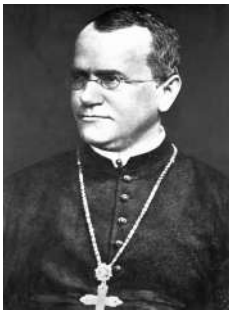
Apomixis

Mendel



(1822–1884)

Background

van Dijks et al, 2022; Vyskot & Jiri, 2022

- Did not know of mitosis, meiosis, or fertilization
- Was not accepted as a biologist (was a breeder)
- Did not interact with other botanists
    - except Nägeli, who worked on plant reproduction

    - 2 Lectures in 1865
    - Published in 1866
        - Discovered in 1900

According to accepted history, could not duplicate his pea work with *Hieracium* (**Nogler 2006**).

- Credited with discouraging his research.
    - Later *Hieracium* was found to be a facultative apomict.
- His order of monks discouraged his work or so the story goes ⇒
    - Became an administrator.
    - His notes were all discarded after his death, making it more difficult to know what his research was all about.

van Dijks et al, 2022

- His monastery had a history of sheep breeding
- Influenced by earlier reports of crosses made by Gärtner, who wrote a review called *Experiments and Observations on Hybridization in the Plant Kingdom*
    - Mendel owned a copy and wrote a lot of marginal notes on it
    - Acquired 34 pea varieties in 1854, probably based on Gärtner's information of crosses in pea

van Dijks et al, 2018 - Rediscovered two 1861 newspaper articles about his public lectures.

- Praised his bean, cucumber and pea varieties for their yield and taste
- Now know his motivation was to find/develop improved plant varieties while hypothesis testing

Van Dijk & Ellis, 2016

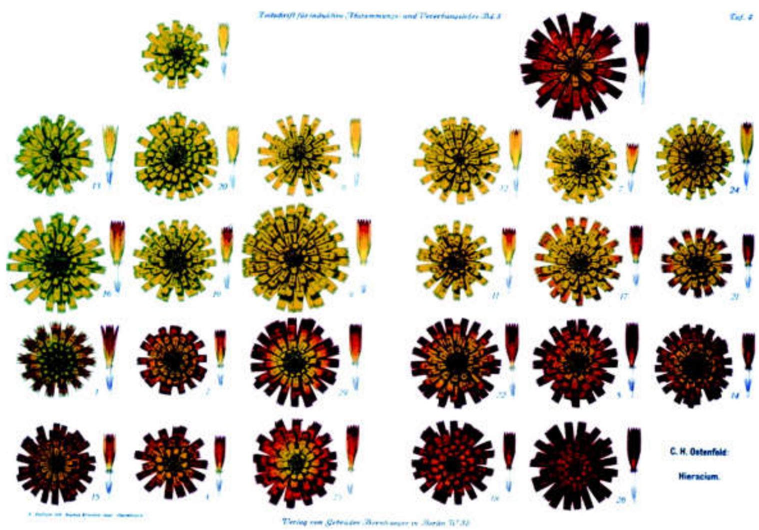
- Mendel verified his pea work in some 20 other spp.
    - He selected these according to
        - Had to be uniform (i.e., selfing and homozygous
        - Had to have lots of polymorphisms

Mendel classified plants into two types

van Dijk & Ellis, 2016; van Dijks et al, 2018

Mendel categorized plants as variable hybrids (F2's in today's language) that segregate, eg, pea

and constant hybrids (apomicts) that segregate in the F1 but not the F2



Hieracium auricola, H. aurantiacum, the F1 progeny from reciprocal crosses. The figure is from Ostenfeld (1910), who recreated Mendel's cross. These species would be true-breeding otherwise.

Marginal notes on Mendel's copy of *Experiments and Observations on Hybridization in the Plant Kingdom*, show he was working on a model for how apomicts work, and he invoked what today would be known as a 2n gamete + parthenogenesis.

However, what was known about his work on *Hieracium* is based on a letter to Nägeli, which is now realized to have pages missing, so his work was taken out of context.

- Created misinterpretations of his work & motivations

Overview & terminology

Based on **Koltunow et al., 1995, Carneiro et al., 2006**

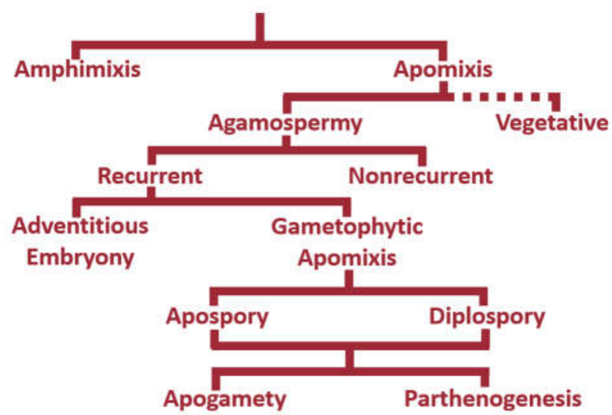
- A/Apo – without
- Amphi – on both sides
- Diplo - two
- Gamo/Gamety - involving a gamete
- Mixis – mixing of chromosomes, I.e., gametic fusion
- Spermy – involving a sperm
- Recurrent – Always happens
- Non-recurrent – Sometimes happens

Apo = without; *mixis* = mixing of chromosomes, i.e., gametic fusion.

- This distinguishes it from *amphimixis*, normal sexual reproduction (from *amphi* = on both sides)

Originally, this term was used to describe any asexual form of reproduction, including vegetative.

- Now the term only refers to asexually produced seed.
- The term agamospermy (without a sperm) was used to distinguish seed-based apomixis from vegetative reproduction.

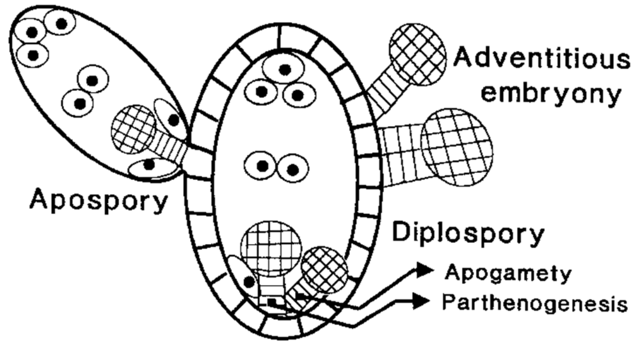


In turn, agamospermy is divided into two types:

- Non-recurrent: Every now and then a haploid sporophyte develops from a cell in the egg sac.
  - This is the source of the haploids that occur in twin seedlings.
- Recurrent: Always happens. This is also divided into two types, gametophytic and adventitious

Recurrent apomixis

Adventitious embryony (somatic embryogenesis)



Types of recurrent apomixis

Somatic embryos form by mitosis from one or more cells of the nucellus; sometimes of the integuments.

- Therefore, it is distinguished by the lack of alternation of generations.
- These embryos do not form inside a female gametophyte
- They also have the same genotype as the parent plant
- A zygotic embryo, along with more than 1 adventitious embryo, can also be present in the same ovule, giving rise to polyembryony
  - Common in citrus, orchids, mango, mangosteen

- In addition, adventitious embryony does not prevent virus transmission through seed.
- It may be difficult to differentiate between seedlings from the zygotic and the somatic embryos.

