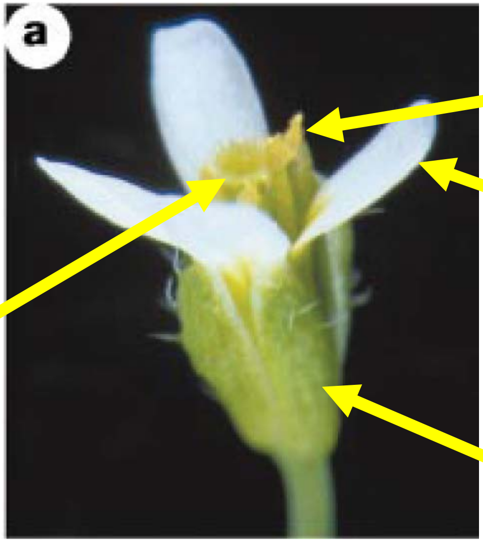
Genetic Specification of floral organ identity

Initiating floral development

Deciding when to initiate flowering - induced mutations

- in Nature

Flower structure of Arabidopsis

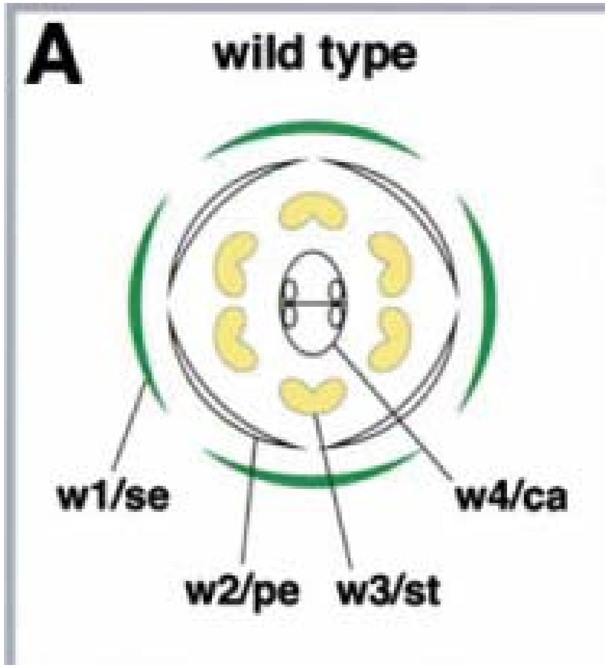


Stamens

Petals

Carpels

Sepals



In Arabidopsis:

Whorl 1	4 sepals
Whorl 2	4 petals
Whorl 3	6 stamens
Whorl 4	2 carpels

Mutations that affect flower development placed in classes

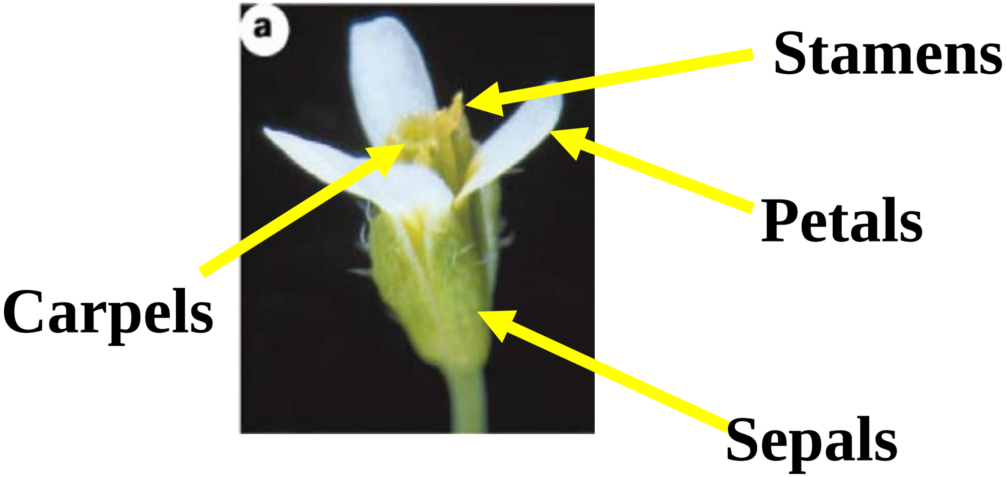
Alter the organs formed in two adjacent whorls

A mutants : affect sepals and petals

B mutants : affect petals and stamens

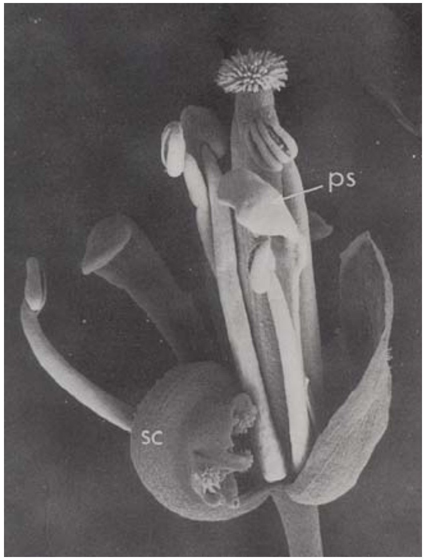
C mutants : affect stamens and carpels

Antirrhinum

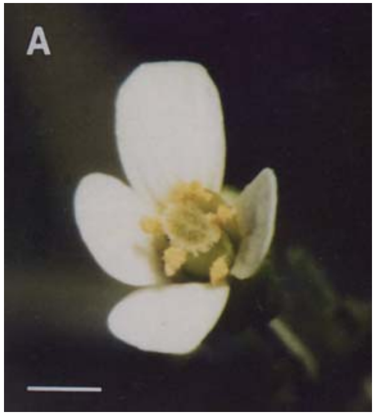


A function mutations affect flower development in Whorl 1 and 2

apetala2



apetala2



Wild-type

Sepals → carpelloid organs

petals → Absent or stamenoid

stamens → normal

carpels → normal

B function mutations affect flower development in Whorl 2 and 3

apetala3

pistillata

Wild-type

Sepals → normal

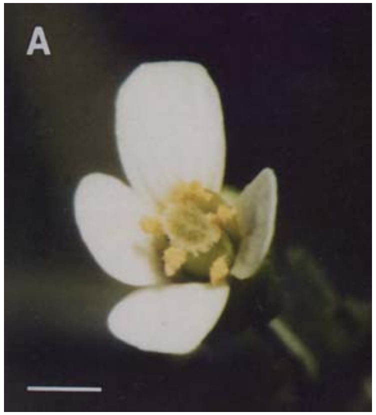
petals → Sepals or absent

stamens → carpelloid

carpels → normal

C function mutations affect flower development in Whorls 3 and 4

agamous



Wild-type

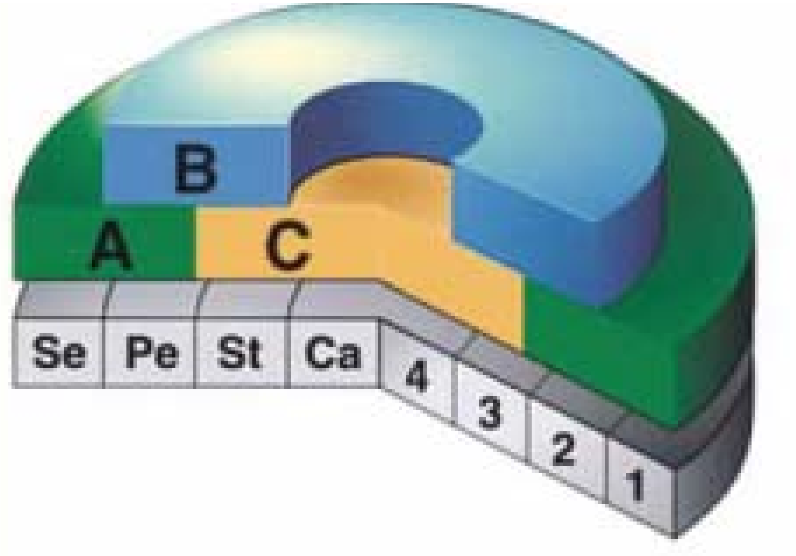
Sepals → normal

petals → normal

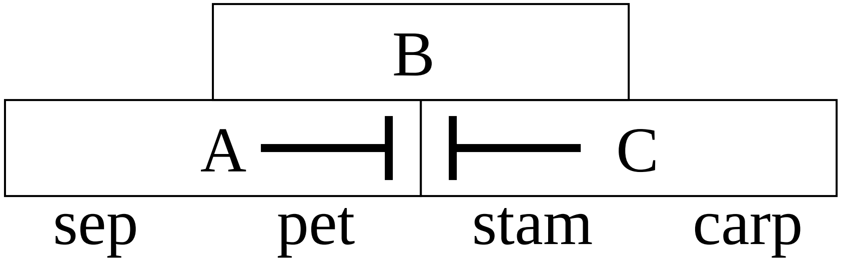
stamens → petals

carpels → Sepals and flower inside a flower

Specification of floral organ identity occurs in four circular domains called whorls and is controlled by three genetic functions called A,B and C



Analysis of mutant phenotypes suggested ABC model in which functions combine to create petal and stamen identities



Model suggests:

