

Meiosis: Apomixis

Section IV-F

1

Modes of reproduction in plants

Sexual

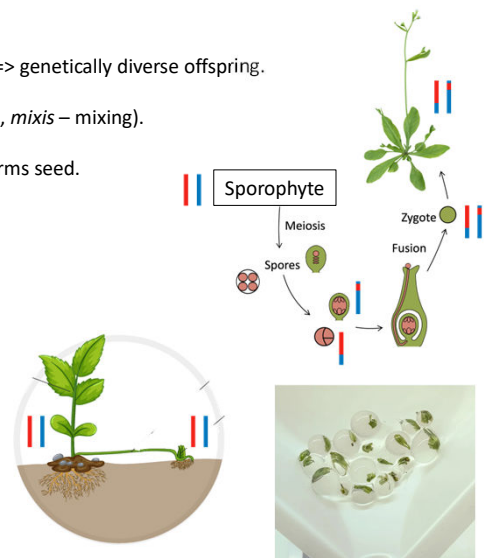
- Gametes produced through meiosis => genetic recombination and reduction => genetically diverse offspring.
- Fertilization: fusion of ♀ and ♂ gametes. (aka Amphimixis, Greek: *Amphi* – both, *mixis* – mixing).
- Zygote develops into embryo, which along with nutritive tissue endosperm forms seed.

Asexual

- No fusion of gametes.
- Progeny genetically identical to parent (clones), propagation through mitosis.
- Two main types :
 1. vegetative propagation (new plants from roots/stems/leaves/buds etc.)
 2. apomixis (through seeds) (Greek: *Apo* – away/without, *mixis* – mixing)

Apomixis: asexual reproduction through seeds.

Synthetic seeds: artificially encapsulated somatic embryos or shoot tips.
can be used for propagation like natural seeds.



byjus.com

reddit/cannabisbreeding (UmamiSeedCo)

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Modes of reproduction in plants

Sexual vs Asexual reproduction

● pro

● con

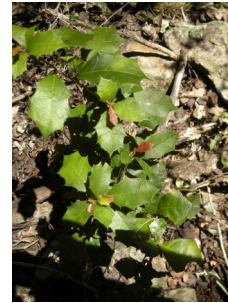
Sexual reproduction	
Genetically diverse progeny	● ●
Seeds: easy dispersal over space and time	●
Needs time & energy	●

Apomixis	
Clonal progeny	● ●
Seeds	●
Needs time & energy	●

Vegetative reproduction	
Clonal progeny	● ●
Vegetative materials: bulky and perishable	●
Fast & less energy intensive	●

Little bit of history.. back in 1841

- *Alchornea ilicifolia*, a dioecious native holly from eastern Australia.
- A young female specimen introduced to Royal Botanical Gardens at Kew, England.
- John Smith (1841):
obtained viable seeds from this specimen (even in absence of male plants).
- First report of apomixis in plants. At that time, it was called parthenogenesis (Greek: *Parthenos* – virgin, *genesis* – birth).



Wikipedia/Ethel Aardvark

The Northern Lads/ Ron McEwen

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History of Apomixis

Mendel Bicknell et al. 2016; Nogler, 2006; Van Dijk and Ellis, 2016, Correns 1905

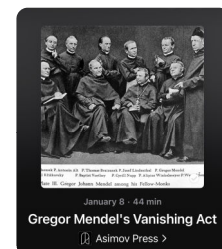
- Three laws of inheritance:
 - Two lectures in 1865, published in 1866, rediscovered in 1900.
 - Made 40 reprints, posted to notable scientists of the time.
 - Although he hoped for a better reception, only Carl von Nägeli showed any real interest.
 - In a letter to Nägeli, he expressed his frustration:

"I attempted to inspire some control experiments, and for that reason discussed the *Pisum* experiments at the meeting of the local society of naturalists. I encountered, as was to be expected, divided opinion; however, as far as I know, no one undertook to repeat the experiments"

- Worked on several species:
 - Bees: for many years, tried to develop a strain that produced more honey.
 - Peter van Dijk rediscovered two newspaper articles (1861): praised his bean, cucumber and pea varieties for their yield and taste.
 - Van Dijk et al. 2018: "We argue that Mendel's initial interests concerned crop improvement, but with time he became more interested in fundamental questions about inheritance, fertilization, and natural hybridization."
- Tried to verify the laws he found with *Pisum*, in ~20 different species. Had great hope for *Hieracium* (hawkweeds) – known for its diversity. In a letter to Nägeli:

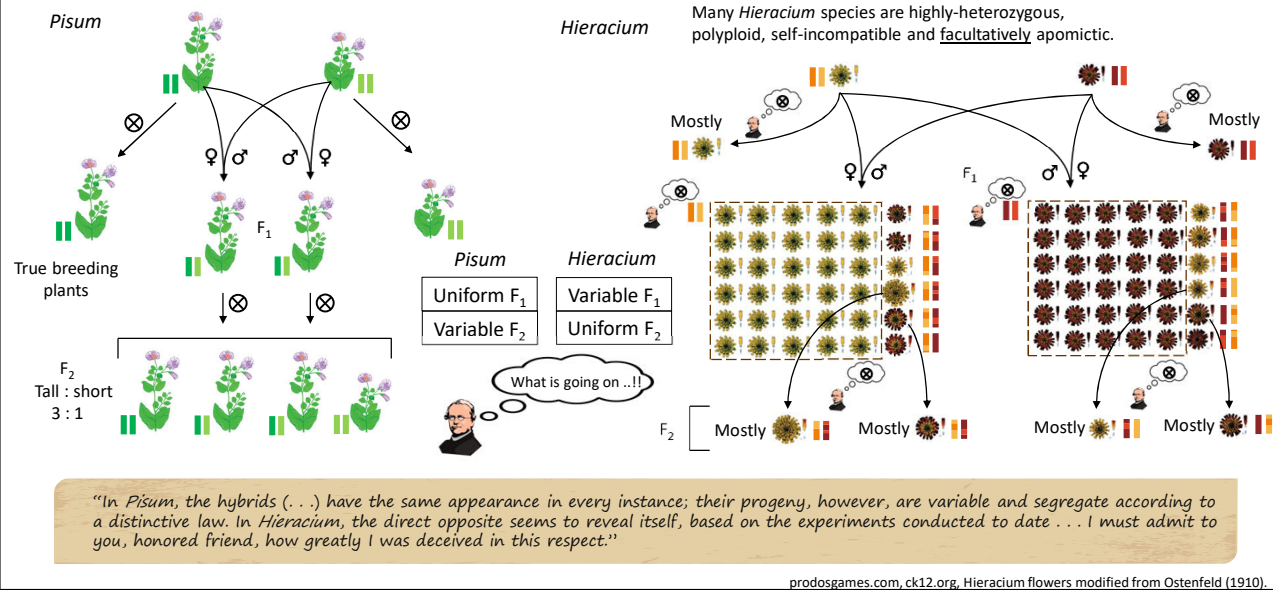
"I knew that the results I obtained were not easily compatible with our contemporary scientific knowledge, and that under the circumstances, publication of one such isolated experiment was doubly dangerous; dangerous for the experimenter and for the cause he represented. Thus, I made every effort to verify, with other plants, the results obtained with *Pisum*"

Podcast



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History of Apomixis



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History of Apomixis

Mendel Bicknell et al. 2016

- Classified inheritance in plants into two types:
 - Pisum* type: **variable hybrids** – hybrids (F_1) which segregate (in F_2).
 - Hieracium* type: **fixed hybrids** – hybrids (F_1) which don't segregate (in F_2).

