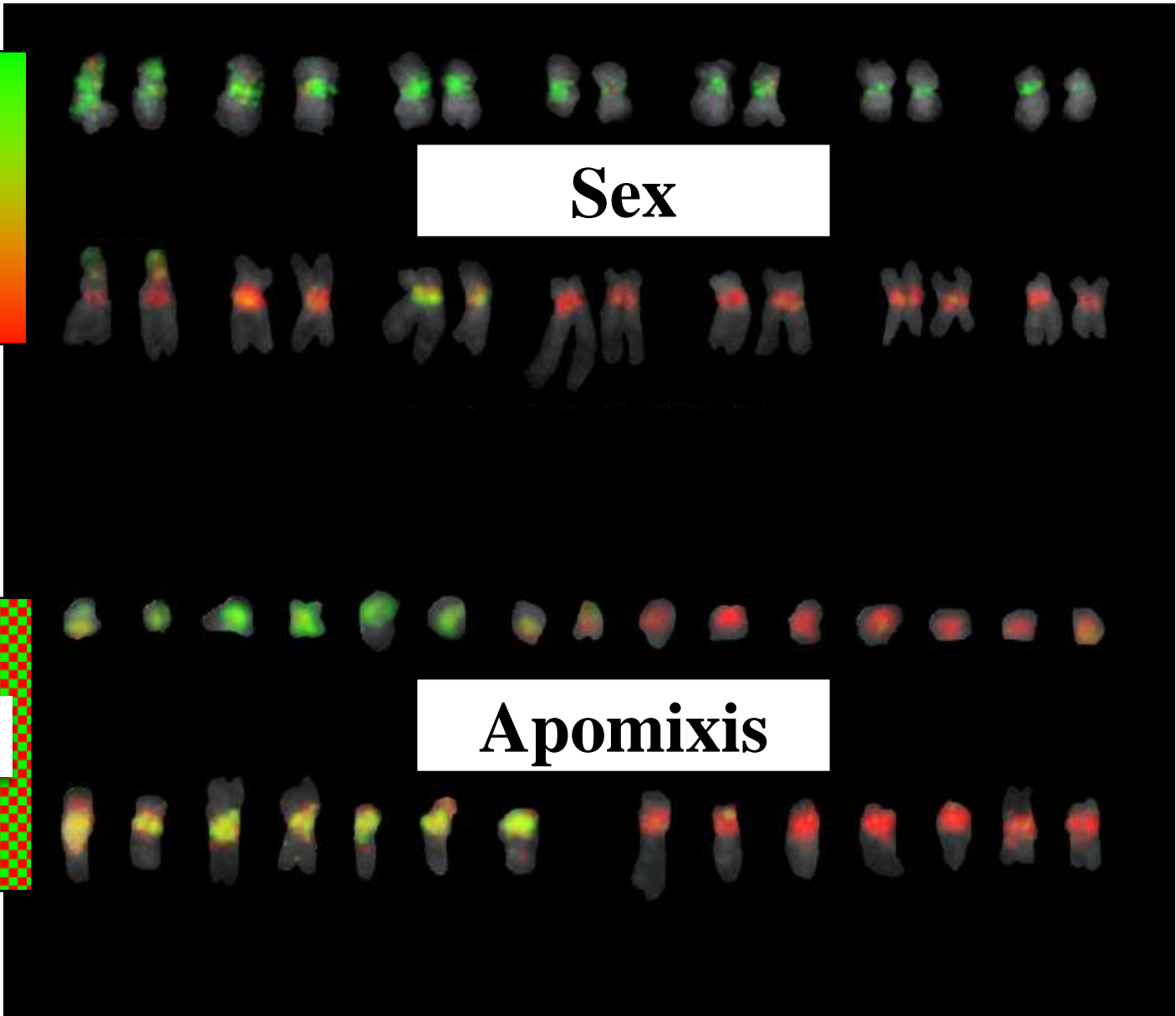


B. holboellii X *B. stricta*

holboellii
X
stricta



divaricarpa



Kantama, L., Sharbel, T.F., H. de Jong *et al.* (PNAS 2007)

How does naturally-occurring apomixis arise and evolve?

