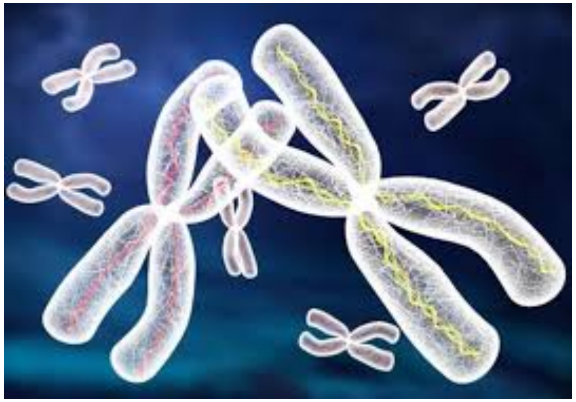
AGR 6322 Advanced Plant Breeding

Fall 2018

Polyploidy in Plant Breeding



Goals for today

Definitions, Auto vs. Allopolyploids, Applications

Syllabus

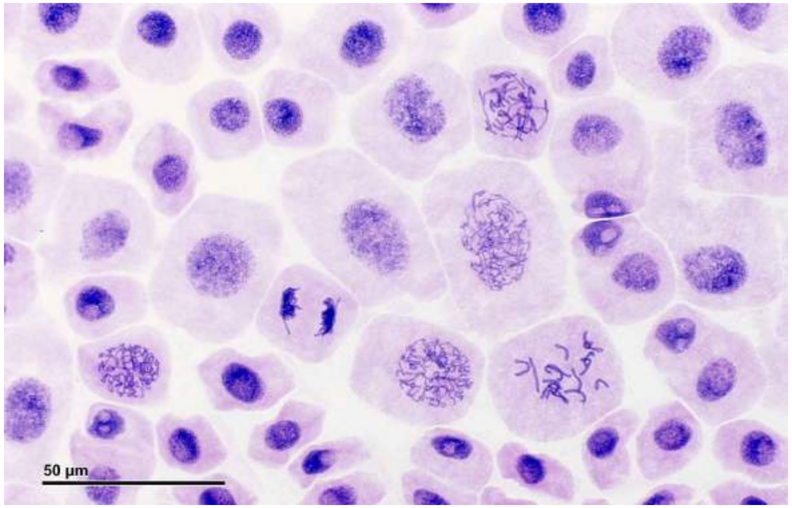
Population development

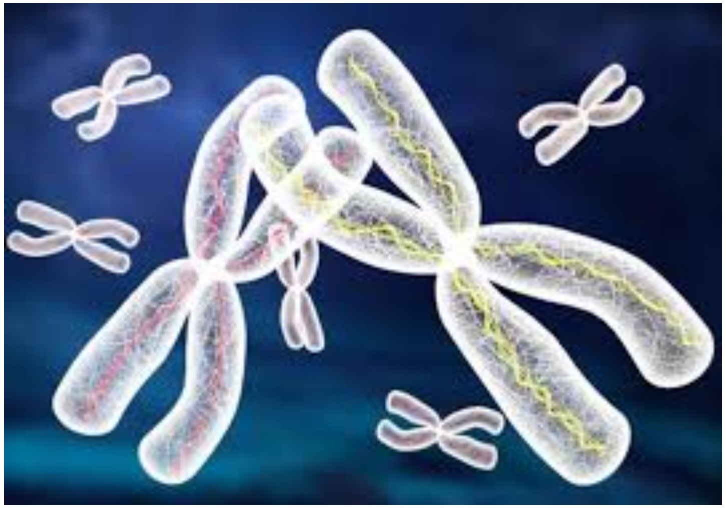
Population Evaluation

Trait Integration

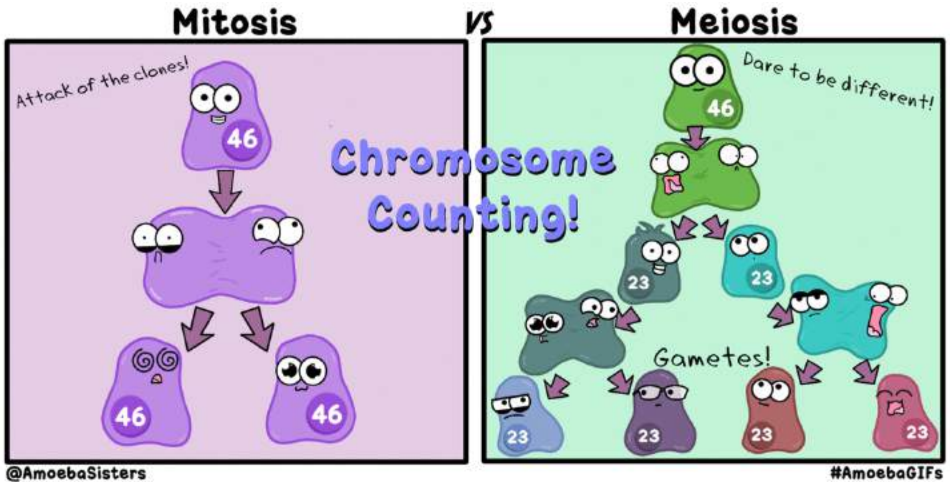
Product Commercialization, Marketing and Supply