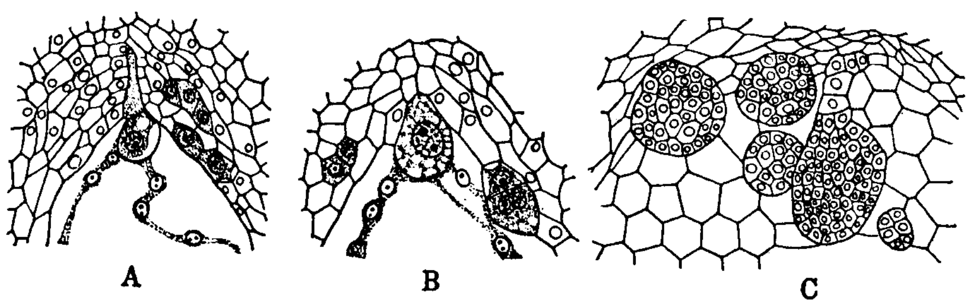
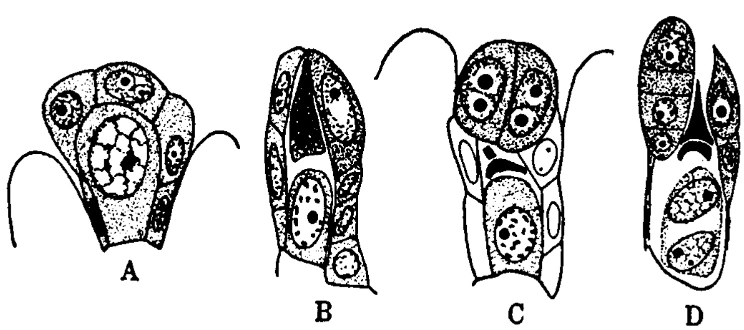


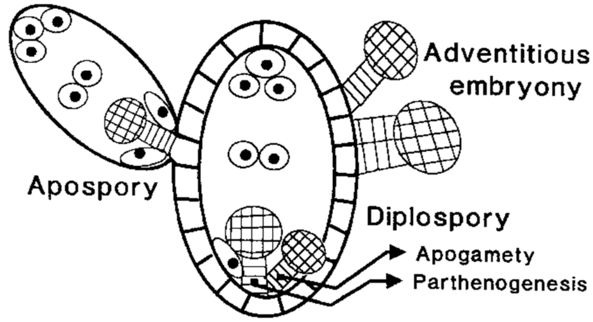
Figure 1. Adventive embryos in Citrus trifoliata. A. Zygote, densely cytoplasmic nucellar cells. B. More advanced. C. Zygotic & somatic embryos growing into endosperm. Osawa, 1912.



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 sac & somatic embryos forming from the nucellus Maheshwari, 1950, from Afzelius, 1928.

Key ID feature: multiple embryos but only 1 egg sac

Gametophytic



Embryos are formed within a female gametophyte

- (Hence alternation of generations is present)
- There are 3 types

Apospory (without a spore)

A nucellar cell forms a female gametophyte by mitosis.

- Hence it has the same genotype as the parent plant

Right: normal (bottom) and aposporous (top) egg sacs of *Hieracium excellens*, (Maheshwari, 1950, from Rosenberg, 1907)

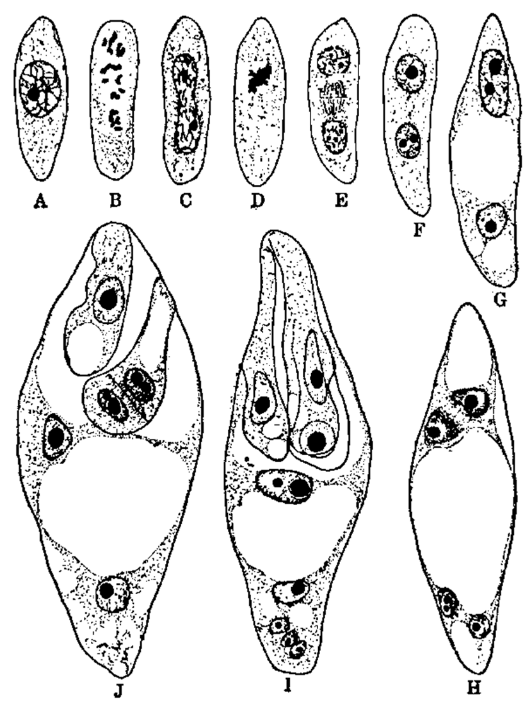
- Found in Asteraceae, and Poaceae, such as *Pennisetum*

Key ID feature: Multiple egg sacs

Diplospory (from a diploid spore)

Forms an egg cell by mitosis, or, usually, from meiosis that begins but fails, resulting in the formation of a 2n spore

- Hence, the term apomeiosis is sometimes used.
- 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, <u>auto segregation</u> also takes place in diplospory, and the genotype is not necessarily the same as that of the parent.



Diplospory in Ixeris dentata. A. Prophase. B. Later stage with 21 I. C. Restitution. D. Metaphase. E. Telophase. F, G. 2-cell stage of gametogenesis. H. 4 celled egg sac. I. Mature gametophyte. J. 2-celled proembryo beginning to form. (Maheswari, 1950, from Okabe, 1932)

Found in composites such as *Taraxacum,* and grasses such as *Elymus* and *Tripsacum*

Automixis

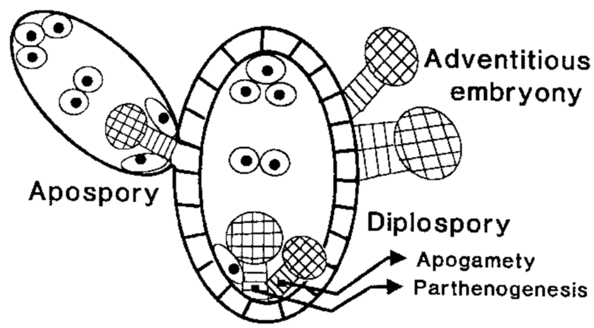
rare- Meiosis is successfully completed, but then two spores restitute to form a diploid spore from which the megagametophyte forms.

- Because of the recombination and segregation that occurs during meiosis, the genotype of the resulting spore will be different than that of the parent plant.
- This is known as autosegregation.

Key ID: 1 egg sac– is determined genetically

2n gametes vs apomixis

The difference between 2n gametes and diplospory/automixis is that a 2n gamete must still be fertilized for it to develop, while diplospory/automixis form an embryo without fertilization.



More terminology

- Parthenogenesis – in apomixis, when the embryo is formed from an unfertilized eg
- Apogamety - (without a gamete): The embryo is formed from a cell other than the egg, usually a synergid; at times an antipodal
- Autonomous - No pollination or fertilization is required
- Pseudogamous - Pollination and fertilization of the central cell (leading to endosperm development) is necessary
- Facultative - both amphimixis and apomixis occur in the same species
- Obligate - all individuals of a species are apomicts.

Note: only apospory and adventitious embryony (the most common types) provide for a genotypic stability

- Diplospory can be stable if meiosis fails before crossing over occurs

Role in evolution

Based on: **Bashaw, 1980; Grossniklaus & van Dijk, 2001, Richards, 2003**

It preserves heterozygosity (usually without segregation) from generation to generation

- Is found in over 400 species from 40 families
  - Is common in grasses, but has never been documented in such economically important families such as the Solanaceae and Leguminoseae
- Found 10% of families and 0.1% of species → not all that common
  - Much more common in marginal areas where pollinators are lacking, such as the arctic.
  - Reported to be 60% of British flora, but that figure is tough to believe
- Has evolved multiple times
- Apomixis is frequently found among polyploids and interspecific hybrids, so it appears to be a mechanism to escape sterility

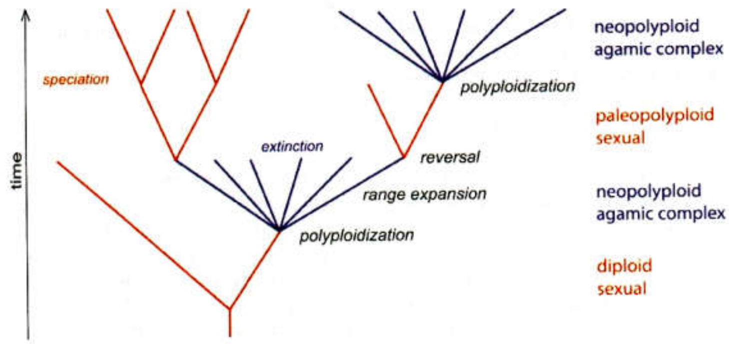
Darlington, 1939: "*Apomixis is an escape from sterility, but an escape into a blind alley of evolution*."