Overlapping functions provide new identities

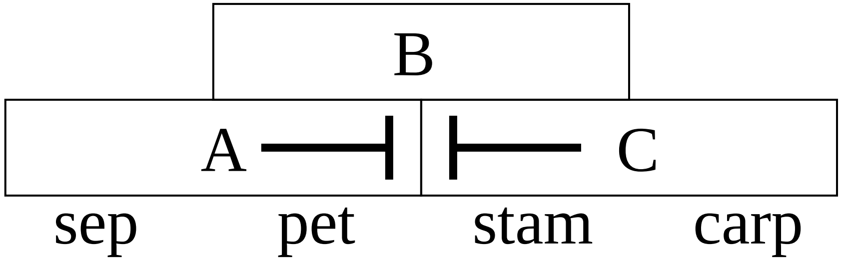
A function must oppose C and C must oppose A

Genes conferring different functions, identified from mutants:

A function - *APETALA1*, *APETALA2*
B function - *APETALA3 PISTILLATA*
C function - *AGAMOUS*

Interpretation of mutant phenotypes according to ABC model

Wild type

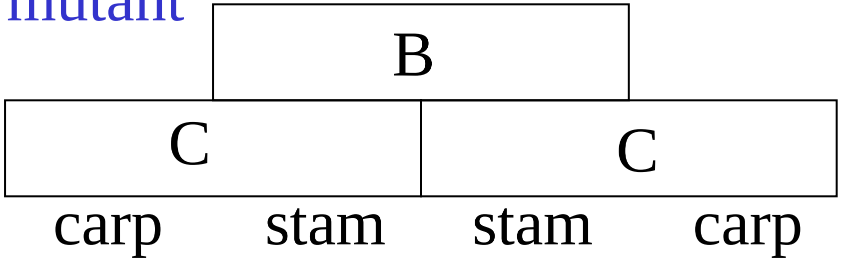


B

A ⊣ ⊢ C

sep pet stam carp

A mutant

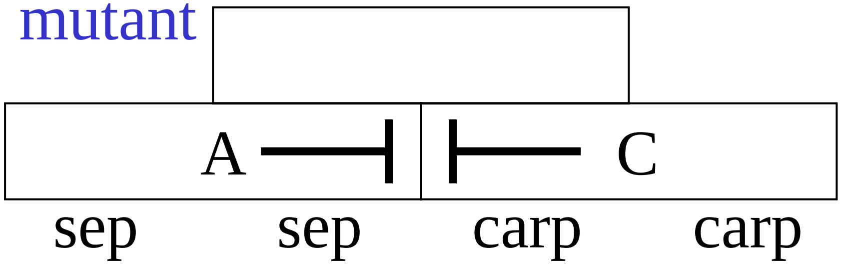


B

C C

carp stam stam carp

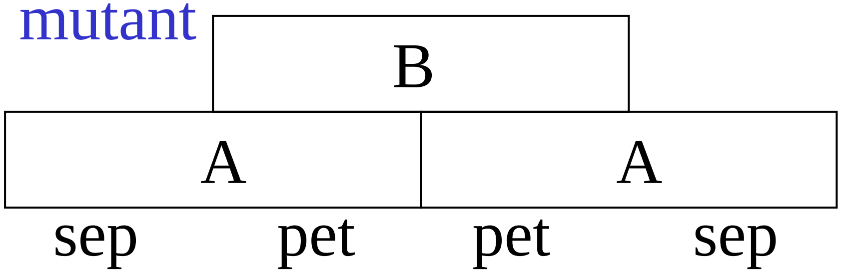
B mutant



A ⊣ ⊢ C

sep sep carp carp

C mutant



B

A A

sep pet pet sep

Double mutants

pi ag double mutant / loss of B and C function
All whorls are sepals
More whorls

Wild-type

ap2 pi double mutant /
loss of A and B function

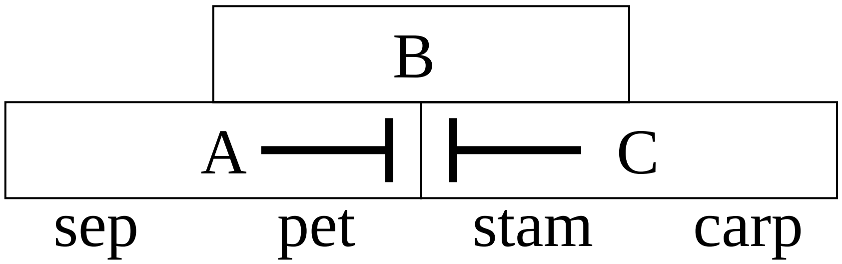
All whorls are carpels

ap2 ag double mutant / loss of A and C

Leaves in 1st and 4th whorl
Modified petals/stamens in second and third
More whorls

Interpretation of double mutant phenotypes according to ABC model

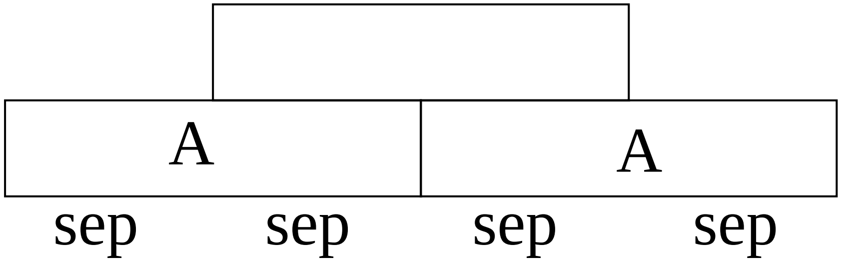
Wild type


B

A ⊣ ⊢ C

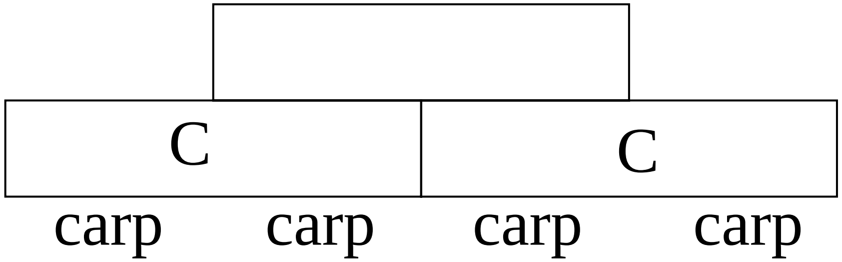
sep pet stam carp

BC mutant


A A

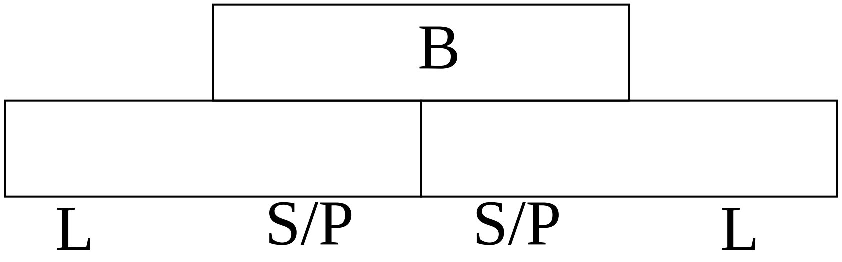
sep sep sep sep

AB mutant


C C

carp carp carp carp

AC mutant

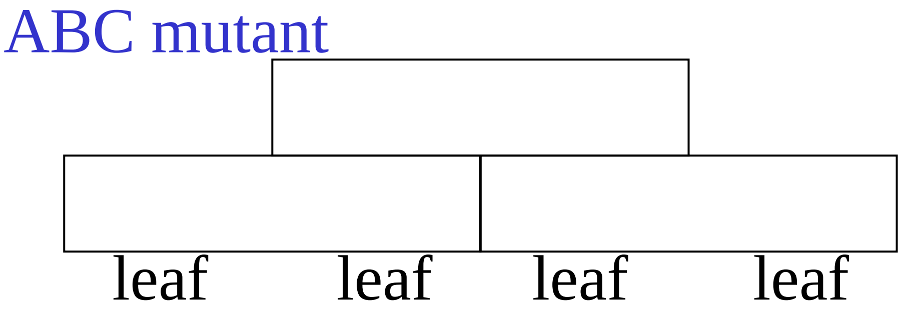

B

L S/P S/P L

ABC triple mutant phenotype

ap2 pi ag triple mutant / loss of ABC function

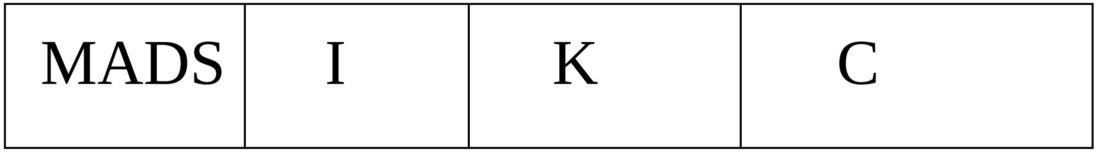
All whorls leaf like



ABC mutant

leaf leaf leaf leaf

Many ABC genes encode MADS box transcription factors
Structure of plant MADS box transcription factors



