

Acepo

A close-up photograph of a purple flower's reproductive parts. The image shows several stamens with bright yellow pollen grains on their anthers. The background is a soft, out-of-focus purple, matching the petals of the flower.

Analysis & Advice
around
Cells & Pollen

Acepo Background Info



- Founded in 2017 by Dr. Iris Heidmann

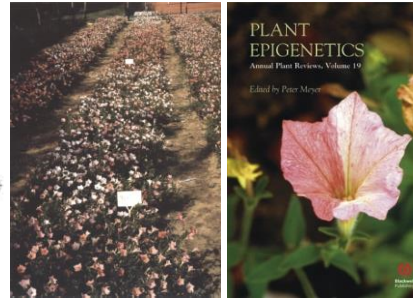
**Institute for Horticultural Plant Breeding
(Ahrensburg, D)**



**Max Planck Institute
for Plant Breeding Research (Cologne, D)**

**A new petunia flower colour
generated by transformation
of a mutant with a maize gene**

Peter Meyer*, Iris Heidmann*, Gert Forkmann†
& Heinz Saedler*



**A protocol for transformation and regeneration of
*Antirrhinum majus***

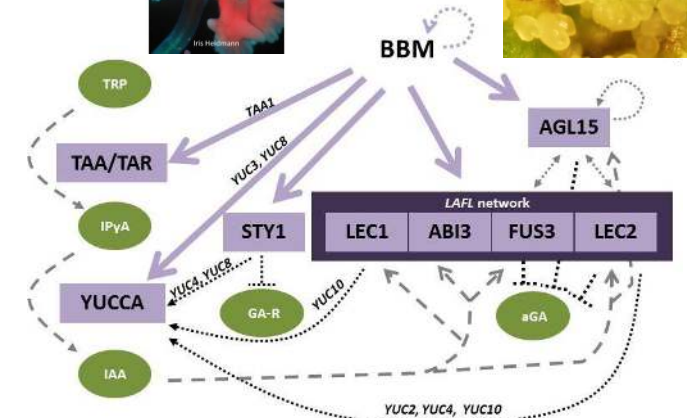
Iris Heidmann, Nadia Efremova, Heinz Saedler and
Zauzsanna Schwarz-Sommer*



**Enza Zaden
(Enkhuizen, NL)**



**WUR/PRI
(Wageningen, NL)**



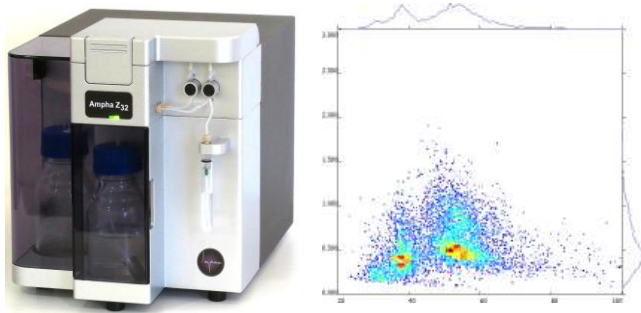
University of Konstanz (D)



Analysis and Advice @Acepo



Cell & Pollen Quality Analysis



- Pollen/Cell viability
 - Pollen germination
 - Ploidy indication
 - Cell counts
- Pollen, flowers, or whole plants can be sent to Acepo
- Acepo travels to the customer

Advice around Cells & Pollen

- Support breeding strategies by pollen analysis
 - Screen for male sterile/fertile lines (GMS/CMS)
- Optimise conditions for pollen production

At customers location:

- Mutagenesis (EMS, irradiation)
- Plant tissue culture
- Embryo rescue
- Doubled haploids
- Plant regeneration
 - Protoplasts
 - Somatic embryogenesis
 - Transformation protocols



Orchids – Challenging Pollen

*Iris Heidmann, Acepo
Amphacademy 2017*

Orchids around the globe



Orchids: Uses and values



Ornamental

- ❖ > 500 million USD
- ❖ Cut and pot plants
- ❖ Vegetative propagation
- ❖ 70 % export NL & Thailand

- Phalaenopsis
- Dendrobium
- Miltoniopsis
- Cambria
- Cymbidium



Condiment

- ❖ 20 - 600 USD/kilo cured Vanilla pod
- ❖ Annual production 7700 tons
- ❖ 75% Madagascar & Indonesia