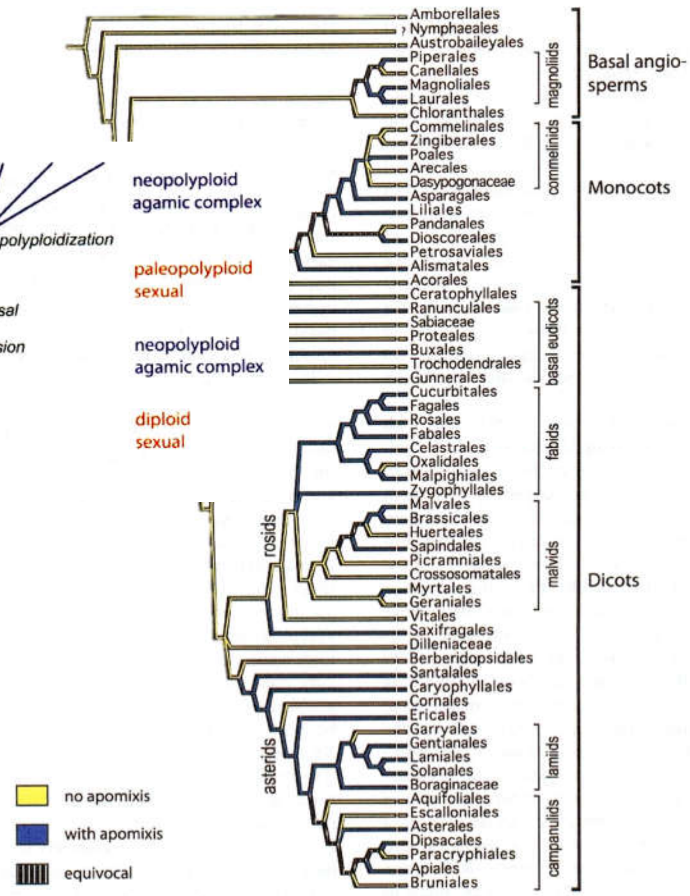
In the short term, apomixis restores fertility to interspecific hybrids and permanently fixes heterosis.

- However, Darlington's view of apomictic species as being evolutionary dead ends is not correct:
  - Apomicts are highly heterozygous, and can act as reservoirs of genetic diversity
  - Apomicts have sexual members of the species, and function as males in apomict x sexual crosses
  - Sexual x apomict crosses can therefore 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

Hörandl and Hojsgaard, 2012

Notice that apomixis has appeared and disappeared in the course of evolution





- Thus the previous views of dead-ends or genetic reservoirs need to be modified as follows:
  - Apospory and diplospory mostly found in the neopolyploid
  - Adventitious embryony are mostly in paleopolyploids

Indicators of apomixis

- 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
- Cytology
- Flow cytometry
- Frequent twin seedlings
- Seeds when no pollen is present (if autonomous)

- Embryo : endosperm genomic ratios via flow cytometry

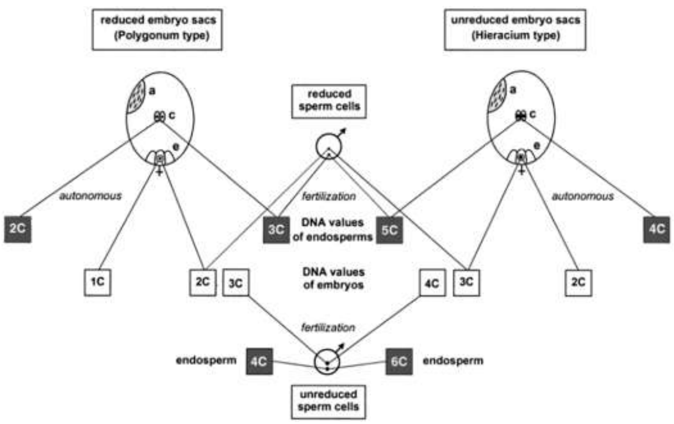


Figure 2. Embryo and endosperm ploidies expected for sexual vs apomixis. ***Matzk et al, 2001***

Species	Mode of reproduction	C values of embryo + (endosperm) expected
Arabidopsis thaliana	Obligate sexual	2C + (3C)
Beta vulgaris	Obligate sexual	2C + (3C)
	Interploidy crosses	2C + (4C)
Brassica napus	Obligate sexual	2C + (3C)
Hieracium pilosella	Facultative autonomous aposporous	2C + (3C) + (4C)
Hypericum patulum	Facultative apomictic	2C + (3C) + (5C)
Hyperic. perforatum	Facultative pseudogamous aposporous	2C + (3C) + (5C)
	Obligate sexual	2C + (3C)
Medicago sativa	Obligate sexual	2C + (3C)
Potentilla argentea	Facultative pseudogamous	2C + (3C) + (5C)

Genetic control of apomixis

The traditional view is that apomixis is the result of an accumulation of mutations. For example, to get diplospory, one would need mutations that condition for:

1) Failure of meiosis, resulting in a 2n gamete
2) Failure of fertilization
3) Ability to develop without fertilization

- For apospory, one would need:

1) Activation of nucellar cells to become embryonic
2) Suppression of the sexual tissues

Two things are evident from this:

1) In a given species, apomixis should be controlled by 2-3 genes
2) One could add mutations to convert a 2n-gamete-producing plant into an apomict

Van Dijk and Bakx-Schotman 2004

Working with apomictic dandelion

- *Diplosporous (Dip*) gene. Single dominant conditions for diplospory
  - *Ddd* or *Dddd* individuals are diplosporous
  - However, these individuals are not able to undergo parthenogenesis

Thus, on the surface, this work appears to support the premise that apomixis results from the accumulation of various meiotic mutations.

- However, most times, apomixis appears to be controlled by a single, usually dominant gene
  - Detection is complicated by the presence of additional genes that modify the process
  - Dominance among meiotic mutants are rare!

As seen in the table below, there is a not too much information on the genetics of apomixis, and that which is available does not necessarily agree with each other.

- The number of genes that have been found to be involved does not prove or disprove the hypothesis that apomixis is a series of mutations.