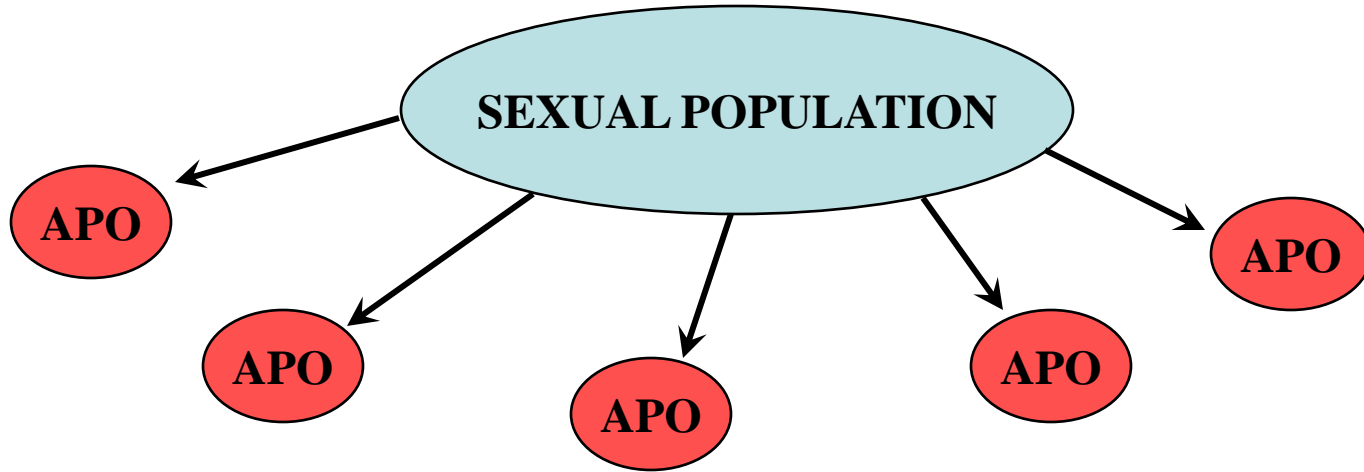
Apomixis origins

- **Repeated origins**
- **Hybridization is key**

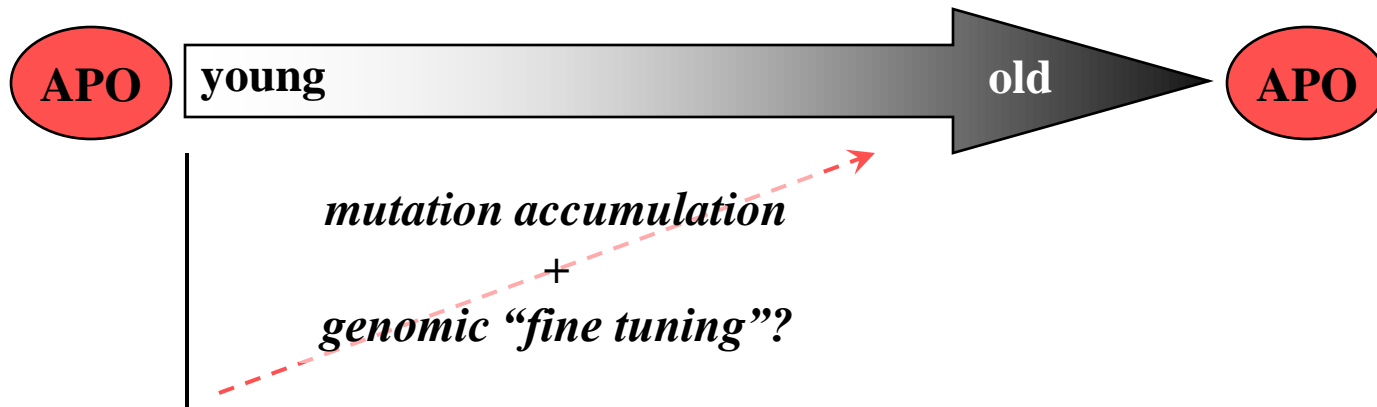
Phenotypic variation

Transcriptomal differences

Asexual (clonal) lineages originate frequently,



and then their genomes accumulate change...



Mutation accumulation and clonal degeneration

Apomictic



Sexual



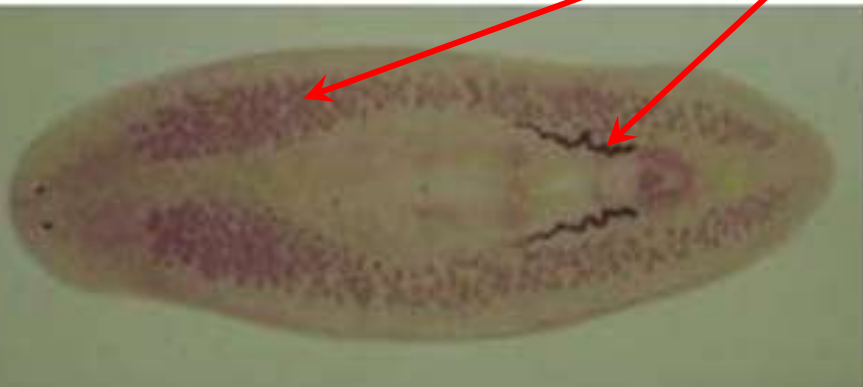
Males are unimportant...

Natural selection relaxed on sperm production in 3n's



Fertilization in animals (e.g. flatworms).