Meiosis analysis for chromosome abnormalities

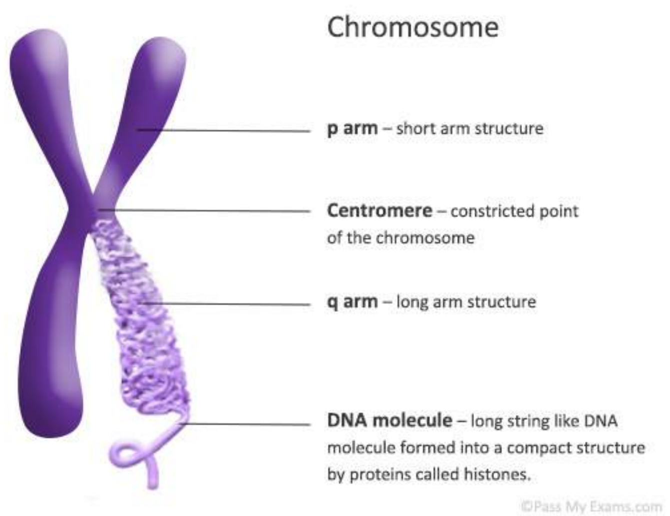




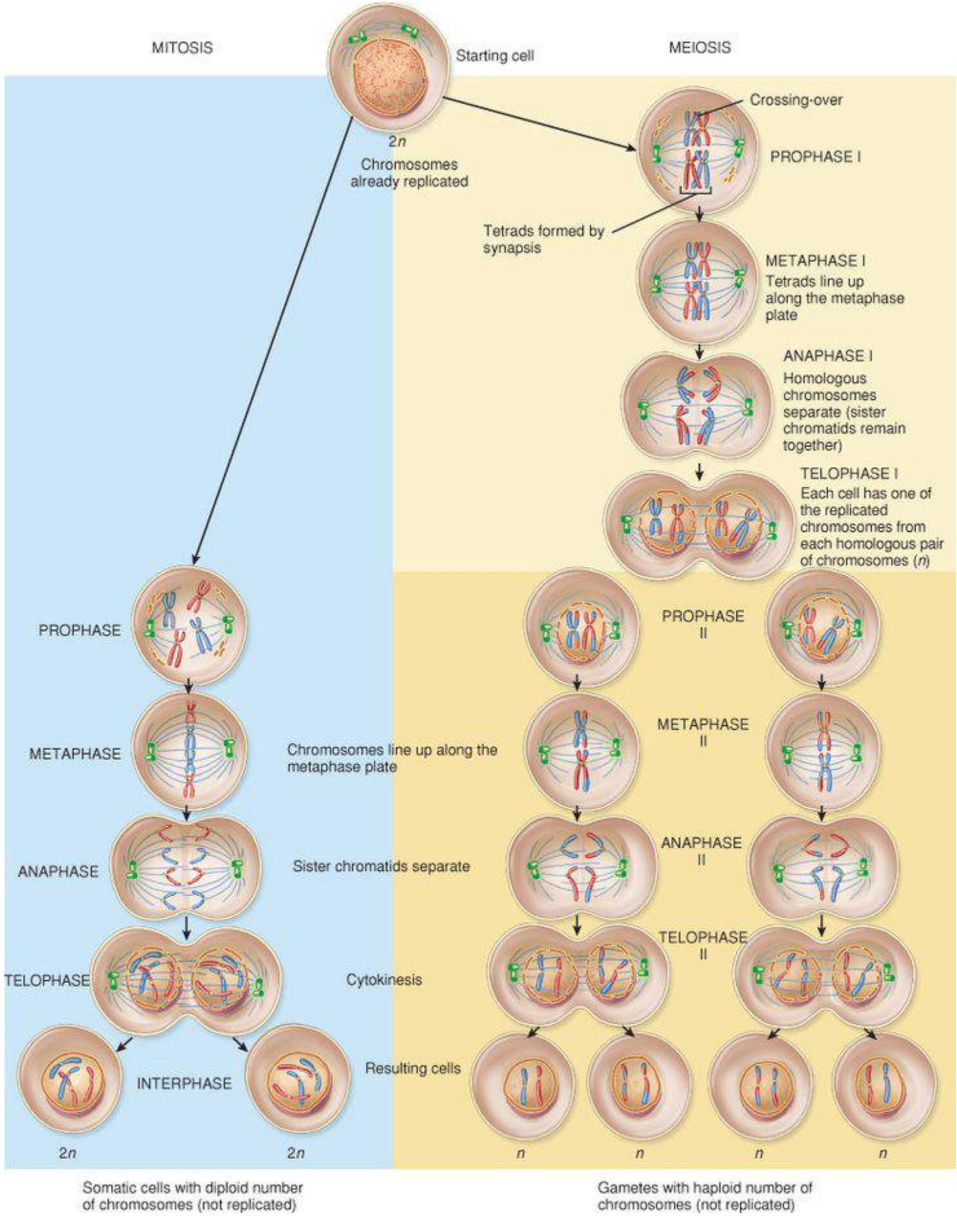
Outline



- ✓ Introduction
- ✓ Mitosis-Meiosis
- ✓ Chromosome features - Karyotype
- ✓ Chromosome Abnormalities
    - Numerical
    - Structural
- ✓ How do chromosome abnormalities happen?