Species	Sexual genotype	Apomictic genotype	Reference
Guayule		*3 recessive genes*	Powers, 1945
Bahiagrass *(Paspalum notatum)*		*aaaa*	Burton & Forbes, 1960
Buffelgrass (*Cenchrum* [formerly *Pennisetum*] *ciliare*	*AaBb*	*Aabb*	Taliaferro & Bashaw, 1966
Bothriochloa - Dicanthium - Capillipedium complex	*AAaa*	*aaaa*	DeWet & Harlan, 1970
Erigeron annuus		*QTL for parthenogenesis* *QTL for diplospory*	Noyes & Rieseberg, 2000
Hieracium	*2 Dominant*	*LOSS OF APOMEIOSIS* *LOSS OF PARTHENOGENSIS*	Koltunow, 2011
Guineagrass (*Panicum maximum*)	*A_B_*	*aabb* *Aabb* *aaBb*	Hanna et al., 1973
Guineagrass (*Panicum maximum*)	*aaaa*	*Aaaa*	Savidan, 1981
Pennisetum glaucum		*single linkage to 1 probe*	Ozias-Akins etal, 1998
Tripsacum		*single linkage to 1 probe*	Grimanelli et al, 1998
Pennisetum glaucum		*BBM1*	Conner et al 2015

Grimanelli et al., 1998

The apparent discrepancy between needing multiple genes but behaving like a single gene can be explained if apomixis is really due to a group of tightly linked genes.

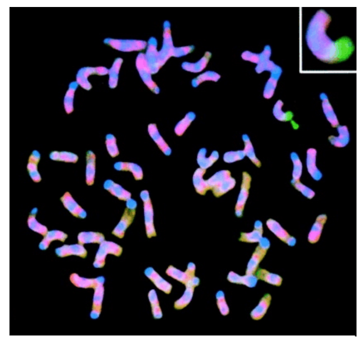
- If so, mutagenesis will probably never induce apomixis

Ozias-Akins et al., 1998; Labombarda et al, 2002
Lack of recombination around apomictic loci also reinforces that the premise that apomixis could be due to a tightly linked group of genes

Labombarda et al., 2002; Goel et al., 2003; Akiyama et al., 2003 & 2011; Conner et al., 2015

Apospory in *Centrus, Paspalum*, and *Pennisetum*, 3 grasses

- The apomixis genomic region (ASGR) is hemizygous
- In the case of *Pennisetum*, the hemizygous region is 50 Mbp-long, – ie, is 1/4 of the chromosome!
    - see photo at right
- The ASGR includes mostly repetitive DNA, particularly retrotransposons



Goel et al. 2003. The apomictic genomic region of pearl millet.

- Arose once in the ancestor of *Centrus* and *Pennisetum*, and has been maintained in descendant species, though its chromosomal position has been moved around.

Conner et al., 2015 –
Working with the aposporic region in *Pennisetum*

- Turns out to have 1 key gene: *Babyboom*-like = APETALA2 transcription factor
- Expressed in egg cell, whereas normally expressed in the pollen

Epigenetic component

Singh et al., 2011

- Expression can differ depending on whether apomixis gene comes from male vs female
- Maize mutants for *ago104*, which plays a role in chromatin methylation
- Meiosis fails, as in diplospory, forming 2n gametes

The proteins that bind small RNAs that are involved in silencing are called argonaut (*ago*) proteins. 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*.

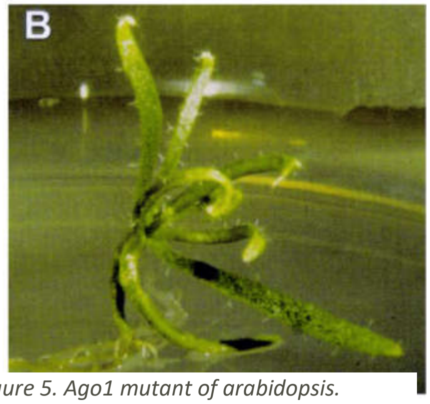


Figure 5. Ago1 mutant of arabidopsis. Bohmert et al, 1998 EMBO J 17:170-180

Figure 4. http://www.scandfish.com/ig/gallery.asp?action=viewimage&categoryid=33&text=&imageid=1210&box=&shownew=

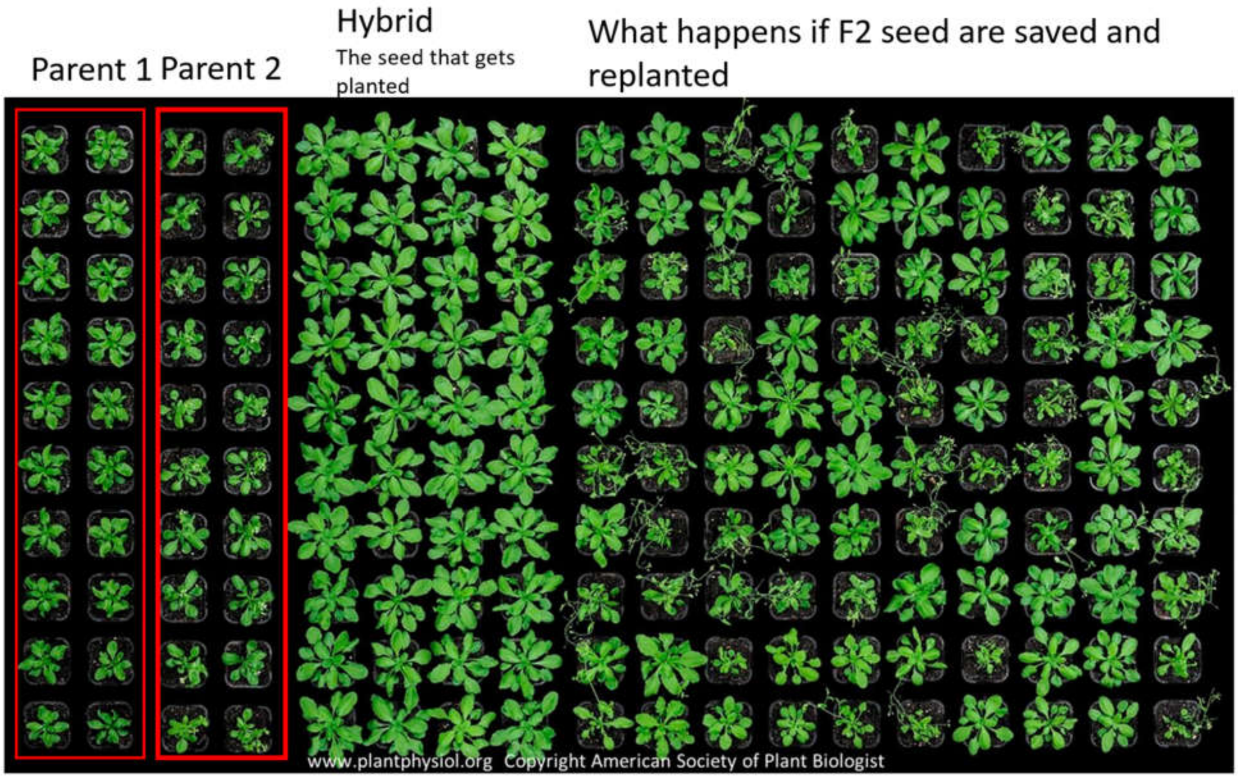
Figure 3. From: 1551 book, "L'Histoire naturelle des estranges poissons marins" http://cavigliascabinetofcuriosities.blogspot.com/2011/06/phylum-mollusca-class-cephalopoda-order.html

Apomixis in breeding

Hybrid seeds

Currently, hybrids cannot be planted from seed, because the seed would not breed true.

- Consequently, new hybrid seed must be produced for each season by crossing inbred parents together



After **Hanna, 1987**

The ability of apomixis to by-pass the effects of meiosis and thus get true-breeding seed has potential to overcome many problems in plant breeding:

- Eliminate the need for progeny testing
- Eliminate the isolation requirements for hybrid seed production and increase female lines
- Eliminate the need for maintaining and increasing male- sterile, maintainer, and restorer lines
- Save space, as hybrid seed production requires field space for male and female lines, but seed is only harvested from the female plants
- Would be useful in species where male-sterile or restorer systems are not known, or in self-pollinated crops which cannot easily be crossed to produce hybrid seed (e.g., wheat)

This would also mean that farmers would not have to buy hybrid seed every year.

