Why are flowering plants so diverse?
Why are flowering plants so diverse?

Evolution of Development (Evo-Devo)
The Logic

- Morphology is the product of development
- Development results from genetic regulatory programs.
- Evolution of diversity is directly related to the evolution of genetic regulatory programs.
- A limited number of genetic pathways have been used and re-used to build different body plans.
Using the Evo-Devo Approach to study floral diversity

Developmental processes that occur in the flowers different taxa.

Infer evolutionary events that led to the differences or similarities.

Floral morphology can be described with different characteristics

- Phyllotaxy
- Symmetry
- Organ Fusion
- Whorl Number
- Organ Elaboration
- Organ Identity
Floral Symmetry

Bilateral Symmetry has evolved many times

L. vulgaris peloric mutations caused by methylation of CYCLOIDEA
Floral Symmetry controlled by *CYCLOIDEA (CYC)* and *DICHOTOMA (DICH)*

*Antirrhinum majus*

WT

Peloric

Mohavea confertiflora - Changes in symmetry with an evolutionary effect

*Cubas 2004*

Hileman *et al.* 2003
M. confertiflora mimics a radial species.

Mohavea confertiflora

Mentzelia involucrata

CYC affects symmetry in Helianthus

Cubas 2004
Changes in *CYC* expression cause “Van Gogh's sunflowers”

**Summary**

- Bilateral symmetry has evolved many times independently.

- *CYC* homologs appear to have been recruited as symmetry genes in all cases of bilateral symmetry within the core eudicots.
Floral Organ Identity

Different phases of shoot growth are regulated by environmental and endogenous signals.
Floral Organ Identity genes are MADS Box genes = Transcription Factors

MADS-Box genes in flowers are important for correct organ identity. HOX genes in animals are important for correct body-plan formation.

MIKC-type MADS-Box genes

- **MADS**
  - Highly conserved 60 amino acid domain involved in DNA binding and protein dimerization.

- **I**
  - “Intervening” domain, plays role in dimerization specificity.

- **K**
  - Alpha-helical K-box, involved in protein dimerization.

- **C**
  - Generally divergent domain with small highly conserved motifs, recently implicated as mediator of ternary protein complex formation.
MIKC-type MADS-Box genes are involved in many aspects of plant development

“ABC” Model of Flower Development

'A' genes control the sepals
'A' and 'B' genes in combination control the petals
'B' and 'C' genes in combination control the stamens
'C' genes control the carpels
Homeotic Mutants – used to study HOX genes in *Drosophila*

HOX Genes

WT

antennapedia

Carroll et al. (2005)

Floral homeotic mutants
How the ABCE Model was determined

“Sliding Boundary” model is one modification to the ‘ABC’ Model

Explaining diversity with the ABC model

Transference of genetic identity from one structure to another
**Pivotal phylogenetic position** - Ranunculales

**High success with gene silencing techniques** (VIGS)

**Many horticultural mutants available**

**Numerous duplications of Floral Organ Identity Genes**

---

**Thalictrum thalictroides** is a good model system for studying Floral Evo-Devo

- **Wildtype**
- **‘Shoaff’s Double’**
- **‘Betty Blake’**
- **‘Green Dragon’**
- **‘Double White’**
C-class and D-class Function in *Thalictrum*

Floral Organ Identity Genes control development along the floral axis
C-class function: conserved carpel and stamen identity across the angiosperms

Arabidopsis C-class gene: *AGAMOUS (AG)*

Kramer *et al*. Genetics 2004

Bowman *et al*. Plant Cell 1989
Riechmann & Meyerowitz. Molec Biol Cell 1997

D-class function: ovule identity is mostly redundant among C- and D-lineage genes

Petunia D-class genes: *FBP7 & FBP11*

Kramer *et al*. Genetics 2004

Angenent *et al*. Plant Cell 1995
Thalictrum has two AG homologs with different expression patterns.

**C-class Genes**
*ThtAGAMOUS1 (ThtAG1)*
*ThtAGAMOUS2 (ThtAG2)*

**No D-class Genes**
*ThdAG1*

Hypothesis

After the duplication event that led to *ThAG1* and *ThAG2*:

- *ThAG1* kept the C-function role in stamen and carpel identity.
- *ThAG2* sub-functionalized to become an ovule identity gene.
Forward Genetics – ‘Double White’ has a defect in *ThAG1*

Reverse Genetics – Down-regulating *ThAG1* creates a ‘double flower’ phenotype
Reverse Genetics – Down-regulating \textit{ThAG1} creates a ‘double flower’ phenotype

\textbf{Conclusion:} \textit{ThtAG1} does have the conserved C-function role in stamen and carpel identity.

\begin{itemize}
  \item \textit{T. thalictroides} WT
  \item \textit{T. thalictroides} ‘Double White’
  \item \textit{T. thalictroides} TRV2\textit{ThAG1}
\end{itemize}

Double Flowers – the first recorded floral mutants

\begin{itemize}
  \item Theophrastus 371-287 BC
  \item \textit{T. thalictroides} WT
  \item \textit{T. thalictroides} ‘Double White’
  \item \textit{Arabidopsis} WT
  \item \textit{Arabidopsis agamous} mutant
\end{itemize}
VIGS of *ThtAG2* does not result in a double-flower

*ThtPDS* Bleaching

*TRV1*

\[ \text{2 x 35S} \quad \text{RdRp} \quad \text{MP} \quad \text{16K} \quad \text{Rz NOS} \]

*TRV2*

\[ \text{2 x 35S} \quad \text{MCS} \quad \text{CP} \quad \text{Rz NOS} \]

*TRV2* _*ThtAG2_* _*ThtPDS*_

\[ \text{2 x 35S} \quad \text{CP} \quad \text{Rz NOS} \]

*ThtAG2*-silenced ovules have a carpel-like morphology and lack an embryo sac.

**Ovule Structure**
- oi = outer integument
- ii = inner integument
- es = embryo sac
- nu = nucellus
- st-l = style-like

**Carpel Structure**
- st = style
- sg = stigma
- ov = ovule
Conclusion: *ThtAG2* has sub-functionalized and is now an ovule identity gene.

**Summary**

- Down-regulation of *ThtAG1* causes a double-flower phenotype.
- The horticultural mutant ‘Double White’ has a transposon disrupting *ThtAG1*.
- Down-regulation of *ThtAG2* causes the loss of the embryo sac and the development of a carpel-like structure in place of the ovule.

**After the duplication that led to *ThtAG1* and *ThtAG2***

- The C-class gene *ThtAG1* kept the conserved C-function role in stamen and carpel development.
- The C-class gene *ThtAG2* has likely sub-functionalized to control ovule development – a role traditionally assigned to the D-class.