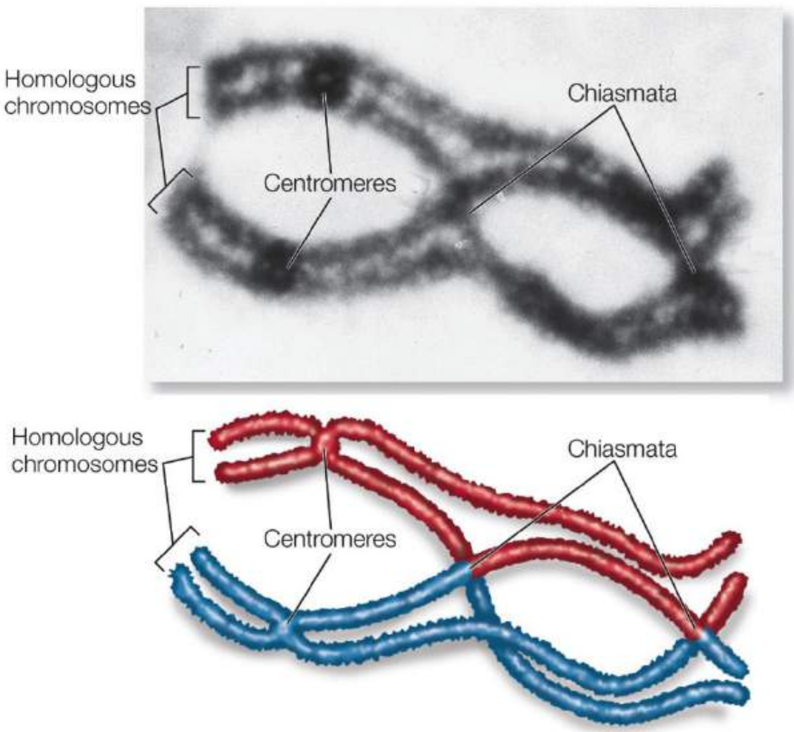
Mitosis - Meiosis

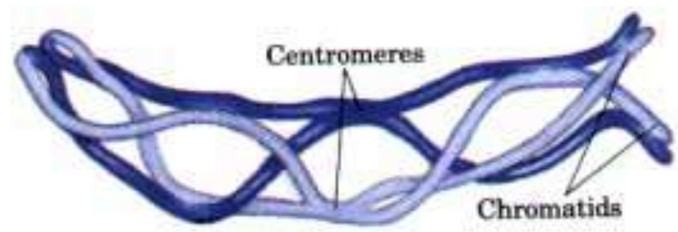


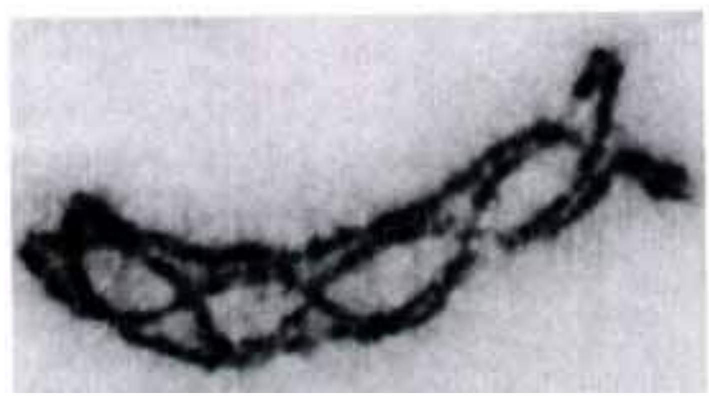
https://www.nature.com/scitable/topicpage/mitosis-and-cell-division-205#

Meiotic analysis

- Study chromosome pairing
  - ➢ Prophase I Diplotene/Diakinesis - For evidence of chiasmata (chromosome crossing over) frequency.





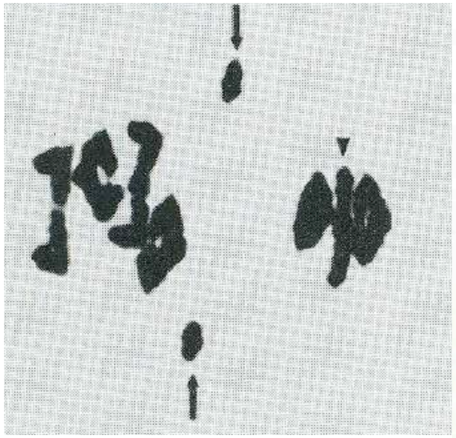


***LIFE 8e*, Figure 9.17**

LIFE: THE SCIENCE OF BIOLOGY, Eighth Edition © 2007 Sinauer Associates, Inc. and W. H. Freeman & Co.

Meiotic analysis

- Study chromosome pairing
  - ➢ At late prophase I or metaphase I for pairing relationships:
    - ✓ Failure to pair indicates some lack of homology.
    - ✓ In interspecific hybrids may identify homologous genomes.



Two Univalents

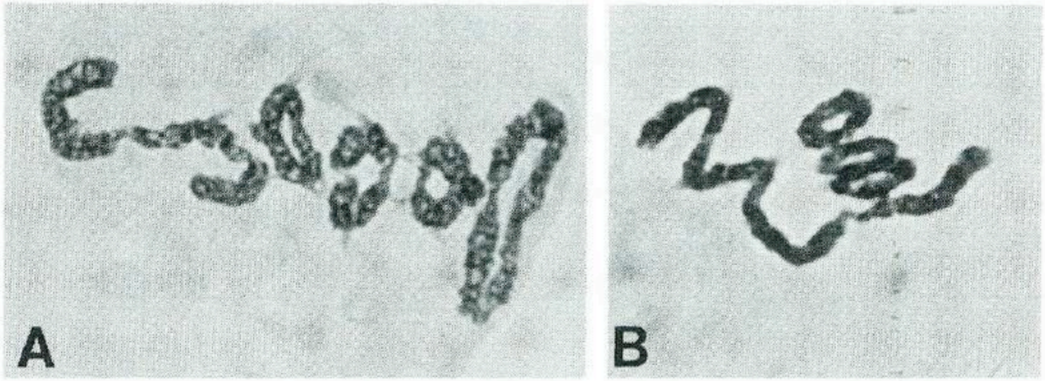


FIGURE 6.16. Meiotic metaphase-I configurations in *Secale* interspecific F_1 hybrids. A, *S. africanum* × *S. vavilovii* (2IV + 3II); B, *S. cereale* × *S. montanum* (1VIII + 3II). (From R. J. Singh, unpublished results.)