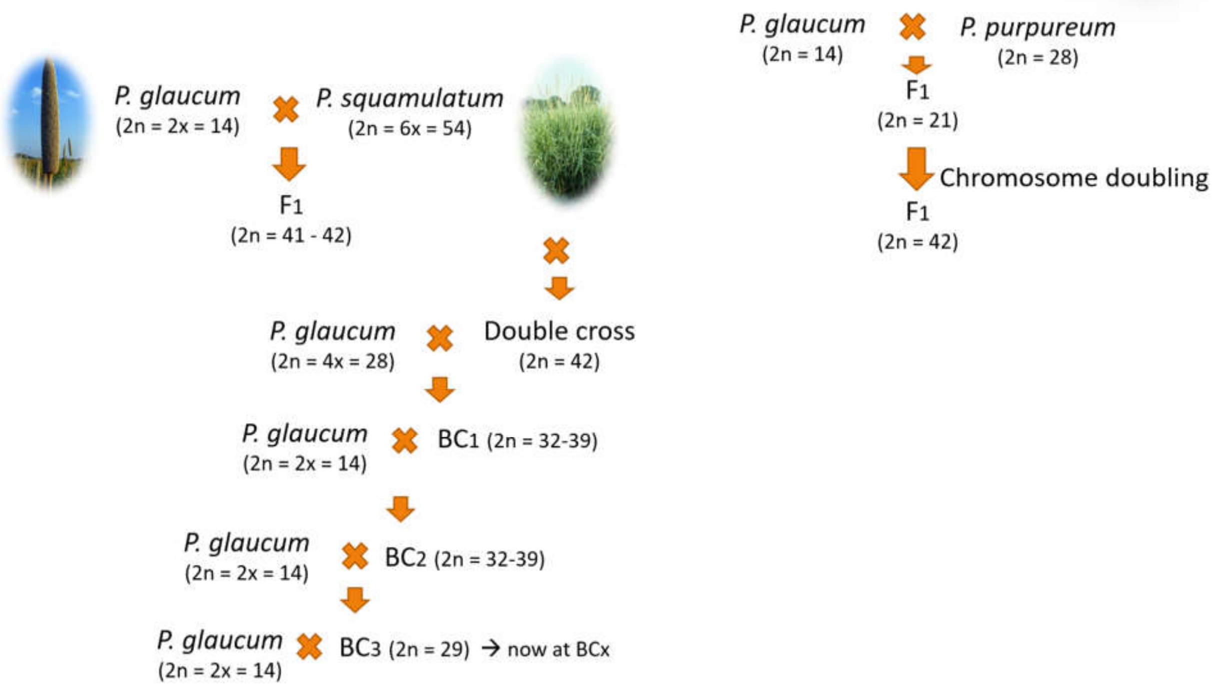
- Instead they could theoretically save some of their own seed

- In the United States, this could destroy the seed industry? – probably not due to IP laws
    - Would lower seed production costs
- In the developing countries, this could help solve many problems associated with lack of infrastructure

The only current commercial applications of apomixis are in Citrus, Kentucky bluegrass (*Poa pratensis*) and buffelgrass

Moving apomixis into pearl millet

After **Hanna, 1987; Dujardin & Hanna, 1983 – 1989**



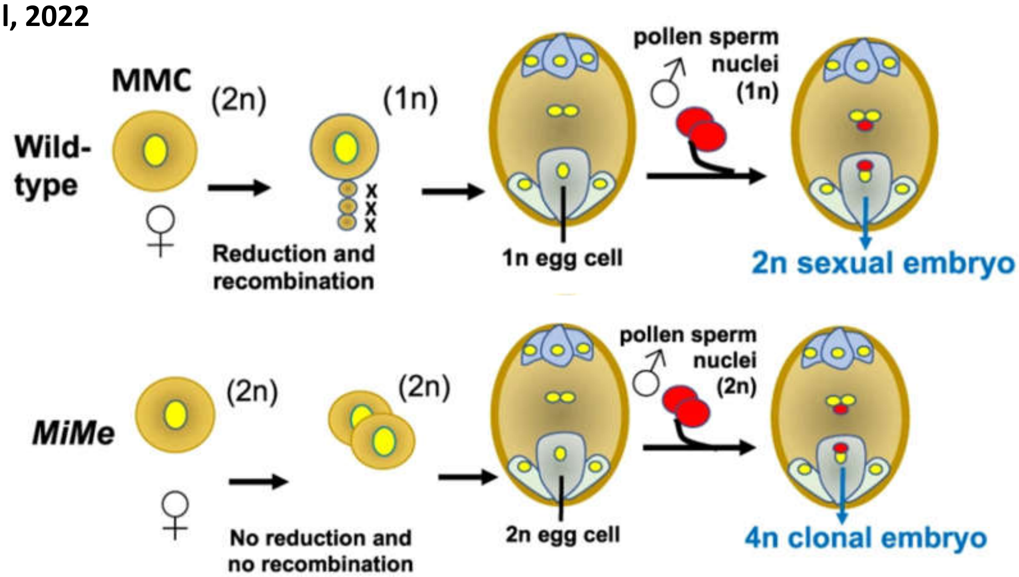
- Selected for obligate apospory and male fertility (as they were dealing with obligate apomixis, male fertility was the only possible way to get sexual transmission of the trait!
- Got one BC3 plant that was partially male fertile and obligately aposporic, from which they got 9 BC4 aposporic plants. Remained so through BC7
- In agreement with the idea that apomixis is controlled by one or few genes linked together, one chromosome from *P. squamulatum* is all that is necessary for apospory

Next options

- Continue backcrossing, and hope to get a cross over due to residual homology between the *glaucum* and *squamulatum* genomes, and thus transfer the trait to pearl millet
- Find pairing genes to permit *glaucum/squamulatum* pairing when inactivated
    - Now that they know the locus is hemizygous, these are not an option
- Use irradiation to translocate the part of the *squamulatum* chromosome coding for apomixis to a *glaucum* chromosome, or
- Get a chromosome substitution line, or
- Get a chromosome addition line, or
- Genetic engineering

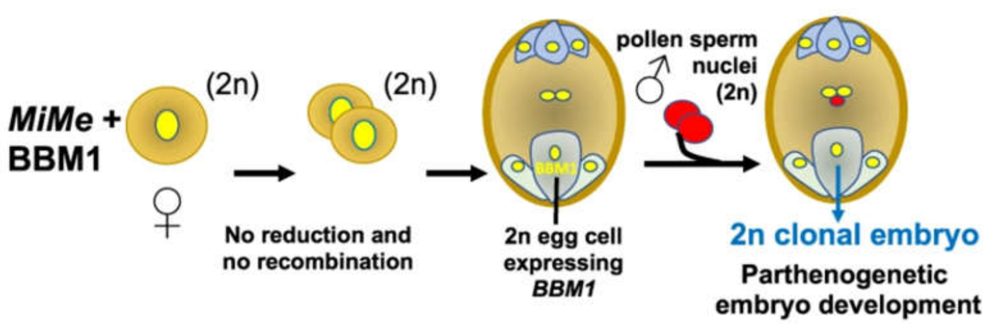
Designing synthetic apomixis

Vernet et al, 2022



Mitosis instead of meiosis mutants produced truly unreduced eggs- in terms of genetics and in terms of chromosome number.