Medicinal

- ❖ TCM
 - ❖ Ayurveda, ITM
 - ❖ Africa
 - ❖ Europe
 - ❖ Australia
-
- aphrodisiac
 - contraceptive
 - pain relieve
 - Diabetics
 - Phytotoxins > cancer (no clinical studies)
 - Malaria treatment in case of resistance to quinine
 - Neuro- and liver protection

Orchid production and breeding challenges

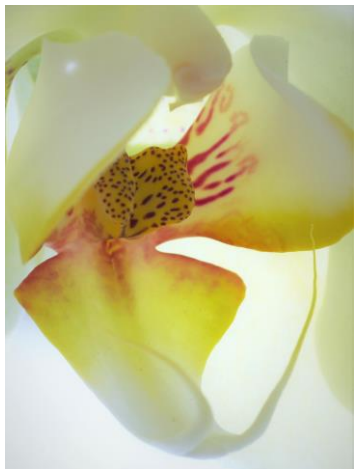


- **Producing pods**
 - All hand pollination
 - Pollination tricky

Time scales

Pollination to fertilisation:
8 days – 40 weeks

Fertilisation to ripe seeds:
up to 48 months



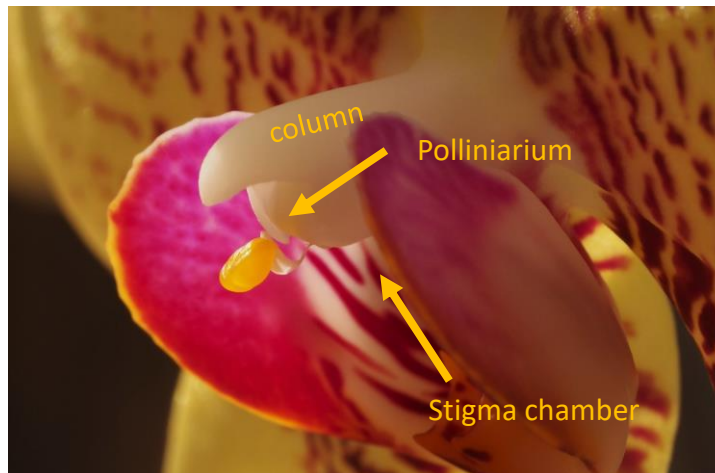
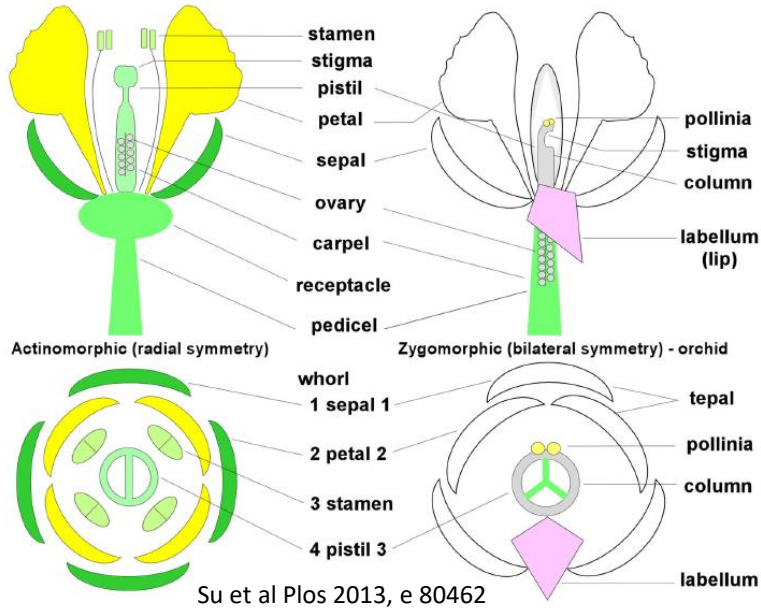
- **Producing flowering plants to market demands**
 - Flowering time: Vernalisation & day length
- **Creating new colour and types**
 - Intra- & interspecific crosses
 - Off-season crosses