<i>Pisum</i>	<i>Hieracium</i>
Uniform F_1	Variable F_1
Variable F_2	Uniform F_2

In a letter to Nägeli:

"At this point, I cannot hold back remarking that it must be noticed that the hybrids of *Hieracium* show an almost opposite behavior when compared with those of *Pisum*. We are here, obviously, confronted with only isolated phenomena, which are the emanation of a higher general law."

- This idea of two types of inheritance persisted even after rediscover of Mendel's work in 1900.
- Embryology studies in plants by Murbeck (1897) and Juel (1898, 1900) and repeat of Mendel's crosses in *Hieracium* by Ostenfeld and Rosenberg (1904, 1906, 1910) established apomixis as a potential mechanism for asexual seed formation in plants.
- Didn't do much experiments in his later years: increasing church responsibilities, unhappy with *Hieracium* results ?, sickness ?

"I am really unhappy about having to neglect my plants and bees so completely. Since I have a little spare time at present, and since I do not know whether I shall have any next spring..."

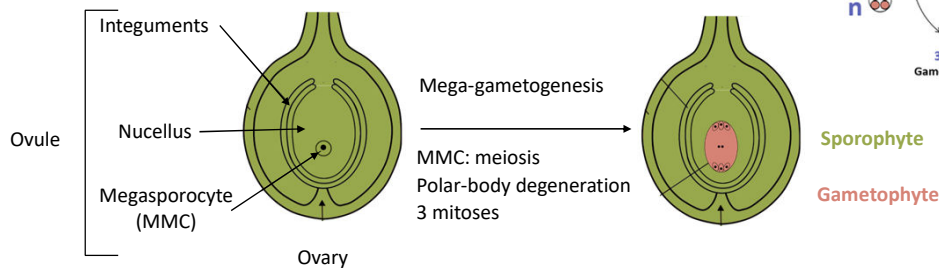
- All his remaining notes were burned after his death (1884), making it more difficult to know what his research was all about.

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Types of Apomixis

Terminology

- Sporophyte
 - Diploid, multicellular stage in the life cycle of plants that produces haploid spores through meiosis.
- Gametophyte
 - Haploid, multicellular stage in the life cycle of plants that produces gametes (sex cells like sperm and egg) through mitosis.
 - Male gametophyte: pollen grain
 - Female gametophyte: embryo sac



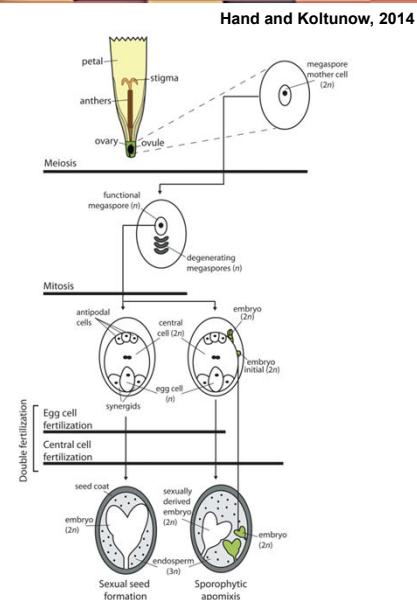
Wikipedia/Tameeria

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Types of Apomixis

Sporophytic apomixis

- Somatic ($2n$) ovule cells surrounding the embryo sac (nucellar or integument cells) undergo an embryonic cell fate. These cells are *aka* Embryo initial cells.
- *aka* adventitious embryony.
- Sexual reproduction (and hence development of a haploid embryo sac) still occurs. Double fertilization to produce haploid embryo and normal endosperm.
- Commonly occurs in: Citrus, mango, mangosteen.
- Does not prevent virus transmission through seed.
- Sexual embryo may or may not mature or germinate.
- It may be difficult to differentiate between seedlings from the zygotic and the somatic embryos.



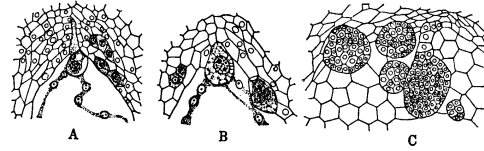
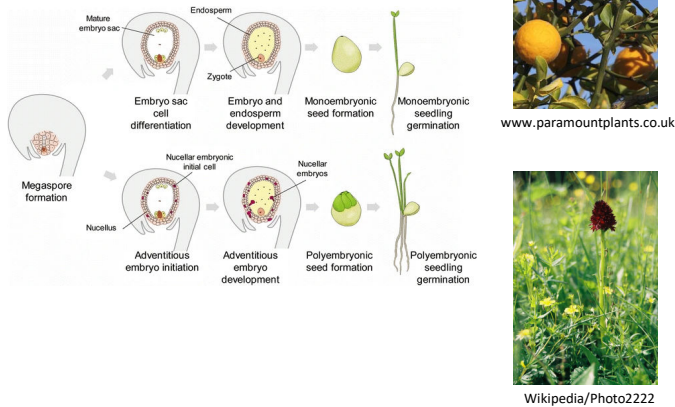
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Types of Apomixis

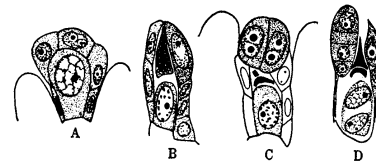
Sporophytic apomixis

Zhang et al., 2018

Citrus



Adventive embryos in *Citrus trifoliata*. A. Zygote, densely cytoplasmic nucellar cells. B. More advanced. C. Zygotic & somatic embryos growing into endosperm. (Maheshwari, 1950 after Osawa, 1912).



Adventitious embryony in *Nigritella nigra*. A. Megaspore mother cell. B. Dyad stage. C. Functional megaspore, degenerating megaspores, and enlarged nucellar cells. D. Two nucleate egg sacs & somatic embryos forming from the nucellus (Maheshwari, 1950 after Afzelius, 1928).

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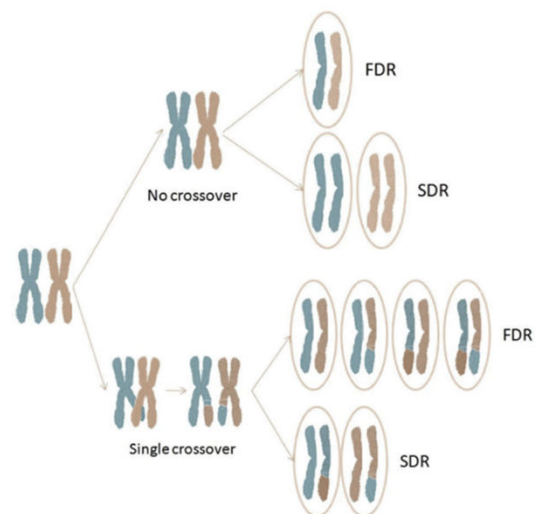
Types of Apomixis

Gametophytic apomixis

- $2n$ gametophyte (embryo sac) is mitotically formed from a diploid cell in the ovule. aka Apomeiosis.
- Divided into two types based on the origin of diploid precursor cell.