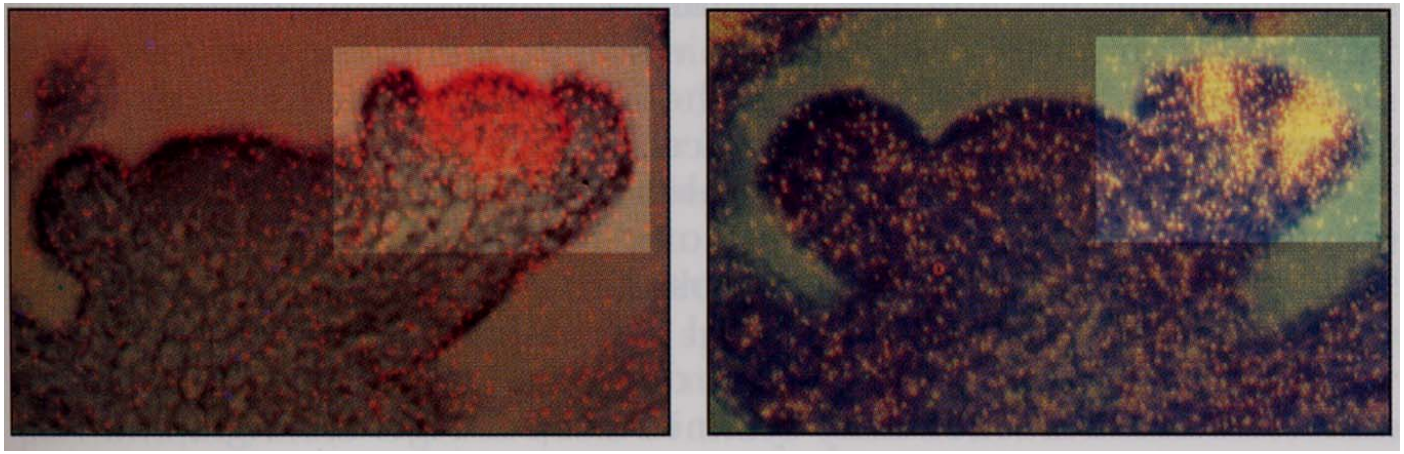
Domains in MADS transcription factors

MADS - DNA binding
I - intervening region
K - protein-protein interactions
C - carboxy-terminal domain

Bind DNA through a conserved DNA sequence called CArG Box – CC(A/T)$_6$GG

AP1, AP3, PI, AG are MADS box transcription factors

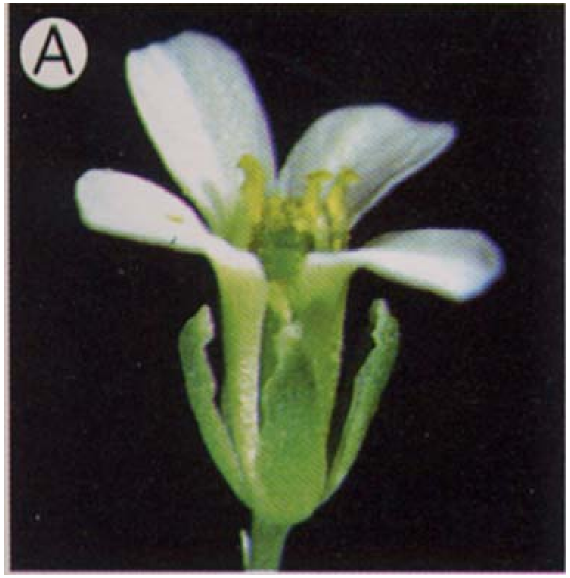
Patterns of expression indicate that spatial control is regulated mainly at the level of transcription



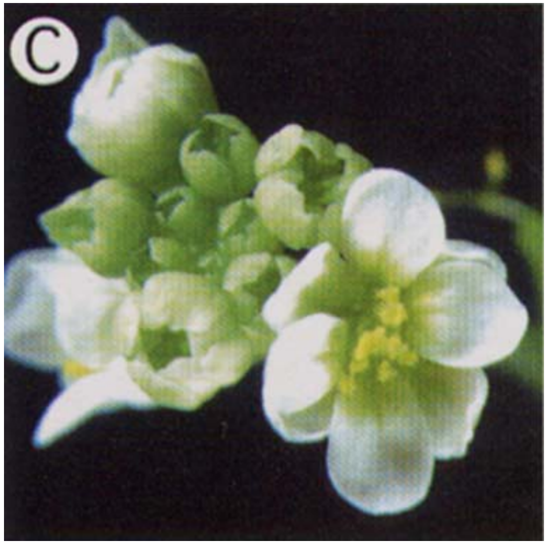
Agamous C function Expressed in whorls 3 and 4	Apetala3 B function Expressed in whorls 2 and 3

Misexpression from the 35S viral promoter in transgenic plants supports proposed roles of B function genes and demonstrates importance of spatial control of transcription

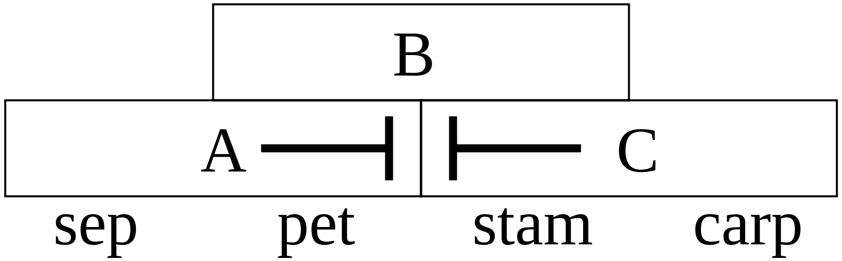
Wild type



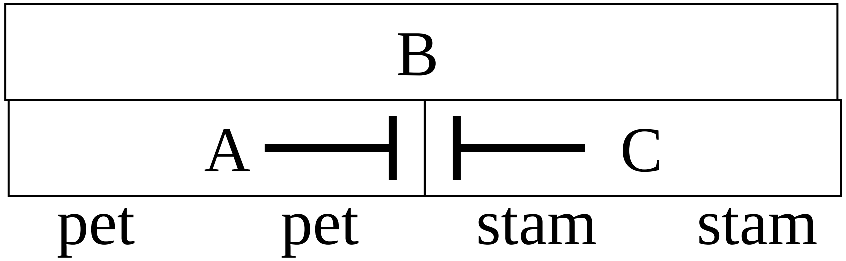
35S::AP3 35S::PI



Wild type



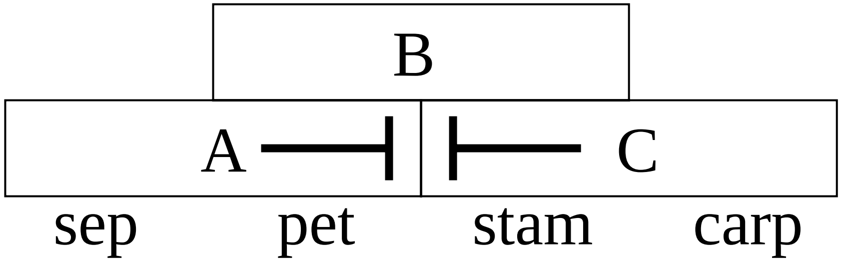
35S::AP3 35S::PI



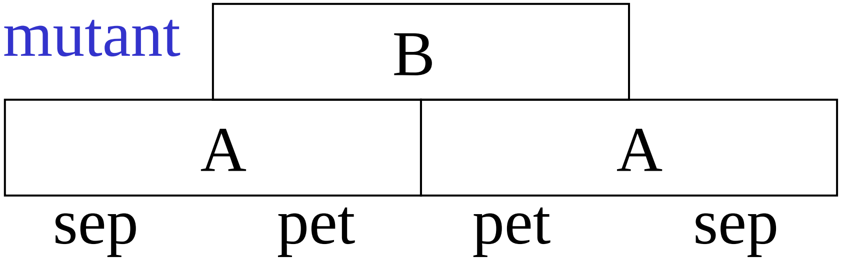
Regulation of Floral organ identity gene expression: C opposes A at the transcriptional level

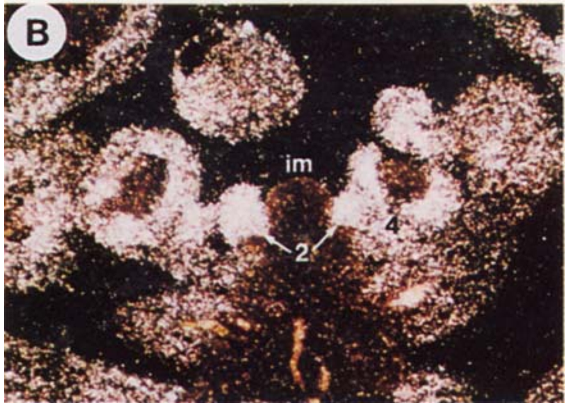
AP1 (A function) misexpression in ag (C function) mutant