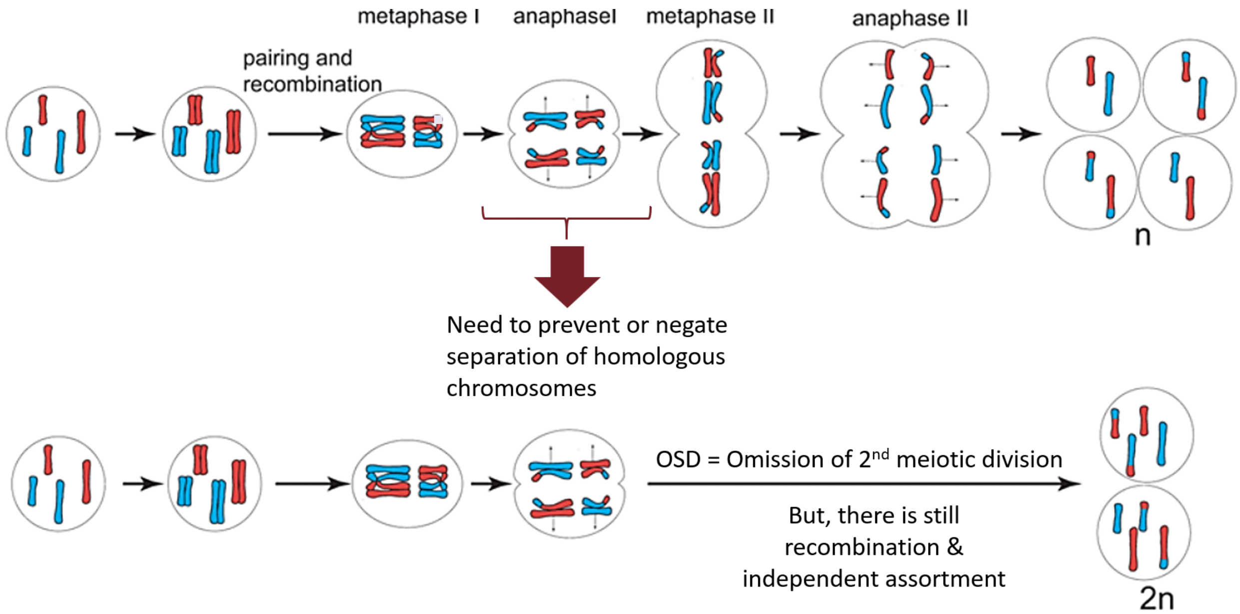
But the egg is still a gamete that requires fertilization, leading to an increase in ploidy with each generation. Parthenogenesis is needed to bypass the problem



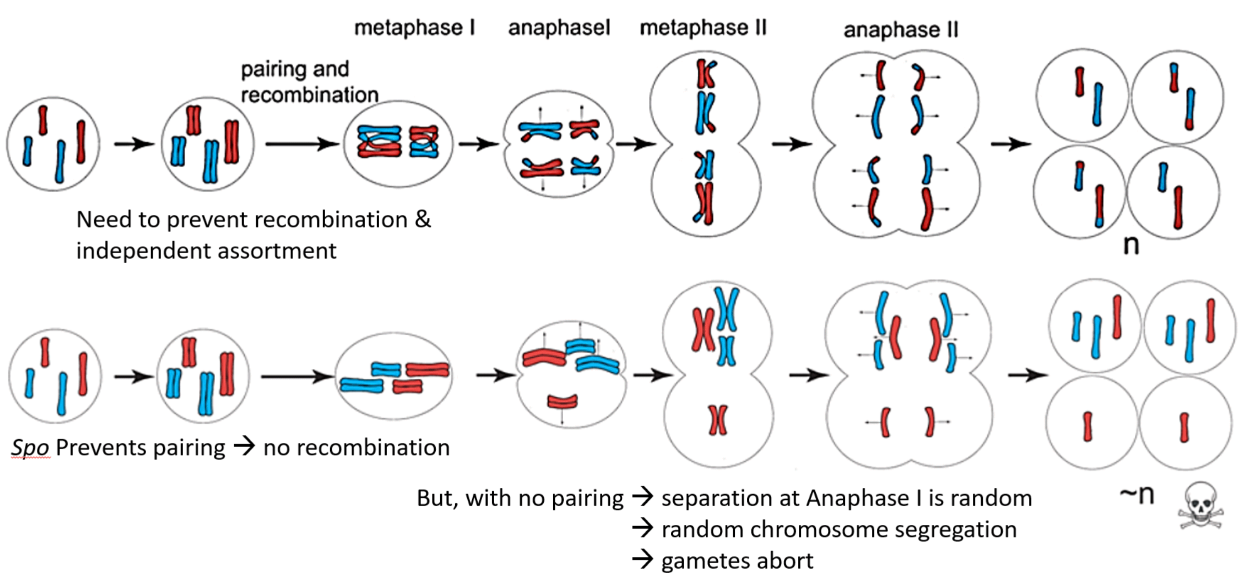
Converting meiosis to mitosis

d'Erfurth et al. 2009

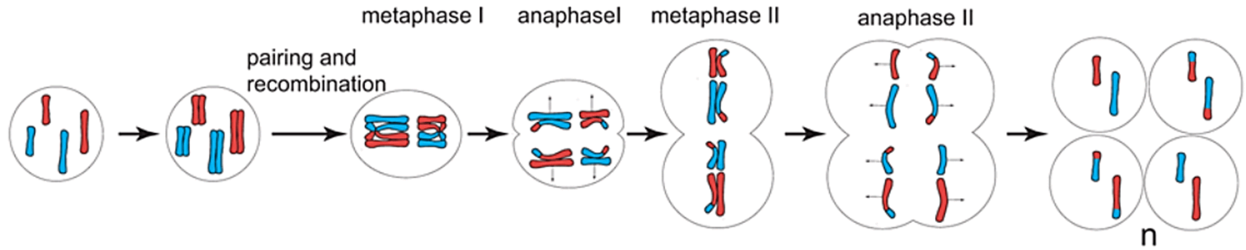
1. Need to stop numerical reduction in chromosome number

