TOPIC 3. **Plant breeding systems and pollen dispersal**

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...with Sílvia Castro SPECIAL GUEST!

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Introduction to breeding systems

Plant mating systems: allogamy, autogamy, geitonogamy

Apomixis: agamospermy, pseudogamy

Mixed mating

P/O ratio, pollen dispersal, secondary pollen presentation

Strategies to limit selfing

temporal, mechanical and physiological control

Reproductive barriers (Sílvia Castro)

Practical approaches…

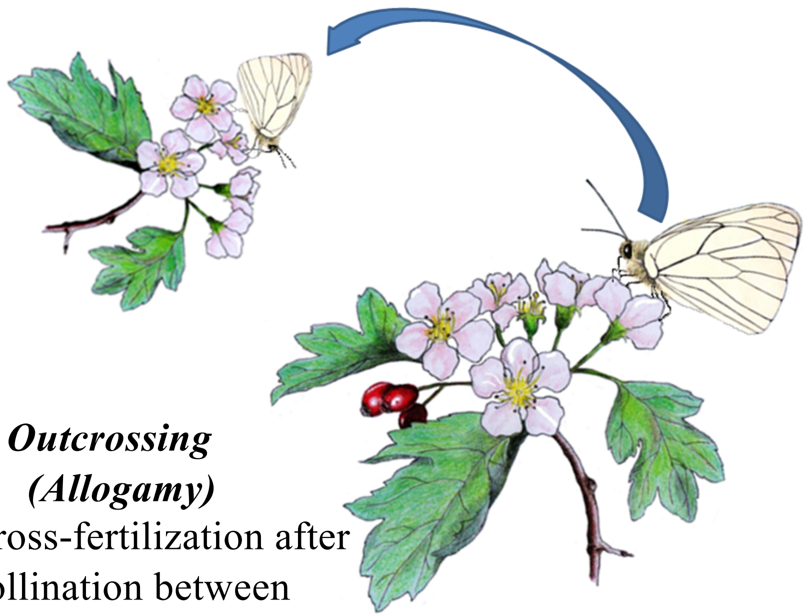
Plant mating systems: allogamy, autogamy, geitonogamy

Asexual reproduction: agamospermy, pseudogamy

Mixed mating

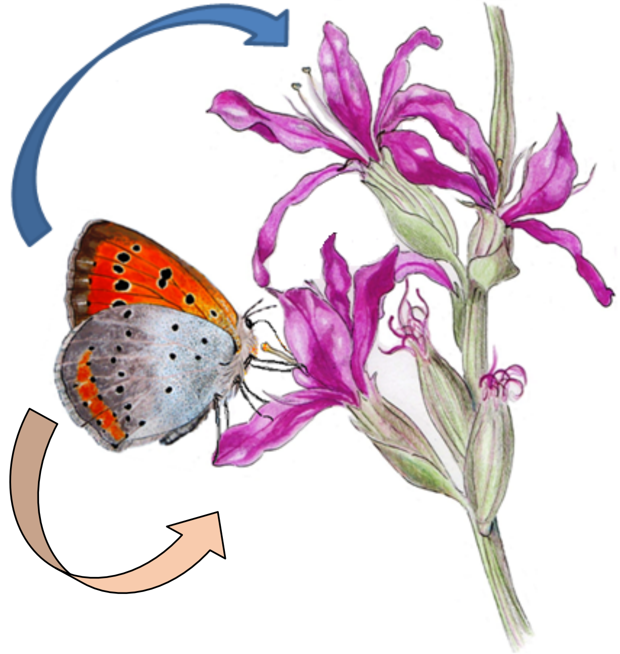
P/O ratio, Pollen dispersal

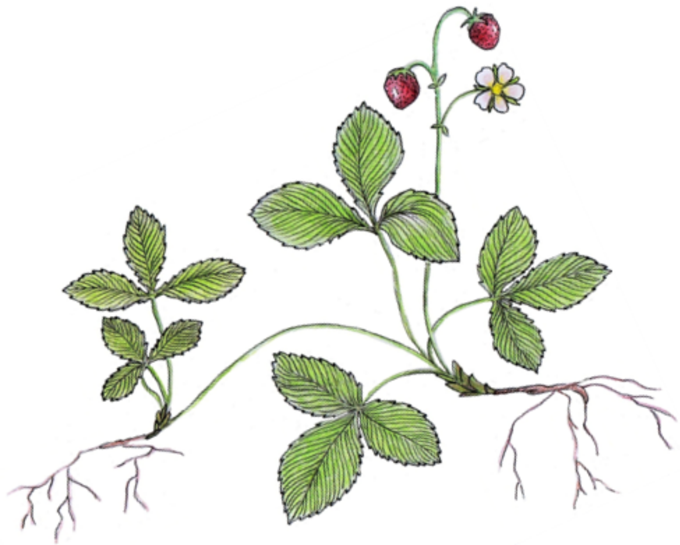
Reproductive systems



1. Outcrossing (Allogamy)
Cross-fertilization after pollination between genetically distinct plants

2. Selfing (Autogamy/ Geitonogamy)
Self-fertilization after pollination within a hermaphroditic flower or between flowers on the same plant





3. Apomixis (sensu lato)
Formation of new individuals by asexual processes

Illustration by Marta Barberis

PLANT BREEDING (REPRODUCTIVE) SYSTEMS

sexual

1.ALLOMIXIS (cross-fertilization, otucrossing)

= cross-fertilization (between 2 different individuals)

2.AUTOMIXIS (self-fertilization, slfing)

= self-fertilization (same individual)

asexual (?!?)

3. APOMIXIS

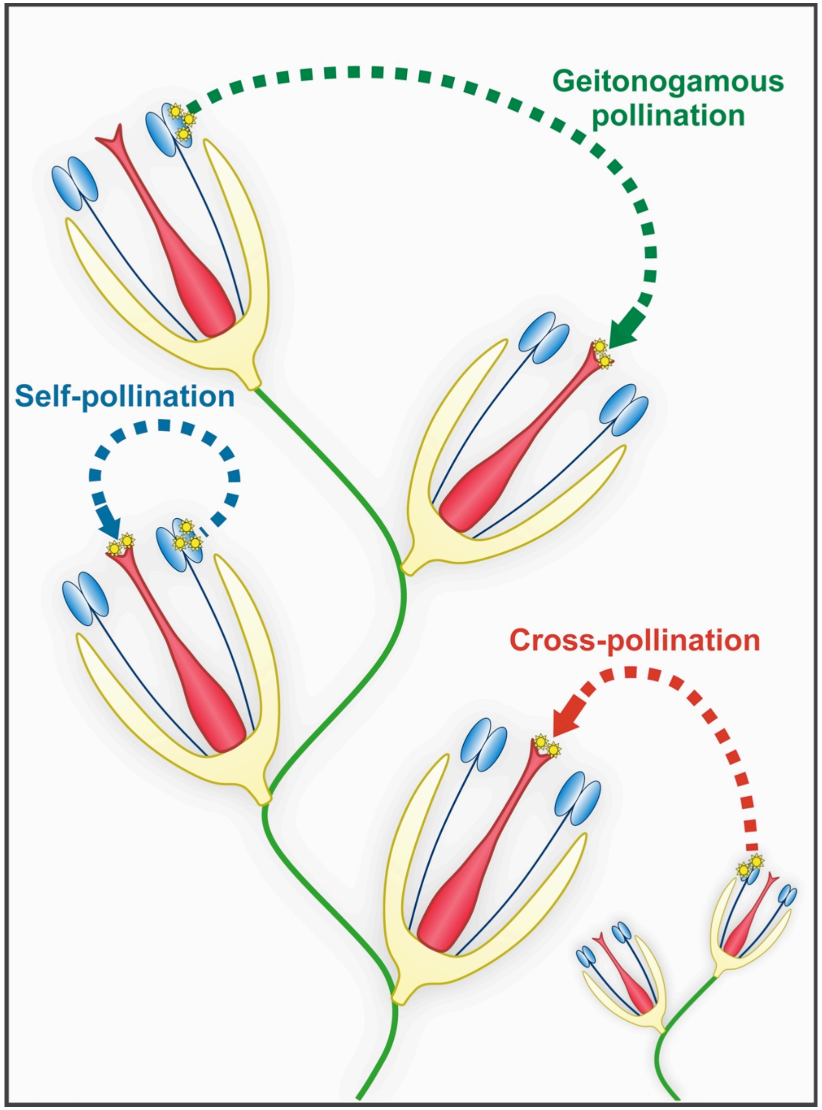
= vegetative apomixis, agamospermy, pseudogamy

asexual propagation, not considered as breeding system

MATING SYSTEMS

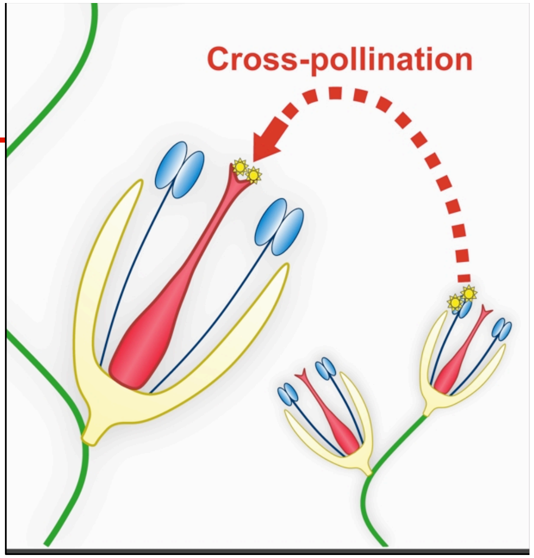
modes of gene transfer from one generation to the next through sexual reproduction.

It is related to the genetic relativeness of the gametes, determined by maternal autogamy rate and male success through pollen grain dispersal.



(Cardoso et al. 2018 and references therein)

1. OUTCROSSING: ALLOGAMY (XENOGAMY)



- Fertilization of gametes from flowers of different individuals

  after cross-pollination

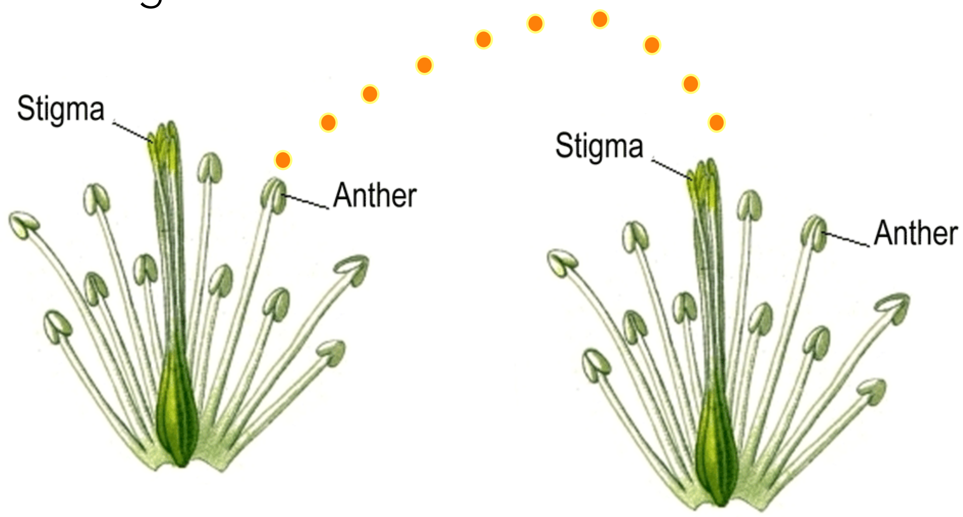
- promotes genetic variability through new combinations
- genetic diversity in the population may allow individuals to adapt to a variety of environmental conditions and increase the likelihood of survival and evolutionary change

- requires pollen vector(s)
- not favoured if individuals are distant or pollinators are scarce

Richards 1997, Simpson 2006

1. OUTCROSSING: ALLOGAMY (XENOGAMY)

- The majority of flowering plants are OUTCROSSERS
- Obligatory in DIOECIUOS species and in hermaphrodite species with incompatibility systems
- In general it is preferential, with different degrees

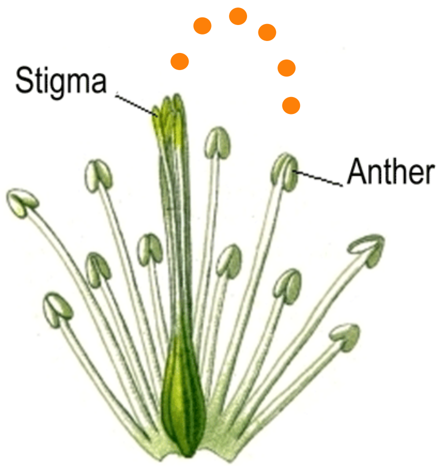


- Gene flow within and among populations largely depends on OUTCROSSING rate
- The level of outcrossing may vary in time and space, being influenced by extrinsic factors
- Plants with bisexual flowers maintain the chance to SELF-FERTILIZE…

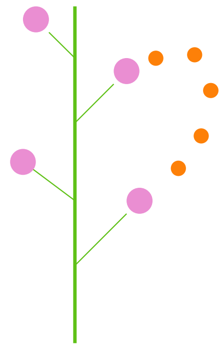
2. SELFING: AUTOGAMY/ GEITONOGAMY

- deposition of (self-)pollen on the stigma of the same flower (autogamy) or of a different flower of the same individual (geitonogamy)

- self-compatibility (breakdown of genetic self-incompatibility)



AUTOGAMY

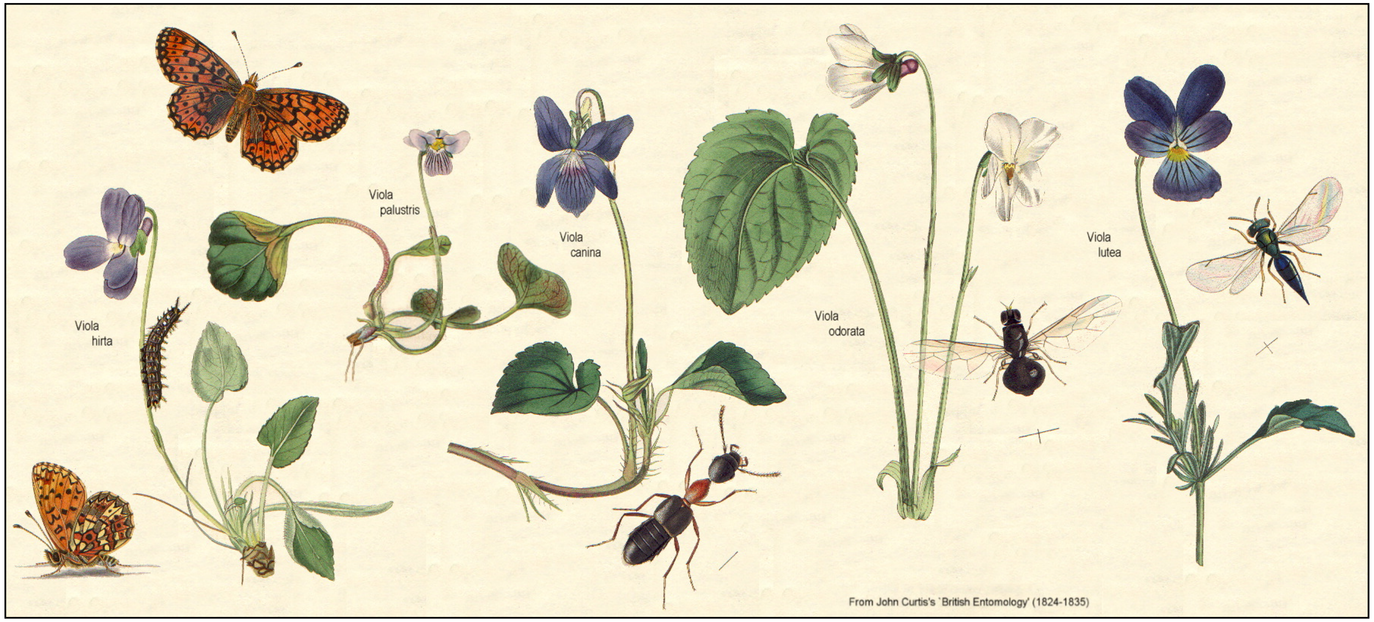


GEITONOGAMY

(Lloyd and Shoen 1992, Barrett 2010, Cardoso et al.2018)

2. SELFING

from facultative to obligate

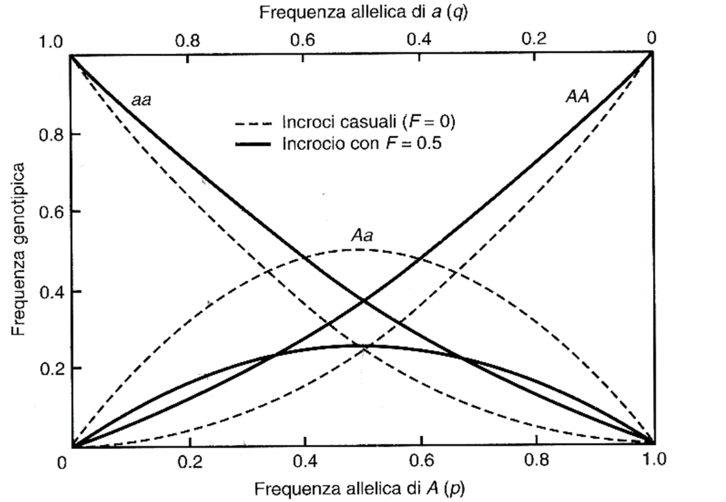


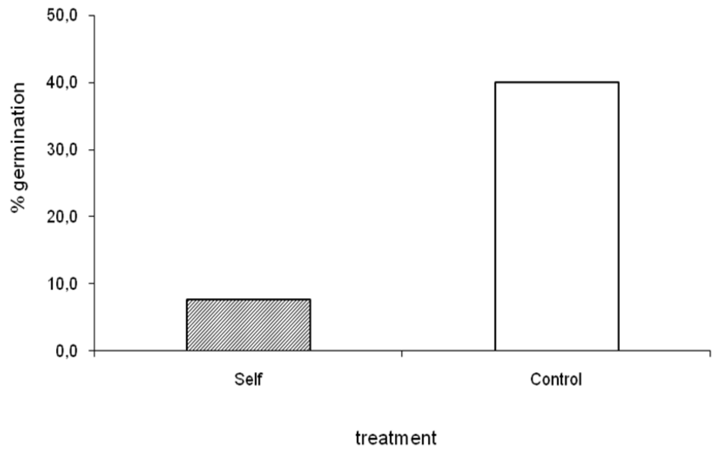
Optional autogamy common in polyploids, first colonizers, annuals

Triticum, Avena
Viola
Oxalis,
Salicornia,
Chenopodium albatum
...