Wild type

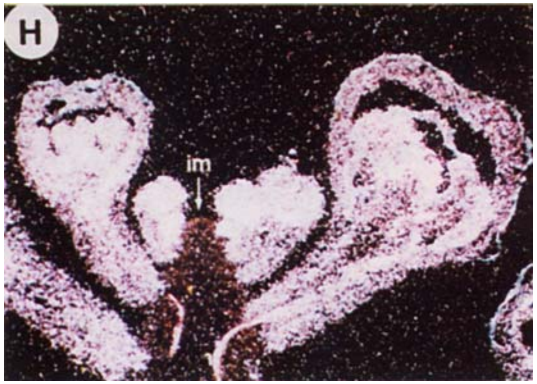


ag
C mutant





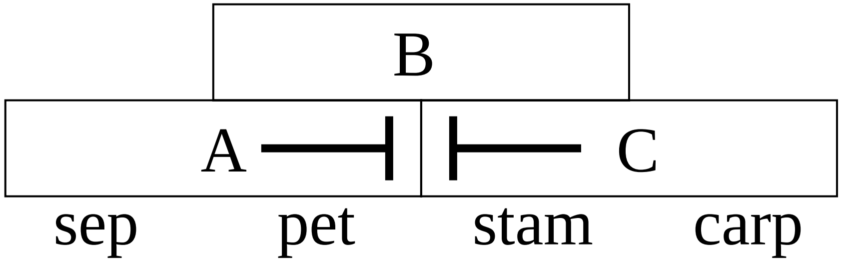
AP1 mRNA in
Wild-type plants
Only in whorl 1 and 2



AP1 mRNA in
ag mutant plants
In all whorls

Further additions to the ABC model : SUPERMAN

Wild type

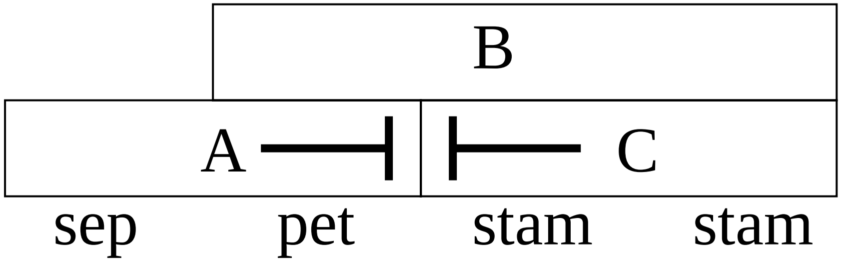


B

A ⊣ ⊢ C

sep pet stam carp

superman



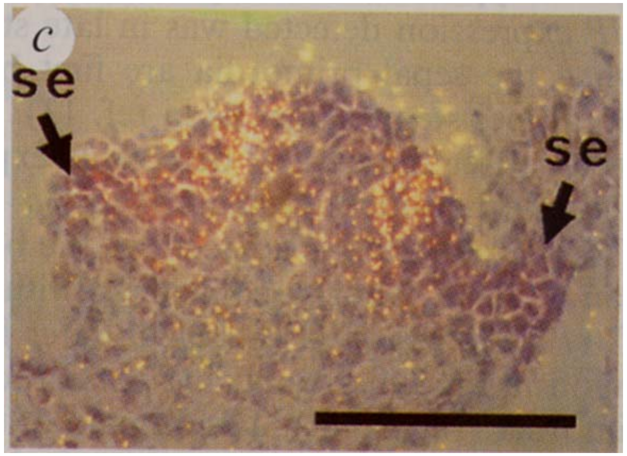
B

A ⊣ ⊢ C

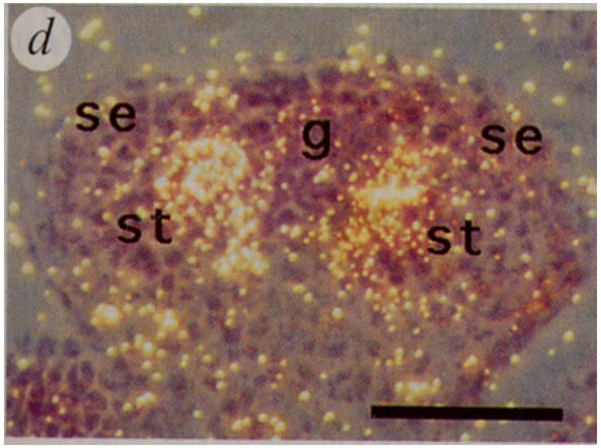
sep pet stam stam

SUPERMAN is expressed at the boundary between the third and fourth whorl

Stage 3



Stage 4

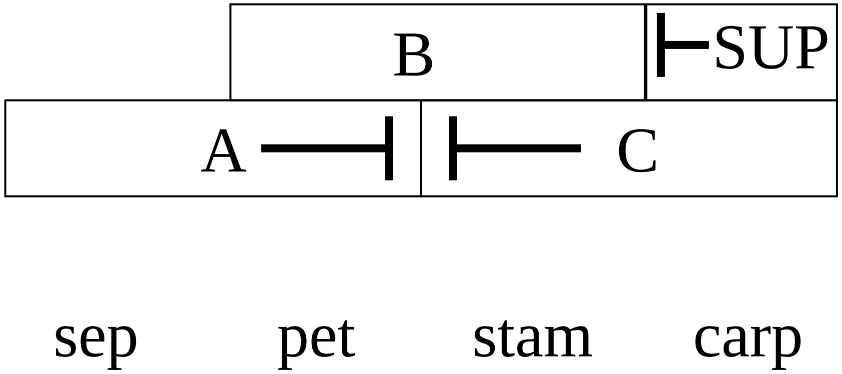


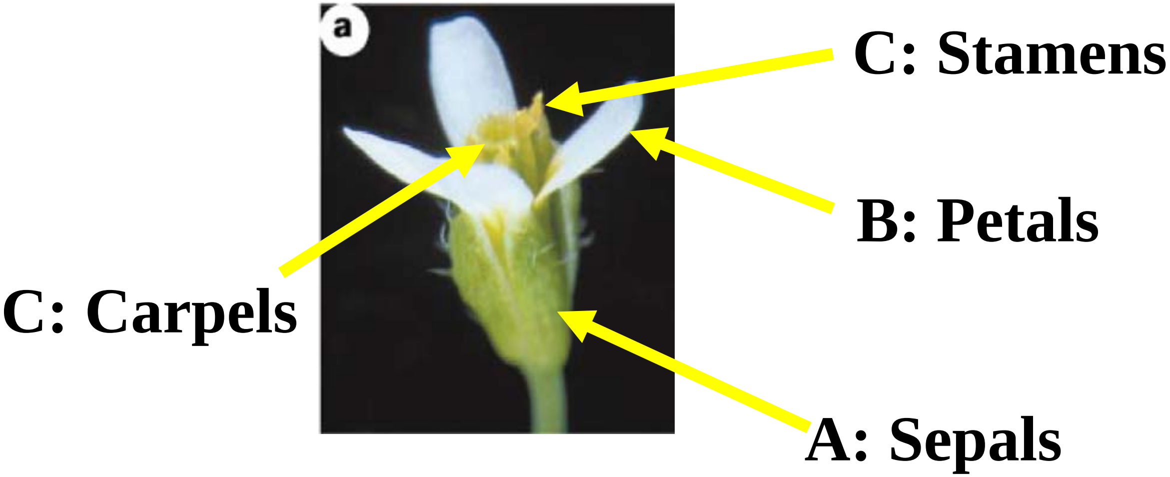
SUP encodes a likely zinc-finger DNA-binding protein containing Cys2 His2 type zinc fingers and a Serine/proline rich activation domain.

ABC model

Summary

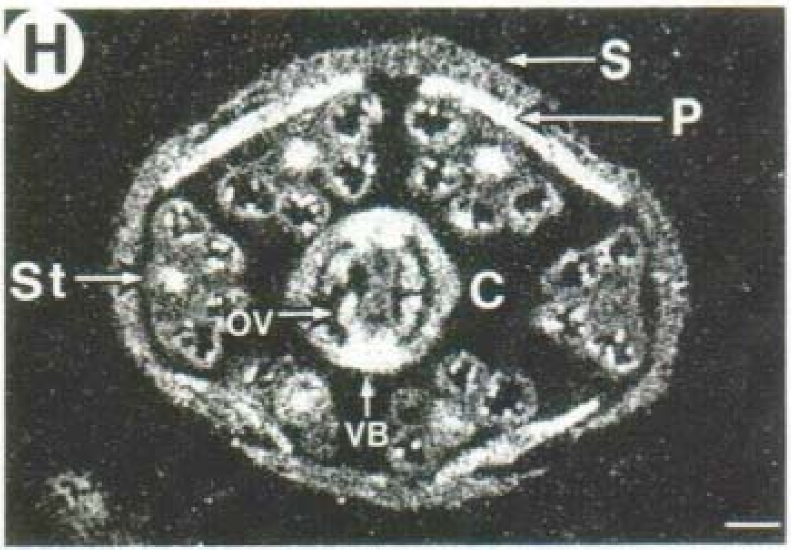
Wild type



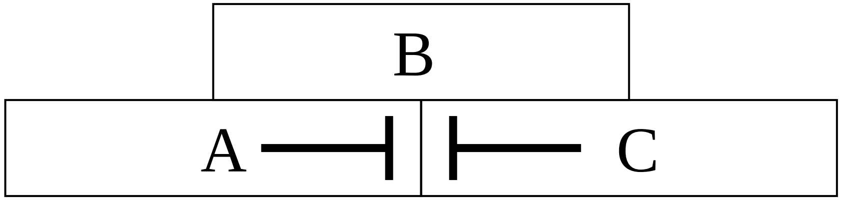


APETALA2 is a class A gene required for whorl 1 and 2, but is expressed in all whorls.