Diplospory

- Diploid precursor cell is megaspore mother cell.
- MMC may enter meiosis and then abort the process OR it may immediately begin mitosis.
- As is the case in $2n$ gamete formation, the resulting genotype depends on whether sister or non-sister chromatids are recovered.
- Even if non-sister chromatids are recovered, genetic reduction has occurred in areas involved in a crossover.
- Hence, autosegregation also takes place in diplospory, and the genotype is not necessarily the same as that of the parent.
- Occurs in: *Taraxacum officinale* (dandelion), *Boechera* spp., *Erigeron annuus*, and *Tripsacum dactyloides*.



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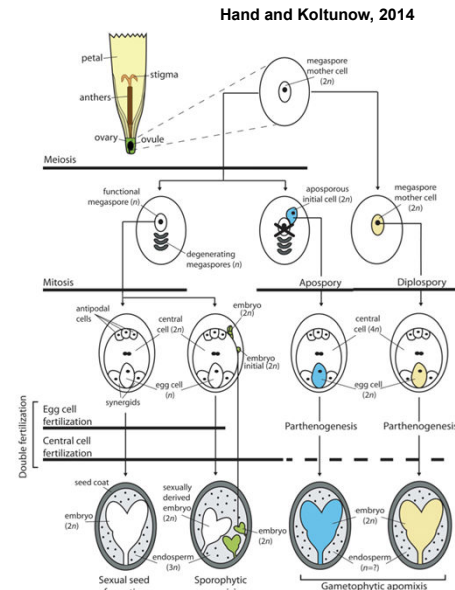
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Apospory

- Diploid precursor cell is other than the megaspore mother cell. *aka* Aposporous Initial cell.
- Sexually derived and aposporous embryo sacs can coexist (e.g. in *Brachiaria* spp.) or the development of later may lead to demise of former (*Hieracium* and *Pennisetum* spp.).
- Embryo development in aposporous and diplosporous embryo sacs occurs without fertilization. *aka* parthenogenesis.
- Endosperm formation may not require fertilization (this is usually rare e.g. in daisy family (Asteraceae)). Autonomous vs pseudogamous.
- Endosperm formed by fertilization – atypical maternal:paternal genome ratios. For e.g. $4m:1p$ as compared to typical $2m:1p$.
- Apomicts have developed multiple strategies to ensure seed viability.

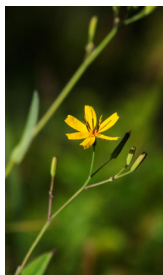


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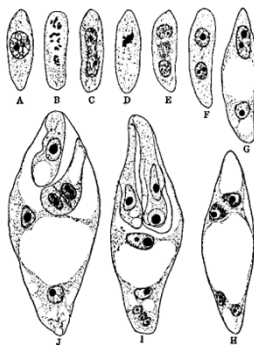
Types of Apomixis

Gametophytic apomixis

Diplospory

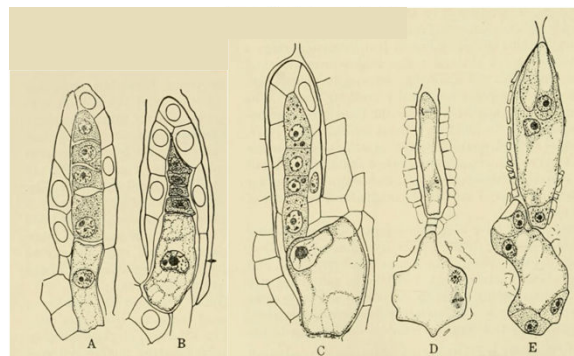


Wikipedia/bastus917



Diplospory in *Ikeris dentata*. A, MMC at Prophase. B, later stage with 21 univalents. C, restitution. D, metaphase. E, telophase. F, G, two-nucleate embryo sac. H, four-nucleate embryo sac. I, mature embryo sac. J, two-celled proembryo. (Maheshwari, 1950 after Okabe, 1932)

Apospory



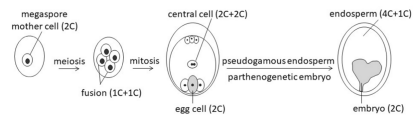
Apospory in *Hieracium excellens*. A, nucellus, showing tetrad of megaspores; note enlargement of cell lying just below chalazal megaspore. B, megaspore tetrad in process of degradation. C, megaspore tetrad and large nucellar cell destined to give rise to embryo sac. D, normal and aposporic embryo growing simultaneously. E, two fully developed embryo sacs; lower is probably of aposporic origin. (Maheshwari, 1950 after Rosenberg, 1907)

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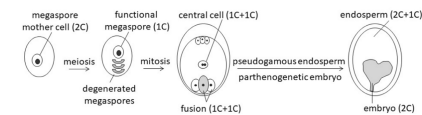
Types of Apomixis

Automixis (rare in plants) Šarhanová et al., 2024

- MMC Meiosis is successfully completed, then
 - two spores reconstitute to form a diploid spore from which the megagametophyte forms.

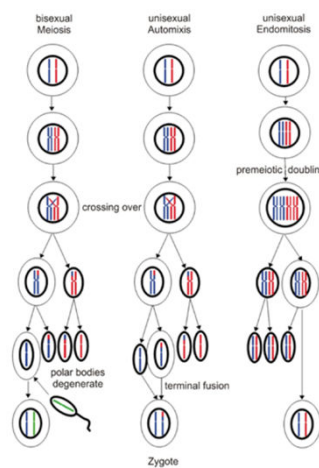


- megaspore forms a reduced embryo sac, where egg cell fuses with other reduced nuclei.



- Because of the recombination and segregation that occurs during meiosis, the genotype of the progeny will be different than that of the parent plant (autosegregation).
- More common in animals.

Wiechmann, 2012



Desert grassland whiptail lizard:
All female populations.