Sperm

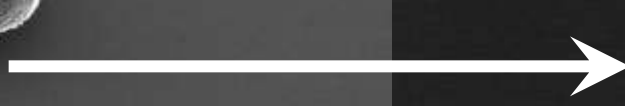


2n sexual



3n apomict

Sex



Apomixis

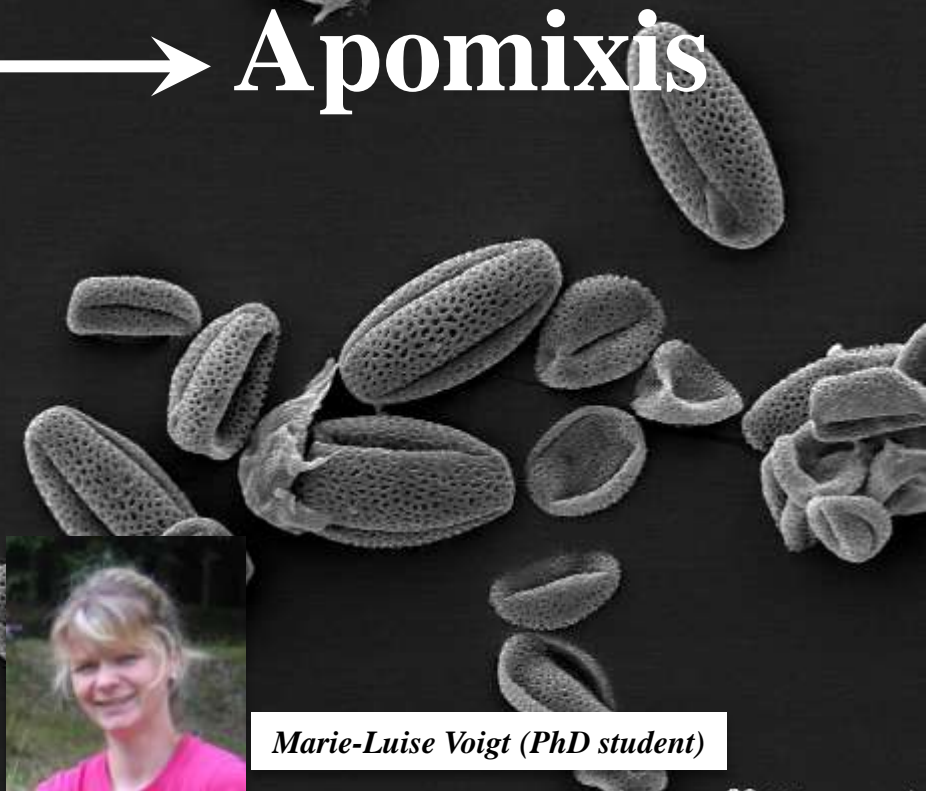


Boechera

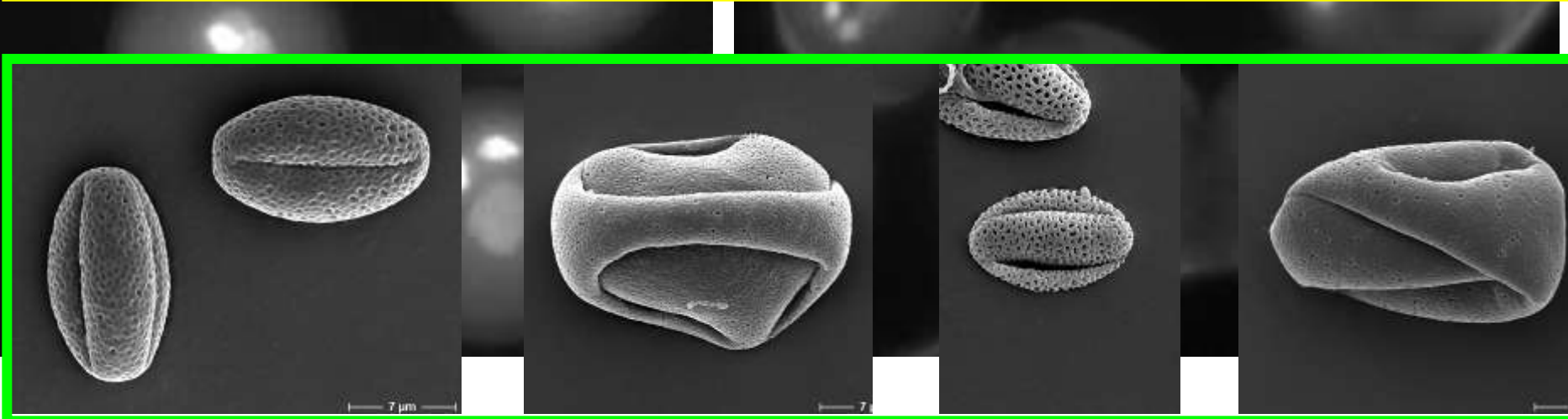
20 μm



Marie-Luise Voigt (PhD student)

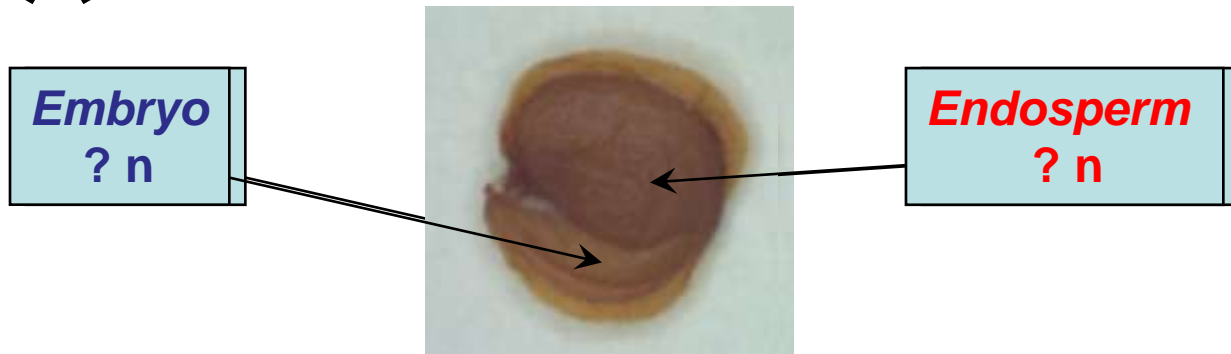
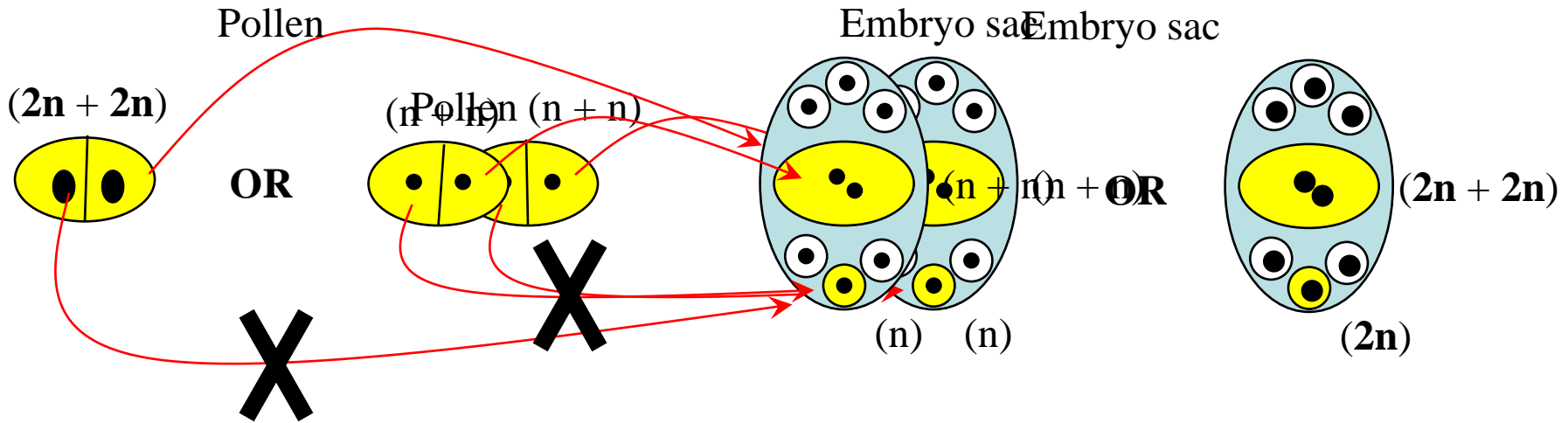


20 μm



We measure apomeiosis by analysing fertilization of the central cell
(using flow cytometry)

