Effects of hybridization on expression of apomixis in the *Ranunculus auricomus* complex

FWF project I 310-B16

Project leader FWF: Elvira Hörandl

Dept. of Systematic Botany

Albrecht von Haller Institute for Plant Sciences

Georg-August-University of Göttingen

Germany

Austrian Research Foundation (FWF)

Project leader DFG: Timothy Sharbel

Dept. of Cytogenetics and Genome Analysis

Leibniz Institute of Plant Genetics and Crop Plant Research

Gatersleben, Germany

IPK GATERSLEBEN

DFG Deutsche Forschungsgemeinschaft

Goals of the project

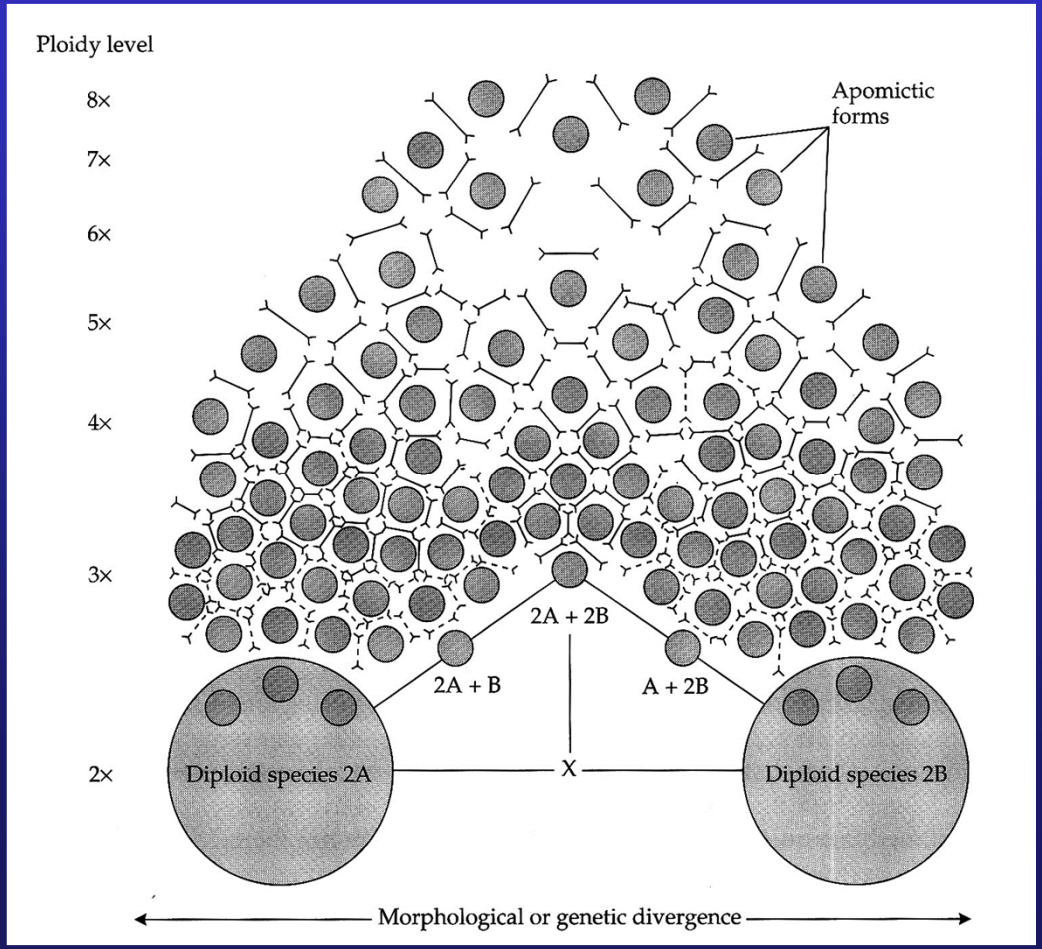
In flowering plants, hybridization and polyploidy are important evolutionary processes and are often connected to changes in the reproductive system.

The aim of the project is to study effects of interspecific hybridization and polyploidy on evolution of apomixis, the asexual reproduction via seed (apomixis) in the *Ranunculus auricomus* complex. We use comparative transcriptomics, NGS technologies, flow cytometry, microscopy and experimental tests.

We are further interested in phylogenetic distribution and biogeography of apomixis in flowering plants.

The evolution of apomictic polyploid complexes

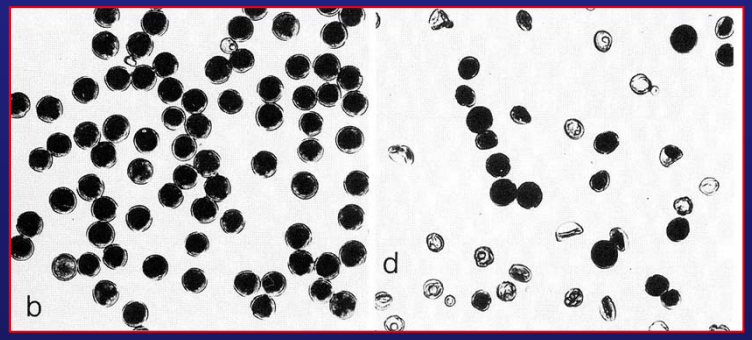
- Apomixis arises in allopolyploid hybrids
- Loss of genetic variation with higher ploid
- Accumulation of deleterious mutations over time (Muller‘s ratchet)