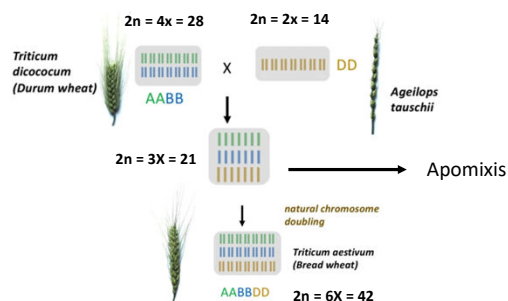
sarawrightnature.wordpress.com

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Apomixis: role in evolution

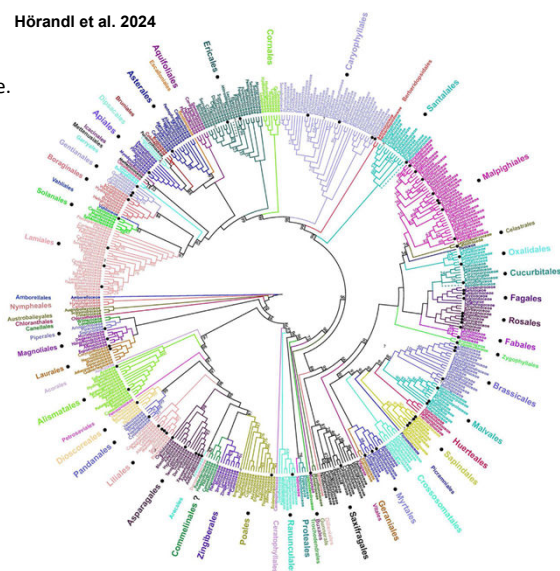
Occurrence Carman, 1997; Ozias-Akins & van Dijk, 2007

- Apomixis occurs in ~400 genera from ~40 plant families, having evolved multiple times within flowering plants. Widespread yet rare.
- Economically important families: observed in Poaceae but not in Solanaceae and Leguminosae.
- Frequently found among polyploids and interspecific hybrids, so it appears to be a mechanism to escape sterility.



Modified from Rosyara et al. 2019

Hörandl et al. 2024



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Apomixis: role in evolution

Occurrence

- Darlington, 1939:** "Apomixis is an escape from sterility, but an escape into a blind alley of evolution."
- However, this view has been found to be incorrect:
 - Apomicts are highly heterozygous and can act as reservoirs of genetic diversity.
 - Apomicts have sexual members of the species, and function as males in sexual x apomict crosses, which can release large amounts of genetic diversity.
 - Agamospecies or Agamic complex: A collection of sexual species and their apomictic hybrids.
 - A lot of gene flow can occur, as well as preservation of successful hybrid genotypes.
 - Some of the most successful species on earth belong to such complexes - e.g. dandelions and bluegrass.



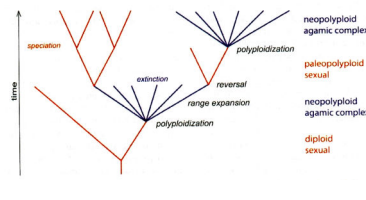
Dandelions (*Taraxacum* spp.)
insideecology.com



Kentucky bluegrass (*Poa pratensis*)
thespruce.com

Hörandl and Hojsgaard, 2012

- Apomixis has appeared and disappeared during the course of evolution.
- Apospory and diplospory mostly found in the neopolyploids.
- Adventitious embryony are mostly in paleopolyploids.



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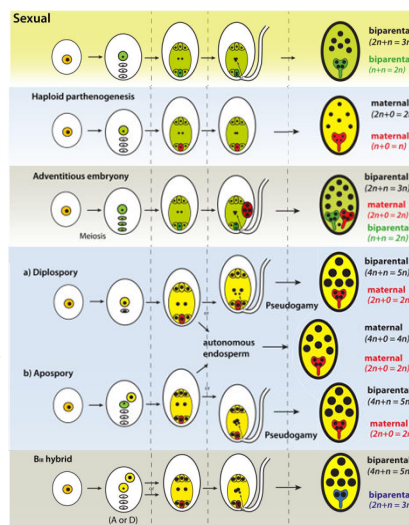
Indicators of Apomixis

Occurrence

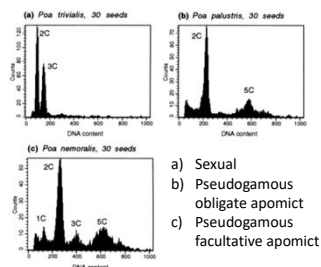
- Most progeny will look like the maternal parent (with only an occasional off-type if facultative).
- Fertility occurs where one would not expect it - such as odd-ploids or wide hybrids.
- Flow cytometry.
- Cytology/histology/microscopy.
- Frequent twin seedlings (adventitious embryony and apospory).
- Seeds when no pollen is present (if autonomous).

	Parthenogenesis	Reduced ♂	Unreduced ♂
Reduced ♀	 Haploid parthenogenesis	 Sexual	 ???
Unreduced ♀	 Apomixis	 B _n hybrid	 Polyploidization

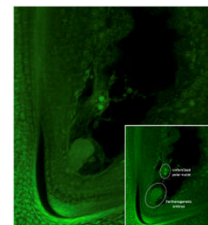
Hojsgaard and Hörandl, 2019



Matzk et al, 2001



Sidhu et al. 2022



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Genetic control of Apomixis

- Sexuality is the ancestral trait: apomixis was derived from sexuality repeatedly during the evolution of angiosperms.
- Suggests that apomixis is the result of an accumulation of mutations. For example, to get diplospory, one would need mutations that condition for:
 - Failure of MMC meiosis, resulting in a $2n$ gamete.
 - Initiation of embryogenesis in $2n$ gamete.

For apospory, one would need:

- Initiation of embryogenic fate in nucellar cells.
- Suppression of the sexual gametophyte.

- A reasonable hypothesis would be:
 - In a given species, apomixis should be controlled by 2-3 genes.
 - One could add these mutations to convert a sexual plant into an apomict.
- However, mostly apomixis is controlled by a single, dominant locus observed as simplex genotype.
- The apparent discrepancy between needing multiple genes but behaving like a single locus can be explained if apomixis is controlled by a group of tightly linked genes. (Grimanelli et al., 1998)

Ozias-Akins & Van Dijk, 2007

Species	Apomixis type	Loci	Genotype	Suppression of recombination
<i>Brachiaria brizantha</i>	Apospory, pseudogamous endosperm	1	Aaaa	—
<i>Cenchrus ciliaris</i>	Apospory, pseudogamous endosperm	1	Aaaa	+
<i>Erigeron annuus</i>	Diplospory, mitotic, autonomous endosperm	2	D/dd ⁺) Fff	+
<i>Hieracium caespitosum</i>	Apospory, autonomous endosperm	2	Aaaa Pppp	—
<i>Panicum maximum</i>	Apospory	1	Aaaa	+
<i>Paspalum notatum</i>	Apospory, pseudogamous endosperm	1	Aaaa	+
<i>Paspalum simplex</i>	Apospory, pseudogamous endosperm	1	Aaaa	+
<i>Pennisetum squamulatum</i>	Apospory, pseudogamous endosperm	1	Aaaa	+
<i>Poa pratensis</i>	Apospory	2	Aaaa Pppp	—
<i>Ranunculus auricomus</i>	Apospory, pseudogamous endosperm	1	Aaaa	??
<i>Taraxacum officinale</i>	Diplospory, meiotic, autonomous endosperm	3	Ddd Ppp	—
<i>Tripsacum dactyloides</i>	Diplospory, mitotic, pseudogamous endosperm	1?	Dddd	+

A: apospory, D: diplospory, P: parthenogenesis, F: fertilization factor

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Genetic control of Apomixis

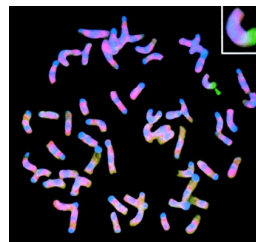
- The apparent discrepancy between needing multiple genes but behaving like a single locus can be explained if apomixis is controlled by a group of tightly linked genes. (Grimanelli et al., 1998)
- Apomictic loci usually have suppressed recombination.