Pollination to fertilisation:
~ 24-48 hours

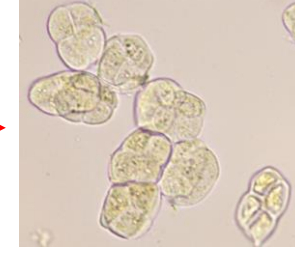
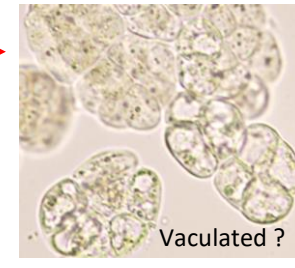
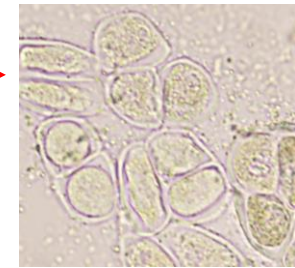
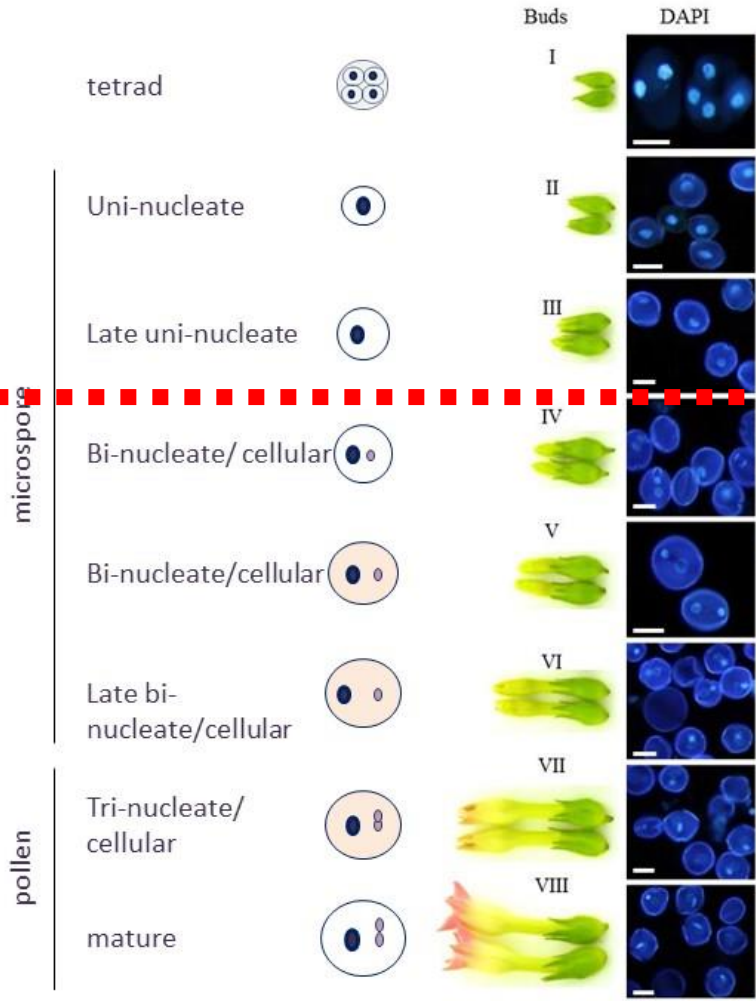
Fertilisation to ripe seeds:
8 - 12 weeks



Orchid flower morphology



Orchid pollen development



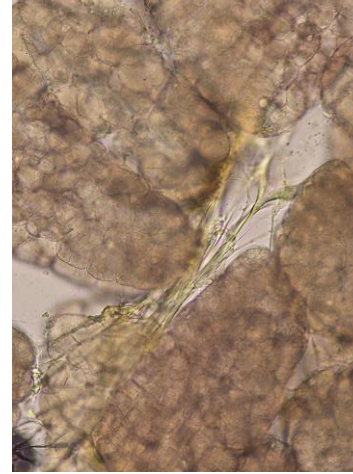
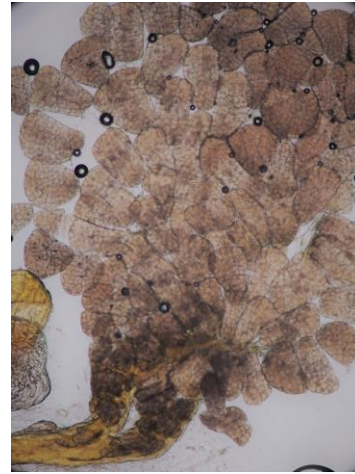
Days after Pollination	Developmental Stage	
	Male Gametophyte	Female Gametophyte
7	Pollen germination	Protuberance differentiation
14	Pollen tubes in ovary	Protuberance development
28	Pollen tubes in ovary	Protuberance branching
42	Pollen tubes in ovary	Ovule primordia differentiation:
56	Generative cell and vegetative nucleus	Archeporsial cell Megaspore mother cell
70	Two sperm and one vegetative nucleus	Prophase of meiosis I
84	Pollen tube in embryo sac	Maturation of female gametophyte
98	Pollen tube degeneration	Two-celled proembryo