Meiotic analysis

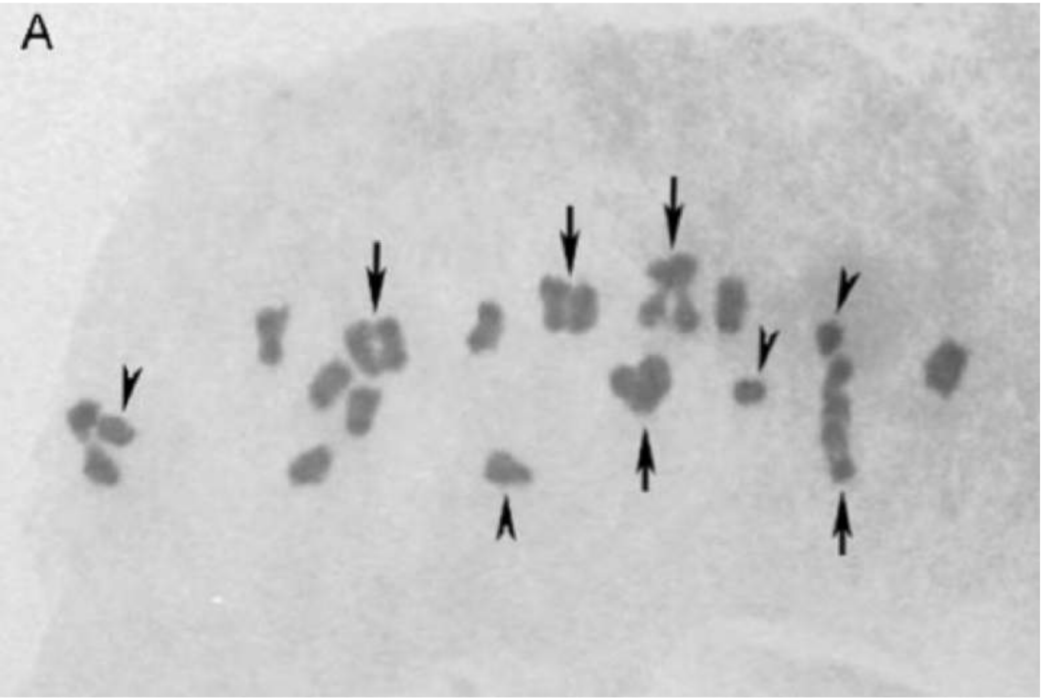
How to record, calculate, and report meiotic pairing data?

a. Determine exact chromosome number
b. Observe and record exact pairing relationships in minimum of 20 cells.
c. Calculate mean pairing frequency by calculating the mean of all configurations.

Microphotography of meiotic chromosome configurations: metaphase I in tetraploid (2n = 40) *P. oteroi* showing

- 4 univalents (arrow heads),
- 8 bivalents
- 5 quadrivalents (arrows)

(Novo et al., 2015)



Chromosome Abnormalities

- Numerical Abnormalities:
  - ✓ Aneuploidy (incomplete/abnormal number of chromosomes). Nomenclature: Nullisomic, Monosomic, Trisomic and Tetrasomic.
  - ✓ Polyploidy
- Structural Abnormalities: a chromosome's structure can be altered in several ways.
  - ✓ Deletions
  - ✓ Duplications
  - ✓ Translocations
  - ✓ Inversions

Polyploidy

2n = 2x = 14

Diploid

Ahloowalia, 1965

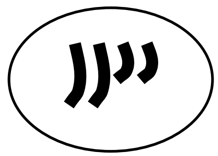
2n = 4x = 28

Tetraploid

Polyploidy

- An individual having more than two sets of the basic chromosome number.
  - **Basic Chromosome Number (x):**
  - **Gamete chromosome number (n)**
  - **Total chromosome number (2n):** normal somatic cell chromosome number.
    - Total number of chromosomes in an organism

Polyploidy

- Ploidy Levels:
  - Monoploid (x): one se
  - Diploid (2x): two sets 
  - Triploid (3x): three sets
  - Tetraploid (4x): four se
  - Pentaploid (5x): five
  - Hexaploid (6x): six set

Ploidy levels in cells, assuming a basic chromosome number of x = 2

Polyploidy

- Counting chromosomes:
  - In a tetraploid
    - Somatic cells (2n)
    - 4 sets of basic chromosomes (4x).
      - **So 2n = 4x**
      - **gametes are n = 2x.**
  - In a hexaploid
    - Somatic cells (2n)
    - 6 sets of basic chromosomes (6x).
      - **So 2n = 6x**
      - **Gametes are n = 3x.**

Polyploidy - Examples

Species	Gamete/Haploid (n)	Basic (x)	Total (2n)
Human *Homo sapiens*	23	23	2n = 2x = 46
Maize *Zea mays*	10	10	2n = 2x = 20
Oats *Avena strigosa*	7	7	2n = 2x = 14
Avena barbata	14	7	2n = 4x = 28
Avena sativa	21	7	2n = 6x = 42
Wheat *Triticum monococcum* (AA)	7	7	2n = 2x = 14
T. turgidum (AABB)	14	7	2n = 4x = 28
T. aestivum (AABBDD)	21	7	2n = 6x = 42
Tall Fescue *Festuca arundinacea*	21	7	2n = 6x = 42
Bermudagrass *Cynodon dactylon*	18	9	2n = 4x = 36
C. dactylon	27	9	2n = 6x = 54
C. transvaalensis	9	9	2n = 2x = 18

Characteristics of Polyploids

Cell enlargement (the ‘gigas’ effect; Stebbins, 1971; Levin, 2002; Knight & Beaulieu, 2008), but less evident effects can also occur.