Simplex genotype → Low recombination → Tight linkage

- Difficulties in genetic mapping and introgression.

Goel et al., 2003; Akiyama et al., 2003 & 2011

- Apospory in *Cenchrus* and *Pennisetum* is controlled by a hemizygous locus named apospory-specific genomic region (ASGR).
- The ASGR includes mostly repetitive DNA, particularly retrotransposons.
- Arose once in the ancestor of *Cenchrus* and *Pennisetum*, and has been maintained in descendant species, though its chromosomal position has been moved around.



Goel et al. 2003. ASGR in *Pennisetum squamulatum* ($2n = 8X = 56$). Mitotic spread. ASGR is ~50 Mb long (almost ¼ of the chromosome).

Ozias-Akins & Van Dijk, 2007

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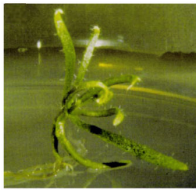
A: apospory, D: diplospory, P: parthenogenesis, F: fertilization factor

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(Epi)genetic control of Apomixis

Singh et al. 2011

- Penetrance and expressivity of the apomixis trait normally differs depending on whether apomixis locus comes from male vs female.
- In maize *ago104* mutants, MMC meiosis fails (like diplospory), generating 2n gametes.
- AGO104 plays a role in chromatin methylation and is a member of the ARGONAUTE family of proteins. Some proteins of this family bind small RNAs during mRNA silencing.
- The first ago mutant found resembled an argonaut, a mollusk that was thought in the Middle Ages to expand a sail, and sail like a ship resembling the Argos.



Ago1 mutant of Arabidopsis
Bohmert et al, 1998

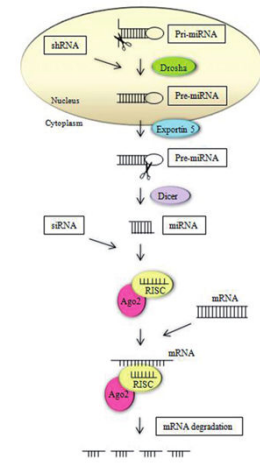


Arganoute (www.scandfish.com)



From: 1551 book, "L'Histoire naturelle des estranges poissons marins"

Small RNA gene silencing mechanism



BETÁKOVÁ and ŠVANČAROVÁ, 2013

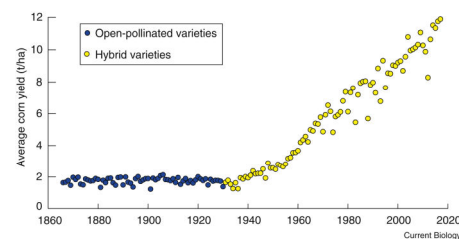
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Why we care about Apomixis?

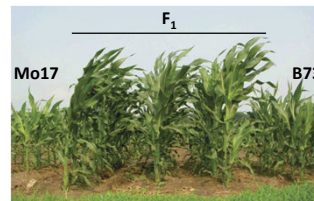
Hybrids

- Hybrids are backbone of modern agriculture
esp. in cross-pollinated crops such as corn.
- Heterosis (*aka* hybrid vigor): tendency of a hybrid offspring to show qualities superior to those of its parents.
- Why are hybrids not so widely adopted in crops other than corn?
 - Self-pollinated crops don't show high heterosis.
Hybrid yield increase: corn (50 – 100 %), rice (10 – 20 %).
 - Hybrid seeds are expensive to produce.

Hochholdinger and Baldauf, 2018



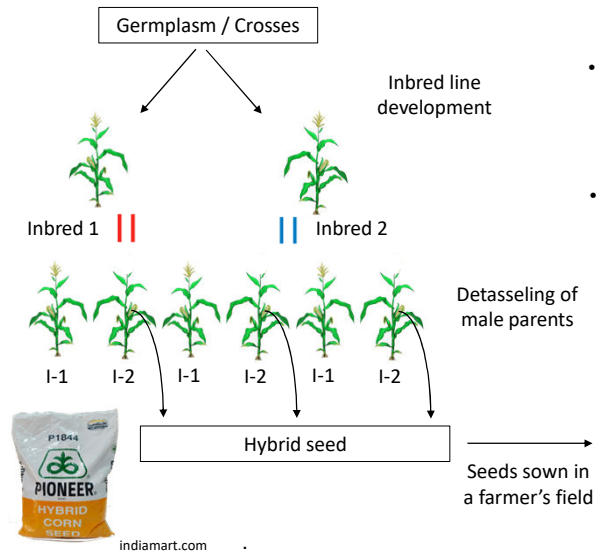
Springer and Stupar, 2007



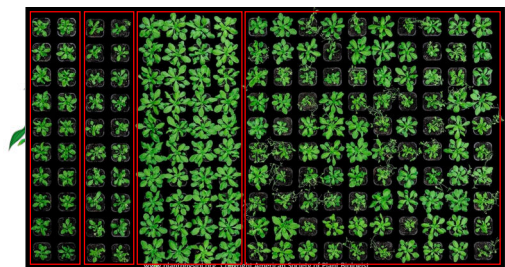
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Why we care about Apomixis?

Hybrid seeds are expensive!



- Inbred Line Development: multiple generations of self-pollination to create stable, homozygous parent lines.
- Detasseling/emasculation:
 - Corn: monoecious – easier (relatively)!
 - Emasculation in other crops (mostly have small hermaphrodite flowers) – its tedious!! – uses male sterility systems.
- Farmers need to buy seed every year:
 - Genetic recombination shuffles the genome.
 - Low adoption in developing countries (small holder farmers).



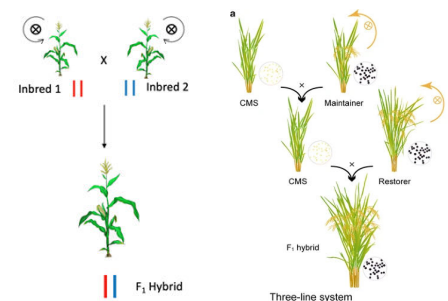
Ian K. Greaves et al. 2015

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Why we care about Apomixis?

Benefits Hanna, 1987

- Heterozygous genotypes are mortal, unless vegetatively propagated (apomixis).
- Eliminate the need for maintaining and increasing male and female lines (for e.g., corn) or male-sterile, maintainer, and restorer lines (for e.g., rice).
- Save space, as hybrid seed production requires field space for male and female lines, but seed is harvested from the female plants only.
- Would be useful in species where male-sterility systems are not known, or in crops where crosses can't be easily performed (e.g., wheat).
- Farmers would not have to buy hybrid seed every year, instead they could save some of their own seed.
- In the United States, could this destroy the seed industry?
 - Probably not due to IP laws
 - Cultivars are replaced after every few years anyways.
 - But would lower seed production costs
- The only current commercial applications of apomixis are in Citrus, Kentucky bluegrass and buffelgrass.
- In the developing countries, this could be a game changer.