Stebbins, Grant

The *Ranunculus auricomus* complex

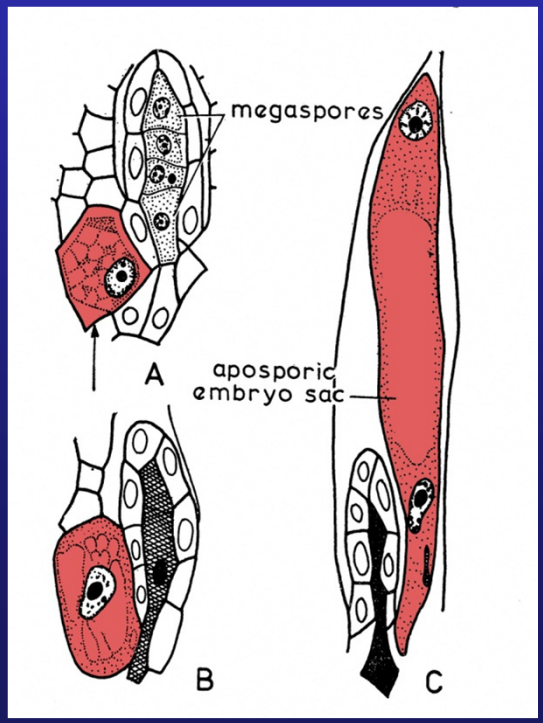
sexual apomicts



Hörandl et al. 1997

- Apospory
- Pseudogamy
- Heritable, genetic control
- Highly facultative

Nogler 1984; Rutishauser 1954



Natural and experimental hybrids

R. notabilis 2n = 16

*lowland, **early** ecotype*
Begin April (8.9°C)

R. carpaticola 2n = 16

*Slovak mountain range, **late** ecotype, May*

R. cassubicifolius 2n = 32

*Pre-Alps, **late** ecotype*
May (10.8°C)

Natural hybrids

Apomictic, 2n = 48

Paun et al. New Phytol 2006

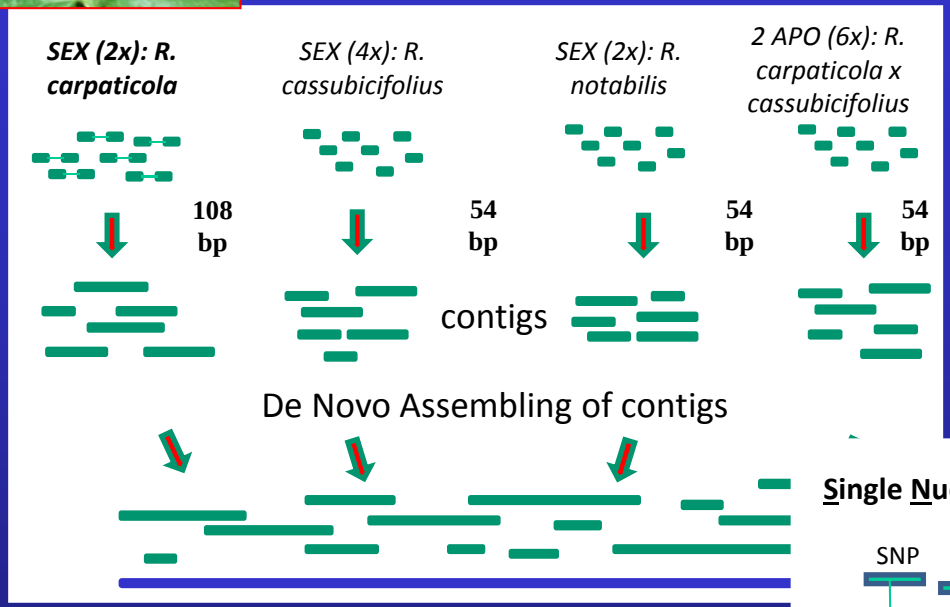
Experimental hybrids

2n = 16, sexual or apomictic?

2n = 24, sexual or apomictic?

Transcriptomics

(Illumina sequencing of RNA in flowering buds)



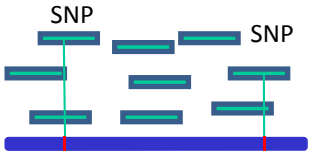
cDNA normalized

R. carpaticola as reference genome (108mer PE)

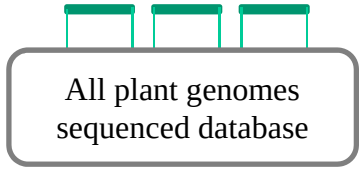
Assembly: CLC genomic workbench

SNP calling using BWA

Single Nucleotide Polymorphisms



Contigs annotation: gene function



SNPs mapping and genomic divergence of cytotypes / species

Pellino et al., Molec Ecol in press

Ranunculus RNA Seq data

172.506 genes in 29.274 contigs

33.986 coding sequences

Overview SNP detection

	apo2915	apo3528	carpaticola	cassub	notabilis	all(SE)	all(BAM)
total SNPs	66.712	59.997	427.739	52.340	61.535	256.105	443.727
filtered SNPs	35.925	32.145	336.559	27.243	34.288	215.317	353.552
CTGs with SNPs	10.924	10.120	44.854	8.781	10.194	42.427	46.978