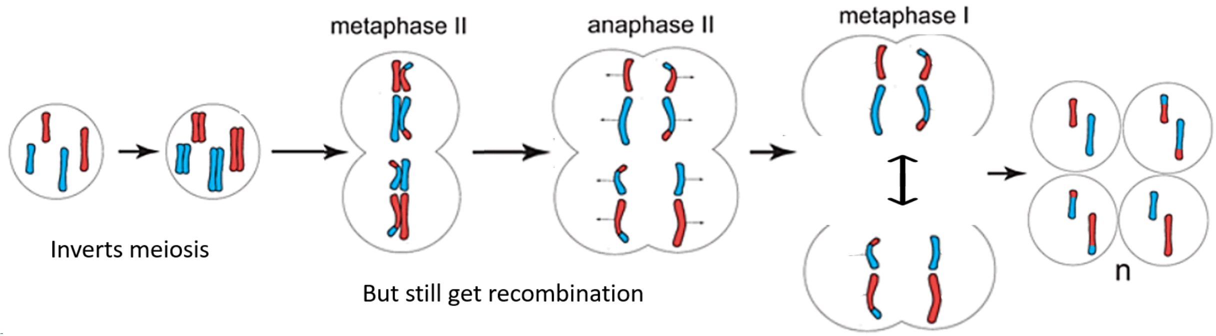


2. Need to prevent recombination



3. Need to invert meiosis

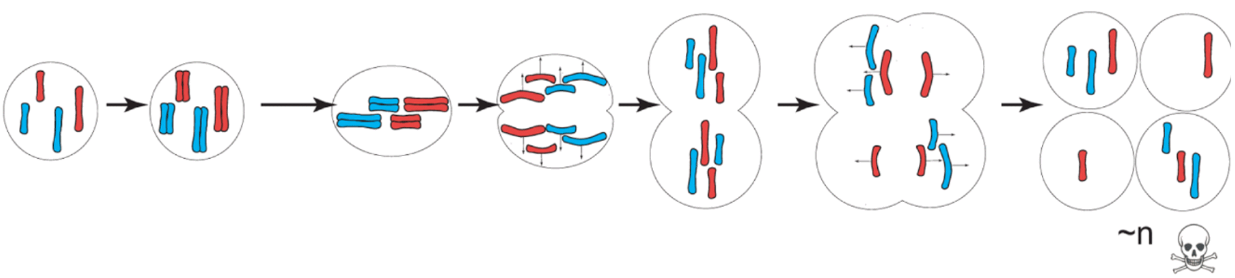




4. Combining spo11-1 with rec85

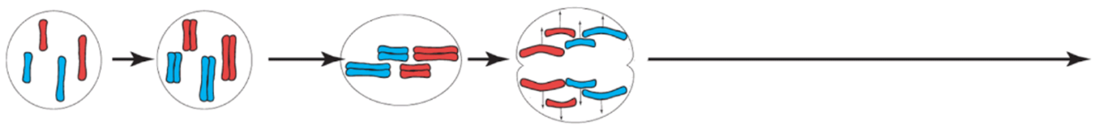
- *Prevents recombination*
- *Prevents independent assortment*

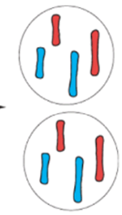
But, does not solve the problem of random chromosome separation @ Ana II



5. Combining spo11-1 with rec8 & osd11 = MiMe

- *Prevents recombination*
- *Prevents independent assortment*
- *Prevents random chromosome separation*

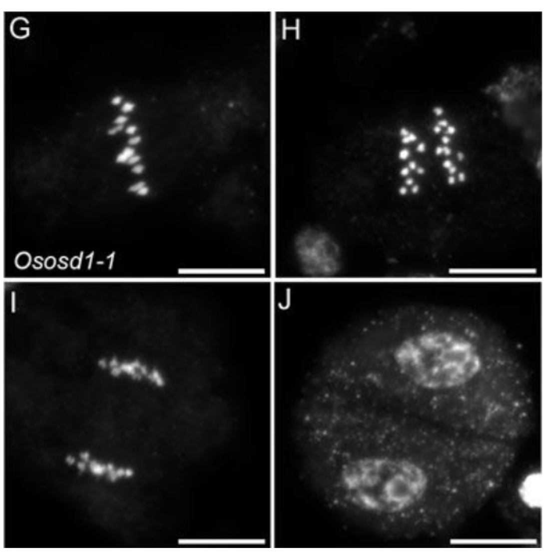




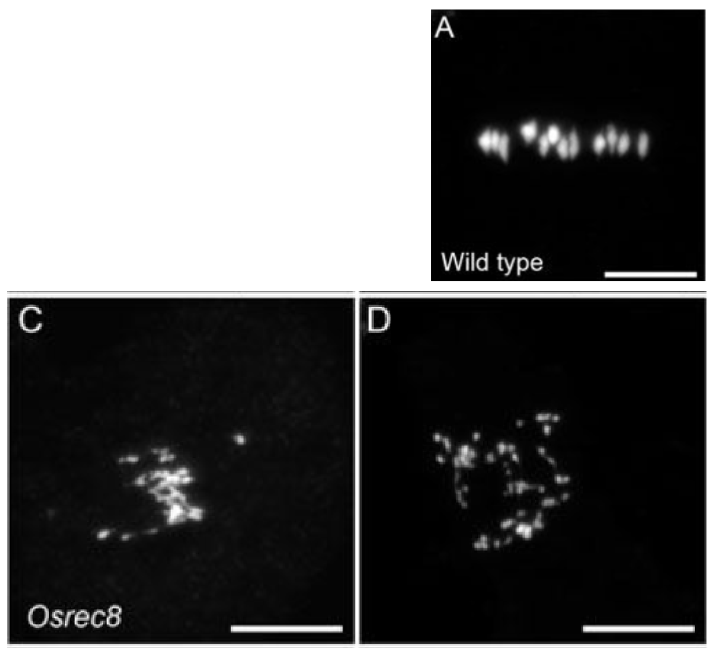
- Gametes are still identical to the sporophyte
  - *But are still gametes*
  - *Require fertilization*

Conversion to MiMe in rice via gene editing

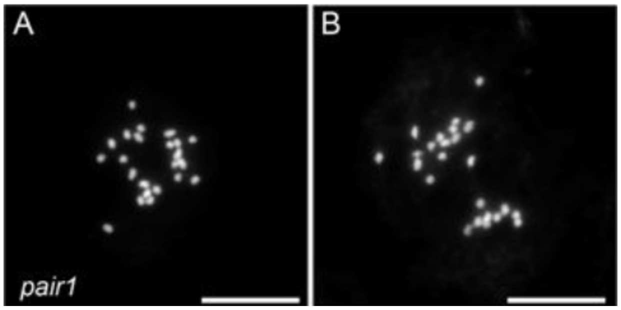
Mieulet et al., 2016



2n gamete formation by omission of second division

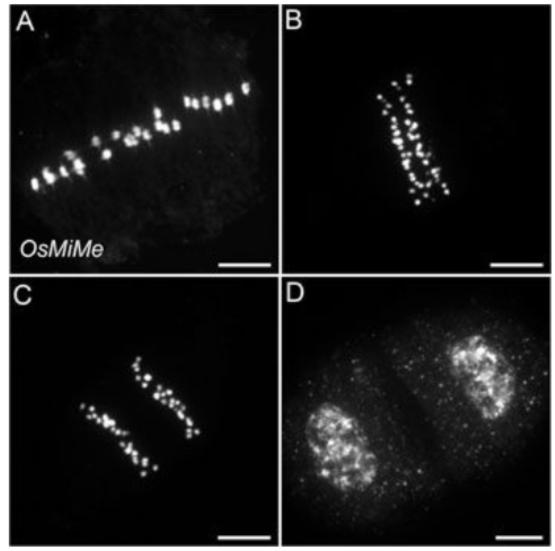


Inverts meiosis. In so doing, SDR becomes FDR



Prevent pairing --> no recombination. Thus the 2n gamete formed is identical genotype to that of the parent