How is AP2 activity restricted to whorls 1 and 2 ?

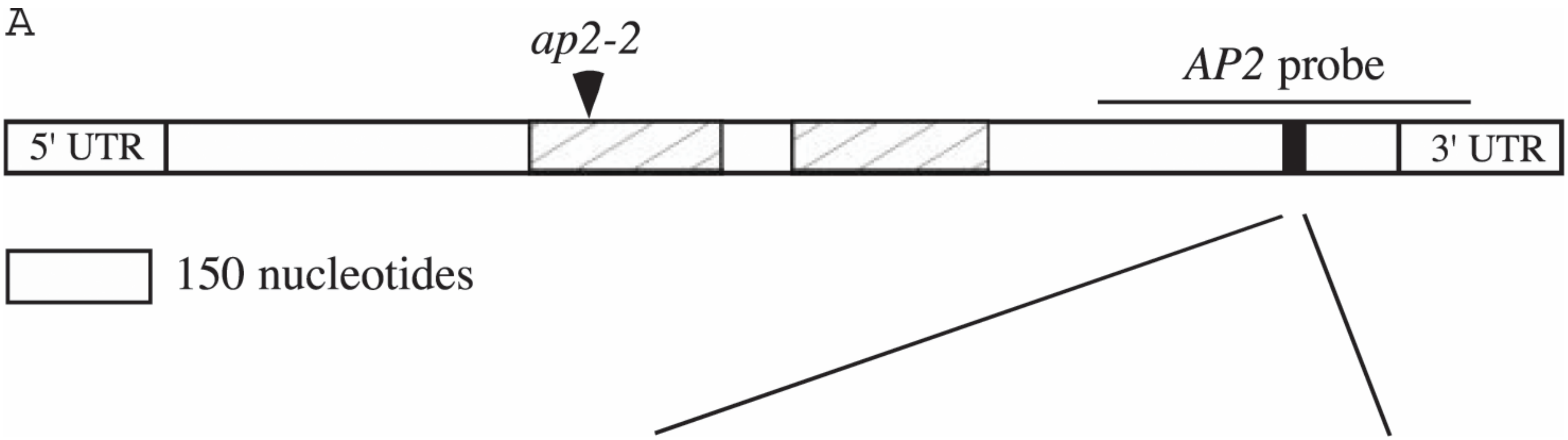


Wild type


B

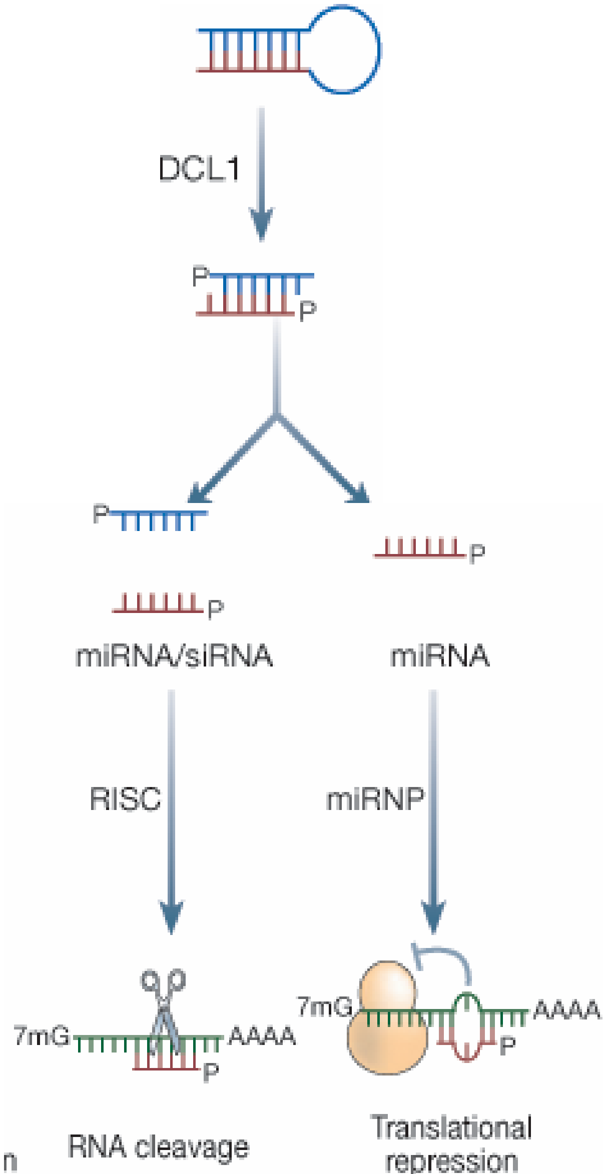
A ⊣ ⊢ C

Arabidopsis microRNA172 has homology to the AP2 gene

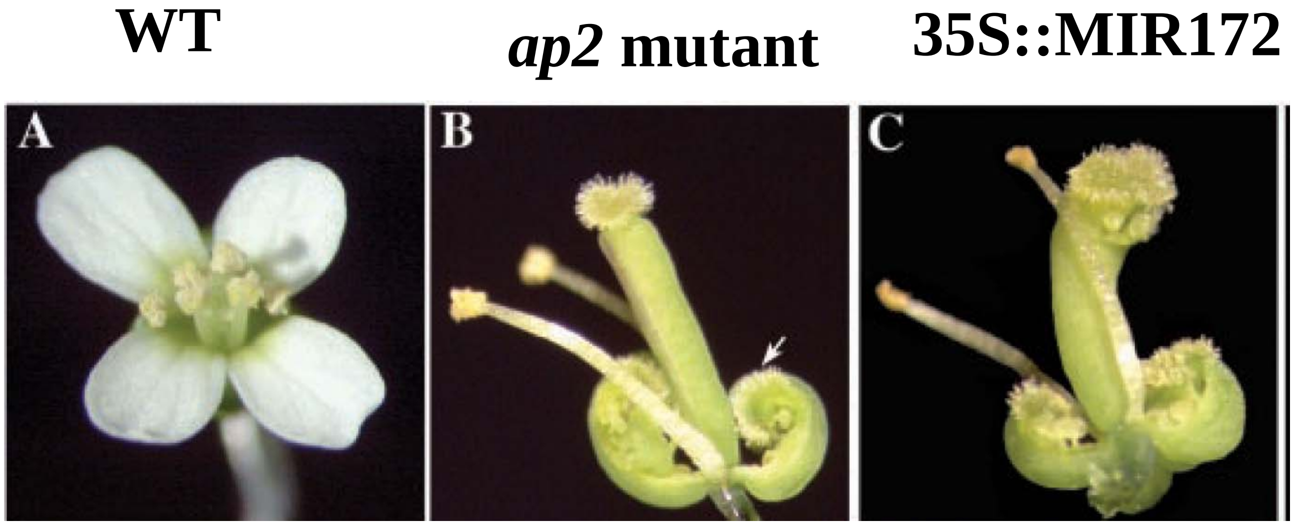


wild-type *AP2* RNA	5' gcu gca gca uca uca gga uuc ucu 3'
miRNA172a-1,-2	3' ua **cgu cgu agu agu ucu aag a** 5'
miRNA172b-1,-2	3' **ga cgu cgu agu agu ucu aag a** 5'
miRNA172c	3' ua **cgu cgu agu agu ucu aag g** 5'

microRNAs repress gene activity by interacting with the mRNAs of target genes either repressing translation or causing degradation of the mRNA

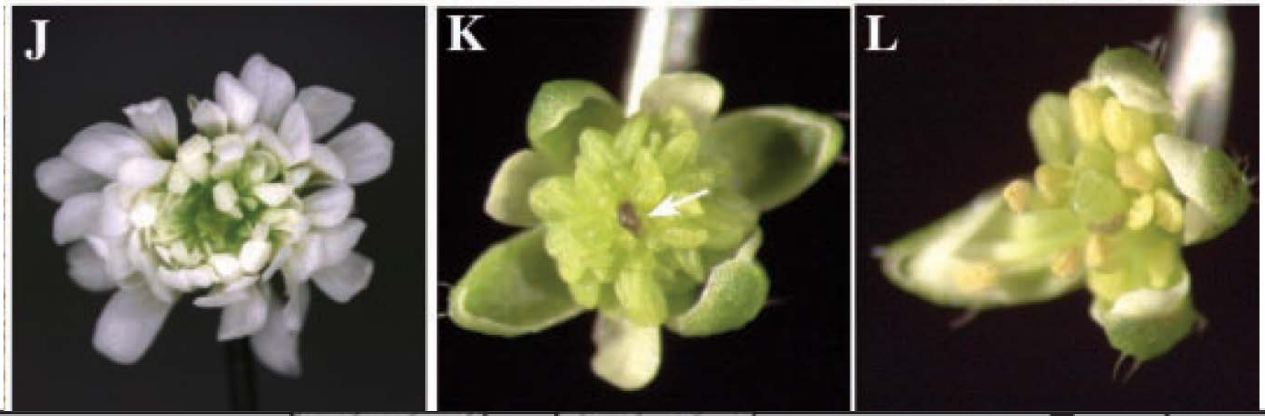


Expression of MIR172 from a viral promoter causes an ap2 mutant phenotype



Expression of MIR172 at high levels in all cells prevents AP2 function

Expression of a mutant form of AP2 mRNA that has reduced homology to MIR172 causes increased petal number and more floral whorls