Gene annotation via BLAST: 1822 contigs (157 chloroplast)

6099 annotated SNPs (possible mutation sites)

1677 phylogenetically informative SNPs

SNP analysis and gene annotation

- No genome-wide mutation accumulation in apomicts
- Apomicts are less than 100,000 years old
- Single hybrid origin
- Genes related to meiosis and gametogenesis are under diversifying selection

Pellino et al., submitted

Sexual species show teh genomic signature of allopatry; autopolyploids differ only slightly from their diploid progenitors

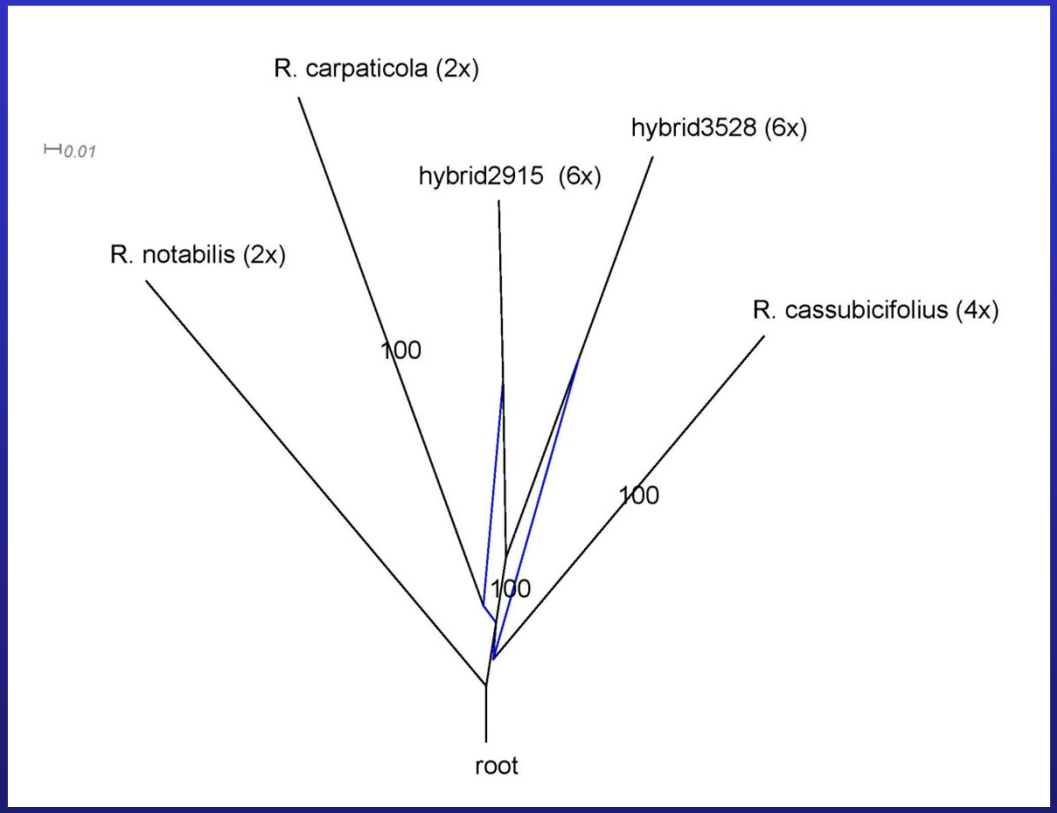
Hojsgaard et al. subm.

SNP analysis: Evolutionary origin

Shared presences (in at least two taxa) of SNPs:
Matrix of 1677 phylogenetically informative sites – Nexus file

Hybridization Network

(Splitstree 4.0)
Huson & Bryant, 2006)