Fan and Zhang, 2017

Hy-Gain for smallholders

BILL & MELINDA GATES foundation

THE UNIVERSITY OF QUEENSLAND AUSTRALIA



Country ->	China	India
Total area under rice cultivation	30 m ha	45 m ha
Rice production (2020 – 21)	210 m tons	120 m tons
%age Hybrid rice	> 50 %	< 10 %

Data source: National Food Security Mission, India & National Bureau of Statistics, China

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Breeding for Apomixis

Introgression from related species

Savidan, 2000; Sokolov and Khatyapova, 2001

Tripsacum dactyloides X *Zea mays*

Carman et al. 1985

Elymus rectisetus X *Triticum*

Hanna, 1987; Dujardin & Hanna, 1983 – 1989, Kaushal et al. 2010

Pennisetum squamulatum X *Pennisetum glaucum*

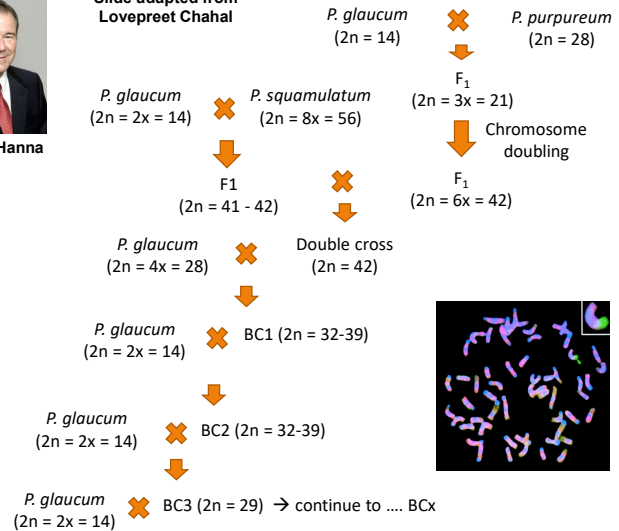


Morgan et al. 1998



Wayne Hanna

Slide adapted from Lovepreet Chahal



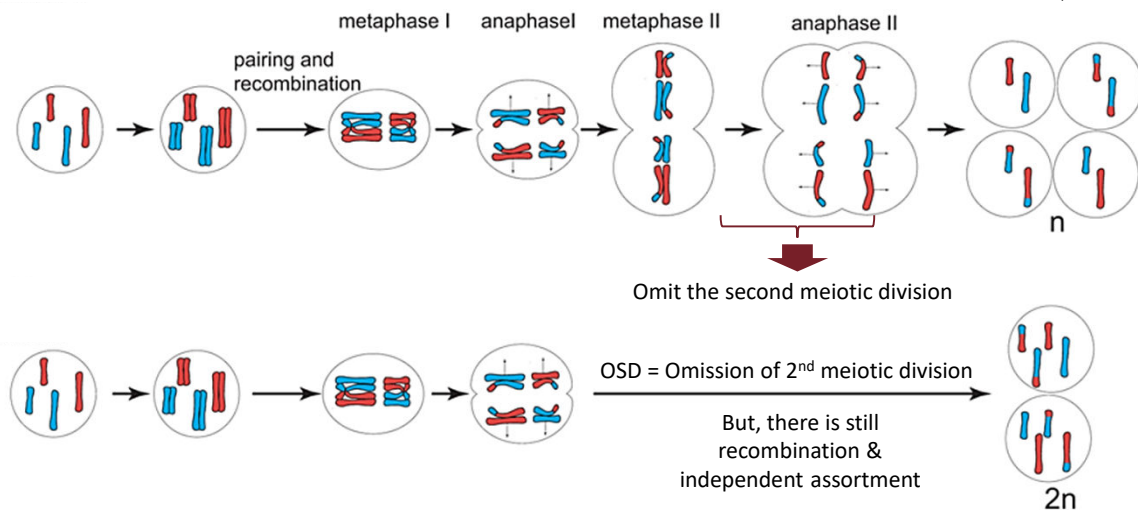
tifton.caes.uga.edu, clearview-farm.com, sellomarket.com, istockphoto.com

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d'Erfurth et al. 2009

Converting meiosis to apomeiosis

1. Need to prevent chromosomal reduction



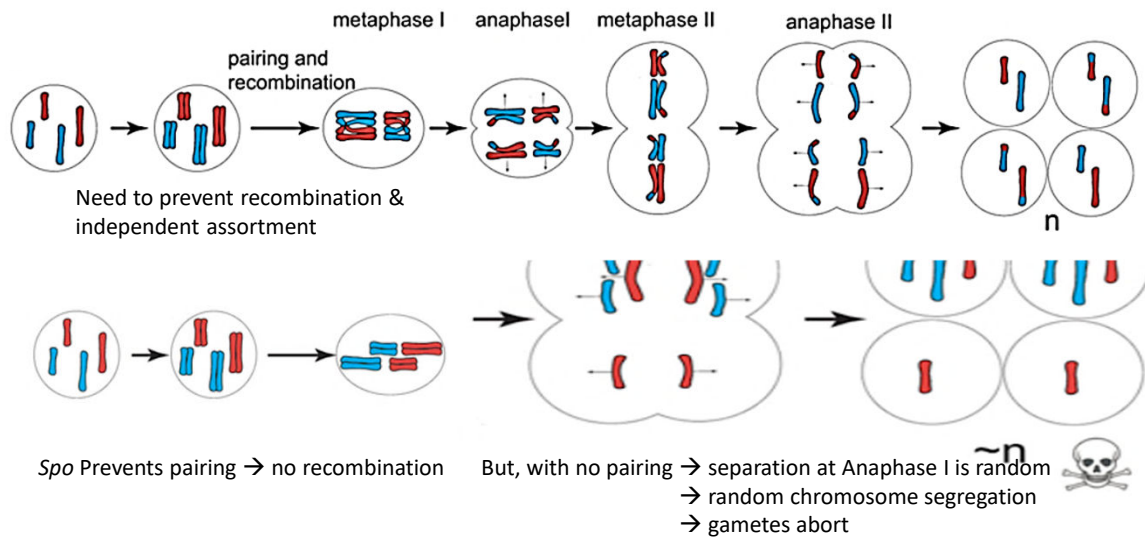
Raphaël Mercier

24

d'Erfurth et al. 2009

Converting meiosis to apomixis – *spo11-1* gene

2. Need to prevent recombination

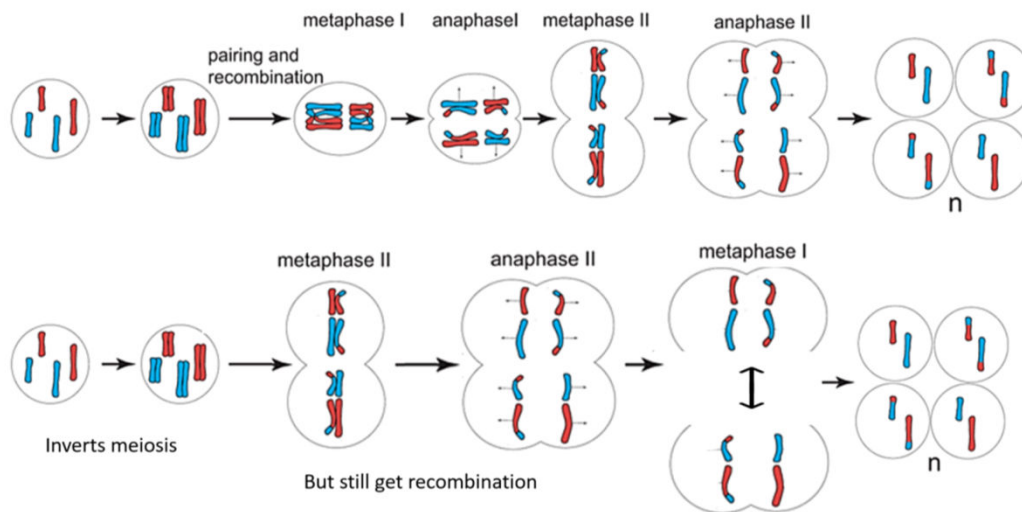


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Converting meiosis to apomeiosis