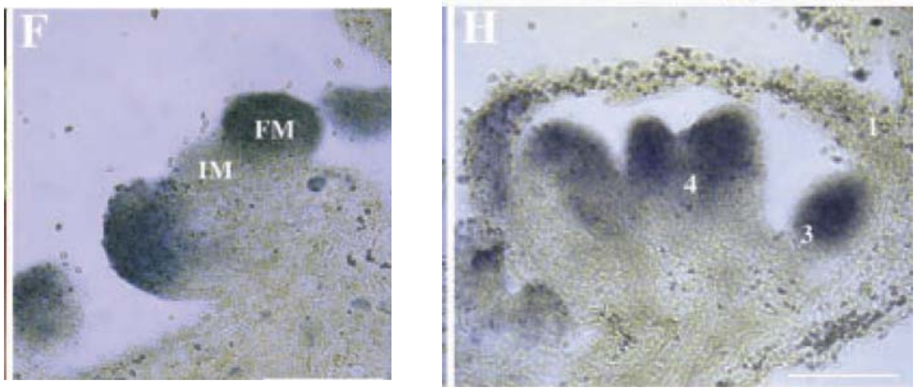
A

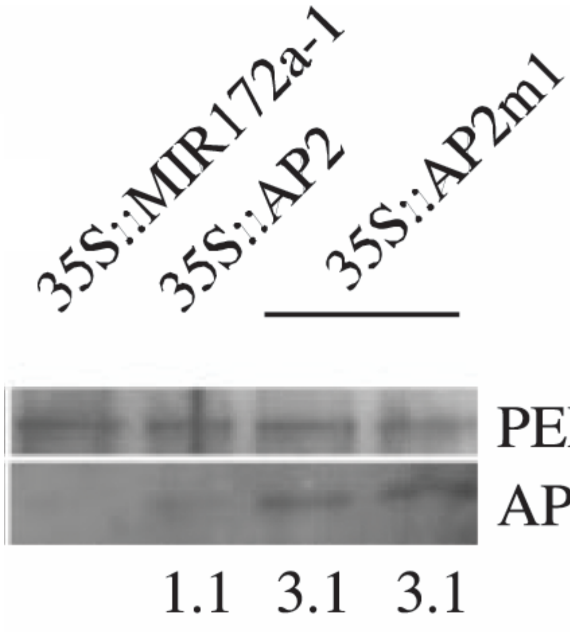
AP2 protein		A	A	A	S	S	G	F	S	
AP2m3 RNA	5'	gca	gcu	gcu	ucc	ucu	ggu	uuc	ucu	3'
AP2m1 RNA	5'	gca	gcu	gcu	ucc	uca	ggu	uua	ucu	3'
wild-type *AP2* RNA	5'	gcu	gca	gca	uca	uca	gga	uuc	ucu	3'
miRNA172a-1,-2	3'	ua **cgu**	**cgu**	**agu**	**agu**	**ucu**	**aag**	**a**	5'	
miRNA172b-1,-2	3'	**ga cgu**	**cgu**	**agu**	**agu**	**ucu**	**aag**	**a**	5'	
miRNA172c	3'	ua **cgu**	**cgu**	**agu**	**agu**	**ucu**	**aag**	**g**	5'	

MIR172 is expressed only in the inner whorls in older floral primordia and reduces AP2 protein levels

Floral meristems

Older, Stage 7 flower



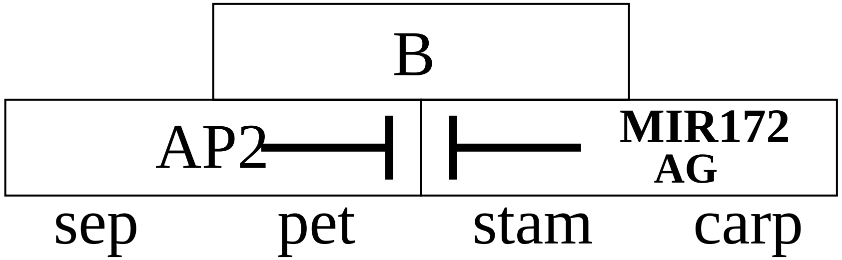


Control protein

protein

MIR172 restricts AP2 activity to the 1 and 2 whorl so that AG expression is prevented in these whorls but can occur in whorls 3 and 4

Wild type



B

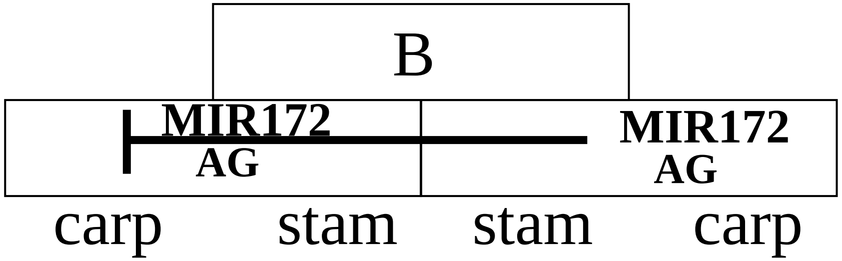
AP2

MIR172

AG

sep pet stam carp

35S::MIR172



B

MIR172

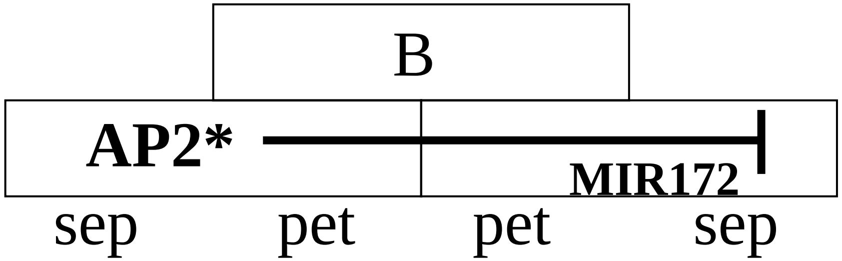
AG

MIR172

AG

carp stam stam carp

35S::AP2* not recognised by MIR172



B

AP2*

MIR172

sep pet pet sep

Misexpression of B or A and B in leaves is not sufficient to convert leaves to petals

Wild-type

B function

35S::AP3 35S::PI

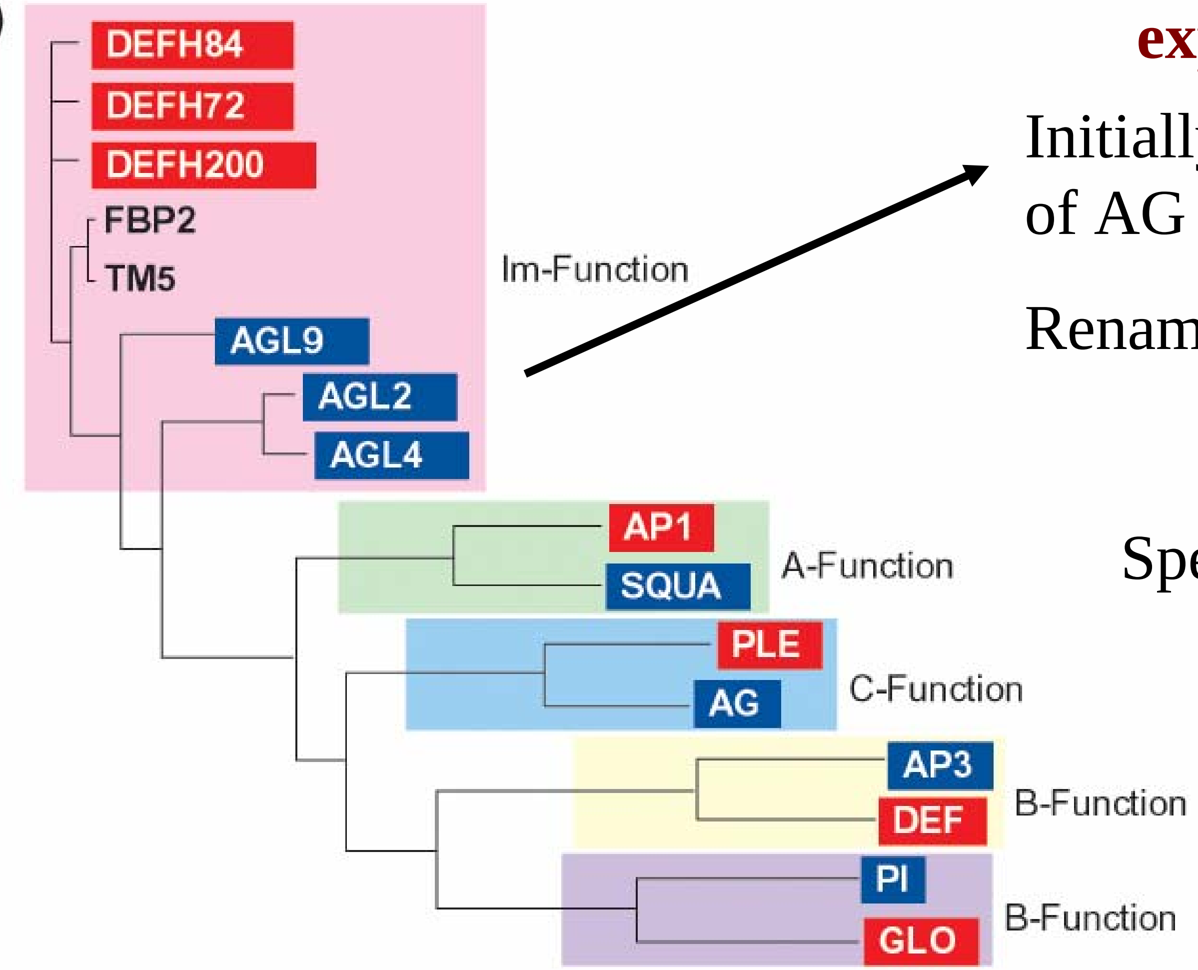
A function

35S::AP1

Suggests another floral-specific factor is absent in the leaves.