Apomicts: max. 66,000 years old

Fit =100%

Rapid divergence of apomictic lineages after their hybrid origin

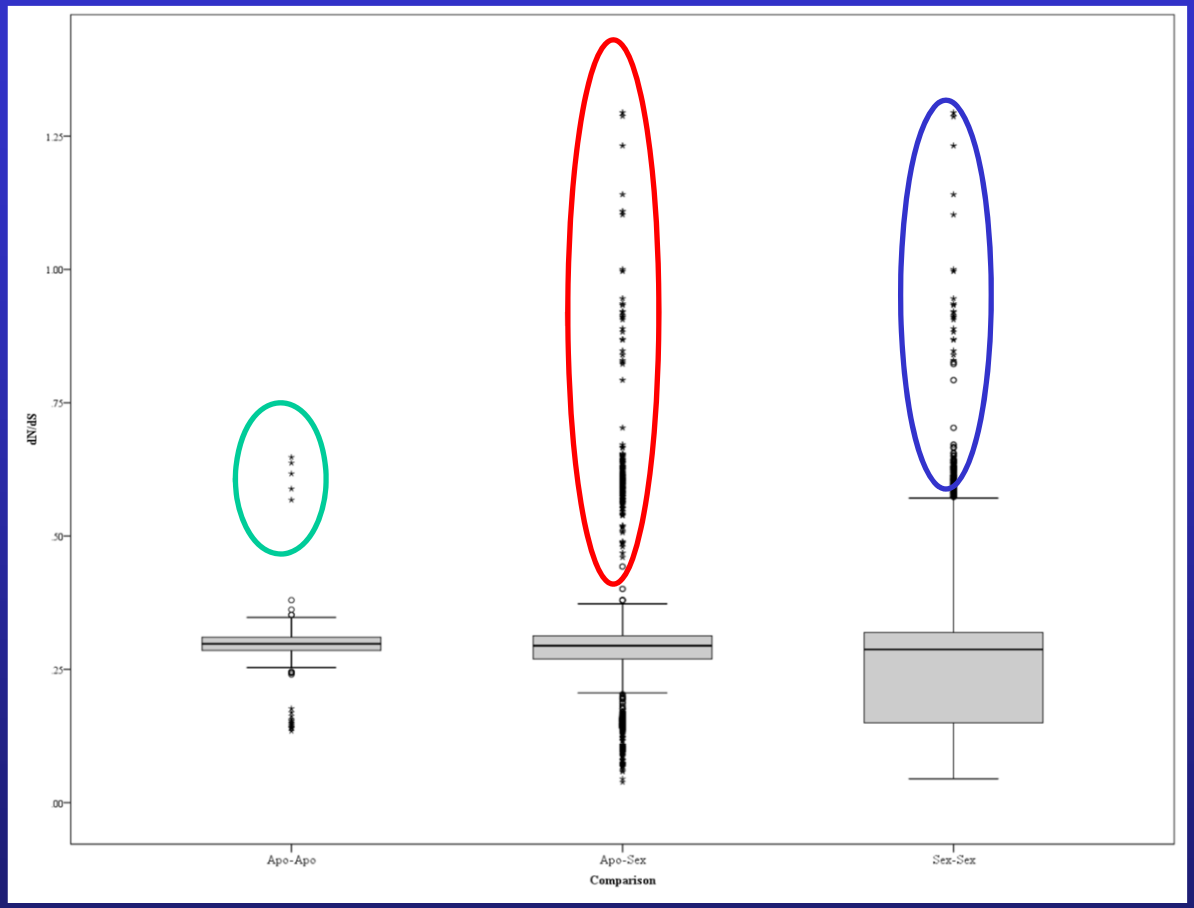
Pellino et al., Molec Ecol in press

SNP analysis: divergence and loci under selection

800 annotated genes analyzed in an open reading frame

ratio non-synon. / synonymous substitutions

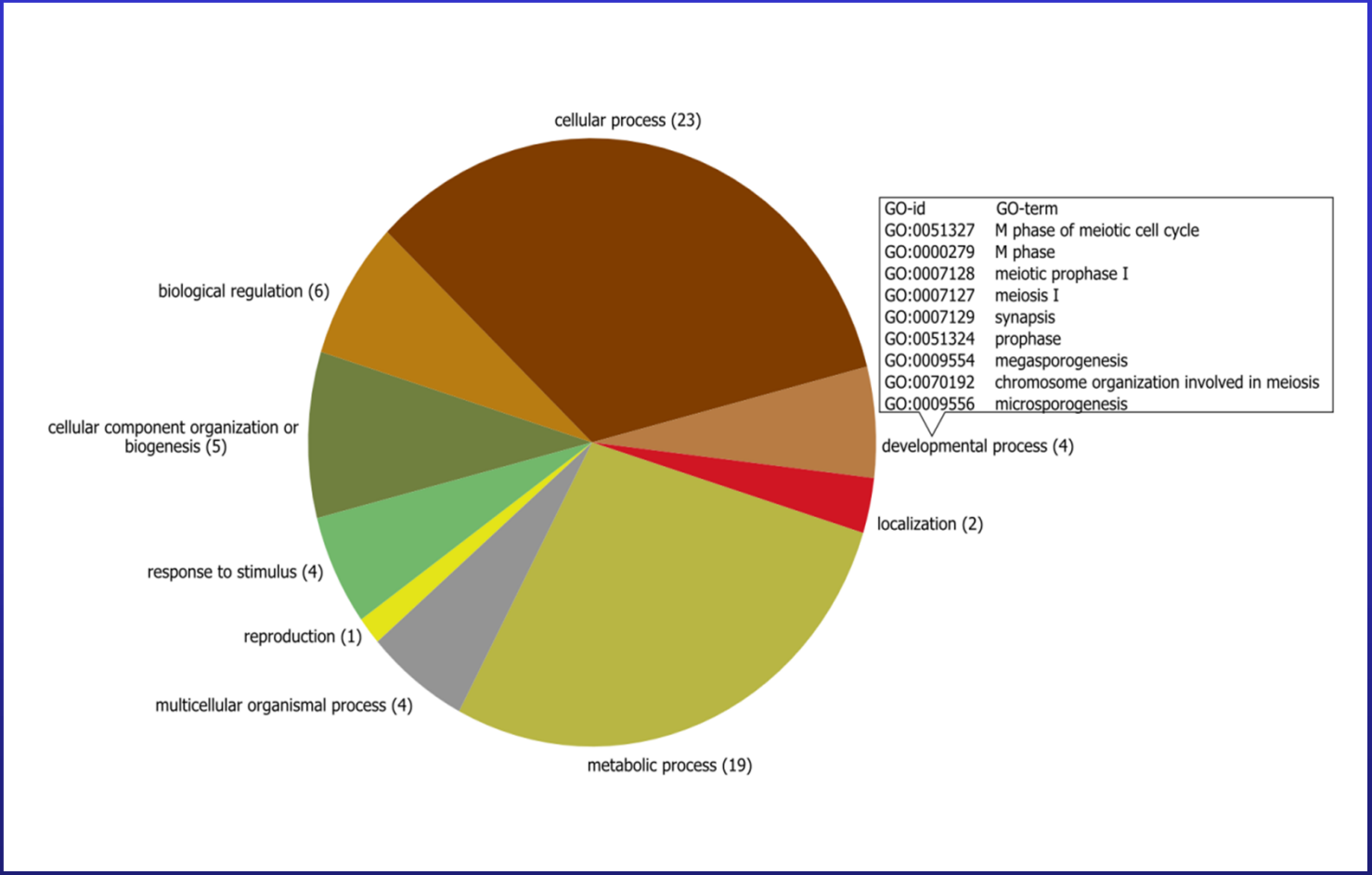
Outlier genes are under diversifying selection