APOMEIOSIS



2:3, 2:4, 2:5, 3:5, 3:6, etc

Deviation 2 embryo 3 embryo endosperm generation ratio

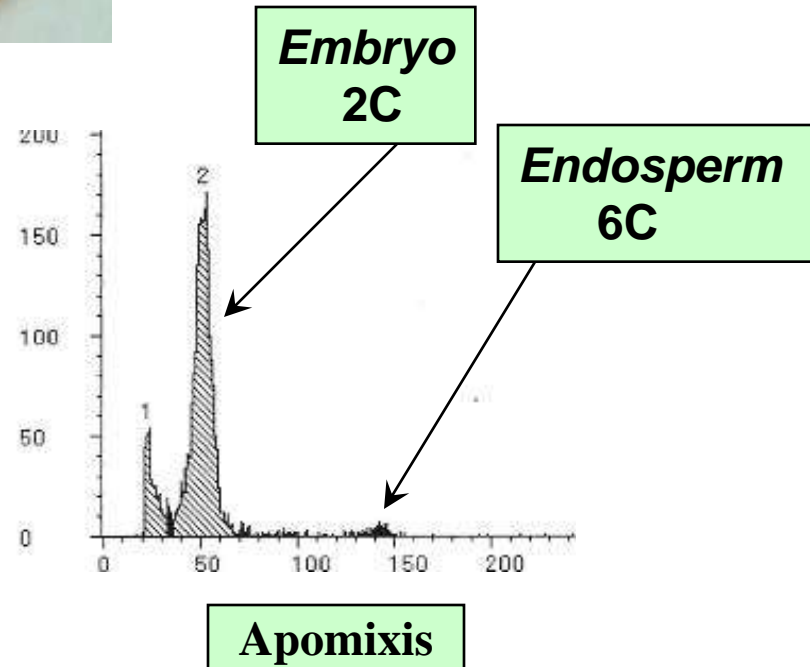
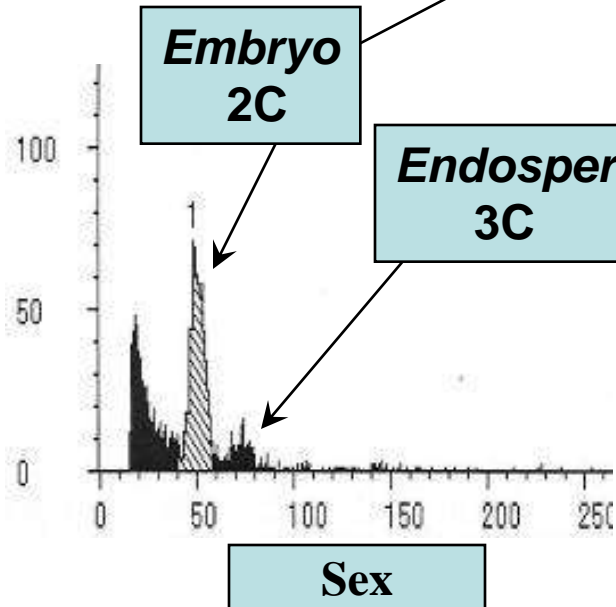


Dr. Olawale Mashood Aliyu

High-throughput phenotyping

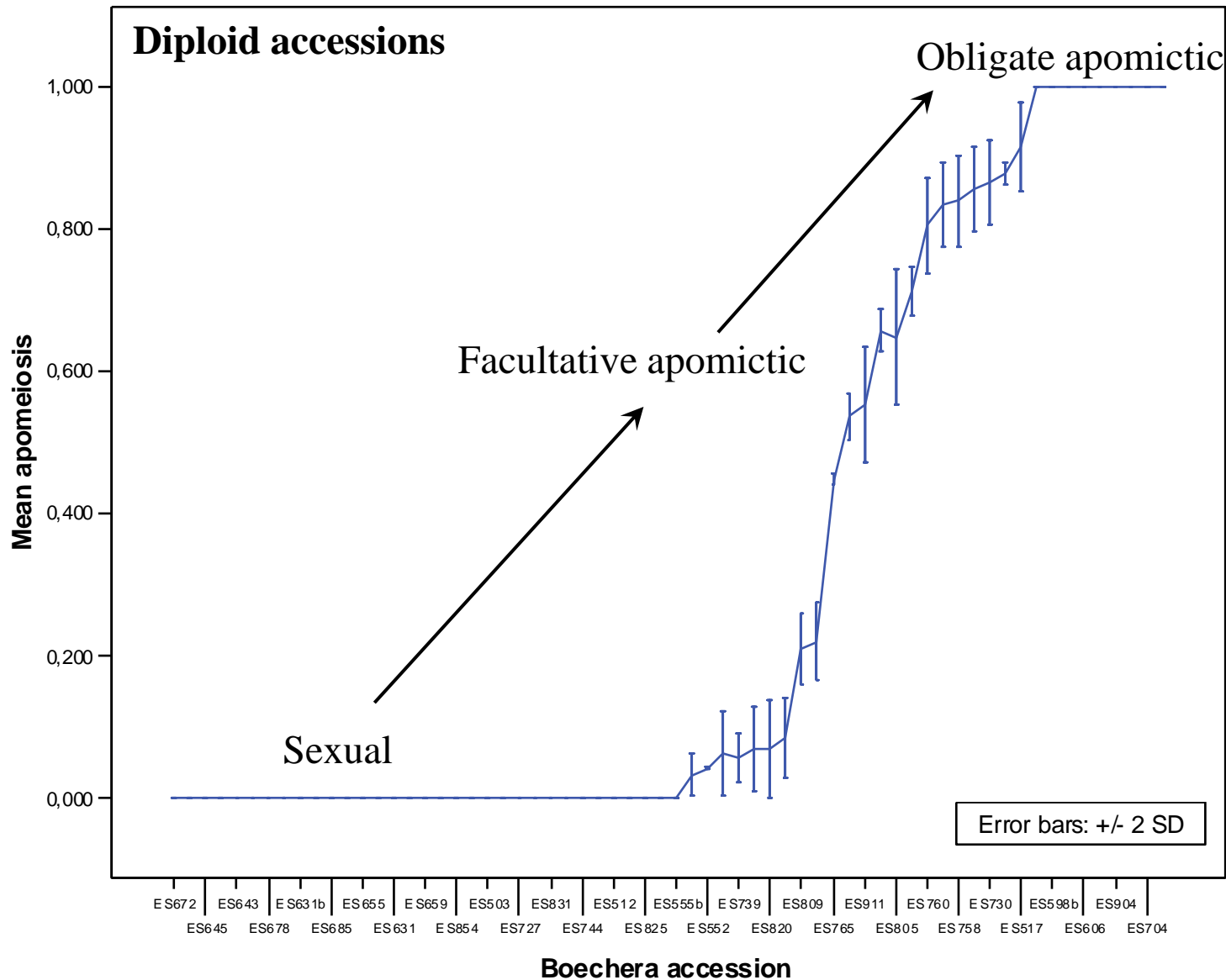
*Flow cytometric single seed ploidy analysis
(around 1000 seeds/day)*

Partec PA II – Robbywell system
(96 samples / 2 hours)



Quantitative variation for apomeiosis expression

(100 accessions, 3 individual per accession, 96 single seeds per individual)



How does naturally-occurring apomixis arise and evolve?