Ecological tolerance have been demonstrated (Levin, 2002).

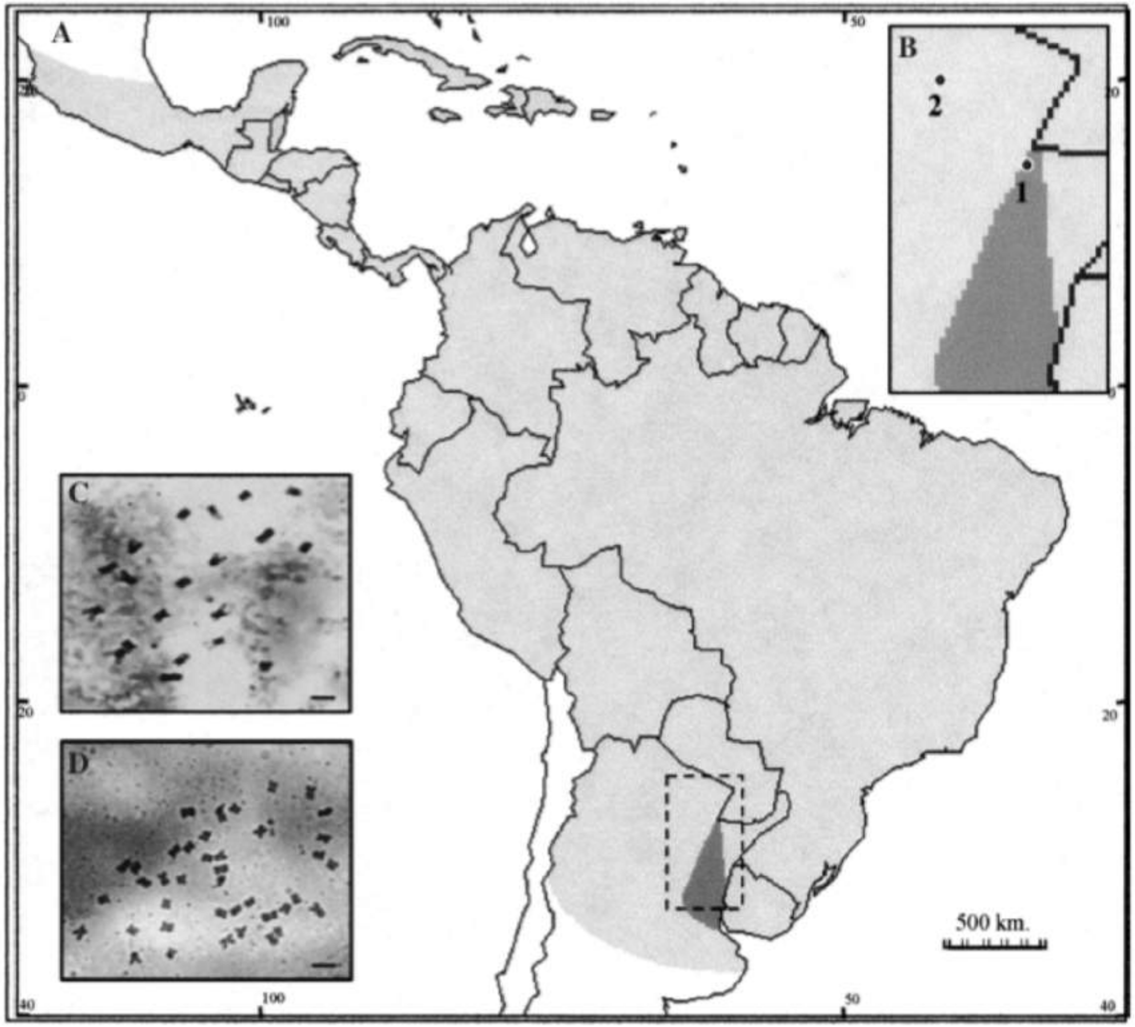
Sometimes polyploids have reproductive organs that are larger than their diploid counterparts, more flowers per inflorescence, and (as incompatibility may be lost) increased selfing (Robertson et al., 2010),

Chromosomal duplications and increases in DNA amount have the potential to alter quantitative plant traits like flower number, plant stature or stomata size.

Polyploid plants not only have larger cells but the plants themselves are often larger. This has led to the deliberate creation of polyploid varieties of plants as forages, grains, watermelons, marigolds, and snapdragons.