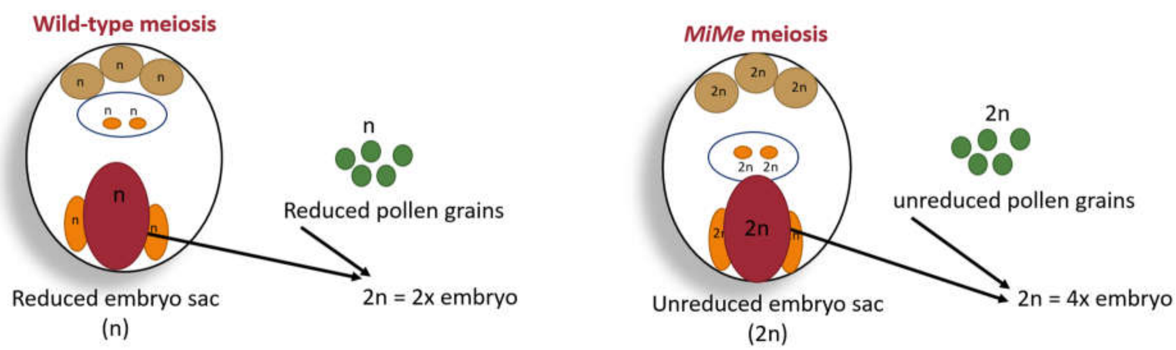
The triple null produces MiMe

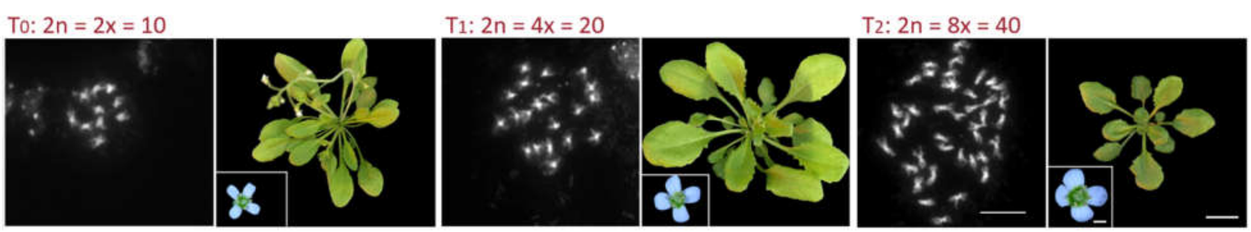


Combining all 3 mutants leads to MiMe (Mitosis instead of Meisois) mutants

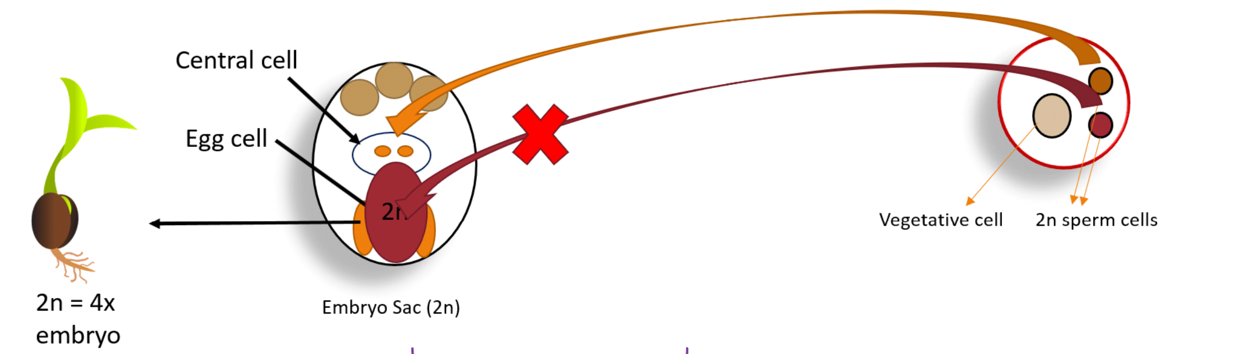
- but, lack parthenogenesis

But, now the ploidy level doubles





To turn MiMe into apomixis, need to add parthenogenesis:



- Prevents ploidy from doubling upon fertilization

Turning MiMe into apomixis

PsASGR-BBML

Conner et al. 2015

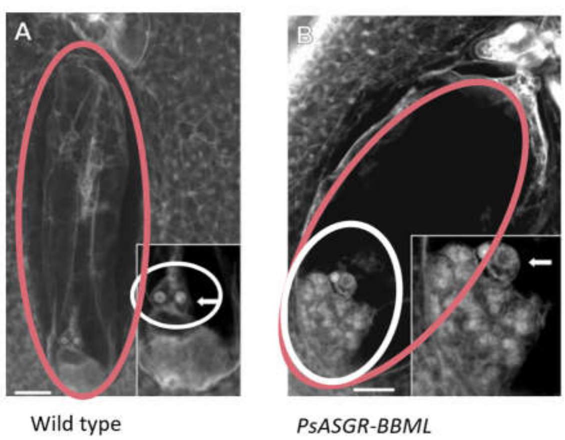


Figure 6. Lovepreet Singh. oziasakinslab.org

PsASGR-BBML can induce parthenogenesis when expressed in the egg

Khanday et al, 2019

The issue is that BBML is normally silenced in the egg and expressed in the pollen.

- The expressed allele enters the egg upon fertilization:

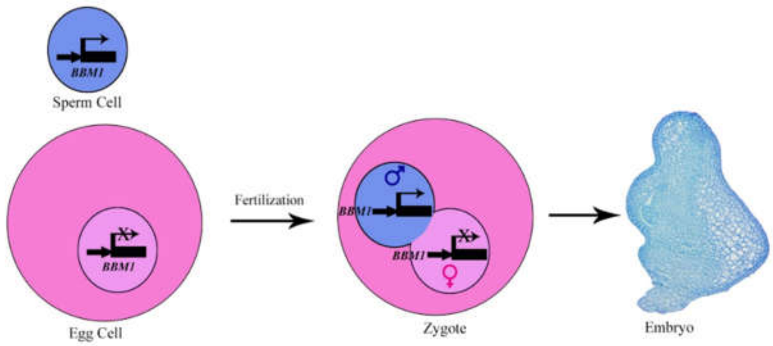
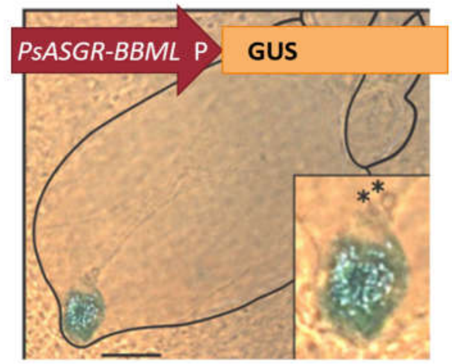


Figure 7http://thenode.biologists.com/its-the-father-paternally-expressed-baby-boom1-initiates-embryogenesis-in-rice/research



The PsASGR-BBML promoter driving transgenic GUS expression in the egg. Conner et al., 2015

Conner et al. 2015

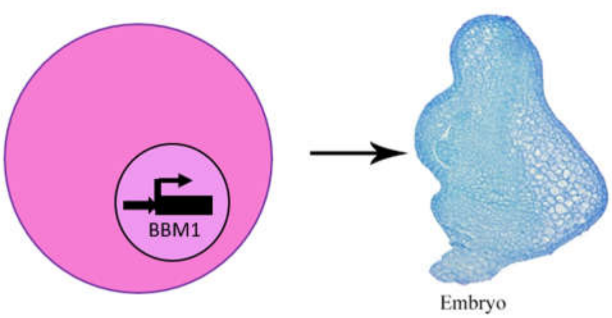
The pearl millet PsASGR-BBML expression is egg-cell-specific due to a mutation that permits expression in the egg

BBMl expression in the eggs

Can now can explain parthenogenesis as having a mutation that permits expression of BBMl in the egg cell

Khanday et al, 2019

Expressing BBMl in the egg cell without the additional mutants leads to haploid production



http://thenode.biologists.com/its-the-father-paternally-expressed-baby-boom1-initiates-embryogenesis-in-rice/research

So, now combine egg-expressed BBMl with MiMe:

So, engineer rice to express BBMl with an egg-specific promoter

- Then use CRISPR to KO the 3 genes to create MiMe
- The result is an apomictic rice but at only 29% frequency

Vernet et al, 2022

Switching from the arabidopsis egg cell-specific promoter its rice ortholog increased the frequency to 60%, with some lines achieving 95% in some environments