This was not identified by initial genetic screens

More MADS box genes expressed in the flower



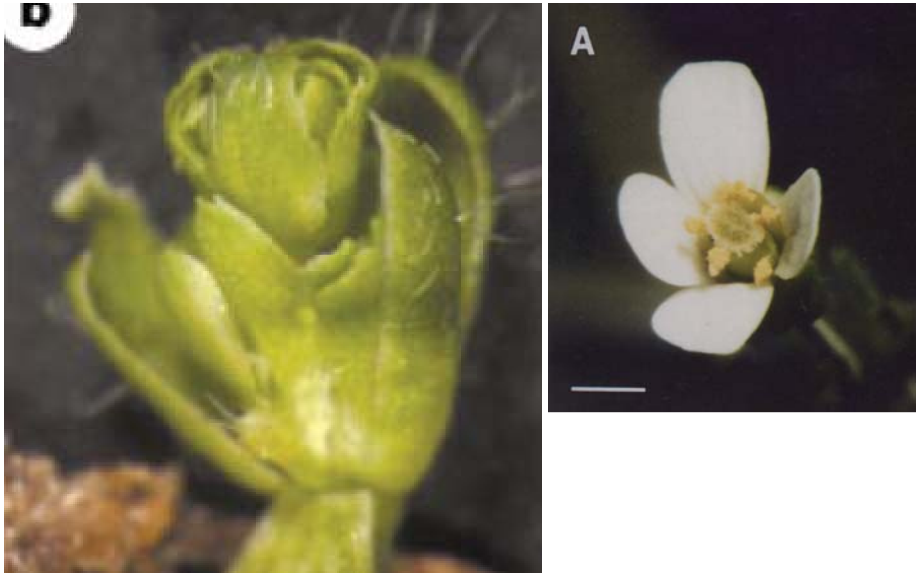
Initially identified as homologues of AG – AGL2, AGL4, AGL9

Renamed sepallata 1,2,3

Specific expression patterns – in whorls 2,3,4, although AGL2 and AGL9 are also expressed in whorl 1 of younger flowers.

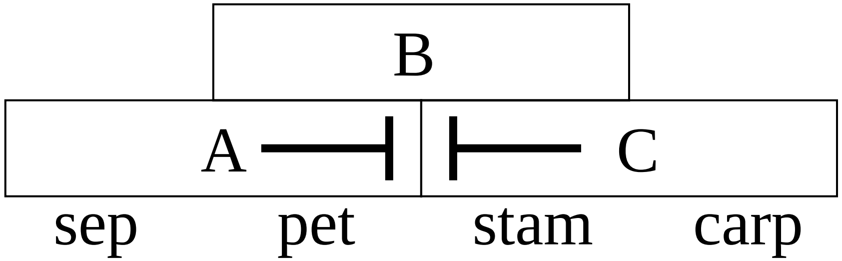
Genes in red from Antirrhinum
Genes in blue from Arabidopsis

Inactivation of SEP1 SEP2 and SEP3 in triple mutants

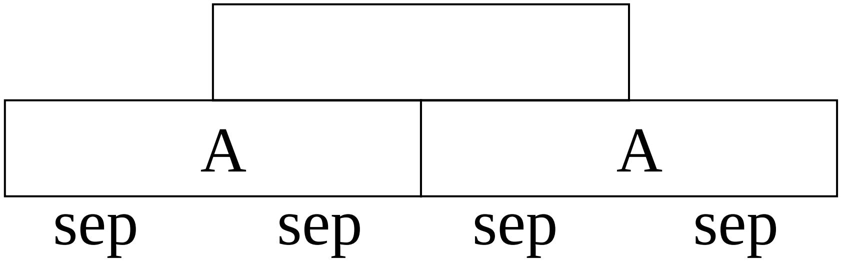


Were not identified in
Original mutant screens
Because of redundancy
Between proteins.