2. AUTOGAMY: cons (disadvantages)

- Low individual genetic diversity
- High frequency of deleterious traits (homozygosity)
  - → < viable seeds
  - → < progeny fitness
- Pollen and seed discounting
- Reduced vigor and fertility





2. AUTOGAMY: pros (advantages)

- Replication of well adaptated genotypes

(marginal, disturbed or extreme habitats)

- Reproductive assurance
  (scarce pollinators, scarse potential mates, pioneer plants)

- More “economic” (< energetic investment : less pollen needed,..)

- More “rapid” (selfer flowers have short lifespan)

Geranium sylvaticum vs. *G. molle*

2. MODES of SELFING

	Facilitated Selfing		Autonomous Selfing		
	Geitonogamy	Autogamy	Prior	Competing	Delayed
Transfer within/between flowers	BETWEEN	WITHIN	WITHIN	WITHIN	WITHIN
Pollen vector involved	YES	YES	NO	NO	NO
Timing (relative to outcross)	SAME	SAME	BEFORE	SAME	AFTER

Lloyd and Shoen (1992)
Dafni, Kevan & Husband (2005)

Mixed mating… is widespread!

Whitehead et al. 2018

literature survey including 741 populations from 105 species HIGHLIGHTS

→ substantial and prevalent among-population variation in the mating system.

→Intermediate outcrossing rates (mixed mating) are **common**: at least in 1 pop in 63% of species

→ influence of ecological and genetic factors on the mating system

may be beneficial in unpredictable habitat

(Goodwillie et al. 2005, Whitehead et al. 2018, Carta et al. 2016)

Information on breeding systems can be directly obtained through the analysis of **resource allocation to sexual function**

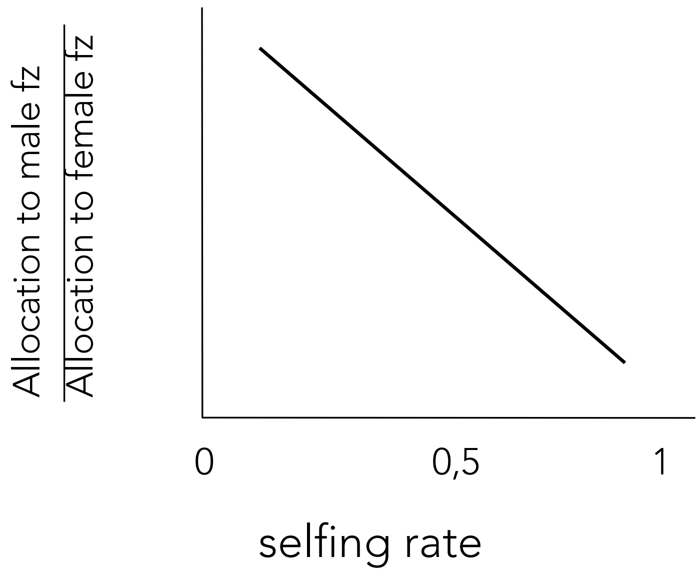
Hermaphrodite Species
- Male function → POLLEN
- Female function → OVULES, SEEDS

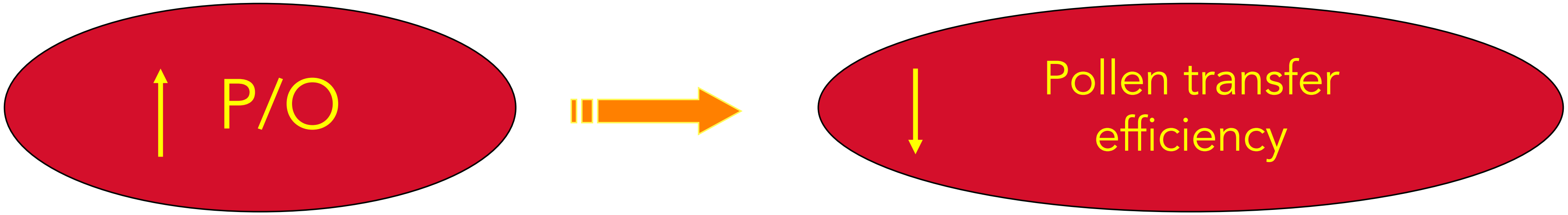
Sex allocation theory:

$$\frac{\text{Allocation to male fz}}{\text{Allocation to female fz}}$$

↓

P/O





Xenogamy : 2108 - 195525

Facultative autogamy : 245- 2588

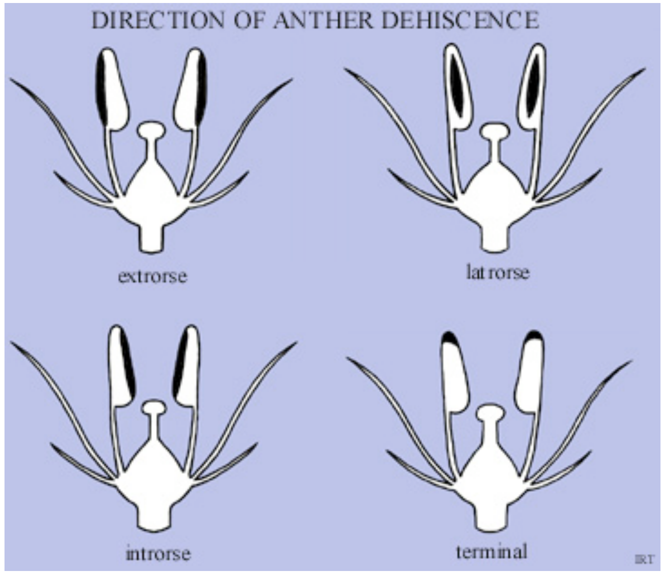
Facultative xenogamy: 32- 396

Autogamy: 18 - 39

Cleistogamy: 3 - 6

(Cruden 1977; Dafni 2005)

Pollen dispersal:
anther opening, pollen presentation



- sudden movement of anther walls launches pollen (anemophyly)
- sudden movement of filaments under tension launches pollen (anemophyly)
- pollen leave the anther as soon as it opens
- pollen launch after explosive opening of closed flowers
- Pollen is not exposed directly in species with pollinaria: these are the dispersing units, this protection enables pollen to survive for long periods

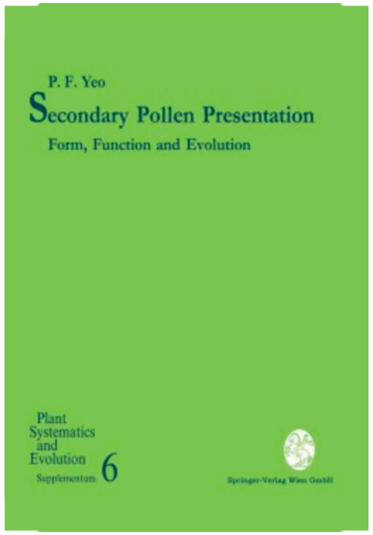
➢ PRIMARY presentation: pollen presented by the anthers

➢ SECONDARY presentation: pollen presented by other floral structures

PRIMARY presentation:
pollen presented by the anthers

SECONDARY presentation:
pollen presented by other floral structures

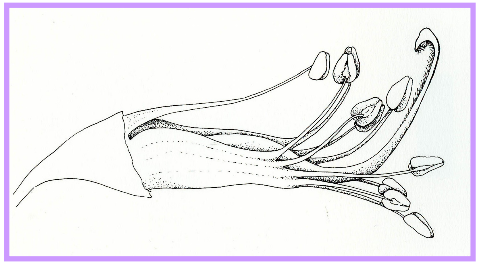
- Perianth
- Calix
- Corolla
- Filaments /staminodes
- Style



Method of issue

- Non-explosive release
- Release in measured doses
- Powered by plan'ts movements in response to touch
- Explosive

....

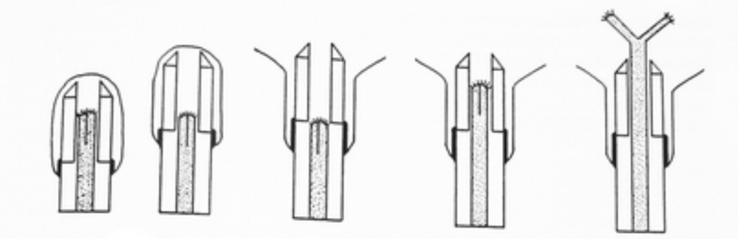


(YEO 1992, Secondary Pollen Presentation. Form, function and evolution)

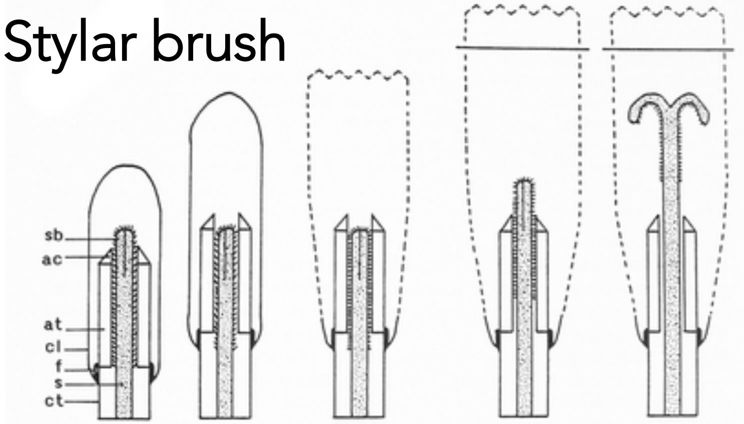
Secondary pollen presentation : Asteraceae

Family trait - 3 main mechanisms

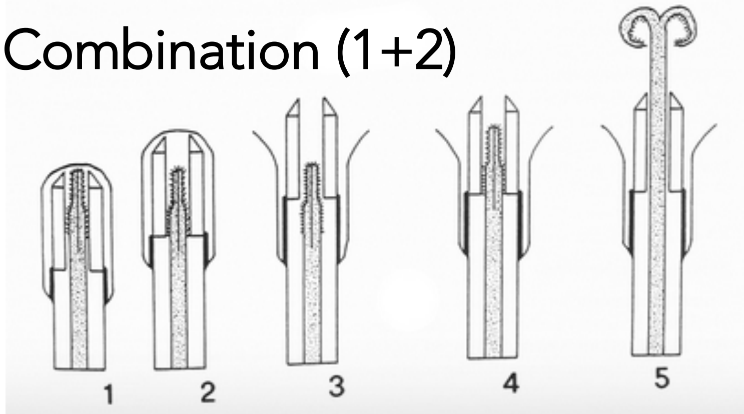
1. Stylar piston

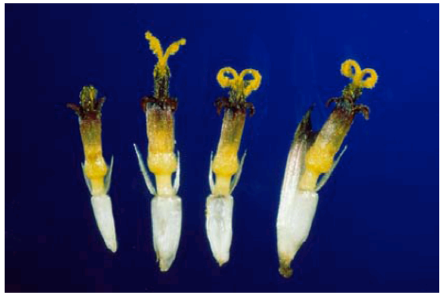


2. Stylar brush



3. Combination (1+2)

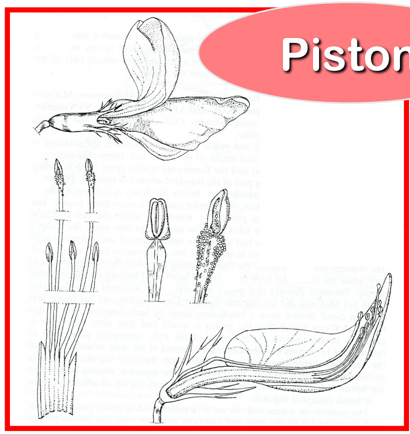




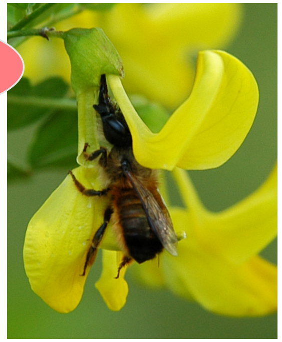
Cichorioideae: type 2. stylar brush

Asteroideae: type 2, type 3
Helianthus annnus: stylar piston + stylar brush

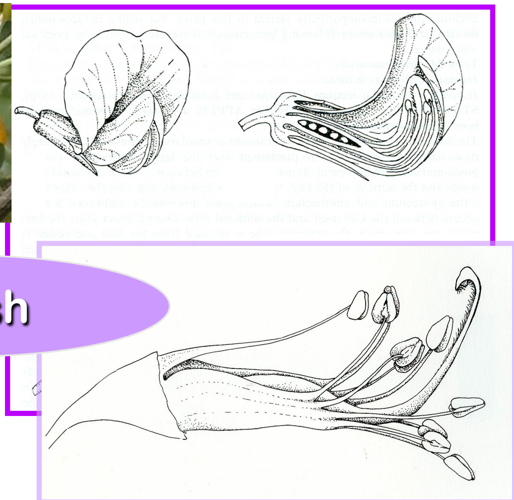
Pollen presentation in Leguminosae



Piston



Loteae
Coronilleae

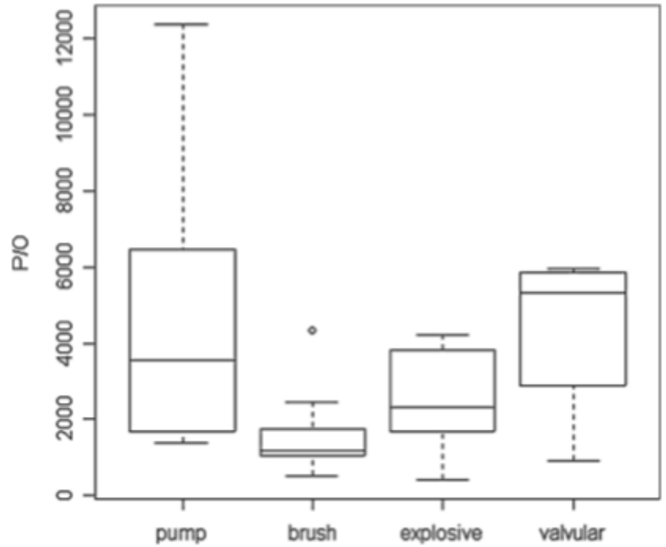


Brush

Vicieae

Explosive

Valvular
(I)



Galloni et al. 2007, 2008

3. APOMIXIS *sensu stricto*

- AGAMOSPERMY : flowers involved
- PSEUDOGAMY : flowers + pollination involved

} SEEDS

Combines «asexual» mode retaining benefits of seed reproduction: dispersion ability, dormancy

Occurs in ca. **78 angiosperm families** and seems to have arisen independently multiple times

3. APOMIXIS asexual & sexual reproduction

- AGAMOSPERMY : flowers involved → SEEDS

1. Gametophytic
   → APOSPORY: vegetative nucellus cells (2n) give origin to a unreduced embryo sac and to a new individual

   → DIPLOSPORY : new embryo originates directly from the macrospore mother cell (non reduced, 2n) or from a "restitution nucleus" **(n+n)**
2. Sporophytic:

   → ADVENTITIOUS EMBRYONY: somatic cells of the ovule (nucellus or integument 2n) develops in parallel with sexual embryo

Taraxacum officinale F. H. Wigg.
Image processed by Thomas Schoepke
www.plant-pictures.de

Commonly found in:

Asteraceae (*Hieracium*), Rosaceae (*Rosa*, *Alchemilla*, *Crataegus*), Poaceae (*Poa*)

(Cardoso et al. 2018, Fei *et al.* 2019)

3. APOMIXIS asexual & sexual reproduction

- AGAMOSPERMY : flowers involved → SEEDS

- PSEUDOGAMY : pollinators involved → SEEDS
  Insects visits are necesary for endosperm fertilization

  Found in: Rosaceae, Poaceae, Orchidaceae,
  Ranunculus auricomus


Taraxacum officinale F. H. Wigg.
Image processed by Thomas Schoepke
www.plant-pictures.de

(Hojsgaard *et al.* 2014)

3. APOMIXIS (AGAMOSPERMY)

Pros…

- Ensures reproductive success where conditions for sexual reproduction are compromised
- Propagation assured also for isolated individuals (scarsity of pollinators and potential mates)
- a single seed may be able to found a new population
- advantages in colonization scenarios

…and Cons

- relatively low genetic variability among individuals : total progeny might be negatively affected by environmental changes BUT… it is not completely so!

nevertheless: apomixis is rarely obligate in Angiosperms, since most of apomictic species produce descendants through sexual and asexual forms

Strategies to limit selfing

- Dichogamy
- Herkogamy and Sexual polymorphisms
- Self-incompatibility
- Inbreeding depression

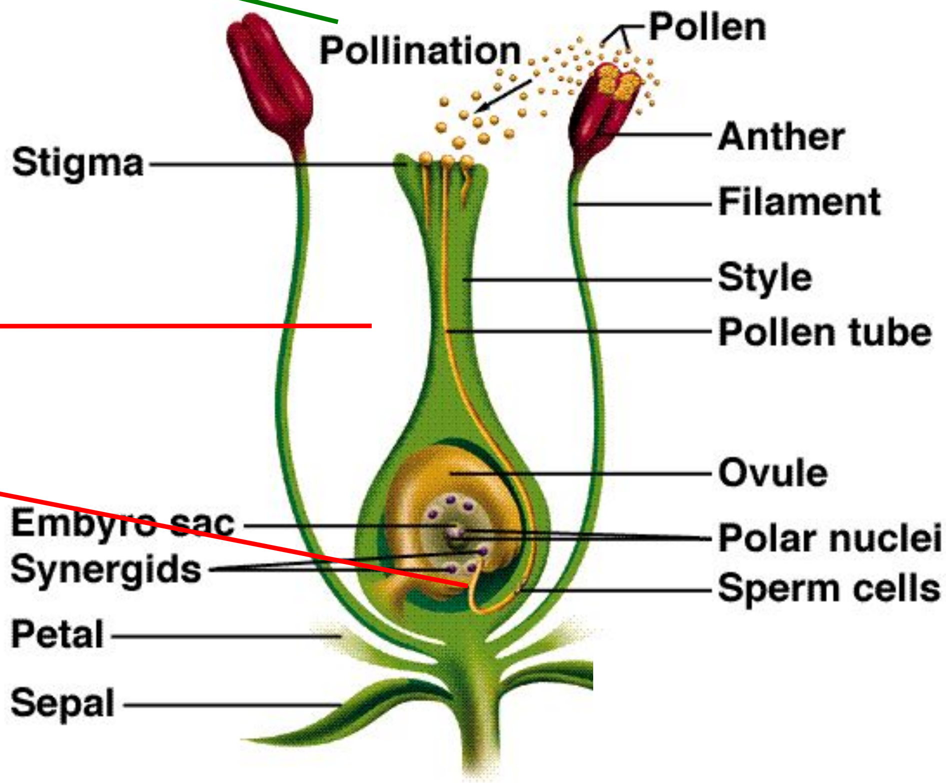
→ **Reproductive barriers** (Silvia Castro)

Pollination

Mechanical and temporal control

Physiological control

1. pollen-stigma
2. pollen tube-style
3. pollen tube-micropyle
4. gamete m-gamete f
5. embyo-endosperm II



Post-pollination control

Fertilization

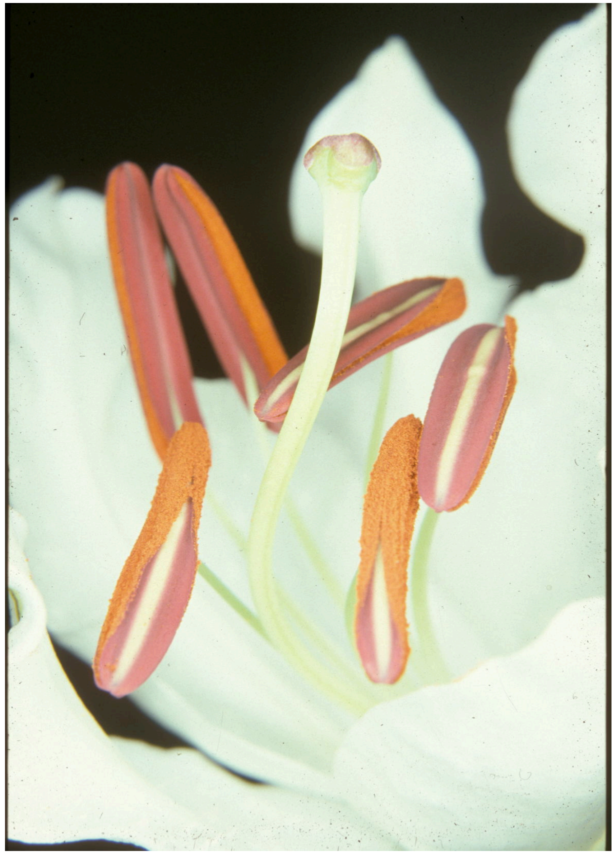
Pre-conditions for pollination

- Pollen: available, mature, viable, reaching a conspecific stigma

  → able to germinate

- Stigma: receptive

  → allows pollen germination



Limitations to selfing

Temporal separation : Dichogamy	→	Protandry, Protogyny
Spatial separation : Herkogamy	→	Ordered Reciprocal: HETEROSTYLY Temporal Flexistyly = reciprocal + dichogamy Movement Enantiostyly: mirror-image flowers
Mechanical barriers	→	Stigmatic cuticle

(Barrett 2010; Willmer 2013)

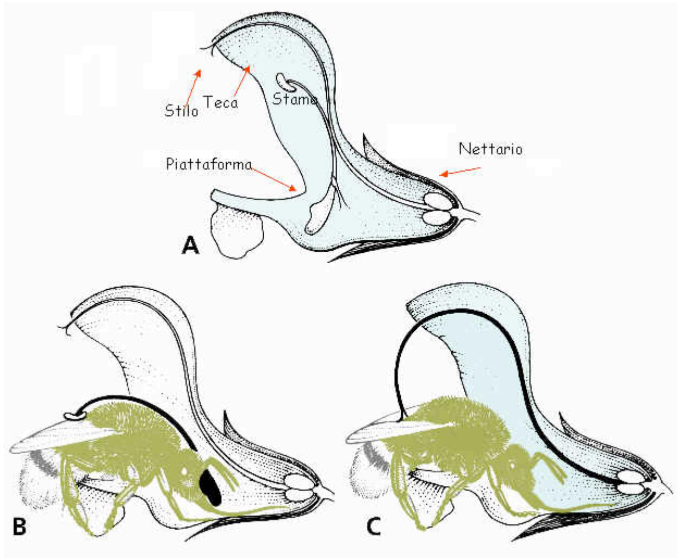
Temporal control

Dichogamy

Protogyny

Aristolochia spp.
Lauraceae (***Persea***)
Magnoliaceae, Piperaceae
Euphorbiaceae

Protandry



Lamiaceae (***Salvia***),
Rutaceae, Leguminosae,
Campanulaceae, Cariophyllaceae,
Asteraceae

Aegonychon purpurocaeruleum (L.) Holub

I
Bud

II
Female

Anthers immature (closed, no pollen)

III
Hermaphrodite young

IV
Hermaphrodite mature

Anthers immature (closed, no pollen)

Echium vulgare L.

Functionally male

1. Anthers dehiscent

Functionally female

2. Stigma rough+stick
Pollen presentation ends

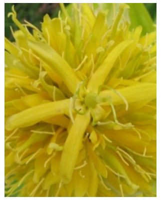
3. Style longer than stamens

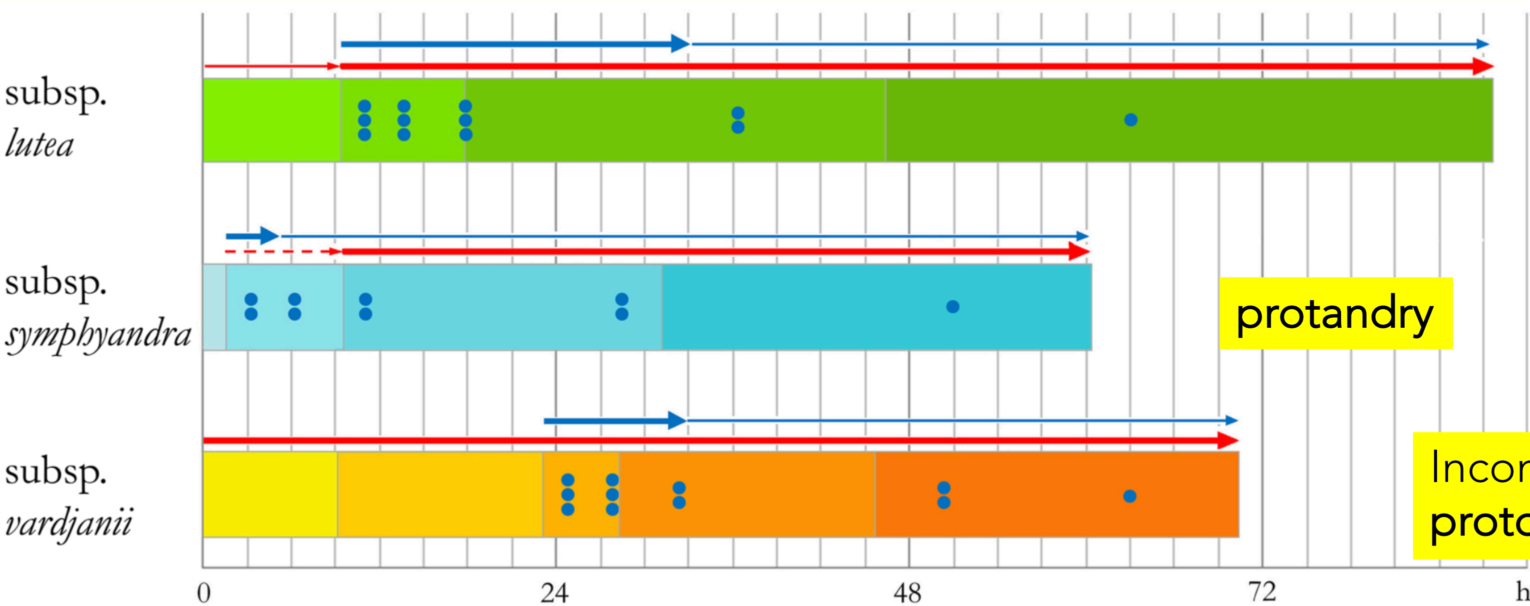
4. Stamens and corolla shrivel[5]

❖ Male: female ratio in the population 1: 9

[5]Corbet S. (1974). Ecological Entomology, 3 25- 37

Gentiana lutea L.





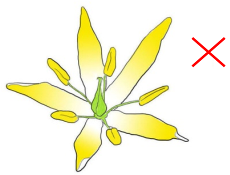
adichogamy

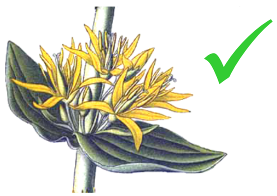
protandry

Incomplete
protogyny

Figure 3. Temporal patterns of flower development (h = hours). Colour gradations indicate the duration of each floral phase, from I (lighter) to V (darker), in subsp. *lutea* (green), *symphyandra* (blue) and *vardjanii* (orange). Red arrows indicate female phases (stigmatic receptivity): thin - class II (dotted line: co-presence of stigma undivided and class II of receptivity); thick - class I. Blue arrows indicate the abundance of exposed pollen: thick - abundant presence of fresh pollen; thin - presence not appreciable. Blue circles indicate pollen viability: 3 circles = I class; 2 circles = II class; 1 circle = III class (see text for details).