6x apo/6x apo | **6x apo/2x sex** | **2x sex/2x sex**

Divergence | Functional shift | Allopatry

Gene annotation: outliers in the dn/ds ratios



Sex / apo comparison:
Nine genes associated to cell cycle are under diversifying selection

Pellino et al., Molec Ecol in press

Gene annotation: outliers in the dn/ds ratios

GO Term Biological function

GO-id	GO-term
GO:0051327	M phase of meiotic cell cycle
GO:0000279	M phase
GO:0007128	meiotic prophase I
GO:0007127	meiosis I
GO:0007129	synapsis
GO:0051324	prophase
GO:0009554	megasporogenesis
GO:0070192	chromosome organisation involved in meiosis
GO:0009556	microsporogenesis

Sex / apo comparison:
Nine genes under diversifying selection are related to sex/apo shift

No overall genome-wide mutation accumulation in apomictic lineages

Key results

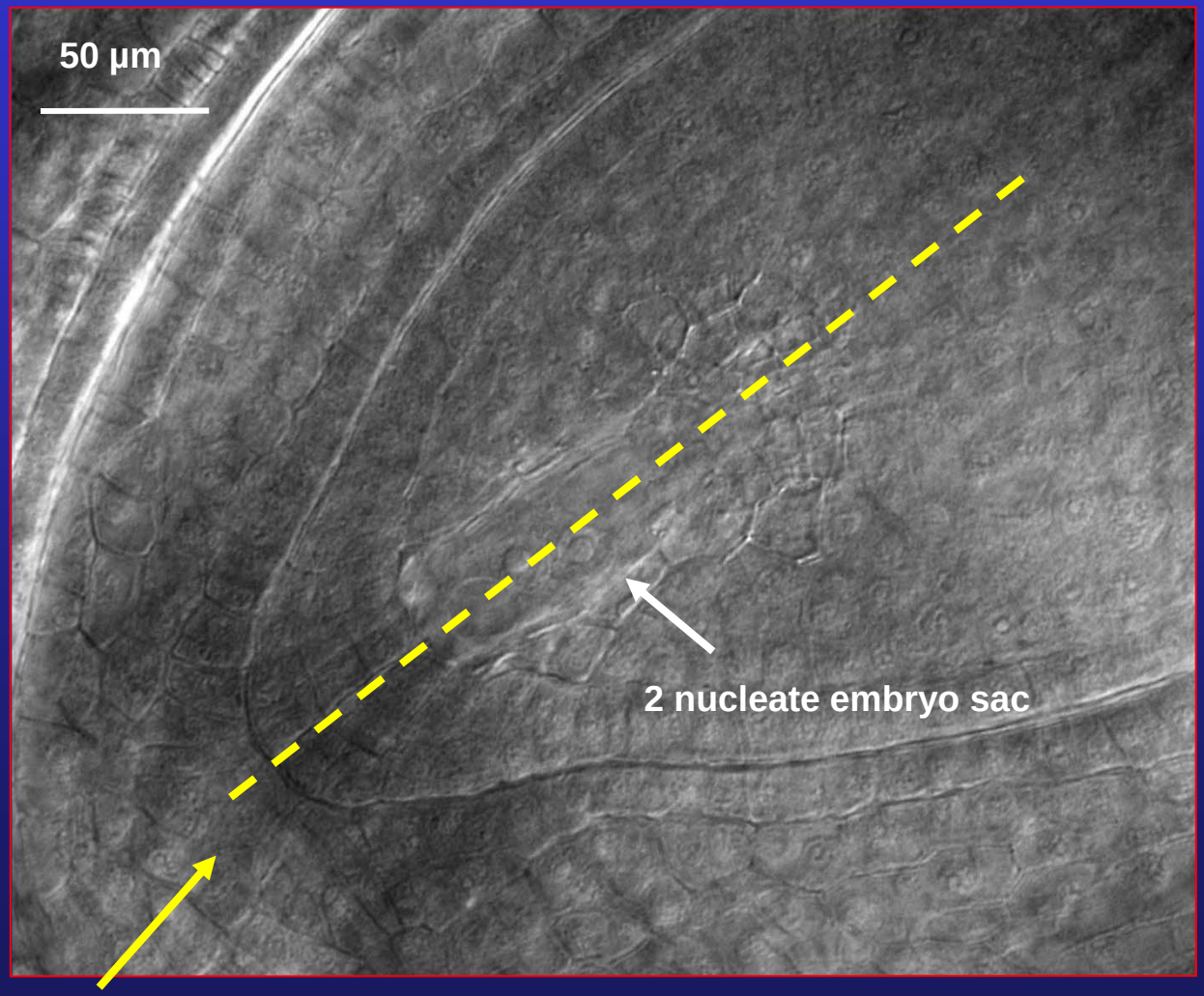
Embryological and developmental studies

- **Experimentally produced F1 hybrids show elements of apomixis (apospory), but not yet functional apomictic seeds**
- **This change is related to asynchrony and shifts in timing of developmental steps: hybrids have a delayed development**

Hojsgaard et al., subm.