Zhang & O'Neill, Plant Cell 1993

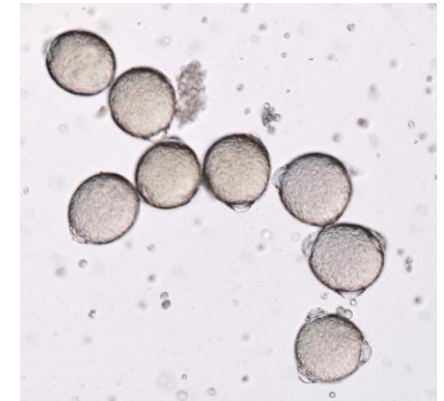
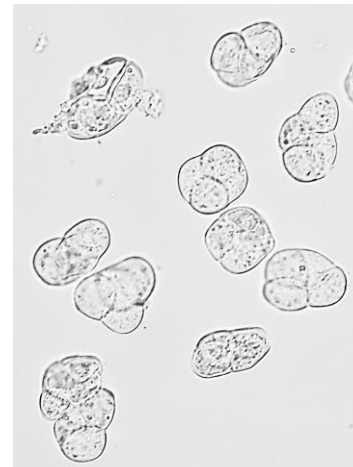
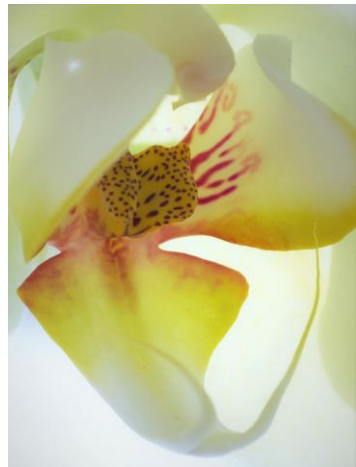
Can orchid pollen be analysed by IFC ?



Soft pollinia



Hard pollinia



Cucumber pollen

System set-up



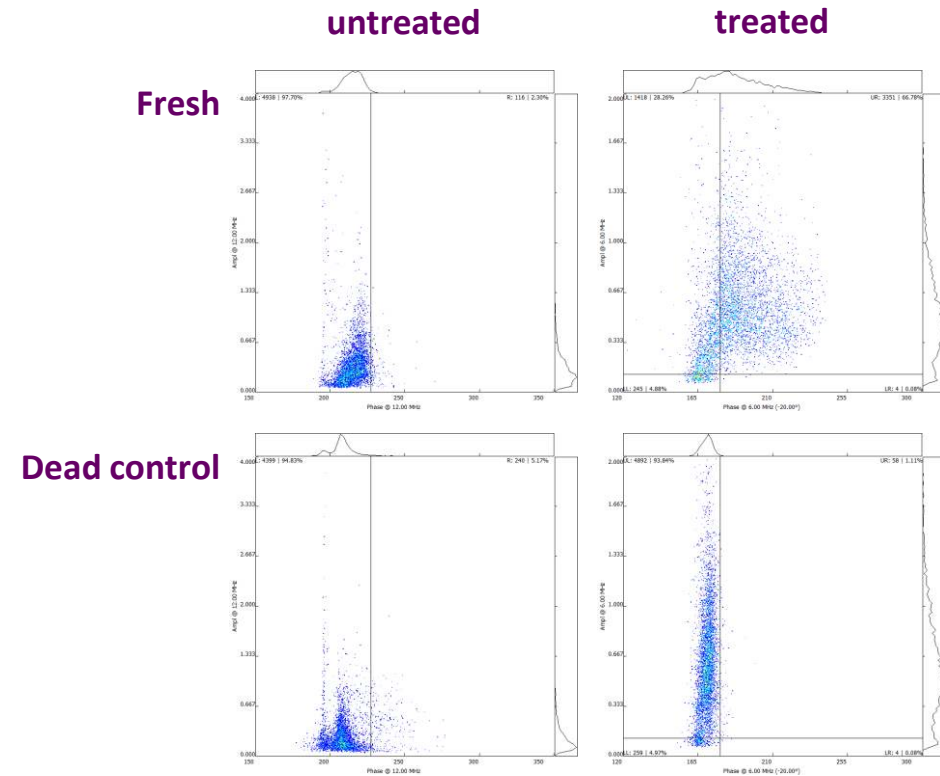
Facts:

- ❖ Orchid pollen is packed in compact pollinia
- ❖ Stays in tetrad form after meiosis
- Develop a non-destructive isolation method
- Right AF buffer
- Correlation to observations

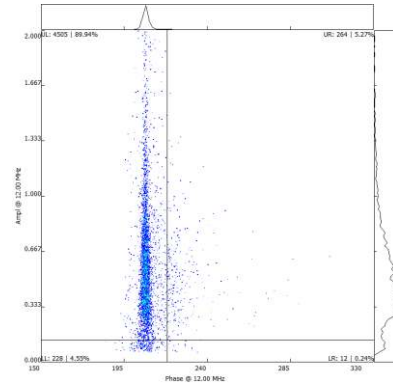
Results:

- Orchid pollen needs a pre-treatment prior measurement

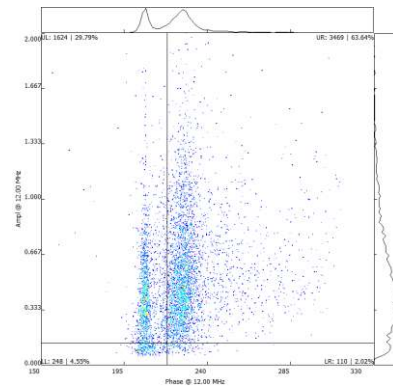
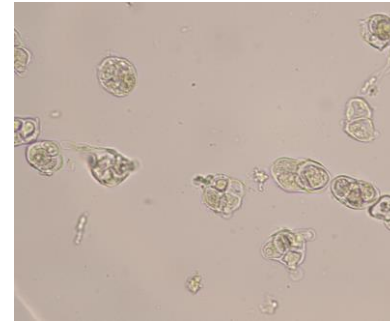
IFC analysis of orchid pollen