Source: Balao et al., 2011: http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2011.03787.x/full

Characteristics of Polyploids



Daurelio et al. 2004. Plant Syst. Evol. 244: 189–
199 (2004) DOI 10.1007/s00606-003-0070-6

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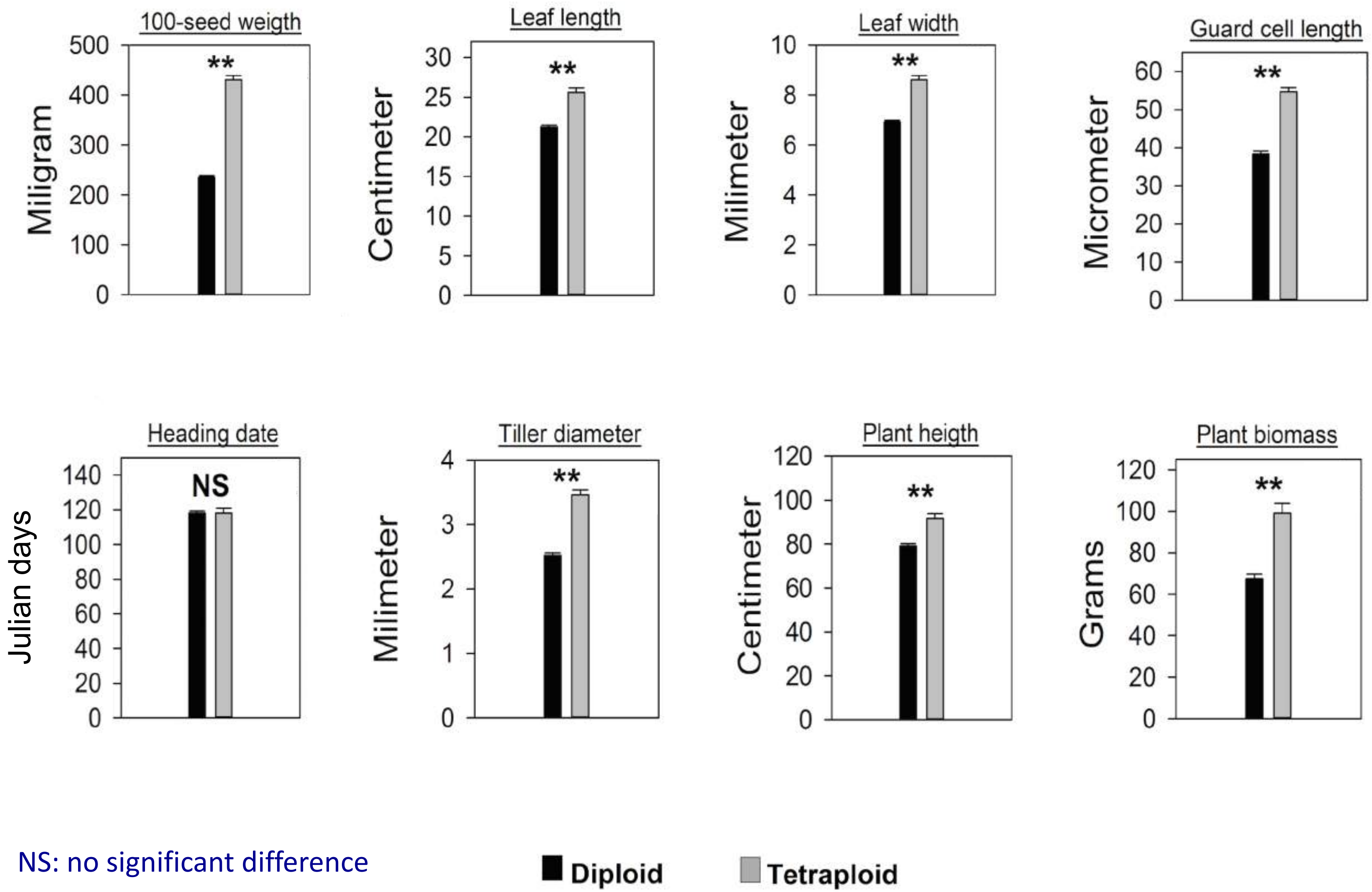


NS: no significant difference

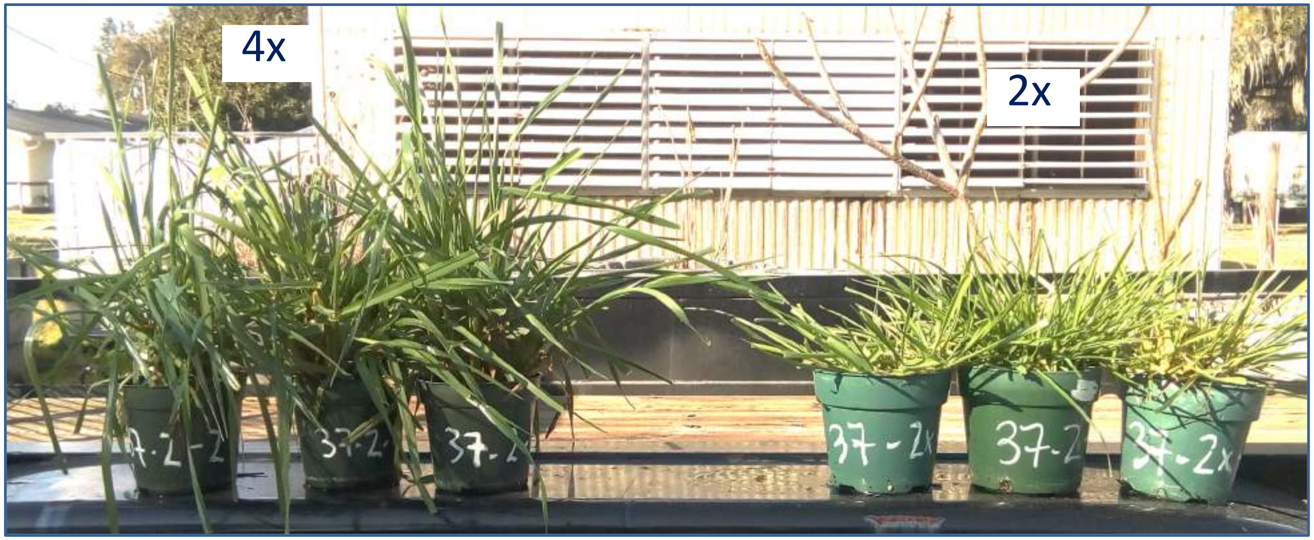
** : significant differences at *P* <0.001