3. Inverting meiosis (mutating *rec8*)

d'Erfurth et al. 2009



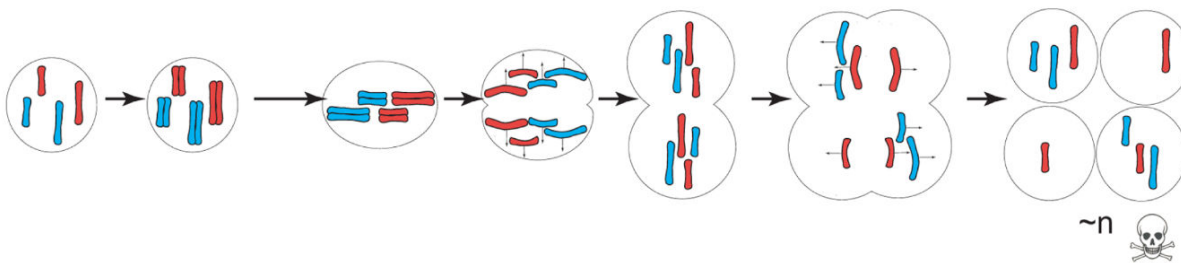
26

Converting meiosis to apomeiosis –

4. Combining *spo11-1* with *rec8*
d'Erfurth et al. 2009

- Prevents recombination
- Prevents independent assortment

But, does not solve the problem of random chromosome separation @ Ana II

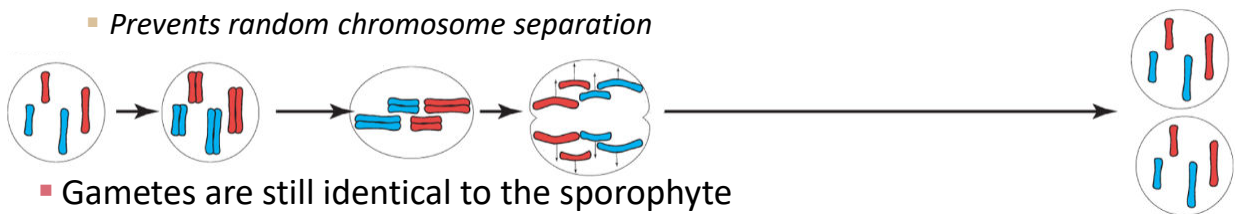


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Converting meiosis to apomeiosis

5. Combining *spo11-1* with *rec8* & *osd11*
d'Erfurth et al. 2009

- Combining *spo11-1* with *rec8* and *osd11*
 - Prevents recombination
 - Prevents independent assortment
 - Prevents random chromosome separation

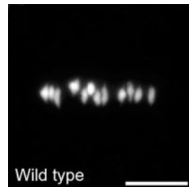


- Gametes are still identical to the sporophyte
 - But are still gametes
 - Require fertilization
 - Doubles each generation

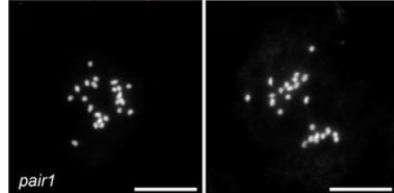
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MiMe in rice via CRISPR

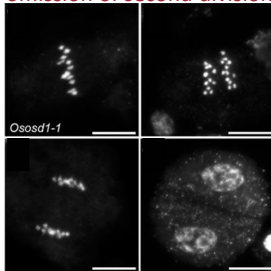
Mieulet et al., 2016



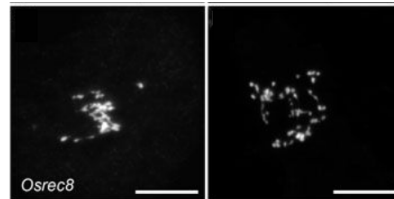
Prevent pairing → no recombination



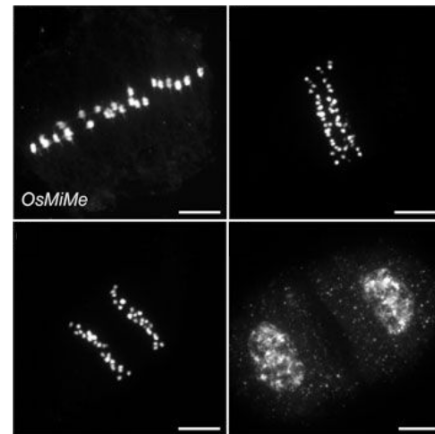
2n gamete formation by omission of second division



Inverts meiosis



3-mutant combo

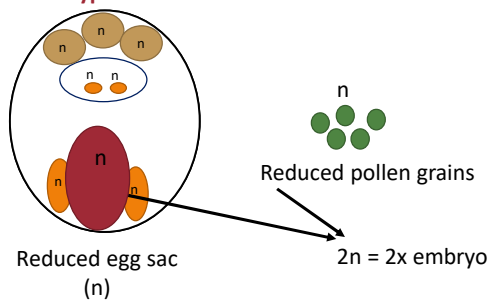


29

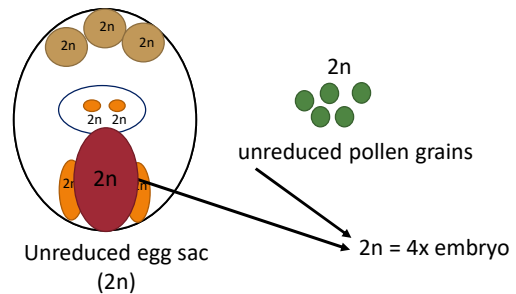
But, now the ploidy level doubles

Slide by Lovepreet Chahal

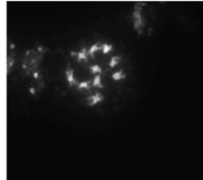
Wild-type meiosis



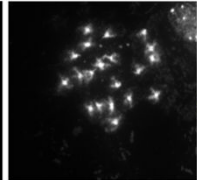
MiMe meiosis



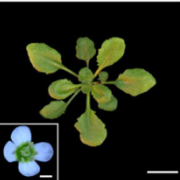
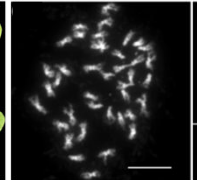
T0: $2n = 2x = 10$