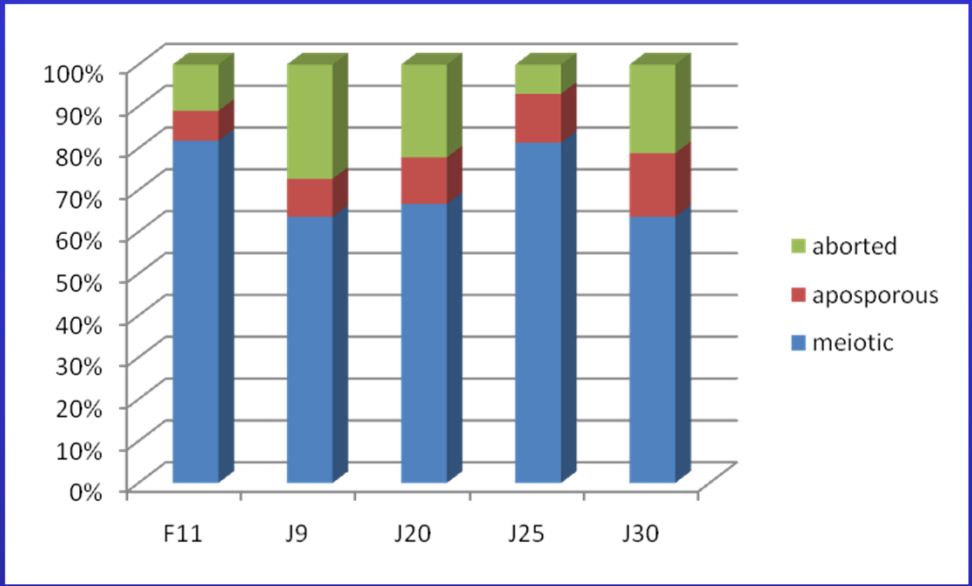
Embryo sac in 2x and 3x F1 hybrids

Immature ES during anthesis

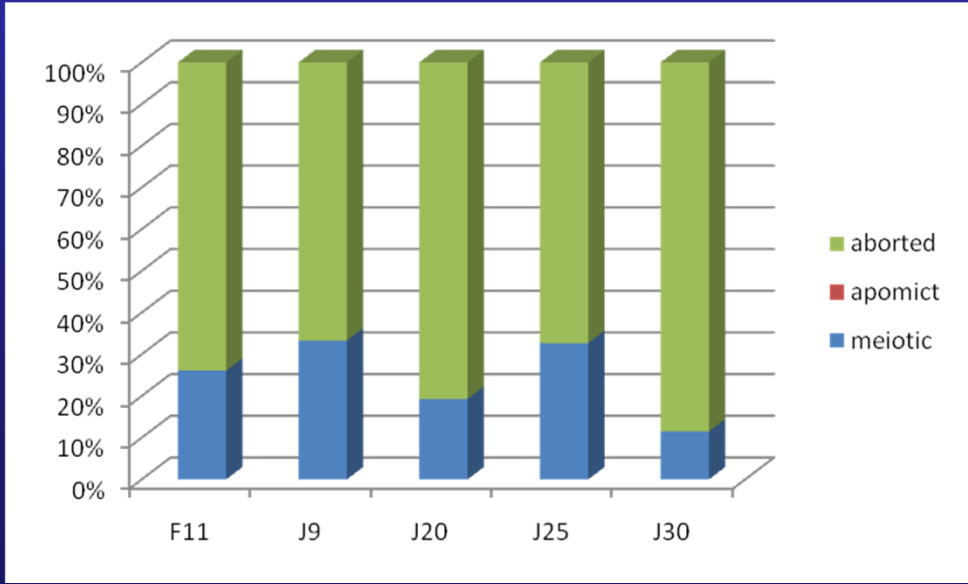


2x hybrid genotypes

Percentages of ovules with functional initial cells:

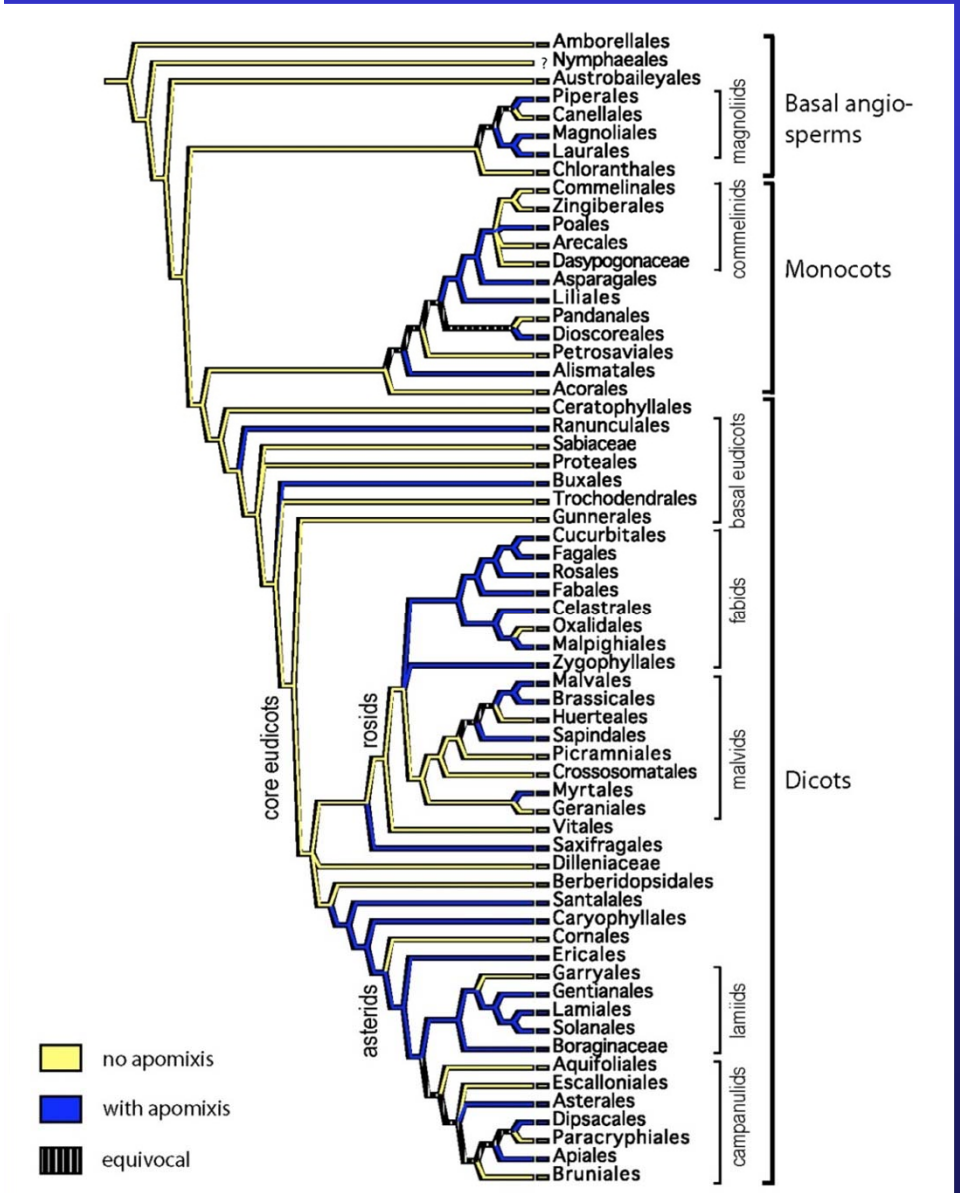


Flow cytometric seed screen: Percentages of seeds of meiotic or apomictic origin:



Key results

Apomixis in angiosperms



Citrus spp.

Hypericum perforatum

Taraxacum

Rubus fruticosus

Poa pratensis

Hörandl & Hojsgaard 2012, Plant Biosystems

Key results

Apomixis in angiosperms

- **A searchable internet database has been installed at**

  http://www.apomixis.uni-goettingen.de

- **Apomixis is widespread and scattered in the phylogeny**
- **Apomixis is associated to diversity and large geographical distribution areas**

Hörandl & Hojsgaard, Plant Biosystems, 2012

Hojsgaard et al., subm.

Further results

Facultative apomixis and environmental stress

- **Apomixis is facultative and could be responsive to environmental stress. We elaborated a theory that oxidative stress and subsequent DNA lesions induce meiosis.**

Hörandl, 2013

Hörandl & Hadacek subm.

We will test for the response of apomixis to light stress conditions with 6x clones of the R. auricomus complex.

Klatt et al. in prep.