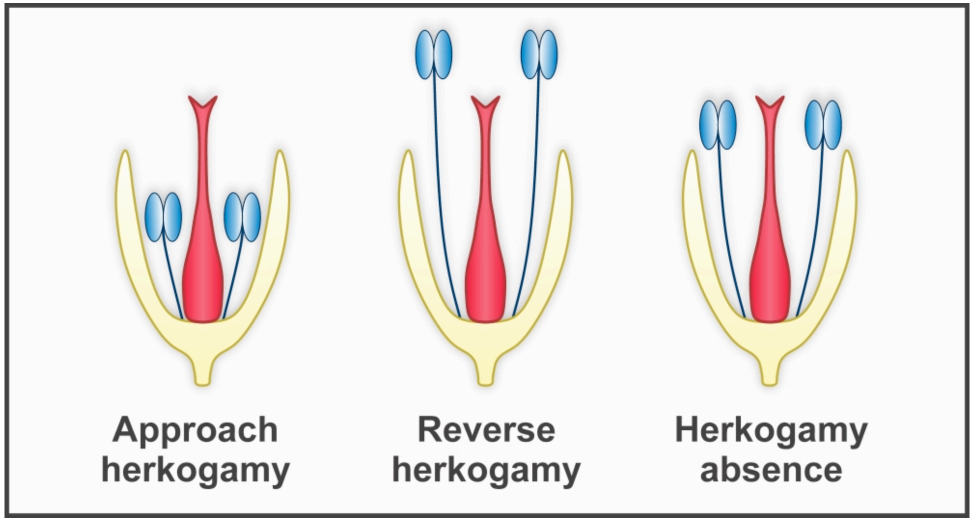
Pollination
unit

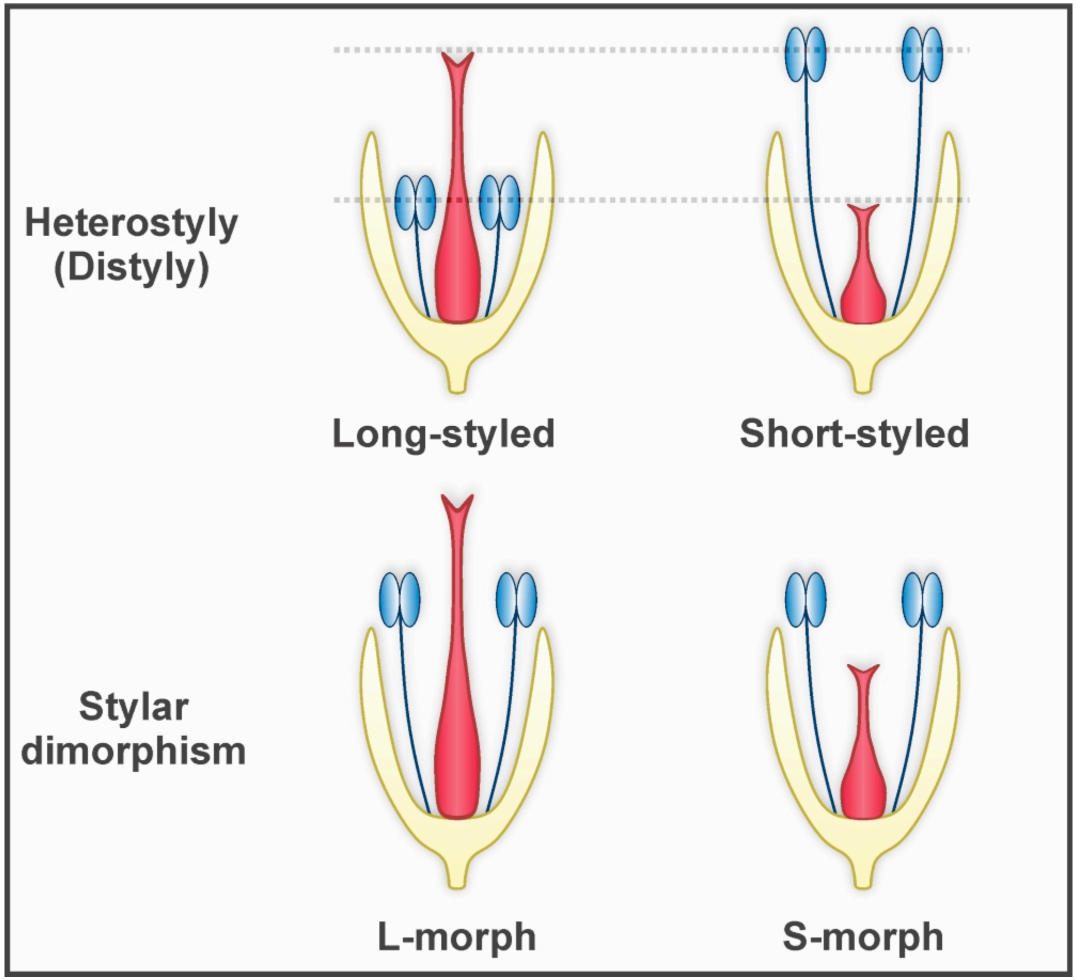




Mechanical control

HERKOGAMY





Mechanical control

HERKOGAMY

Reciprocal: Heterostyly

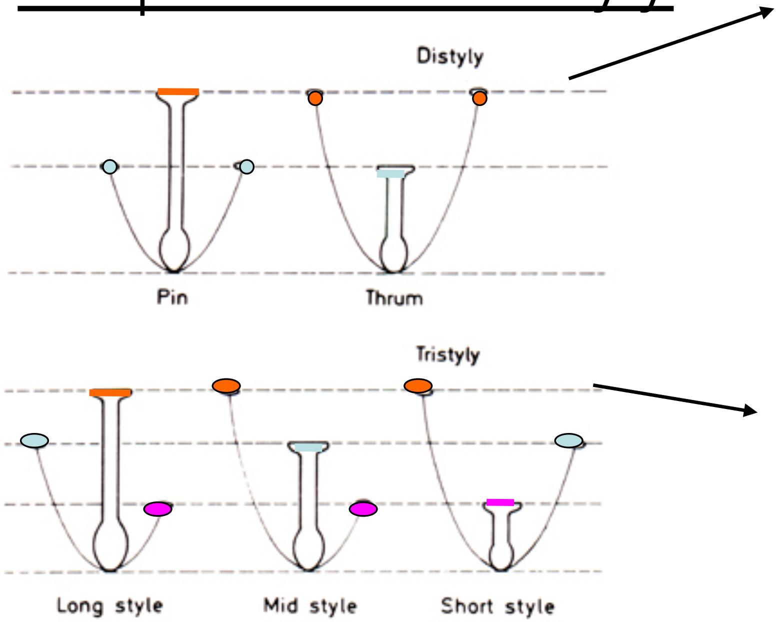
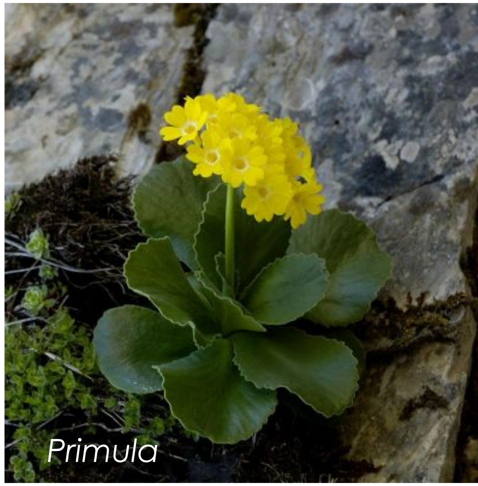
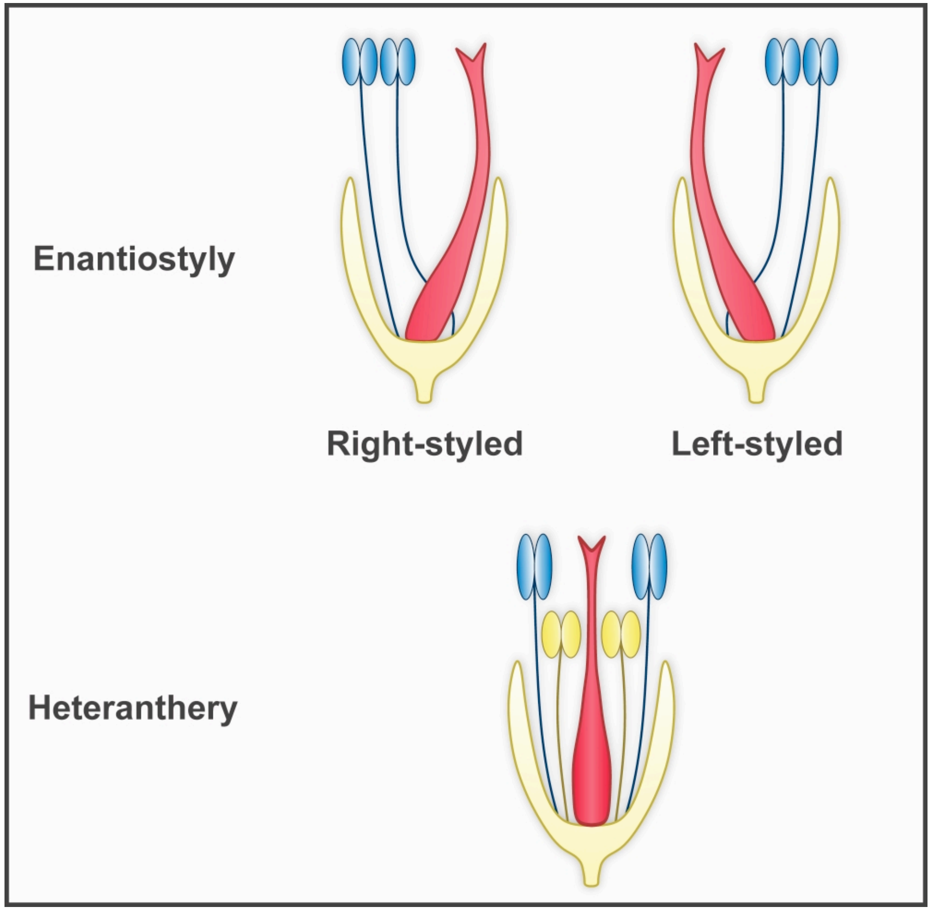
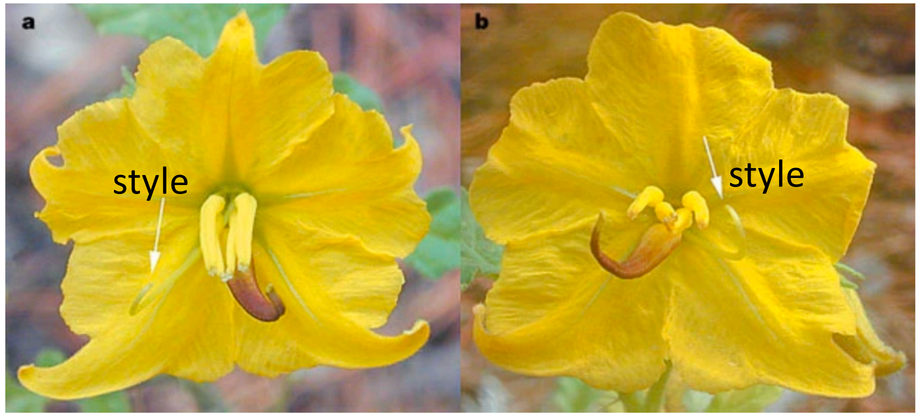


Fig. 7.3. Symbolic representation of distyly and tristyly. In each system, the compatible pollinations only involve anthers and styles at the same level, and therefore the following are incompatible combinations: pin × pin, thrum × thrum, long × long, mid × mid, and short × short. (From De Nettancourt, 1977.) Darwin (1877) listed 14 families in which heterostyly had been confirmed. This list has now been extended to *c.* 25 families (Barrett, 1992).

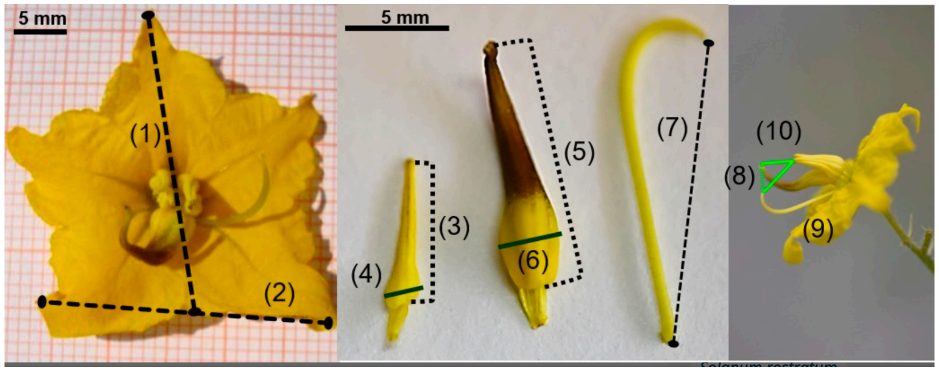
Generally accompanied by physiological incompatibility







The 'mirror-image' flowers of *Solanum rostratum*



(Jessen & Barrett 2002, Solis-Montero & Vallejo-Marin 2017)

Mechanical and temporal control

Dictamnus albus L.

temporal herkogamy
+ dichogamy

Phase ♂: 3.20 ± 0.20 gg

Phase ♀: 2.70 ± 0.75 gg

Intra-flower pollination avoided

Male phase

Female phase

5-6 days

Mechanical control

Stigmatic cuticle or “membrane”

- Flower buds
- Not manipulated/ unvisited flowers

- Open /visited flowers
- Hand-manipulated flower buds

Cytisophyllum sessilifolium

Species	Stigma receptivity			Stigmatic cuticule
	Stage 1	Stage 2	Stage 3	
Coronilla emerus L.	–	–	+	+
Coronilla varia L.	–	–	+	+
Hippocrepis comosa L.	–	+	+	+
Cytisophyllum sessilifolium (L.) Lang	–	+	+	+
Cytisus hirsutus (L.) Link	–	+	+	+
Cytisus scoparius (L.) Link	–	+/–	+	+
Genista cilentina Valsecchi	+/–	+	+	Nt
Genista januensis Viv.	–	+	+	+
Genista radiata (L.) Scop	–	+	+	Nt
Genista tinctoria L.	–	+	+	+
Laburnum anagyroides Medicus	–	+/–	+	+
Spartium junceum L.	–	+	+	Nt
Astragalus glycyphyllos L.	–	+/–	+	+
Colutea arborescens L.	–	–	+	+
Hedysarum coronarium L.	–	–	+	+
Onobrychis viciifolia Scop.	–	–	+	+
Anthyllis vulneraria L.	–	–	+/–	+
Dorycnium hirsutum (L.) Ser.	–	–	+	+
Lotus corniculatus L.	–	+	+	+
Securigera securidaca (L.) Deg. & Dörfl.	–	–	+	+
Robinia pseudoacacia L.	–	+	+	+
Medicago arborea L.	+/–	+	+	+
Ononis masquillierii Bertol.	–	+	+	+
Ononis natrix L.	+/–	+	+	+
Trifolium alpinum L.	+	+	+	+
Lathyrus aphaca L.	+	+	+	–
Lathyrus hirsutus L.	–	+	+	+
Lathyrus latifolius L.	–	+/–	+	+
Lathyrus pannonicus L.	+/–	+	+	+
Vicia cracca L.	+	+	+	–
Vicia hybrida L.	+	+	+/–	–
Vicia sativa L. var. *angustifolia*	–	–	+/–	–

Galloni et al. 2007; Dafni et al. 2005

Limitations to selfing

Temporal separation : Dichogamy → Protandry, Protogyny

Spatial separation : Herkogamy →
- Ordered
- Reciprocal: HETEROSTYLY
- Temporal
- Flexistyly = reciprocal + dichogamy
- Movement
- Enantiostyly: mirror-image flowers

Mechanical barriers → Stigmatic cuticle

Physiological barriers →
- Pre-zygotic (SSI, GSI)
- Post-zygotic (OSI)

(Barrett 2010; Willmer 2013)

Self-Incompatibily systems(SI)

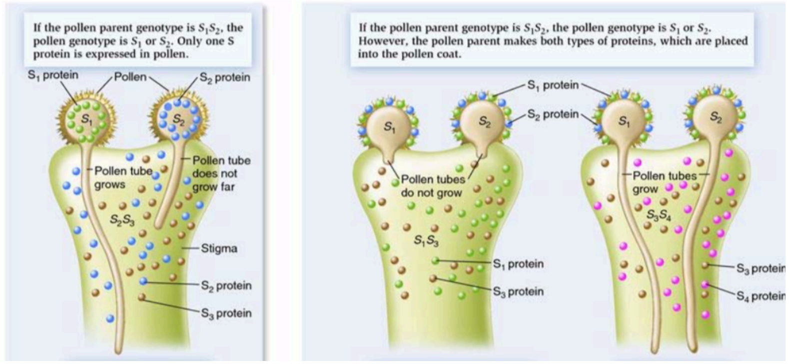
Post-pollination

= inability of a bisexual plant to produce zygotes with its own pollen
= genetic based inability of plants to produce fertile seeds after undergoing some level of selfing

genetically controlled by the multiallelic S gene

biochemical reaction that results in the interruption of pollen tube growth, fertilization or embryogenesis when pollen grains come from the same flower, flowers present in the same individual or flowers from the same morph (intramorph cross-pollination, as in heterostyly)

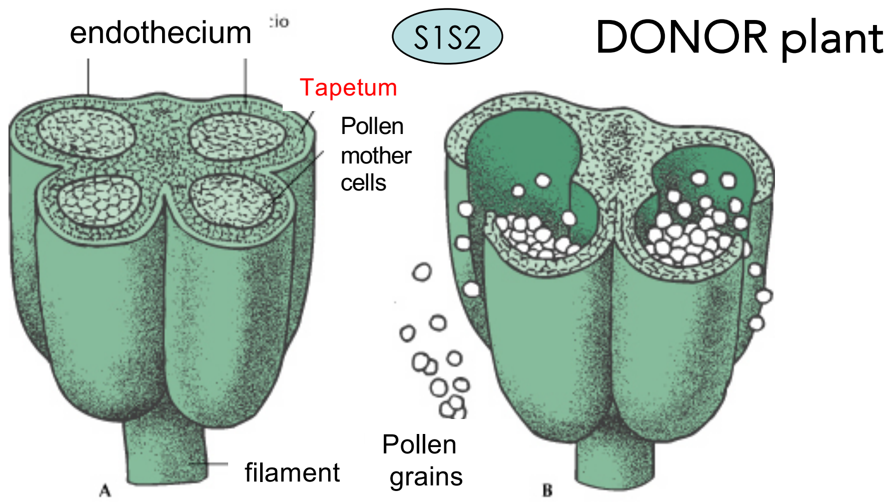
- Pre-zygotic (Sporophytic SI, Gametophytic SI)
- Post-zygotic (Late-acting: LSI, OSI)