T1: $2n = 4x = 20$



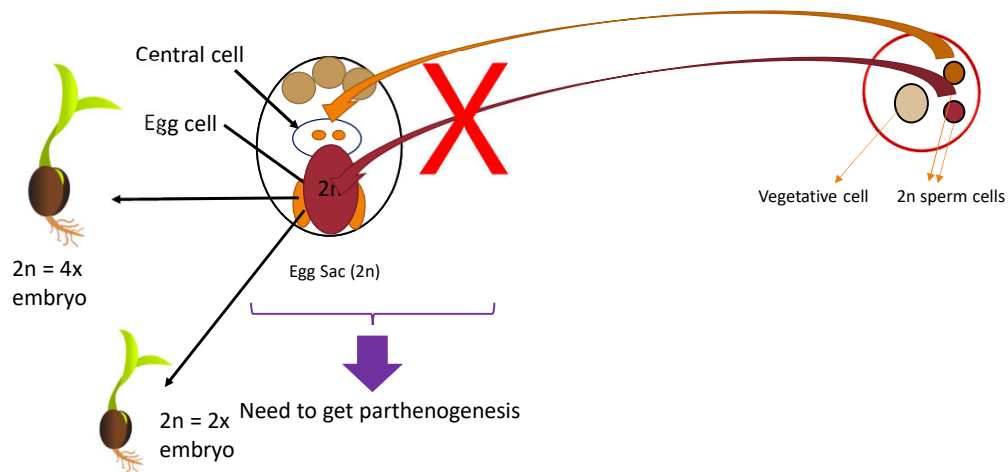
T2: $2n = 8x = 40$



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Turning MiMe into apomixis

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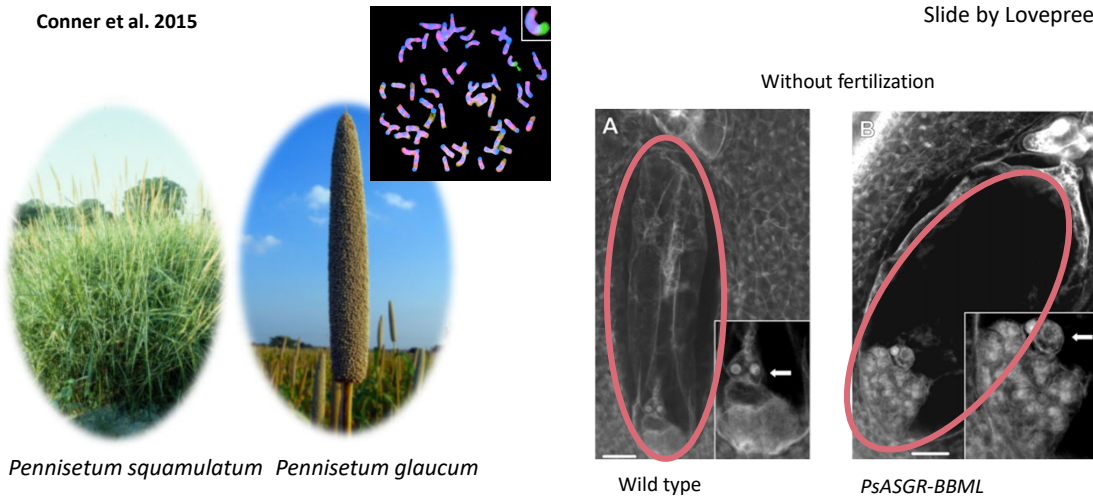


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PsASGR-BBML can induce parthenogenesis

Conner et al. 2015

Slide by Lovepreet Chahal



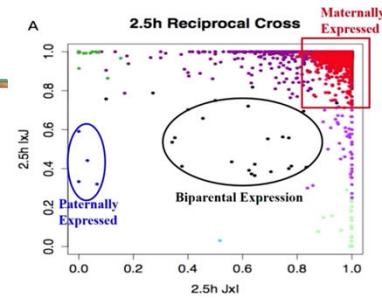
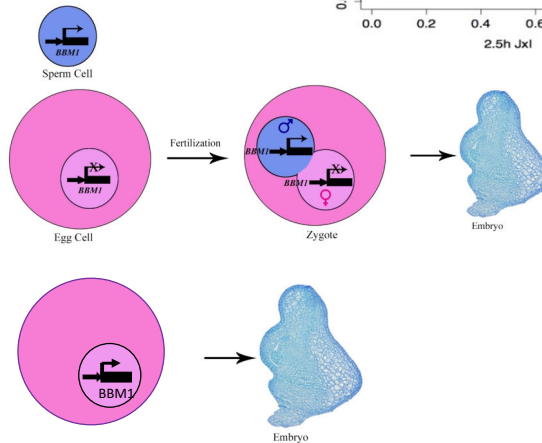
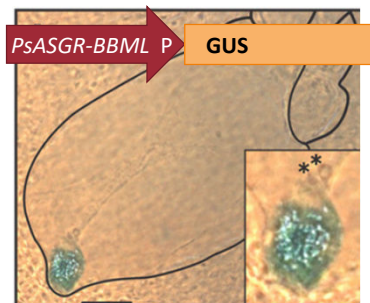
oziasakinslab.org; cdn.pixabay.com

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Baby boom like genes

Conner et al. 2015

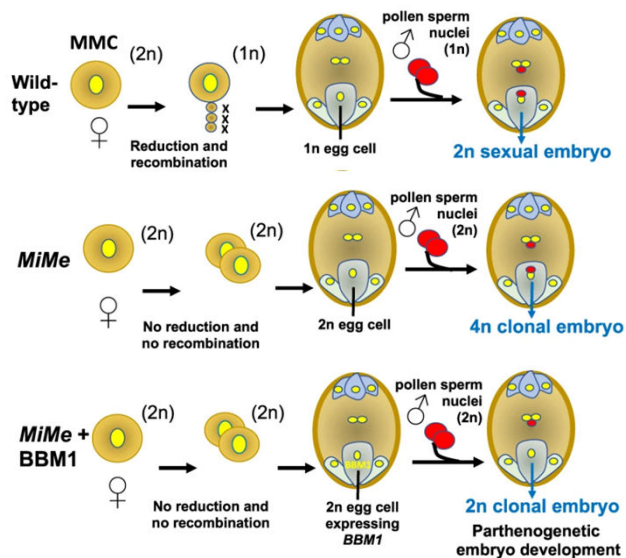
Khanday et al, 2019



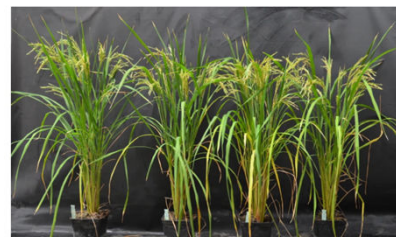
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Synthetic apomixis in Rice

Khanday et al, 2019; Vernet et al, 2022



F1 — F2 progeny —



F1 — T1 apomictic progeny —

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