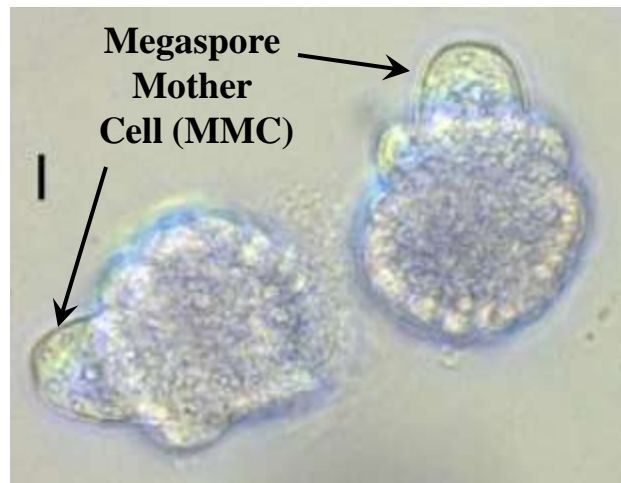
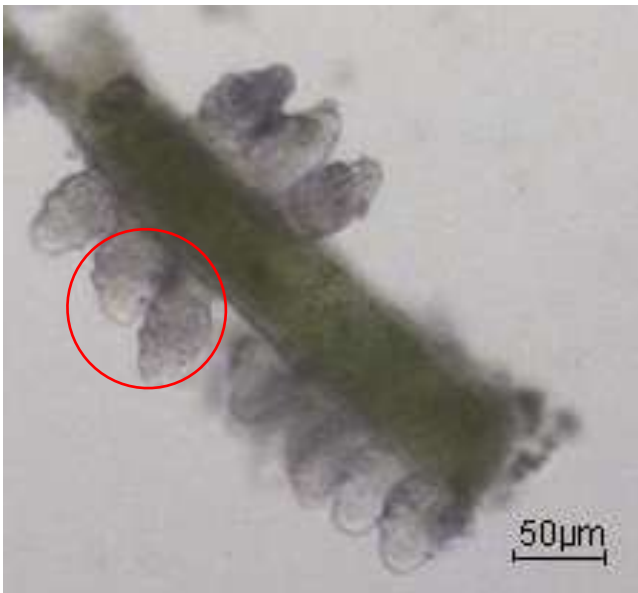
Apomixis origins

- **Repeated origins**
- **Hybridization is key**

Phenotypic variation

- **Quantitative variation**
- **Lineage (genotype) specific**

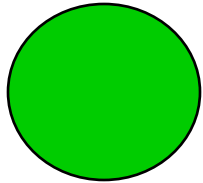
Transcriptomal differences



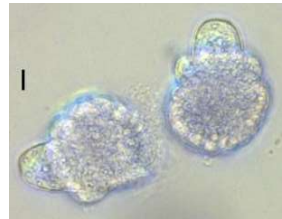
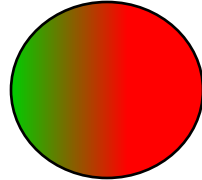
**10 live ovules
per individual**

Transcriptomic comparison of 2 sexual and 2 apomictic accessions

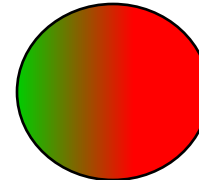
B. holboellii
SEX



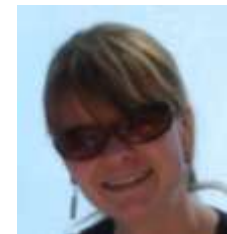
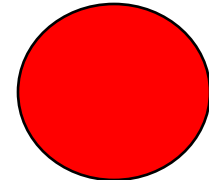
B. divaricarpa3
APO