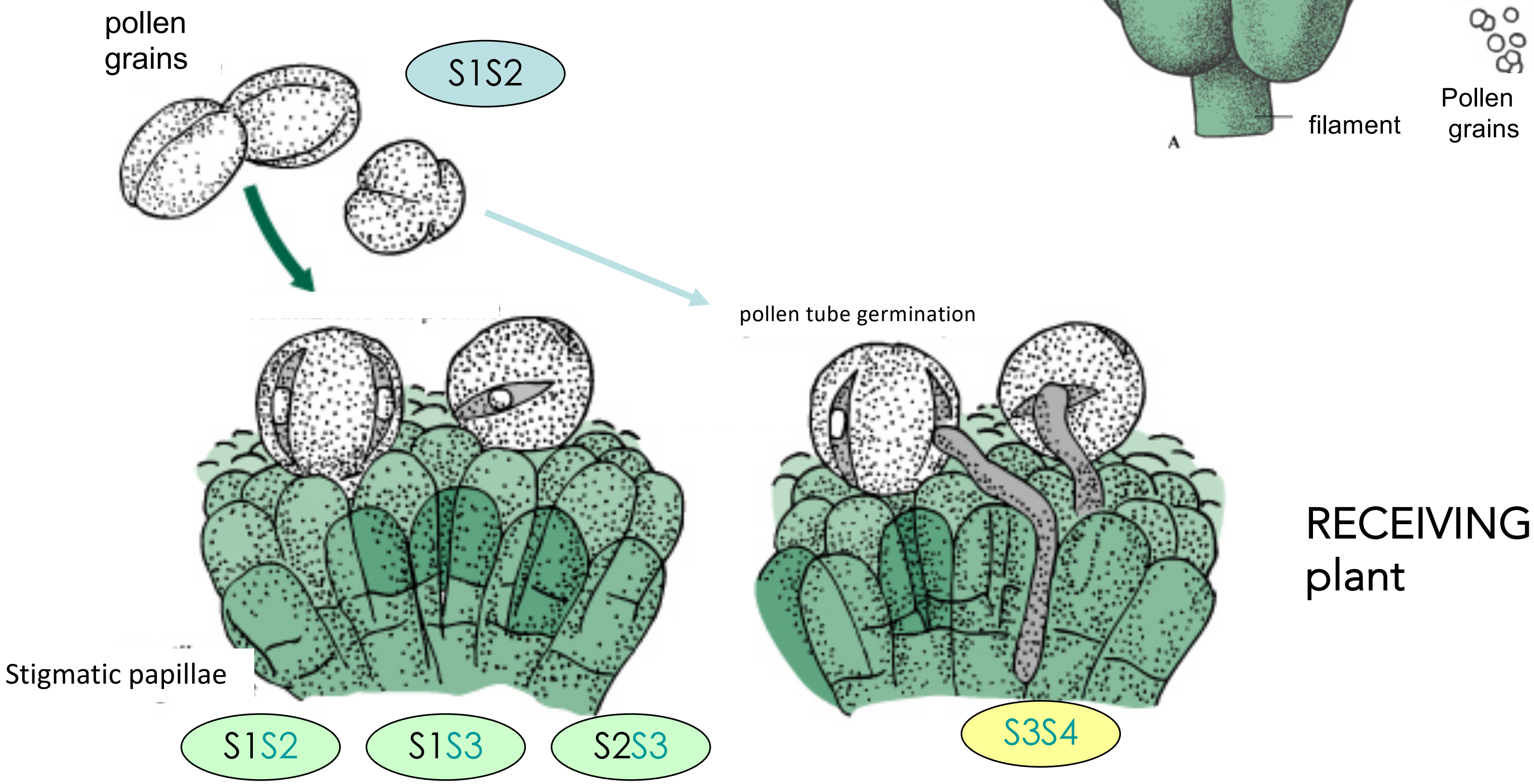


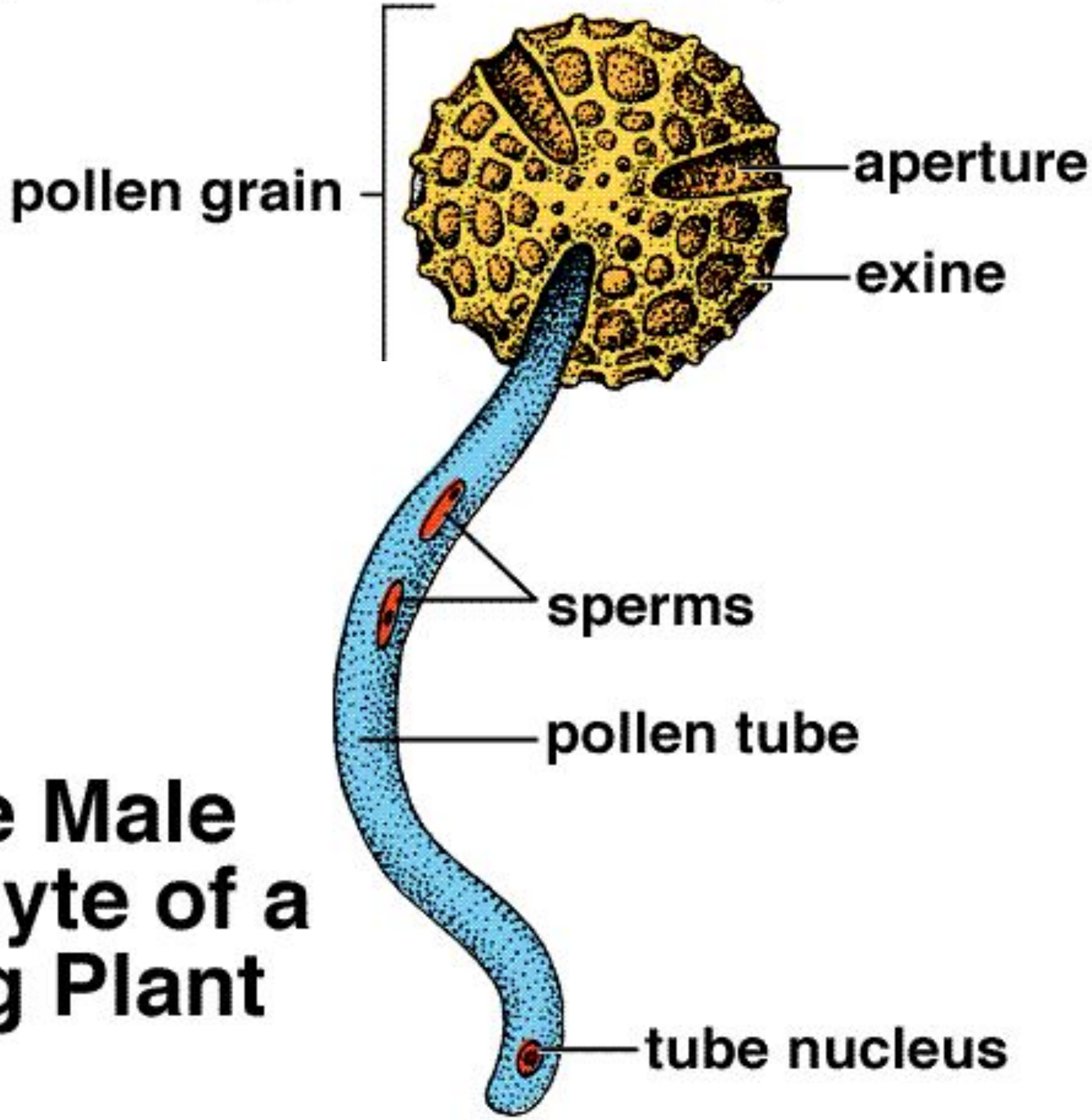
Widely distributed along angiosperms lineages: **in about 71 families, 60% spp.**

Physiological control

Sporophytic Incompatibility



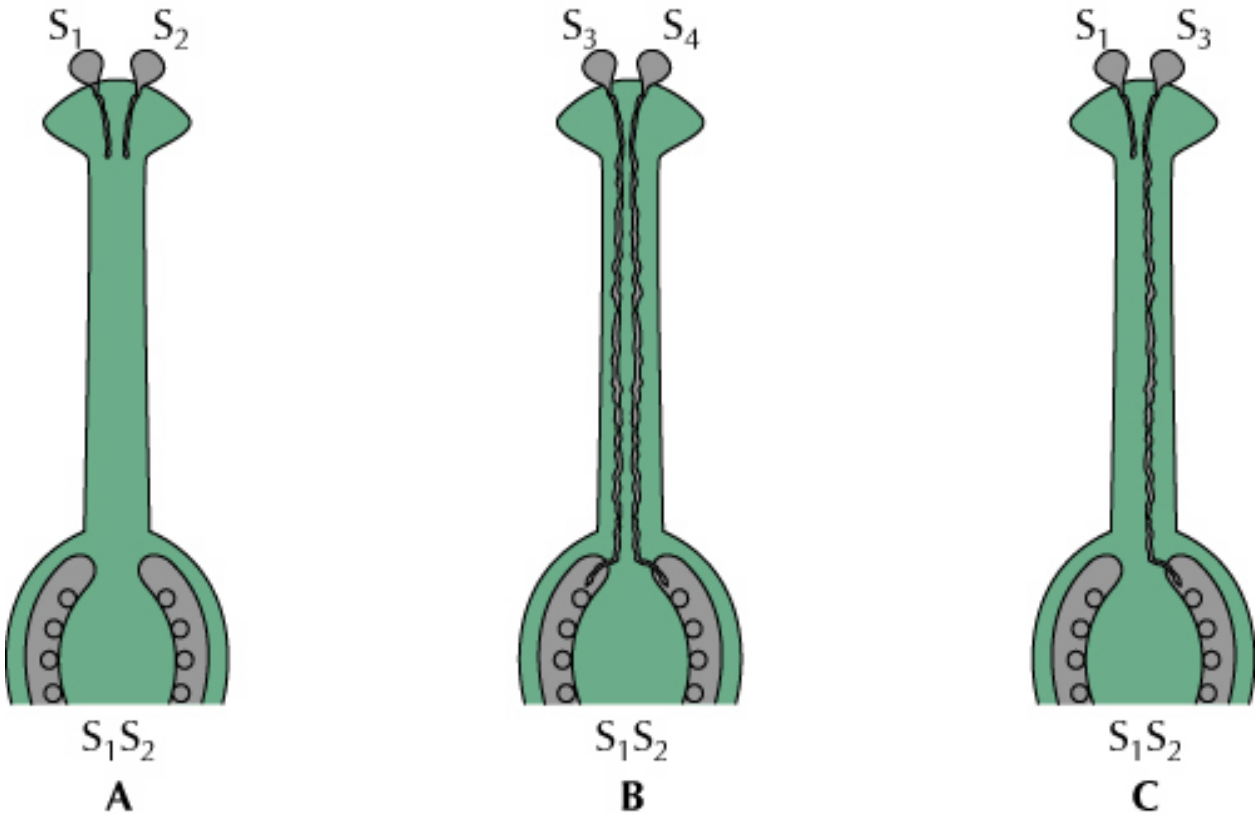




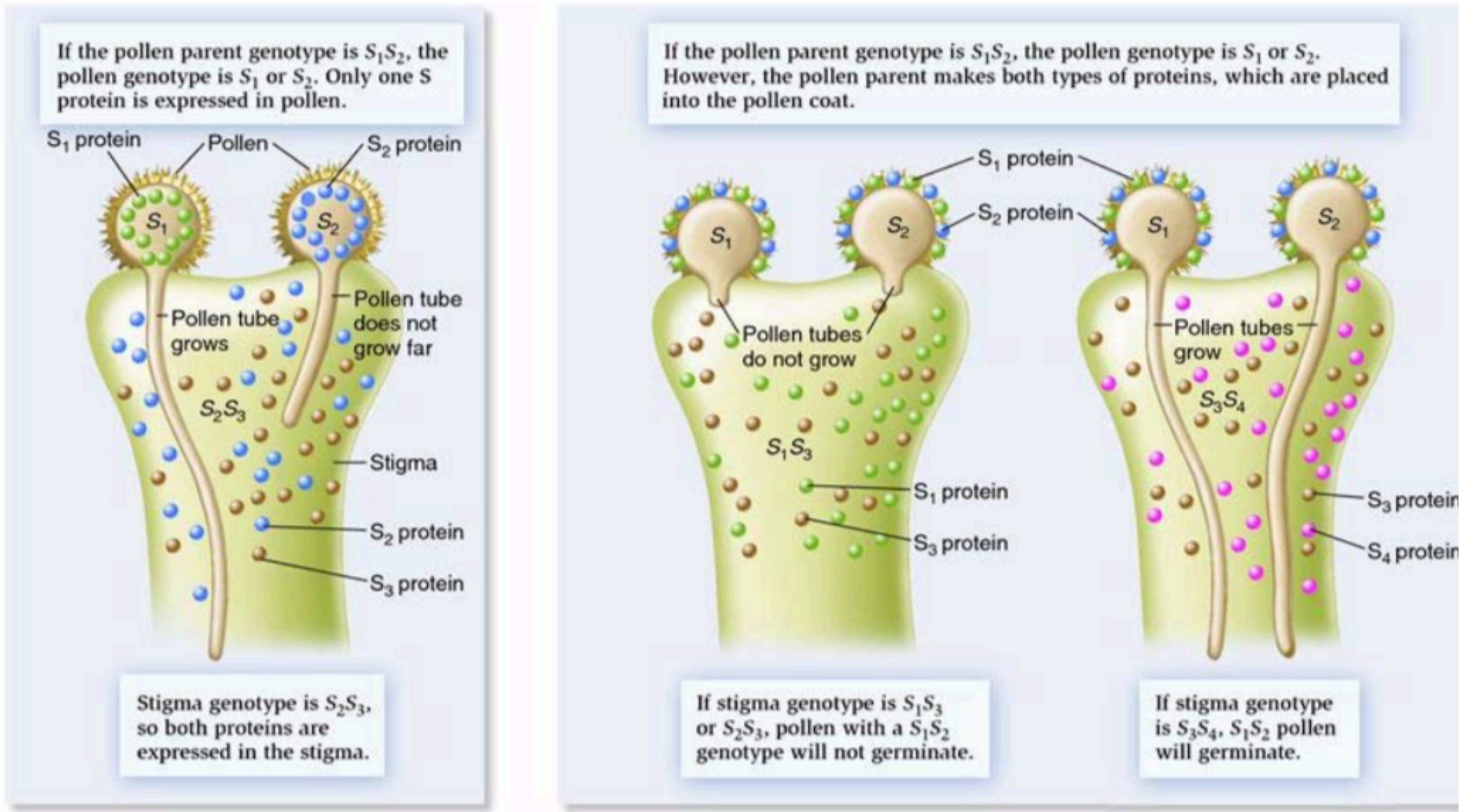
A Mature Male Gametophyte of a Flowering Plant

Physiological control

Gametophytic Self-incompatibility

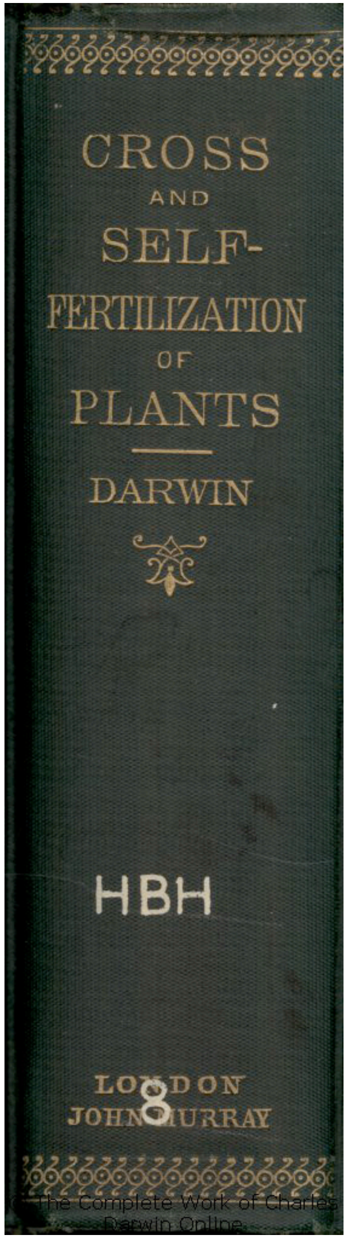


Gametophytic   Sporophytic



(a) Gametophytic SI: If pollen *S* allele does not match either stigma allele, pollen will germinate.

(b) Sporophytic SI: If pollen coat S proteins do not match either stigma S protein, pollen tubes will grow.