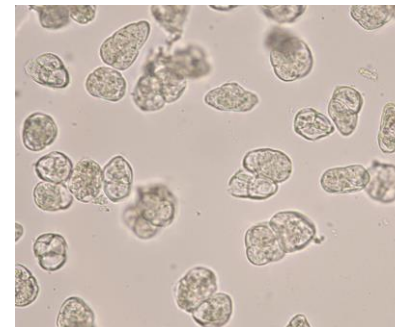
Pollen viability in orchids



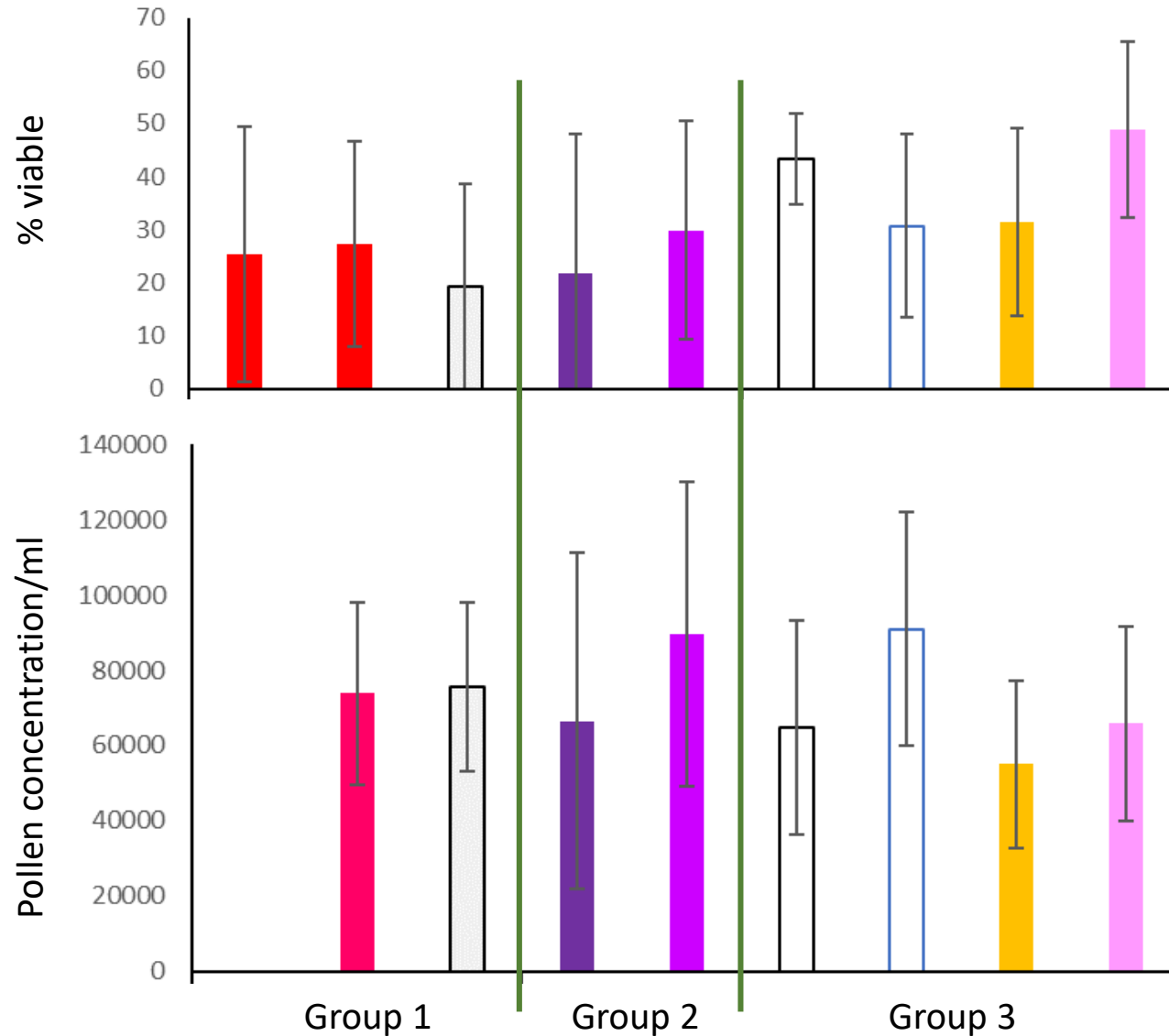
Not well developed