B. divaricarpa8
APO



B. stricta
SEX



M-L. Voigt



Dr. J.M. Corral

SuperSAGE: Serial Analysis of Gene Expression

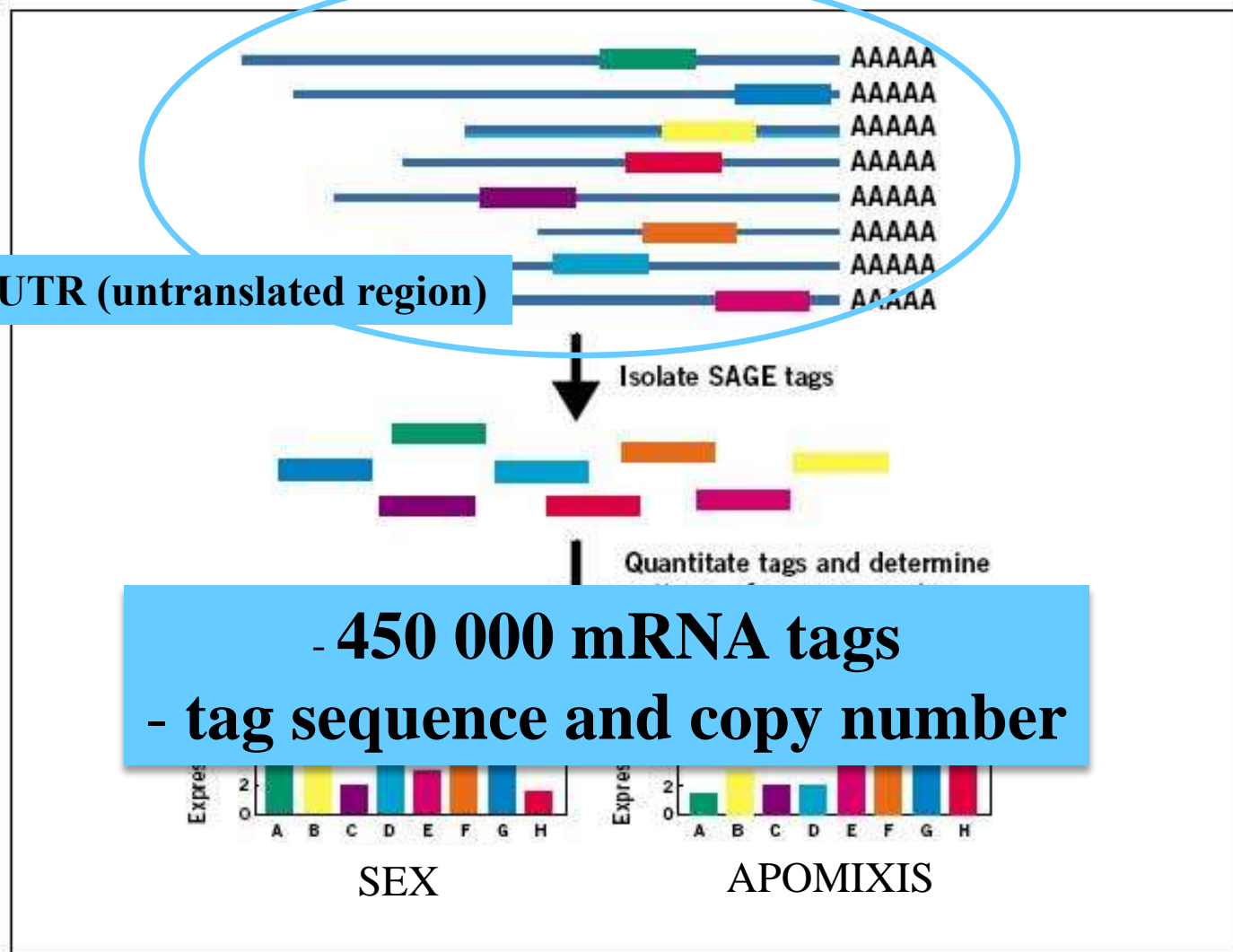


Figure 1. Schematic of SAGE method (Courtesy of sagenet.org)³

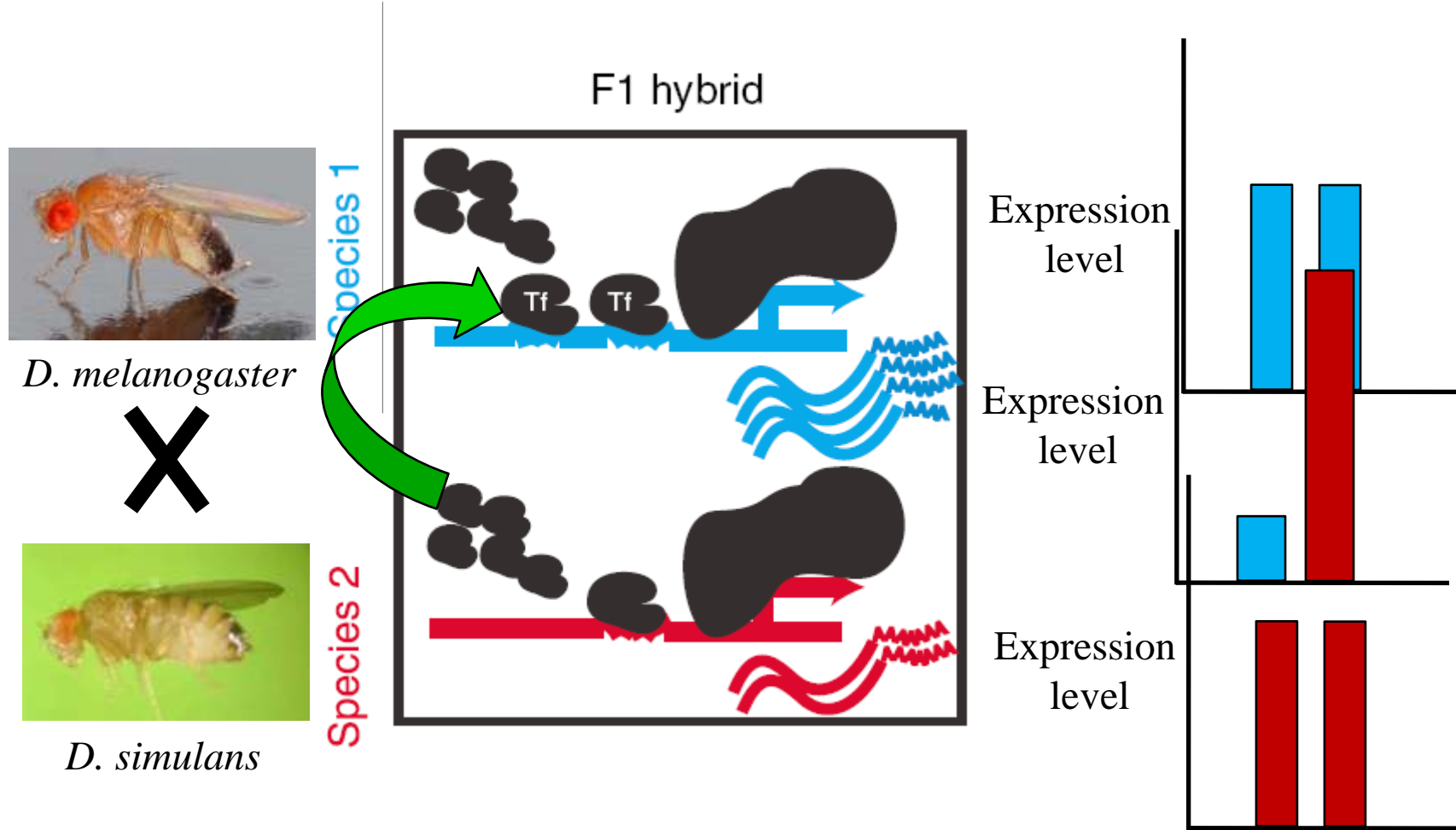
26 bp mRNA tag

Table 2. Overview of flower-specific transcriptome sequencing

| 454 Metrics | Sex | Apomixis |
|-----------------------|--------|----------|
| Number bp (million) | 12.4 | 14.6 |
| Number of contigs | 30 237 | 35 362 |
| Avg. contig size (bp) | 858 | 869 |
| Largest contig (bp) | 3067 | 4980 |