1876 http://darwin-online.org.uk

The **number and quality of offspring produced by a plant can be regulated even after fertilization**. Such post-zygotic control of reproduction occurs through the differential survival of whole fruits or individual seeds

A particular **important and well-studied form of post-zygotic selection** is

INBREEDING DEPRESSION, the **reduced fitness of inbred offspring relative to outbred offspring**

traits subject to inbreeding depression

- pollen quantity
- pollen viability
- number of ovules
- amount of seeds
- germination rate
- growth rate
- competitive ability

Practical approach

- Phenological surveys
- controlled pollination experiments
- Resource sexual allocation (P/O)
- Self-pollination and Self-compatibility Index
- *In-vivo* pollen germination experiment
- Inbreeding depression index

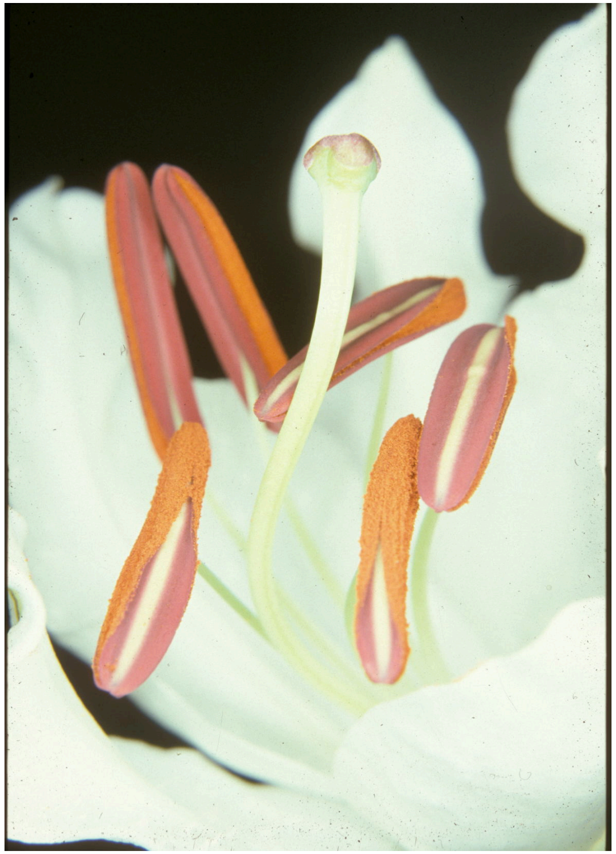
Pre-conditions for pollination

- Pollen: available, mature, viable, reaching a conspecific stigma

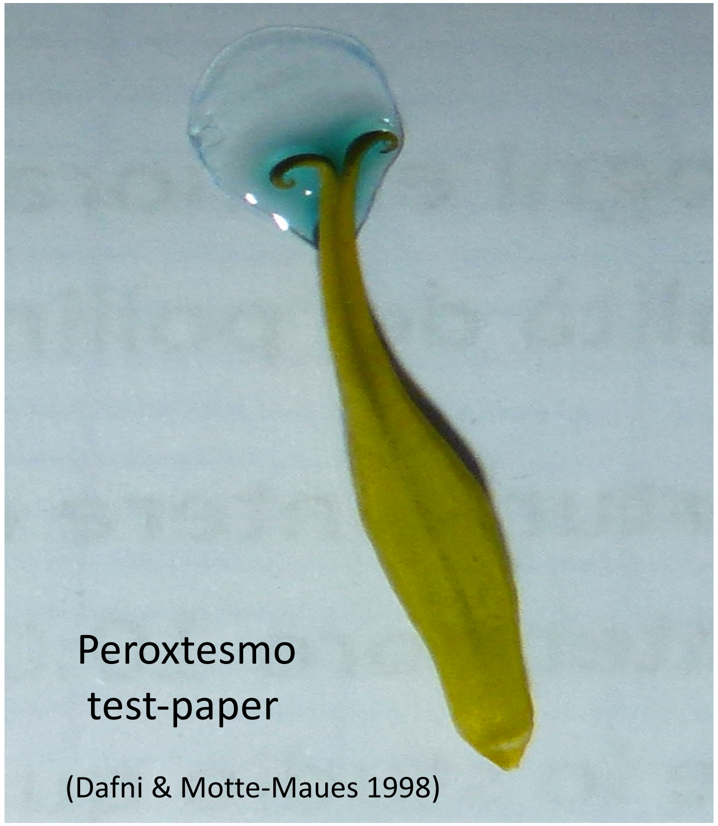
  → able to germinate

- Stigma: receptive

  → allows pollen germination



Stigma receptivity



Stigmatic receptivity: crucial stage in the maturation of the flower can be defined as the "capacity to support pollen germination" or the "readiness to mantain pollen germination"

Each method should be calibrated on the plant species

It is studied for various purposes:

… to **identify opimal flower age for artificial pollination**

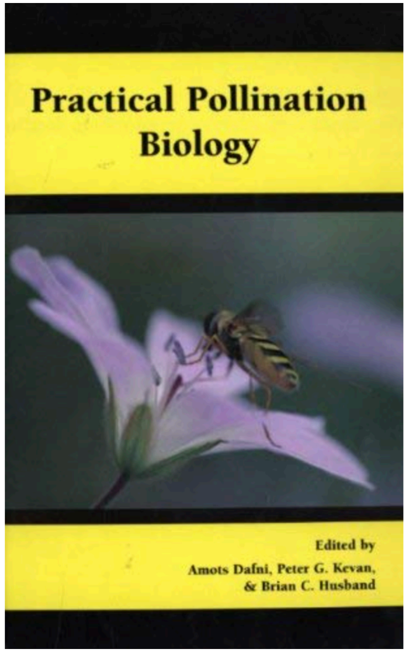
… to investigate pollination efficiency

… to investigate **interference between male and female functions**

… to **study breeding systems**

… to determine relative effectiveness of pollinator visits to flowers at different anthesic stages

Collecting phenological data



Phenology = timing of biological events - can be studied at different scales:

- SINGLE FLOWER → timing of maturation of sexual structures
- INDIVIDUAL PLANT
- POPULATION

Events in the single flower

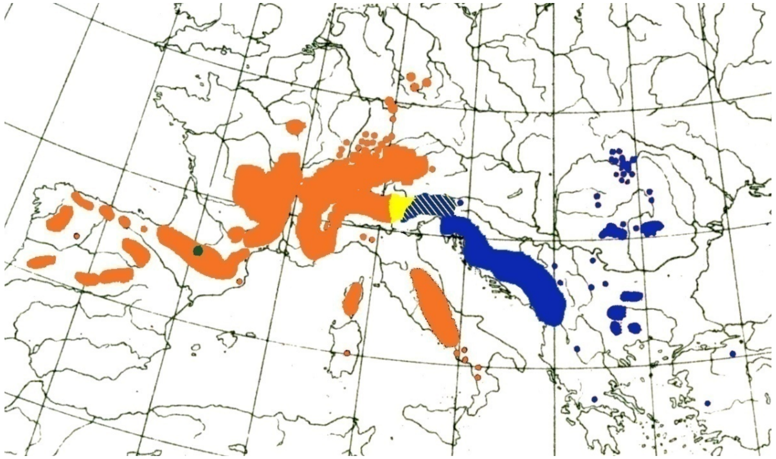
Materials:

- coloured tags/ flower markers durable under field conditions
- Recording data sheet

Method:

- Mark at least 5 flowers x 5 plants at bud stage on different parts of the plant, each with distinctive marker
- Observe flowering progress in the field regularly (every day or *n* hours, depending on floral anthesis duration)
- Complete full set of data for each flower, including general infos on species, pop, habitat, time,… and the flower stage (previously identified)

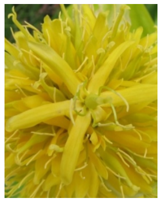
Gentiana lutea L.



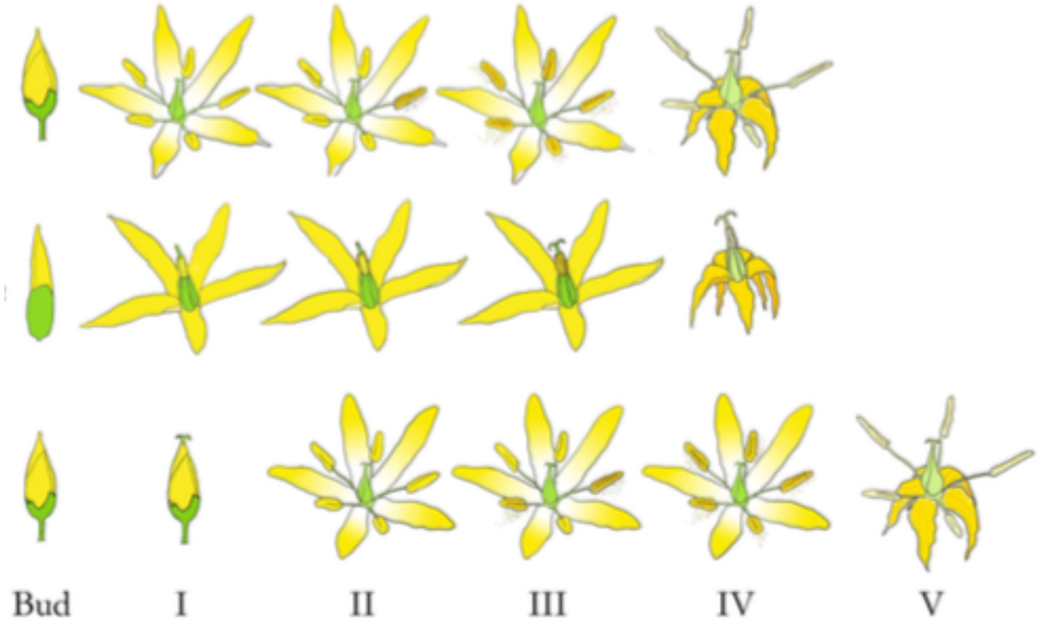
- *G. lutea* ssp. *lutea* L.
- *G. lutea* ssp. *Symphyandra* Murb. (Hayek)
- *G. lutea* ssp. *vardjanii* Wraber

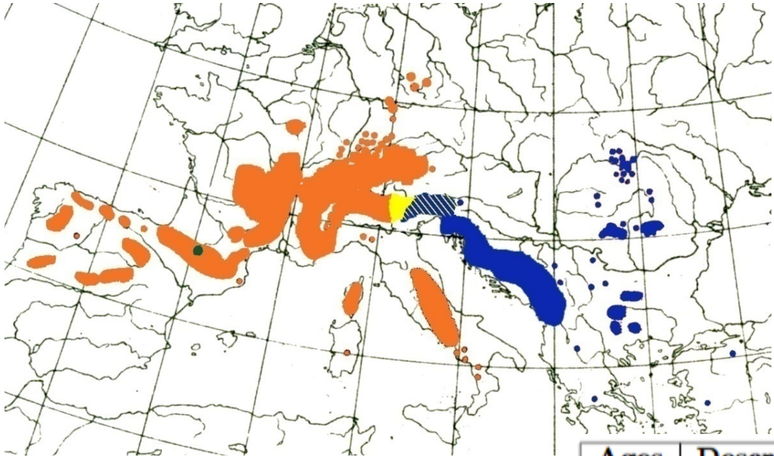
subsp.
lutea

subsp.
symphyandra



subsp.
vardjanii





Gentiana lutea L.

- *G. lutea* ssp. *lutea* L.
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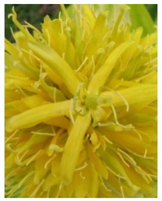
- *G. lutea* ssp. *vardjanii* Wraber

subsp. *lutea*

subsp. *symphyandra*



subsp. *vardjanii*

Ages	Description	fl (st)
	subsp. *lutea*	
	Bud	-
I	Open flower, stigma hardly bilamellate	17 (8)
II	Open flower, stigma bilamellate, 1-4 dehisced anthers	26 (8)
III	Open flower, stigma bilamellate, complete anthers dehiscence	26 (8)
IV	Perianth withered	12 (4)
	subsp. *symphyandra*	
	Bud	
I	Open flower	10 (4)
II	Open flower, stigma undivided or hardly bilamellate, 1-4 dehisced anthers	20 (5)
III	Open flower, stigma bilamellate, complete anthers dehiscence	23 (5)
IV	Perianth withered	17 (4)
	subsp. *vardjanii*	
	Bud	-
I	Bud, stigma bilamellate poked out through the top of the corolla	21 (9)
II	Open flower, stigma bilamellate	28 (9)
III	Open flower, stigma bilamellate, 1-4 dehisced anther	33 (10)
IV	Open flower, stigma bilamellate, complete anther dehiscence	24 (7)
V	Perianth withered	11 (3)

Characterizing plant breeding systems: experimental techniques

- Controlled pollination in the field:

- SPONTANEOUS SELFING
- HAND-SELF POLLINATION
- HAND-CROSS POLLINATION
- CONTROL (NATURAL CONDITION)

- POLLEN SUPPLEMENTATION

- Comparison

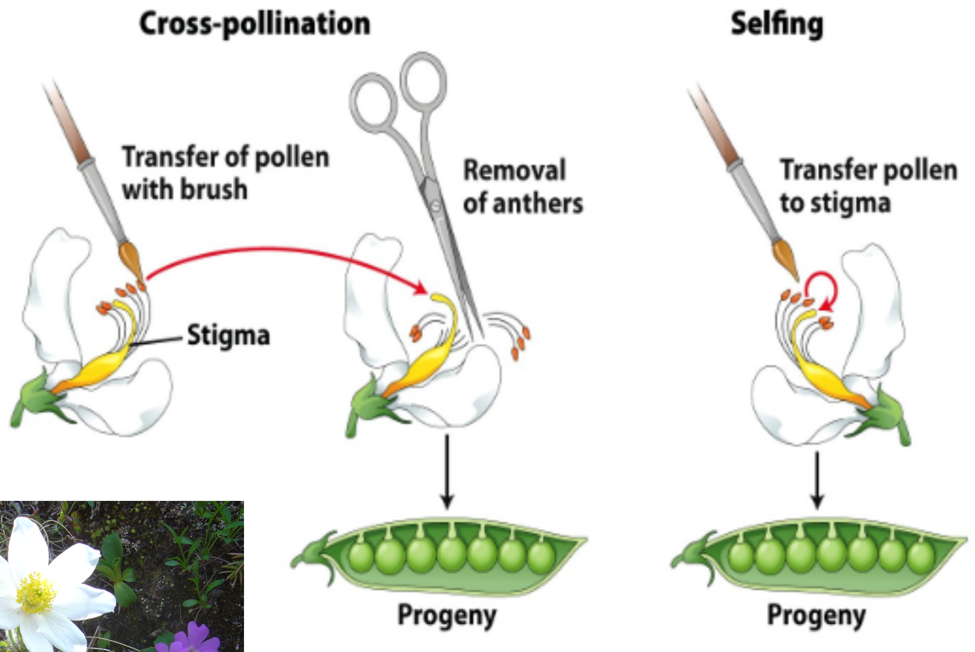
Fruit set = fruits/flowers

Seed set = seeds/ovules

INVESTIGATING PLANT BREEDING SYSTEMS

- Controlled pollinations in the field:

- SPONTANEOUS SELFING (SS)
- HAND SELF-POLLINATION (HS)
- HAND CROSS-POLLINATION (X)
- EMASCULATION→ AGAMOSPERMY (A)
- CONTROL (C)

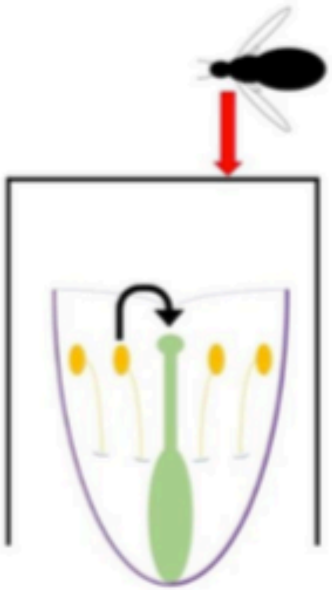


INVESTIGATING PLANT BREEDING SYSTEMS

- ➢ Controlled pollinations in the field:

- SPONTANEOUS SELFING (SS)

♂



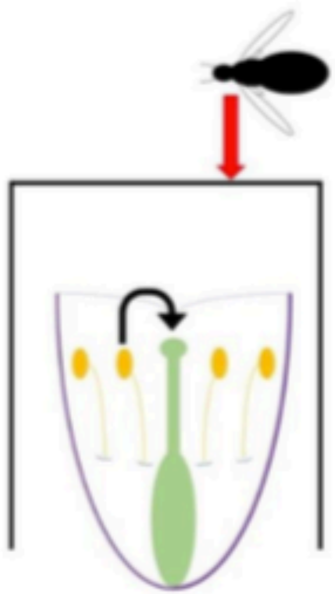
- "Pollination bags"
- No manipulation

INVESTIGATING PLANT BREEDING SYSTEMS

➢ Controlled pollinations in the field:

- SPONTANEOUS SELF-POLLINATION (SS)

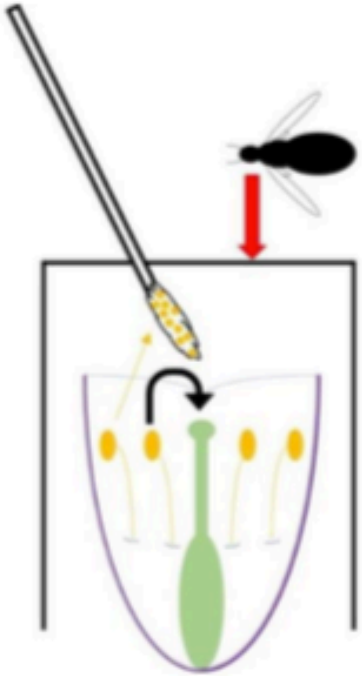
- randomly mark flowers
- in bud stage (not opened yet)
- put bag /net to avoid insect visits and forget the flowers for a while…
- (you can remove the bag after anthesis… and replace it before fruit opening)
- Monitor developing fruits
- Possibly put again bags to collect fruits and seeds

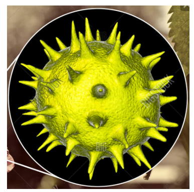


INVESTIGATING PLANT BREEDING SYSTEMS

- ➢ Controlled pollinations in the field:

- HAND SELF-POLLINATION (HS)





→ to assess SELF-COMPATIBILITY

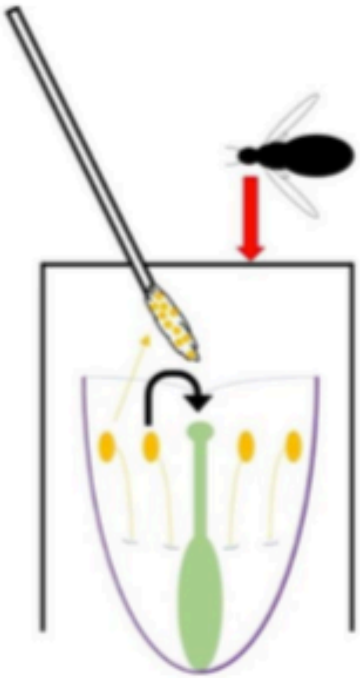
- “Pollination bags”
- Brush/ dehiscent anther (manipulation)