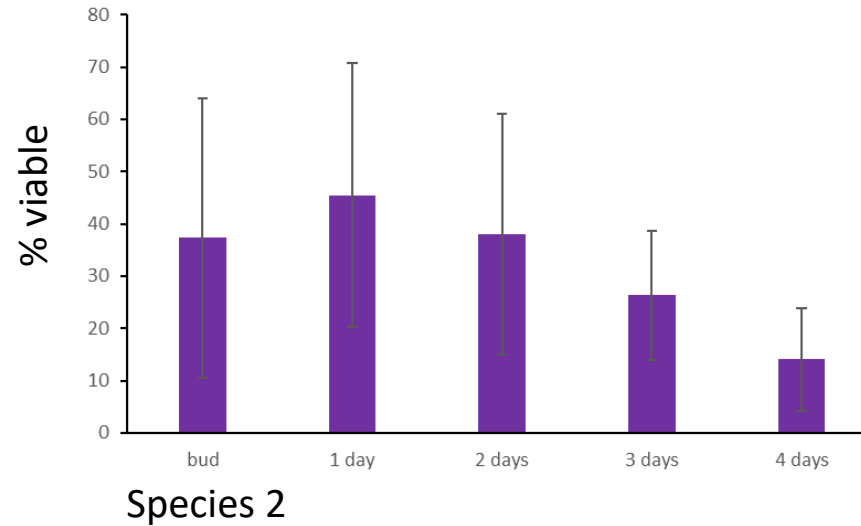
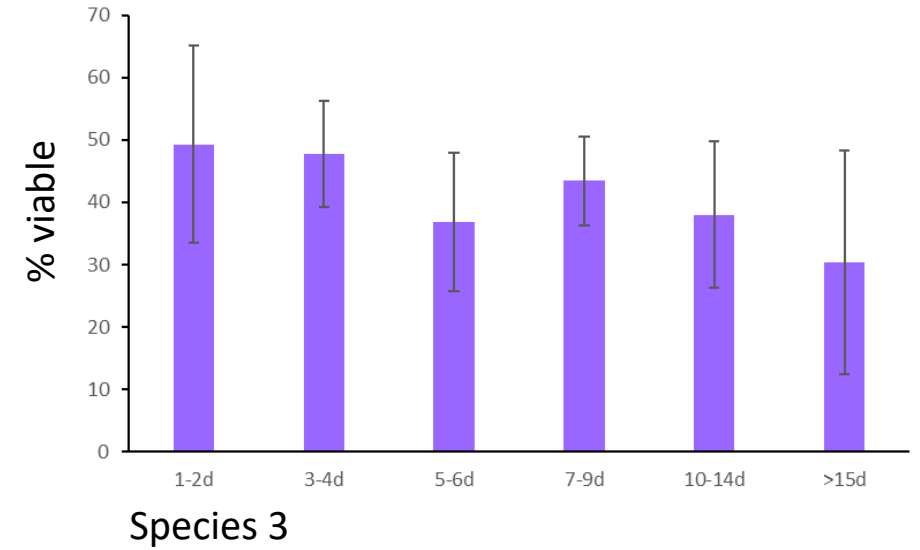
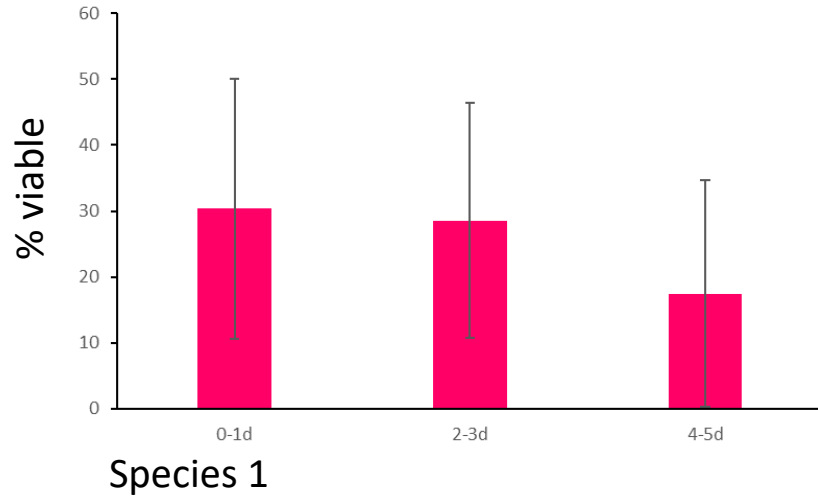
Well developed