Further results

Evolution of apomictic hybrids

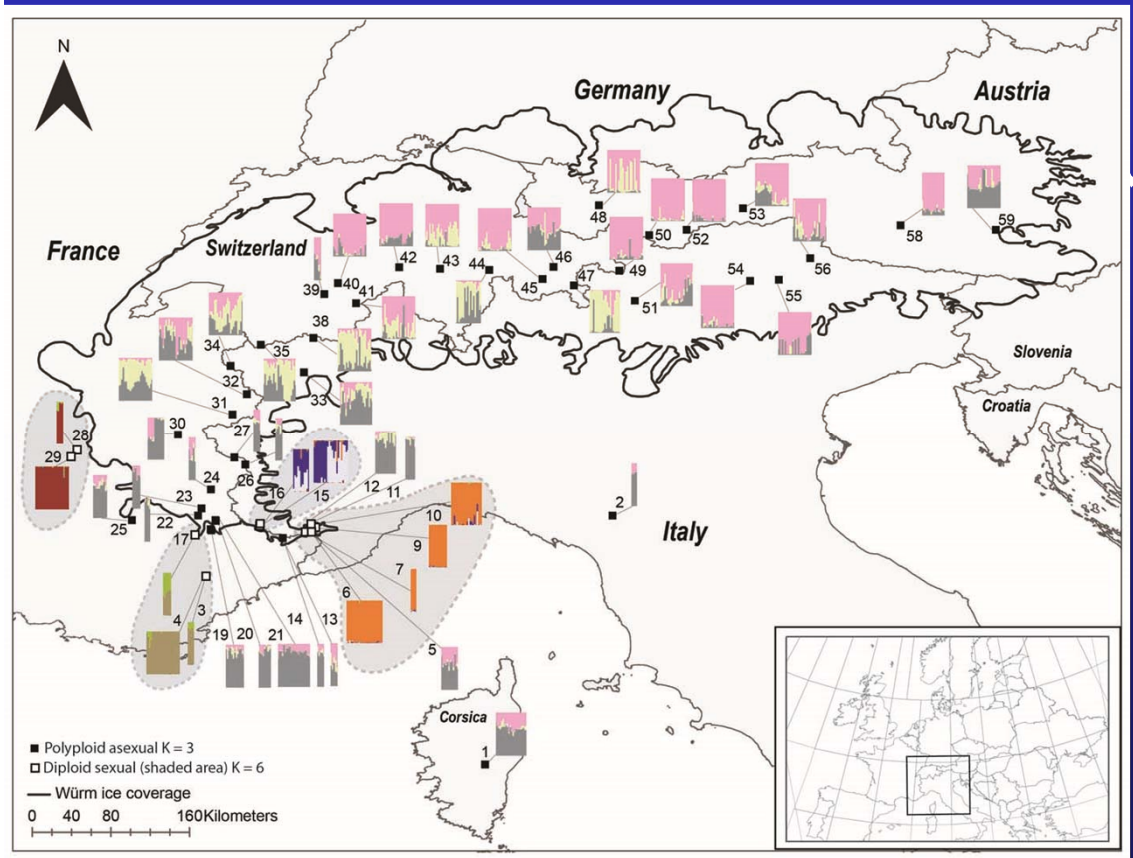
- **Experimental hybrids *R. carpaticola* x *R. notabilis* resemble morphologically the natural 4x hybrid *R. variabilis* (widespread in Austria)**
- **ITS sequence polymorphisms and geometric morphometrics support a hypothesis of hybrid origin of *R. variabilis***

Hodac et al., in prep.

Further results

Geographical parthenogenesis

- **Apomictic plants are better colonizers than related sexuals**
- **Population genetic studies suggest that facultative apomixis preserves genetic diversity, but allows for rapid expansion into remote areas (Baker‘s law)**



Cosendai et al. **2013**

Hörandl et al. 2011

Publications

(Published and submitted)

Cosendai AC, Wagner J, Ladinig U, Rosche C, Hörandl E (2013) Geographical parthenogenesis and population genetic structure in the alpine species Ranunculus kuepferi (Ranunculaceae). HEREDITY, 110: 560-569.

Emadzade, K., Gehrke, B., Linder, H.P., Hörandl, E. 2011. The biogeographical history of the cosmopolitan genus Ranunculus L. (Ranunculaceae) in the temperate to meridional zones. MOLEC. PHYLOG. EVOL. 58: 4-21.

Hörandl E, Emadzade K (2012) Evolutionary classification: A case study on the diverse plant genus Ranunculus L. (Ranunculaceae). PERSPECT PLANT ECOL, 14: 310-324.

Hörandl E, Hojsgaard D (2012) The evolution of apomixis in Angiosperms: a reappraisal. PLANT BIOSYSTEMS, 146: 681-693.

Hörandl, E., Dobes, C., Suda, J., Vit, P., Urfus, T., Temsch, E. M., Cosendai, A.-C., Wagner, J., Ladinig, U. 2011. Apomixis is not prevalent in subnival to nival plants of the European Alps. ANNALS OF BOTANY 108: 381–390.

Hörandl E. 2013. Meiosis and the paradox of sex in nature. In: Bernstein C, Bernstein, H. (ed) Meiosis. InTech, Rijeka, Croatia (in press).

Hojsgaard D., Klatt, S., Baier, R. Carman, J., Hörandl, E. submitted. Apomixis in angiosperms: taxonomic distribution, associations to biodiversity, and biogeographical traits.

Hojsgaard D, Pellino M, Schmutzer T, Scholz U, Vogel H, Sharbel TF, Hörandl E, 2013. .Genome evolution and development in sexual diploids and tetraploids of the Ranunculus auricomus complex. Under revision.

Pellino M, Hojsgaard D, Schmutzer T, Scholz U, Hörandl E, Vogel H, Sharbel TF. 2013. Asexual genome evolution in the apomictic Ranunculus auricomus complex: examining the effects of hybridization and mutation accumulation. MOLEC ECOL. In press.

Acknowledgments

Postdocs and students in Göttingen

- Diego Hojsgaard
- Simone Klatt
- Ladislav Hodac
- Armin Scheben

In Gatersleben:

- Thomas Schmutzer
- Marco Pellino
- Uwe Scholz

Further collaborations

- *Johanna Wagner (Innsbruck)*
- *Stefan Dullinger (Vienna)*
- *John Carman (Logan, Utah, U.S.)*
- *Franz Hadacek (Göttingen, Germany)*
- *Johann Greilhuber (Vienna, Austria)*
- *Camilo Quarin (Corrientes, Argentine)*
- *Fulvio Pupilli (Perugia, Italy)*

Further support by the
EU COST program “HAPRECI”