Rios et al., 2015

Characteristics of Polyploids

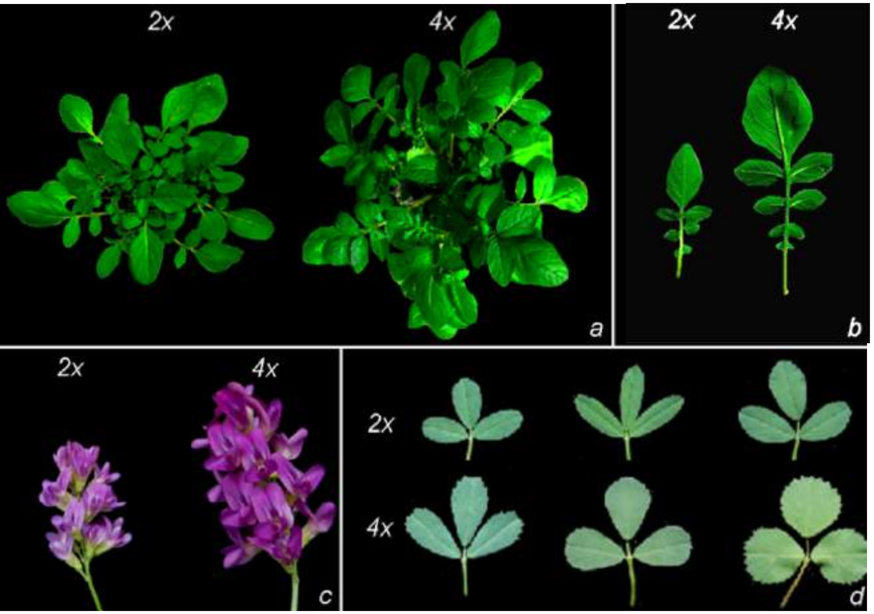
<u>Ploidy level in annual ryegrass</u>

- ○ Follow up study: chromosome doubling
- ✓ Morphological and physiological traits between ploidy levels
  - ▪ Jennifer Timmers (PhD student with Dr. Erickson)
- ✓ Development of 4x cultivars



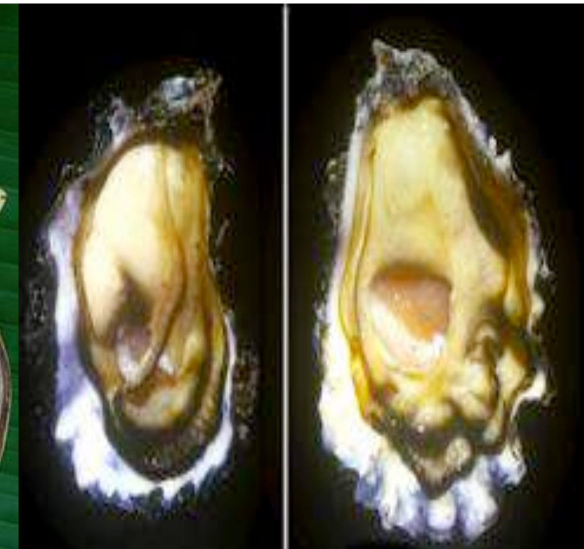
Characteristics of Polyploids

The “gigas” effect (Stebbins, 1950)



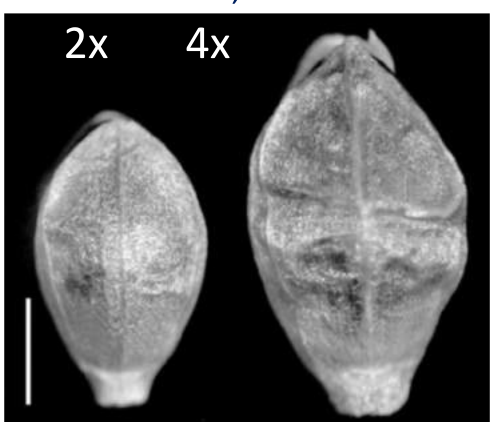
Aversano et al., 2012

www.abc.net.au

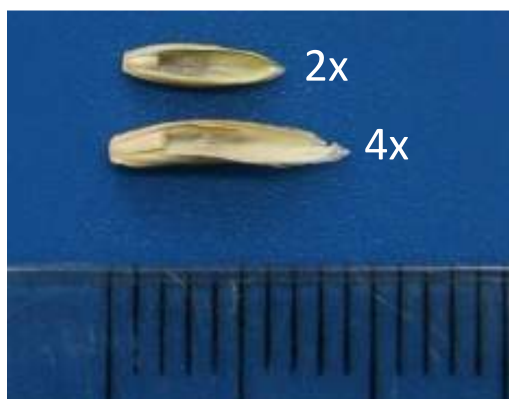


2x 3x

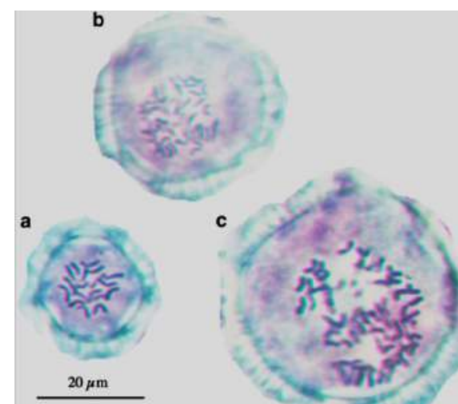
www.vims.edu



Sartor et al., 2008



www.agriseeds.co.nz



Ramsey, 2006

Types: Autopolyploids and Allopolyploids

Most early workers distinguished two types of polyploids (Müntzing, 1936; Darlington, 1937; Clausen et al., 1945):

- Autopolyploids: multiple sets of chromosomes are identical.
- Allopolyploids: multiple sets of chromosomes are different.