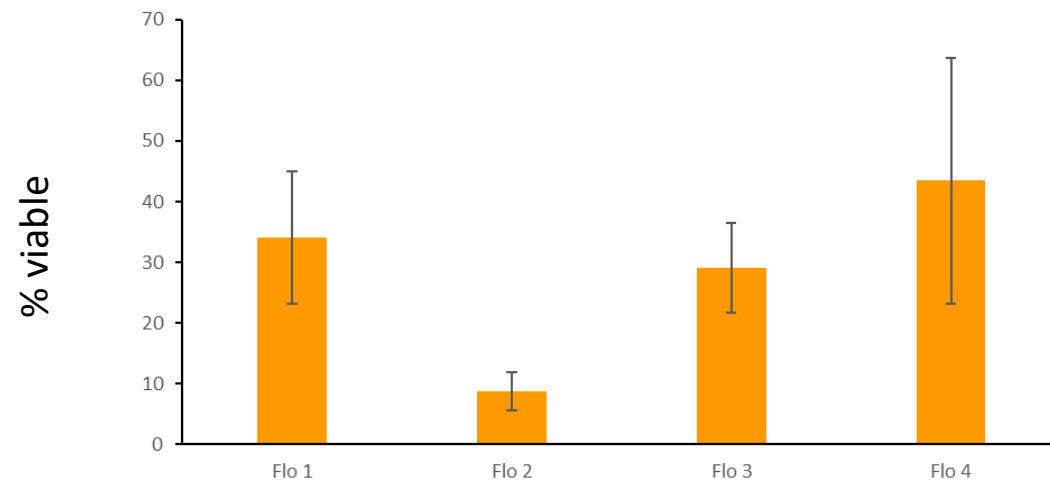
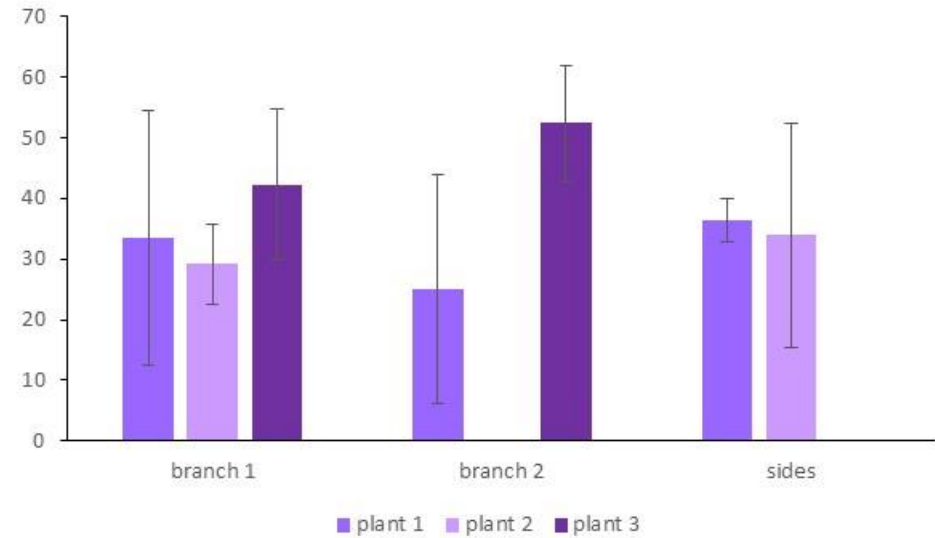
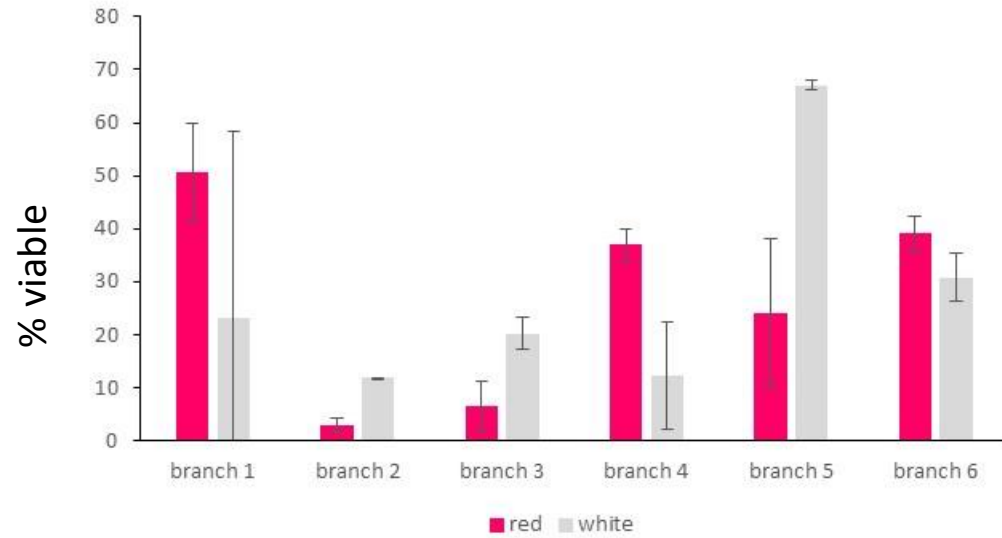
Pollen number and viability in orchids



Pollen viability and floral longevity



Orchid pollen viability can vary per branch



Flower position along branch

Conclusion

- Orchid pollen can be analysed by IFC
- Pre-treatment required
- High variation of viability
- Difference per species/line, even per branch/flower
- Condition and genotype dependent?
- Relation to pollen germination ?
- Successful crosses ?
- Other orchids





Questions ?