Boechera apomictic transcriptome

Trans-acting gene regulation in hybrids

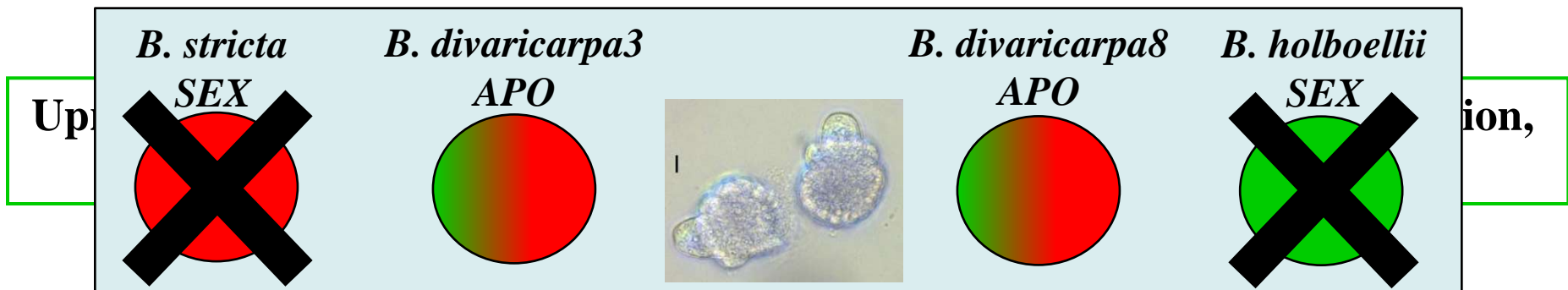
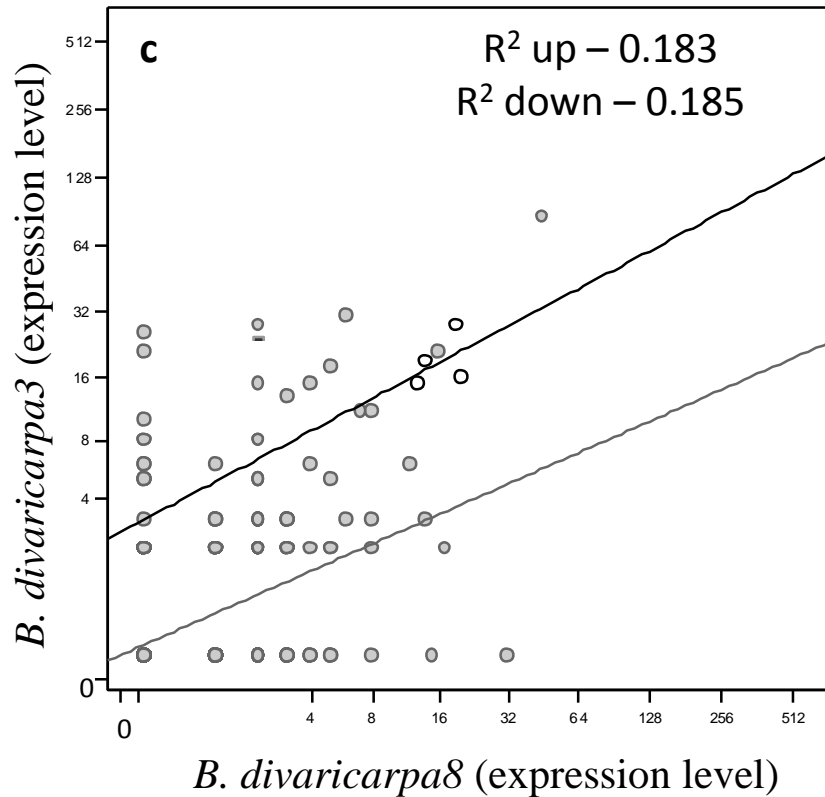


Compensatory *cis-trans* Evolution and the Dysregulation of Gene Expression in Interspecific Hybrids of *Drosophila*

C R Landry et al. (Genetics 2005)

Trans-expression of species-specific alleles in the hybrid genome

B. stricta alleles (n=525)



Heterochrony

“...c
deve
relativ

Apomixis results from deregulation of the sexual process

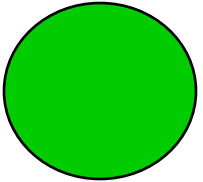
Nogler GA.1971. Genetik der Aposporie bei *Ranunculus auricomuss. l. W. Koch.* I. Embryologie. *Ber. Schweiz. Bot. Ges.* 81:139–79

rate of
etarded
ncestor's

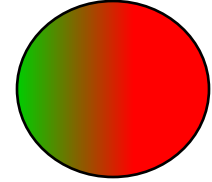


Tiger salamander
Ambystoma tigrinum

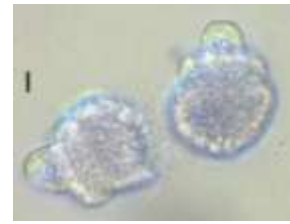
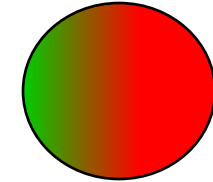
B. nebulosella
SEX



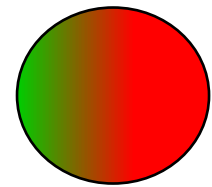
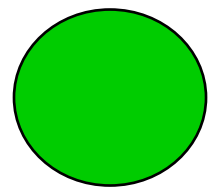
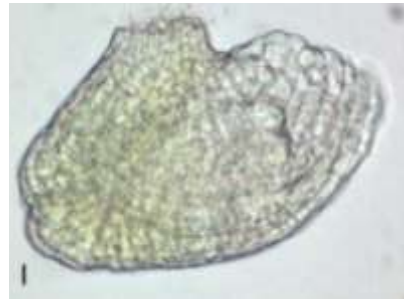
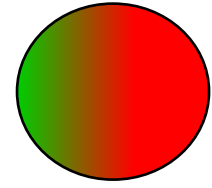
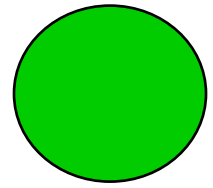
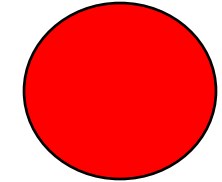
B. divaricarpa3
APO