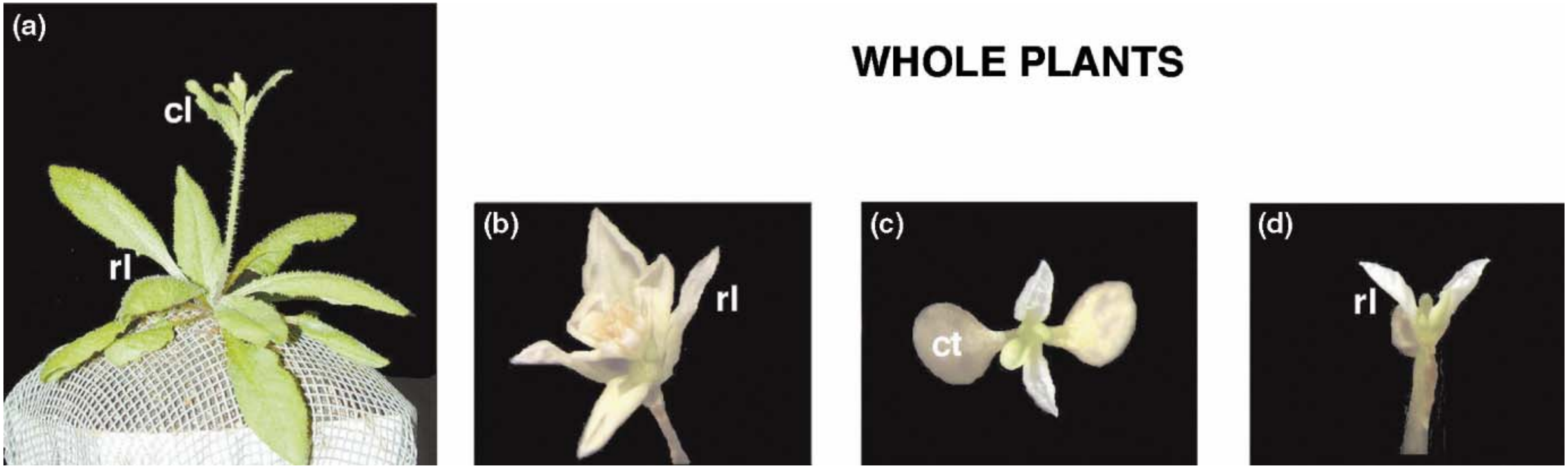
Wild type



sep1 sep2 sep3

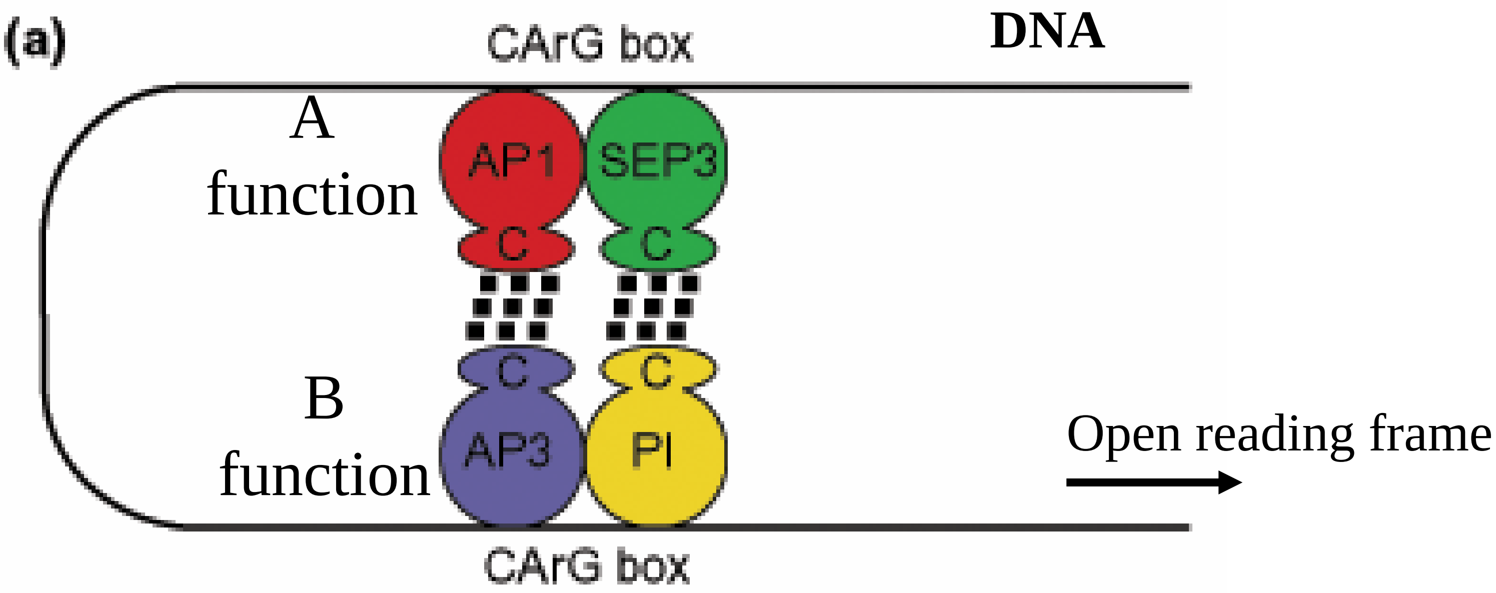


Misexpression of SEP1 AP1 PI and AP3 in leaves creates petals

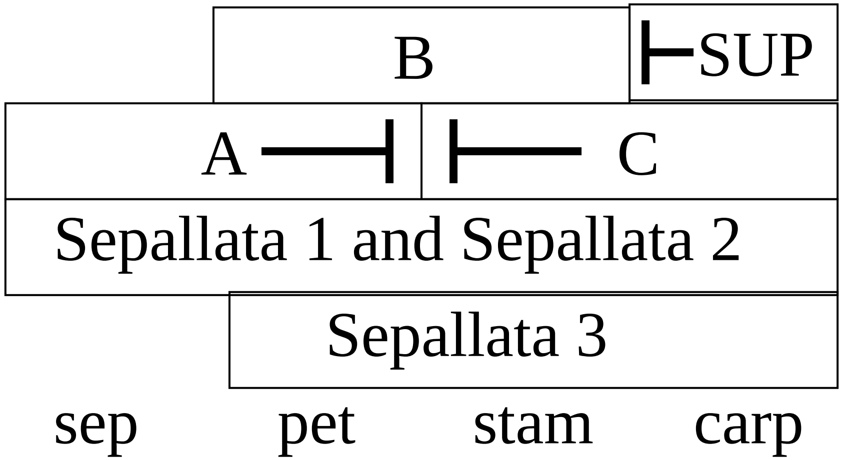


Plants carrying combination of
35S::AP1 35S::SEP2 35S::PI 35S::AP3

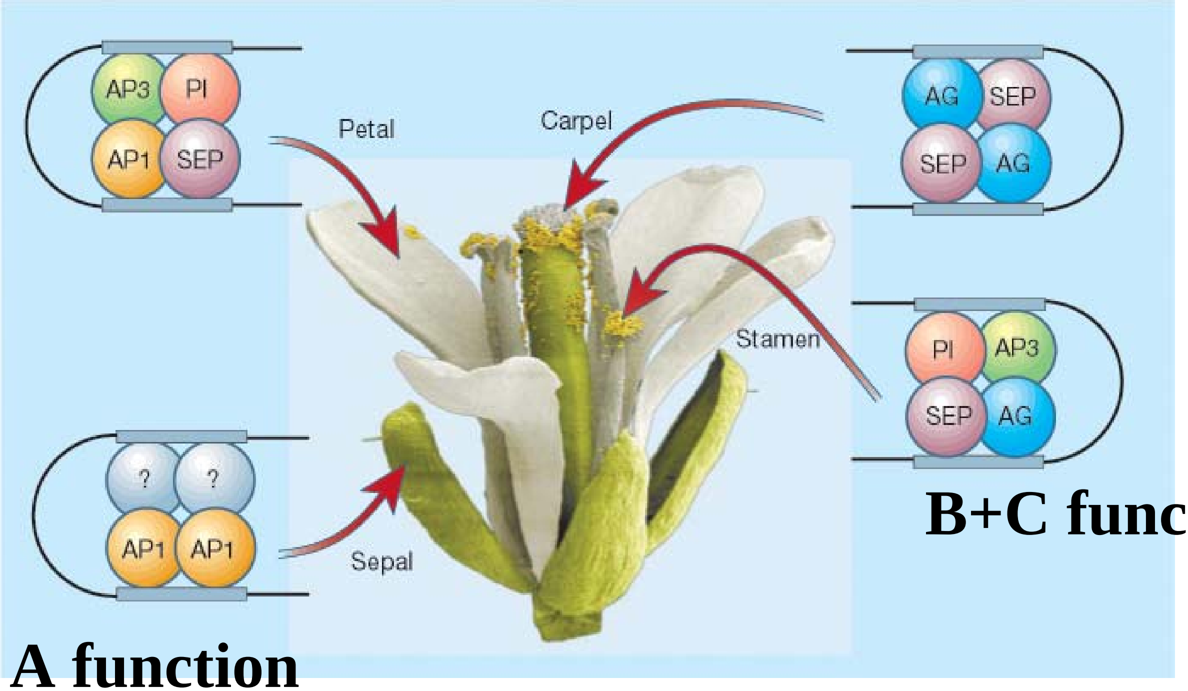
Therefore a combination of AP1 PI AP3 SEP is sufficient To confer B function.



Model explains how A function (AP1) and B function (AP3/PI) Combine to specify the second whorl – petals.



Similar multimeric complexes of MADS box proteins are proposed to specifiy the other whorls



A+B function

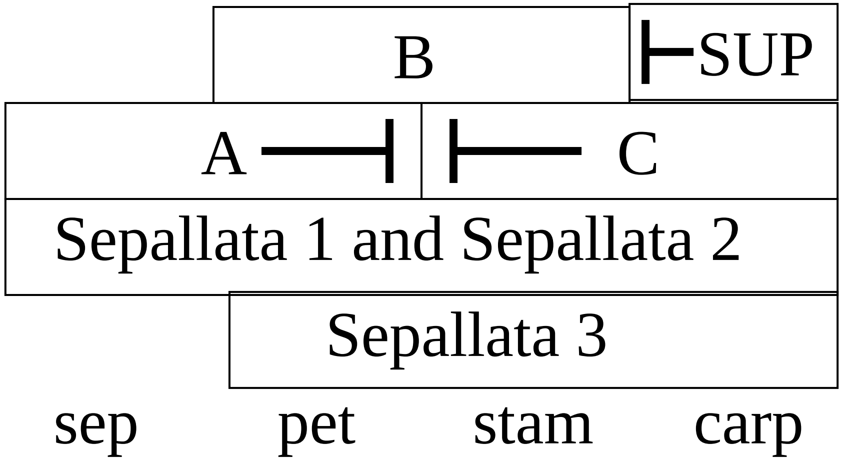
C function

B+C function

A function

SEP proteins mediate multimeric complexes between PI/AP3 and AG or between PI/AP3 and AP1

Wild type

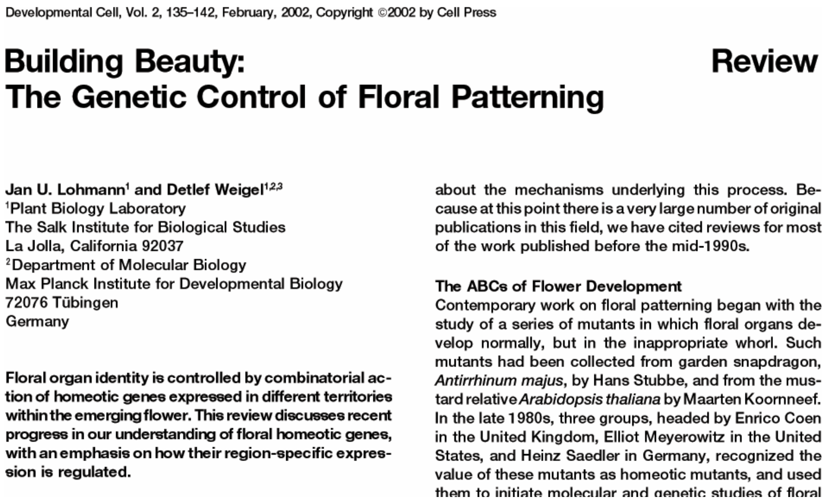


Formation of multimeric complexes suggests mechanism for combining A/B and B/C functions within the ABC model.

Web site

- this presentation

- PDFs



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Building Beauty:
The Genetic Control of Floral Patterning

Review

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Floral organ identity is controlled by combinatorial action of homeotic genes expressed in different territories within the emerging flower. This review discusses recent progress in our understanding of floral homeotic genes, with an emphasis on how their region-specific expression is regulated.

about the mechanisms underlying this process. Because at this point there is a very large number of original publications in this field, we have cited reviews for most of the work published before the mid-1990s.

The ABCs of Flower Development

Contemporary work on floral patterning began with the study of a series of mutants in which floral organs develop normally, but in the inappropriate whorl. Such mutants had been collected from garden snapdragon, *Antirrhinum majus*, by Hans Stubbe, and from the mustard relative *Arabidopsis thaliana* by Maarten Koornneef. In the late 1980s, three groups, headed by Enrico Coen in the United Kingdom, Elliot Meyerowitz in the United States, and Heinz Saedler in Germany, recognized the value of these mutants as homeotic mutants, and used them to initiate molecular and genetic studies of floral

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Forschung

Abt. Entwicklungsbiologie de Pflanzen

George Coupland