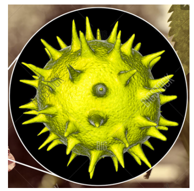
INVESTIGATING PLANT BREEDING SYSTEMS

➢ Controlled pollinations in the field:

- HAND SELF-POLLINATION (HS)

  - randomly mark flowers
  - in bud stage (not opened yet)
  - put bag or net to avoid insect visits
  - Once flowers will open (…), pollinate the marked flowers with self-pollen (from same individual)
  - Remove the bag at the end of flowering (developing fruits)
  - Monitor developing fruits (if there are any)
  - Eventually put again bags to collect fruits and seeds

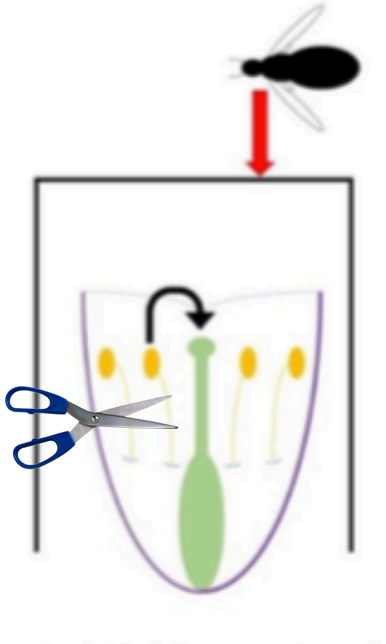




INVESTIGATING PLANT BREEDING SYSTEMS

➢ Controlled pollinations in the field:

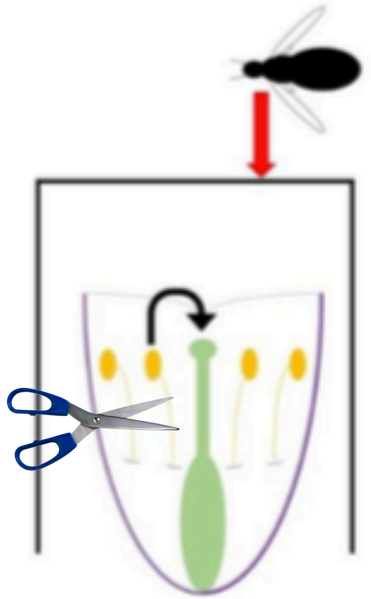
- EMASCULATION→ AGAMOSPERMY (A)



INVESTIGATING PLANT MATING SYSTEMS

- Controlled pollinations in the field:

- EMASCULATION→ AGAMOSPERMY (A)

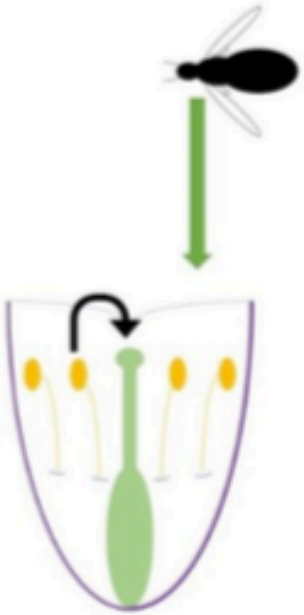


- Remove the stamens before anther dehiscence
- Avoid insect visits
- In some cases, due to flower morphology it might be difficult to cut the stamens → cut the style instead

INVESTIGATING PLANT BREEDING SYSTEMS

- Controlled pollinations in the field:

- CONTROL (C)



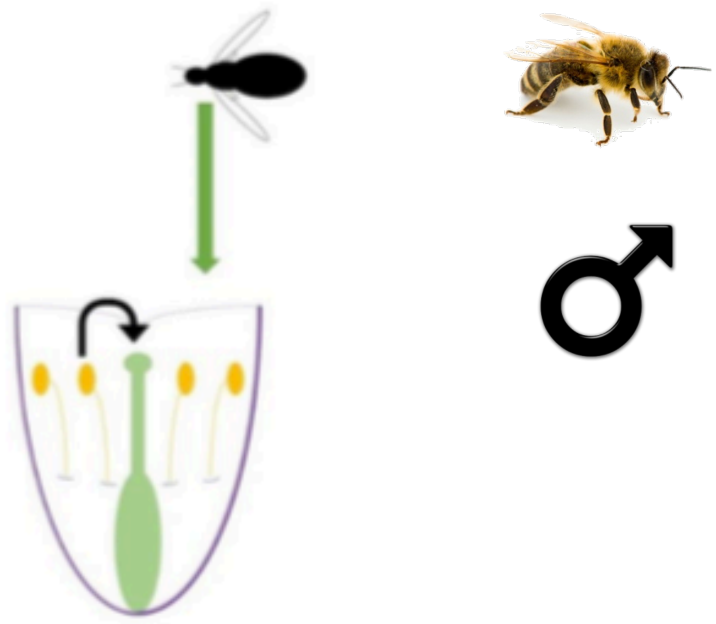
♂

No bags
Flowers free to be visited by insects

INVESTIGATING PLANT BREEDING SYSTEMS

- Controlled pollinations in the field:

- CONTROL (C)



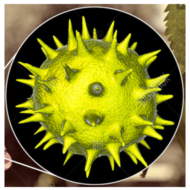
- randomly mark flowers - at the same anthesic stage as those chosen for the other tests - similar number as those chosen for other tests (avoid sample bias)

- No bags
- Flowers free to be visited by insects
- Pollination and reproductive success in natural conditions

Assessing Pollination Limitation

- Hand cross-pollen supplementation

♂



- No bags
- Flowers free to be visited by insects + manually CROSS-POLLINATED

Assessing Pollination Limitation

- Hand cross-pollen supplementation

randomly mark flowers

- at the same anthesic stage as CONTROL flowers

- sample similar to CONTROLS

- try to pollinate effectively…

- No bags
- Flowers free to be visited by insects + manually CROSS-POLLINATED

MATERIAL

-Paint brush(es): clean, thin

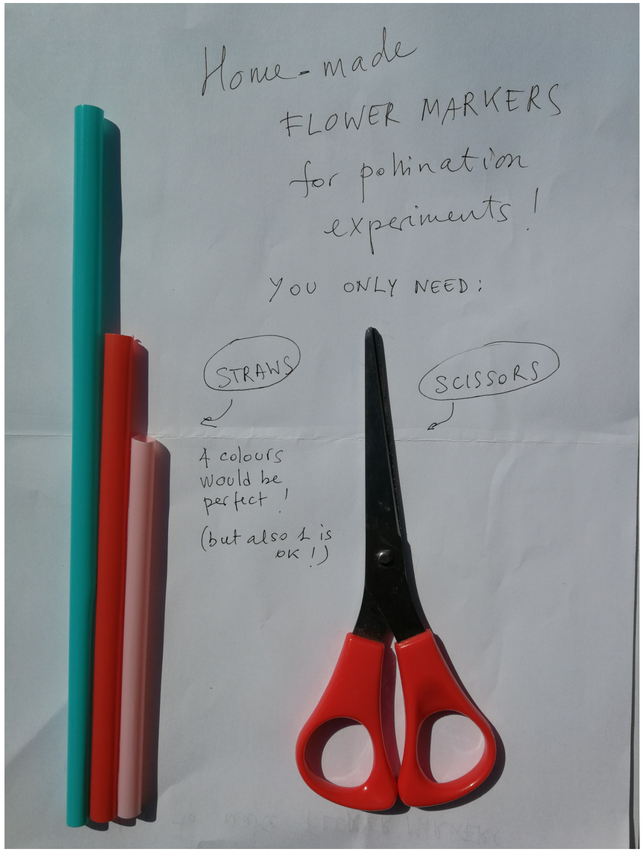
-tweezers

- scissors

-Nylon/tnt/ tulle bag,
nylon socks

-Wire, ribbon, cord
(something to tie the bag)

-Flower markers



SAMPLE size and design

How many flowers?

How many plants?

How many flowers per stem / plant?

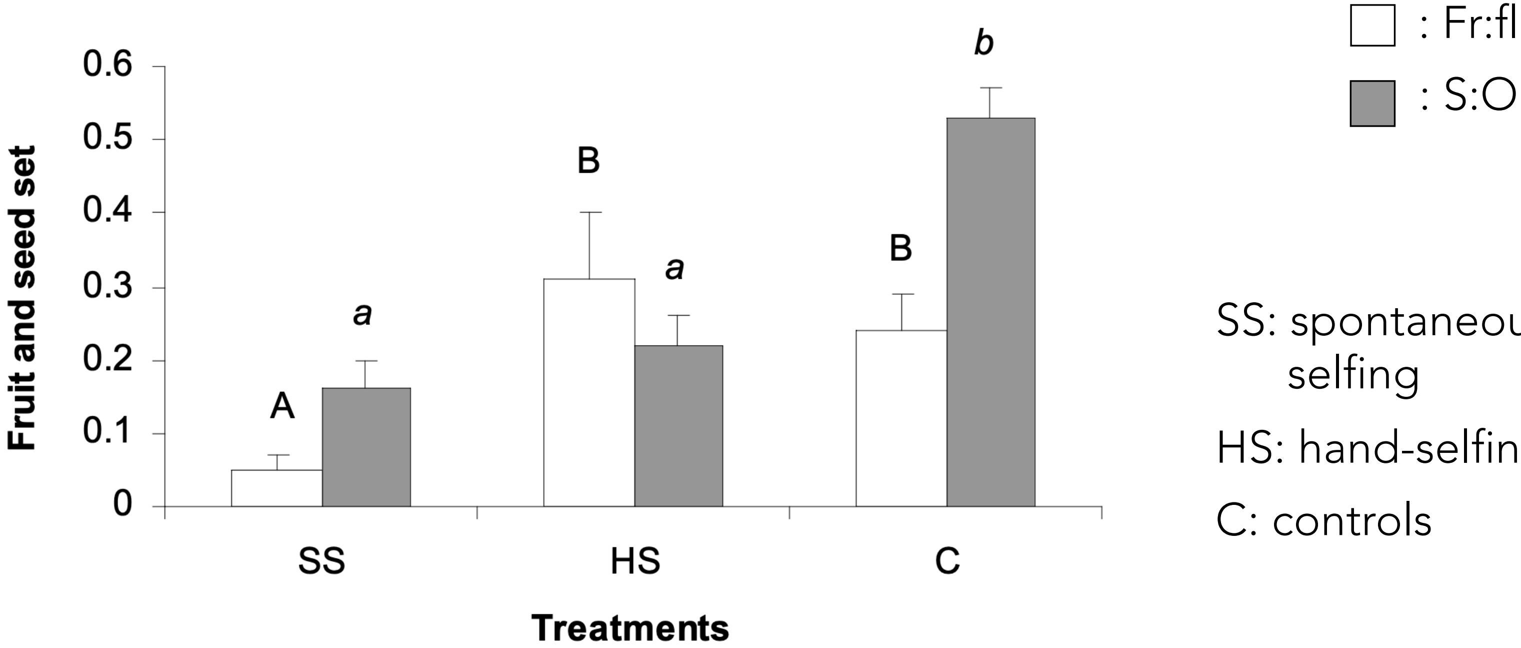
How many tests on the same plant?

And… how many populations?

WHEN and HOW to collect FRUITS and SEEDS?

Reproductive success

- No agamospermy



SS: spontaneous selfing

HS: hand-selfing

C: controls

Fr:fl → Significant differences: SS vs HS (p=0.02); SS vs C (p<0.002)

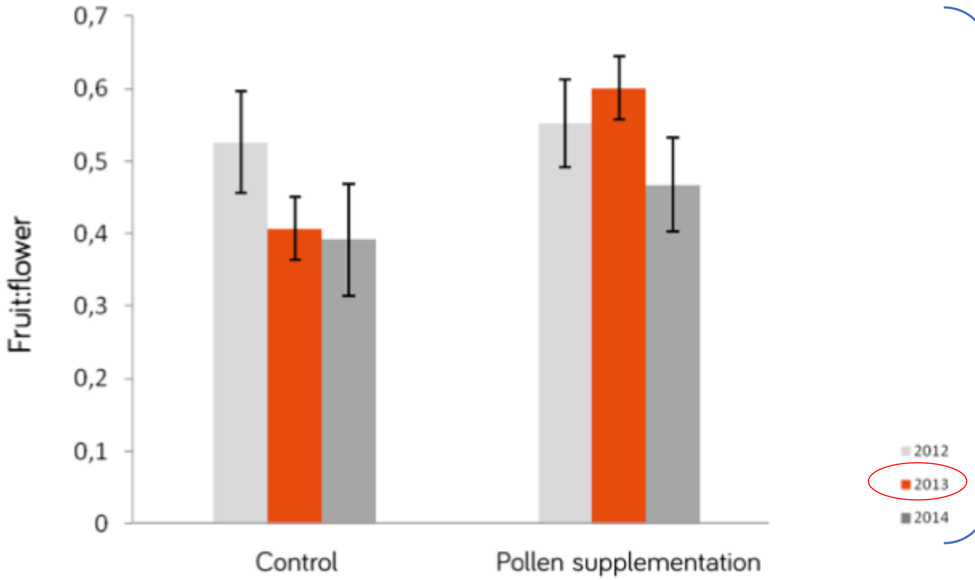
S:O → Significant differences : SS vs C (p<0.002); HS vs C (p<0.001)

Assessing Pollination Limitation

Hand cross-pollen supplementation

Control vs. Supplemented

- Collect fruits
- Count unfertilized or aborted ovules and mature seeds/fruit
- FRUIT SET = fruits/flowers
- SEED SET = seeds/ovules

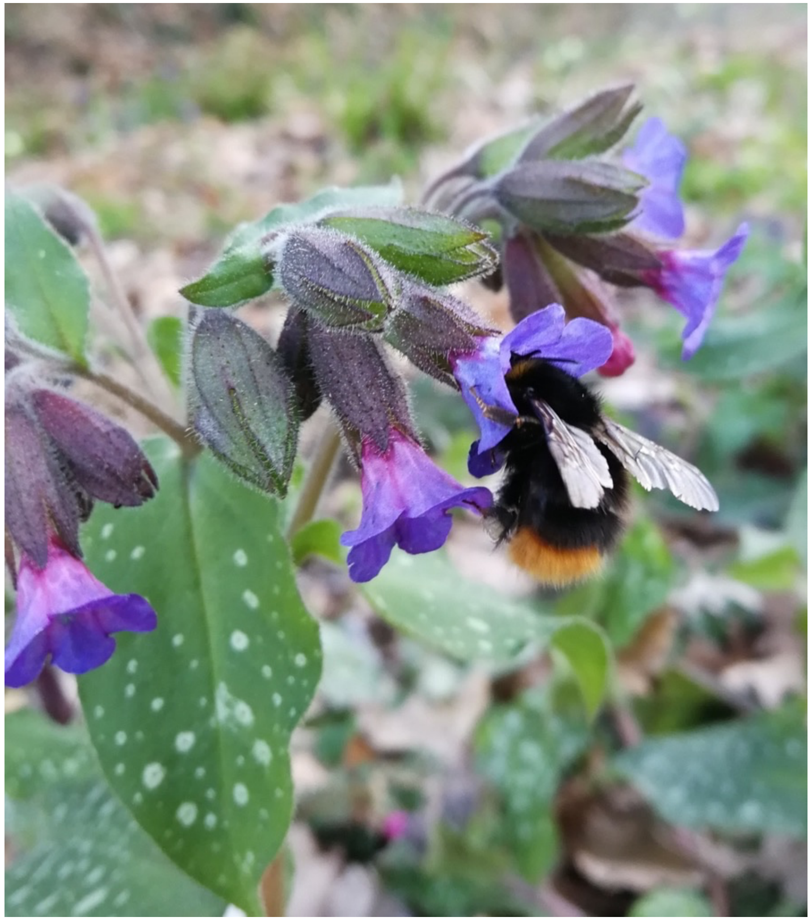


FRUIT SET
Pollinator limitation
only in in 2013

n.b. LIMITATION can be due
either to pollen transfer (pollinator limitation)
or to pollen quality (pollen limitation)

How you know you did pollinate correctly?

Any attempt to characterize plant breeding systems would require some previous knowledge on the temporal and spatial aspects of reproductive phenology, and so the evaluation of stigmatic receptivity and pollen viability.



You can't be sure, but you can increase the likeliness of pollination …

1. POLLEN: visual assessment of **anther dehiscence,** presence of pollen grains, colour, aspect. Make **preliminary observations**: observe flowers in different age stages.