B. divaricarpa8
APO



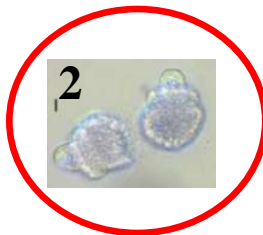
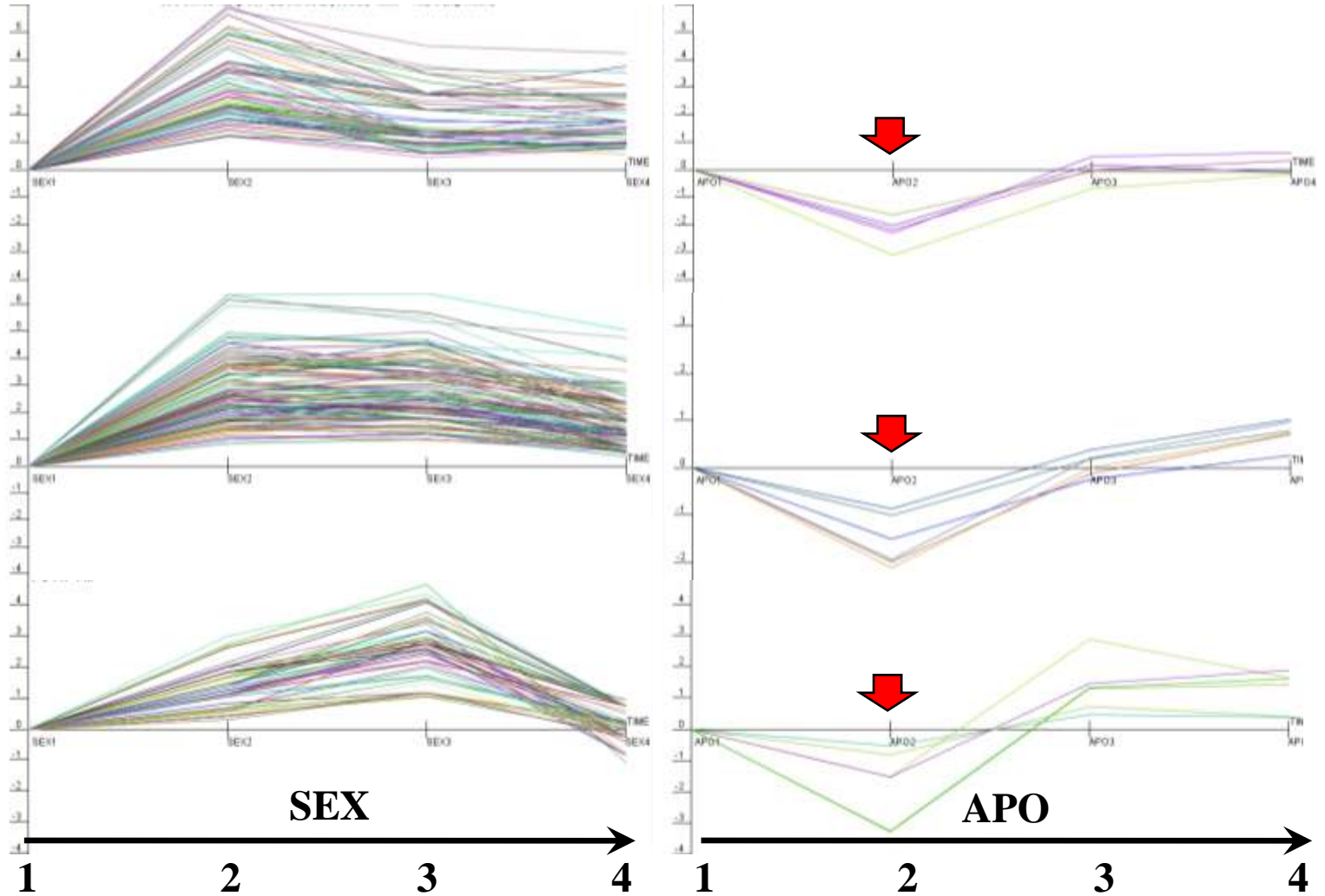
B. stricta
SEX



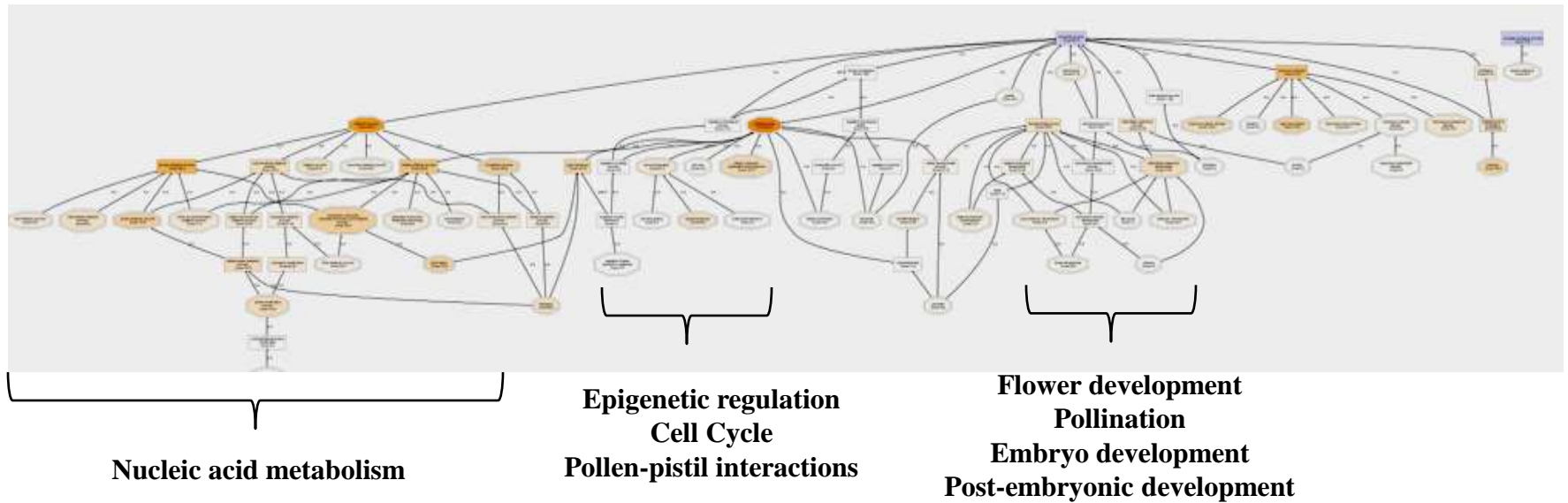
2.2 MILLION sequenced tags

Change accentuated at megaspore mother cell stage

Expression level



Apomixis = differential regulation of multiple reproductive genes



How does naturally-occurring apomixis arise and evolve?

Apomixis origins

- **Repeated origins**
- **Hybridization is key**

Phenotypic variation

- **Quantitative variation**
- **Lineage (genotype) specific**

Transcriptomal differences

- **Heterochrony**
- **Ancient 'parent of origin' effect**

Apomixis research group



Dr. Jochen Kumlehn (IPK)

Dr. Heiko Vogel (Max Planck Institute for Chemical Ecology)

Dr. Björn Rotter (GenXPro)

Dr. Falk Schreiber (IPK)