2. Use **pollen from different flowers** and from different plants (sufficiently distant from the pollen-receiving plant)

3. Be quick and accurate! Don't use "old" pollen (collected the day before)

4. STIGMA: make **observations** to understand the development of female structure (style lenght, stigma aspect, colour,…) during flower lifespan

5. Repeat the pollination at least **twice** (two different days)

↑ P/O → ↓ Pollen transfer efficiency

- Collect *n* flower buds (ANTHERS close to dehiscence but still CLOSED) from different individuals/population
- Count ovules number per ovary using a stereo microscope and manual counter;
- Store anthers in microcentrifuge tube with 400 µl preserving solution (½ glycerin + ½ ethanol 70%)
- collect P from anthers using an ultrasonic water bath for 30' and remove pollen-free anthers
- Add 200 µl (vol depending on P number) of preserving solution to obtain a diluted suspension
- Put an aliquot (2 µl) on microscope slide, add 10 µl Calberla, mount with cover glass
- Count grains with optical microscope and manual counter
- Total n° of pollen grains is given by the result obtained multiplied by the dilution factor

Xenogamy : 2108 - 195525

Facultative autogamy : 245- 2588

Facultative xenogamy: 32- 396

Autogamy: 18 - 39

Cleistogamy: 3 - 6

Cruden 1977; Dafni 2005; Galloni et al. 2007

IAS = Index of Automatic Self-Pollination

$$IAS = Fr{:}Fl_{SS} / Fr{:}Fl_{HS}$$

- IAS = 1 : fully autgamous
- 0 < IAS < 1 : self-compatible
- IAS = 0 : mechanical prevented

SCI = Index of Self-Compatibility

$$SCI = S{:}O_{HS} / S{:}O_{X}$$

- SCI = 1 : completely self-compatible
- 0.2 < SCI < 1 : incompletely compatible
- SCI <= 0.2 : self-incompatible

Zapata and Arroyo (1978); Rossi et al. 2016, Ramirez&Nassar 2017

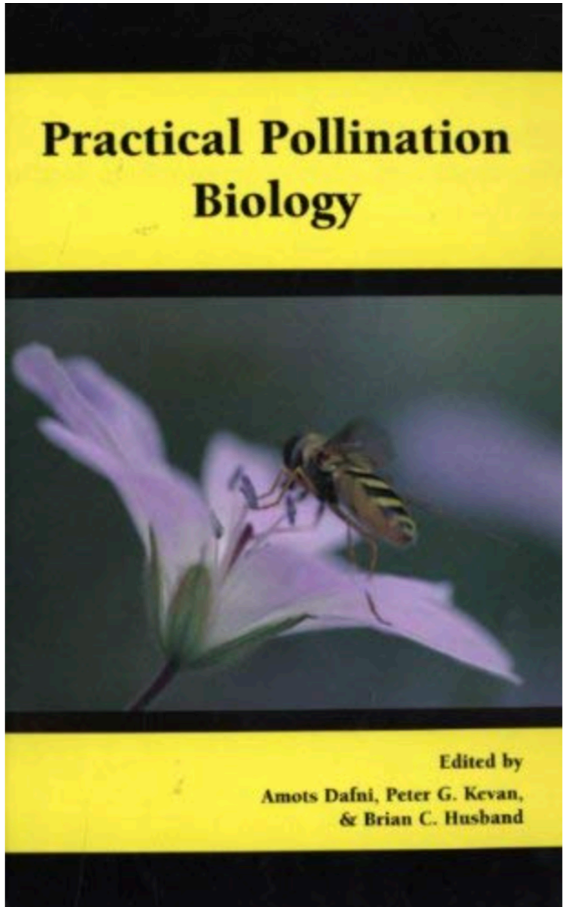
Detection of pollen tubes in the style: Pollen gemination *in vivo*

Materials:

- FPA solution (40% formalin: concentrated propionic acid: 50% ethanol, 5:5:90 respectively) - or FAA
- 8 N Sodium hydroxide
- 0.1 N potassium acetate
- Aniline blue

Method:

- Perform **pollination treatments** (…) in the field
- Collect gynoecia 24 h after the treatments
- Fix gynoecia in FPA solution or FAA
- Bring to the laboratory: soften the styles for 5 (1-12) h in sodium hydroxide. Rinse in tap water for 1-3 h to remove the sodium hydroxide
- mount on microscope slides, stain with 0.1% aniline blue and observe using a fluorescence microscope. Both P tube and callose plugs should show a distinct bright fluorescence

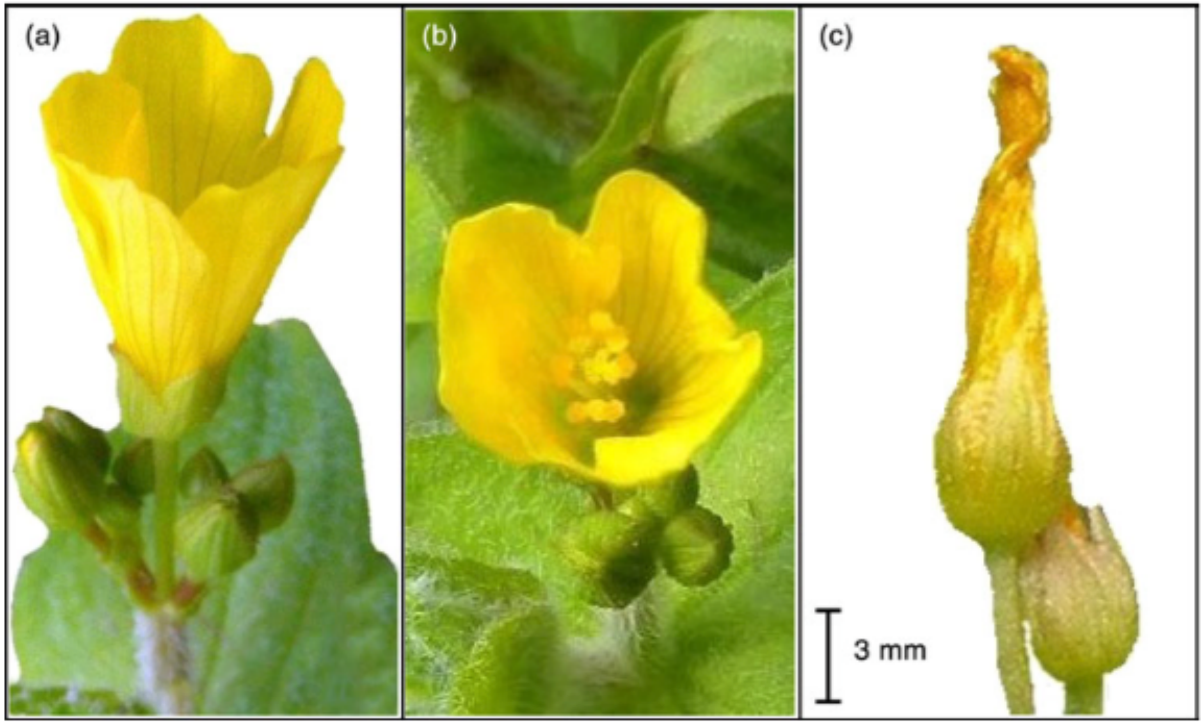


Williams and Knox 1982, Dafni et al. 2005

Hypericum elodes L. in Italy CRITICALLY ENDANGERED

NO HERKOGAMY
NO DICHOGAMY
→ selfing?

One-day lasting flowers



Beginning of anthesis (12 am)

Top view: No herkogamy

Corolla folds up: end of anthesis (5 pm)

Hypericum elodes L.

Phenology indicates possible SELFING: prior, competing or delayed?

Pollination tests

- ✓ APOMIXIS (apo)
- ✓ NATURAL POLLINATION (Open, low and high density flowers)
- ✓ AUTONOMOUS SELF-POLLINATION (ss)
- ✓ HAND SELF-POLLINATION (hs)
- ✓ HAND CROSS-POLLINATION (x, far and near pollen donors)
- ✓ OBLIGATE CROSS-POLLINATION (Open + emasculated)

In vivo pollen germination test

- ✓ Prior flower opening
- ✓ 1 -2,5 - 4 -24 h after pollination (Hand Self vs Hand cross)

Seed germination tests

Mixed mating system !

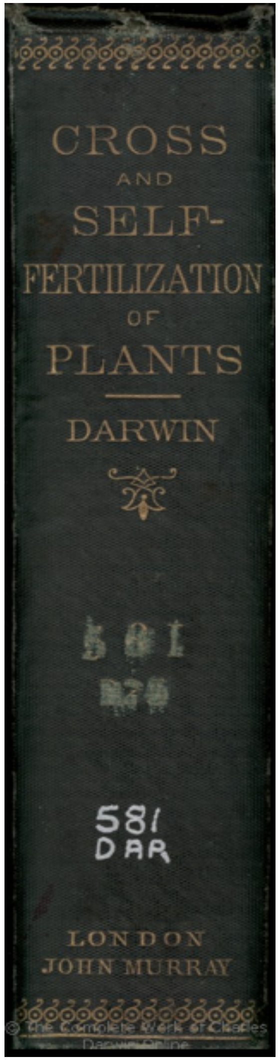
- ➢ Self Compatibility Index (SCI) = $S{:}O_{hs}/S{:}O_x$ = **0.75** → **SELF-COMPATIBLE** (Lloyd & Schoen 1992)
- ➢ Cumulative inbreeding depression **δ=0.57** (mainly pre-dispersal)
- ➢ No pollen tubes in styles from floral buds
- ➢ germinated tubes only in styles collected **after 24h**

- ➢ COMPETING SELFING

  but NOT affecting plant's outcross siring success

(Carta et al. 2016)

Post-zygotic regulation: Inbreeding depression



Darwin 1876

"…I was led to make, during eleven years, the numerous experiments recorded in this volume, by a mere accidental observation; and indeed it required the accident to be repeated before my attention was thoroughly aroused to the remarkable fact that ***seedlings of self-fertilised parentage are inferior, even in the first generation, in height and vigour to seedlings of cross-fertilised parentage…"***

Ws, Wo: mean fitness of **s**elfed and **o**utcrossed offspring

$\delta = 1 - (Ws/Wo)$ $\quad$ $Ws < Wo$

$\delta = (Wo/Ws) - 1$ $\quad$ $Wo < Ws$

Cumulative inbreeding depression calculated considering the overall fitness values, obtained by multiplying the performance of each life stage (P, O, n°Seeds, germination rate, growth rate..) .

Agren &Shemske 1993, Ramsey & Vaughton 1996

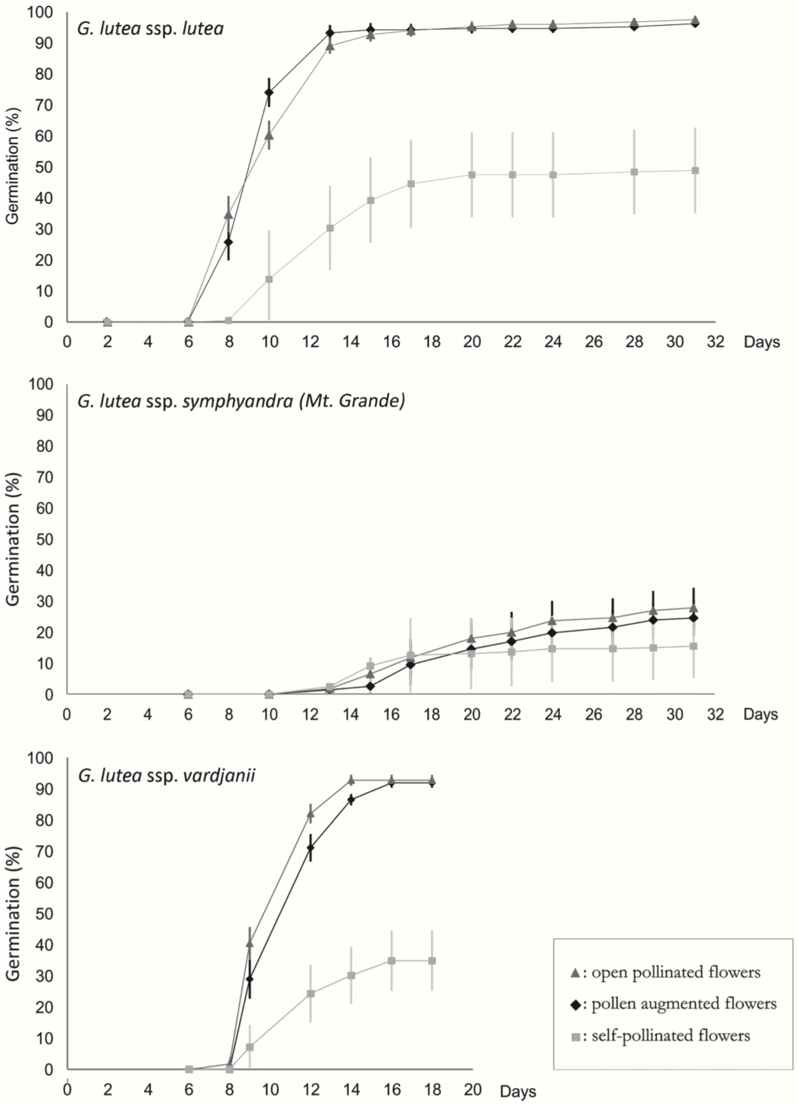


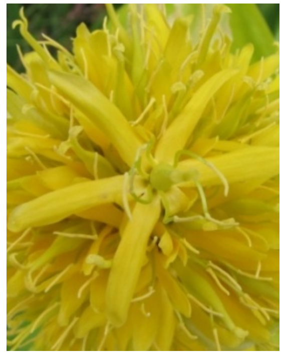
Figure 1. Curves of germination performance of seeds resulting from open-pollinated flowers (black diamonds), pollen-augmented flowers (dark grey triangles) and self-pollinated flowers (light grey squares). Germination percentages are given as mean ± exact binomial 95% confidential interval.

index of inbreeding depression

	Subsp. *lutea* 2011		
	self	cross	δ
Fruit set	0.33	1	0.67
Seed set	0.30 (0.18)	0.71 (0.04)	0.57
Seed weight (mg)	0.81 (0.12)	0.78 (0.02)	–0.30
Germination (%)	48.4 (13.7)	96.5 (0.9)	0.50
Total	5.49	53.36	0.91

	Subsp. *symphyandra* (a) 2010		
	self	cross	δ
Fruit set	0.53	1	0.47
Seed set	0.16 (0.04)	0.74 (0.05)	0.79
Seed weight (mg)	0.61 (0.06)	0.59 (0.03)	–0.02
Germination (%)	5.8 (10.5)	28.4 (6.6)	0.44
Total	0.82	12.53	0.94

	Subsp. *vardjanii* 2009		
	self	cross	δ
Fruit set	0.77	1	0.23
Seed set	0.14 (0.03)	0.83 (0.03)	0.83
Seed weight (mg)	0.91 (0.04)	1.10 (0.05)	0.18
Germination (%)	34.8 (9.7)	92.8 (1.7)	0.63
Total	3.38	84.63	0.96



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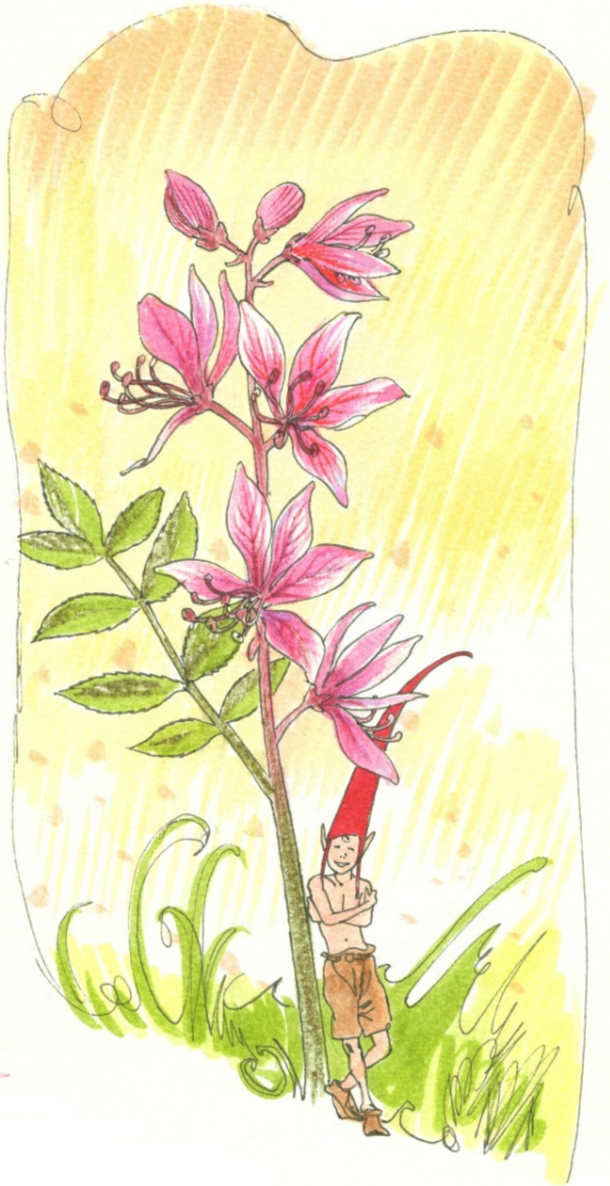

Thanks
for
your attention!