Grant (1981) Chapter 23: Types of Polyploids. His excellent review of this topic remains relevant today.

Principal criteria for distinguishing between autopolyploids and allopolyploids are chromosome behavior, fertility, segregation ratios, and morphology.

Source: Soltis et al., 2003: http://onlinelibrary.wiley.com/doi/10.1046/j.1469-8137.2003.00948.x/full

Grant V. 1981. *Plant speciation*. New York, USA: Columbia University Press.

Types: Autopolyploids and Allopolyploids

Autopolyploidy and allopolyploidy are the extreme members of a graded series:

I. Autopolyploids

- Strict autopolyploid AAAA

II. Allopolyploids

- Segmental allopolyploid $A_sA_sA_tA_t$
- Genomic allopolyploid AABB
- Autoallopolyploid AAAABB

Source: Soltis et al., 2003: http://onlinelibrary.wiley.com/doi/10.1046/j.1469-8137.2003.00948.x/full

Grant V. 1981. *Plant speciation*. New York, USA: Columbia University Press.

Types: Autopolyploids and Allopolyploids

- Autopolyploids
  - Each additional set of chromosomes is identical to the parent species.
  - If **A** represents a haploid set of chromosomes, then a normal diploid would be **AA**.
  - **Autotriploids – AAA**
  - **Autotetraploids - AAAA**

Importantly, autopolyploids should exhibit <u>tetrasomic or higher level inheritance, rather than disomic inheritance</u>.

Although it is now clear that autopolyploids are much more common than traditionally maintained, they are probably not as common as allopolyploids (we still have no firm estimate of the relative abundance of autopolyploids).

<u>Source:</u> Soltis et al., 2003: http://onlinelibrary.wiley.com/doi/10.1046/j.1469-8137.2003.00948.x/full

Grant V. 1981. *Plant speciation*. New York, USA: Columbia University Press.

Types: Autopolyploids and Allopolyploids

- Autotetraploids
  - More fertile because balanced gametes are produced.
  - Endoreduplication: Chromosomes replicate, but the cell never divides resulting in a doubled chromosome number.
  - Example:
    - Tetraploid cells from diploid cells.
      1. Apply a cold or heat shock to cells undergoing meiosis.
      2. Colchicine (chemical) – interferes with spindle formation
         - » Application to somatic cells undergoing mitosis.
         - » Spindle fiber interference (disjunction does not occur – spindle fiber errors)
         - » Cell re-enters interphase – sister chromatids separate – chromosomes doubled.

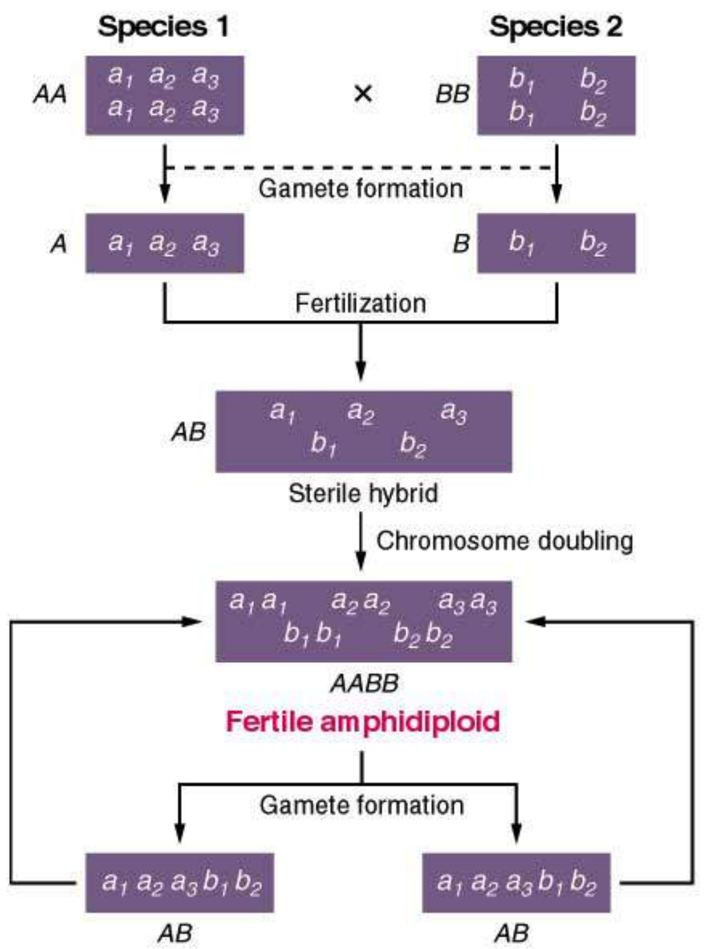


Types: Autopolyploids and Allopolyploids

- Allopolyploid: two closely related species are hybridized resulting in a plant with two different genomes.
  - Egg
    - Species with chromosome sets AA (A genome)
  - Pollen
    - Species with chromosome sets BB (B genome)
  - Fertilization
    - **AB Sterile hybrid.**
      - No homology between genomes
      - Lack of pairing in meiosis
      - Unbalanced gametes.
  - Natural or induced chromosome doubling results in two copies of each genome (AABB – tetraploid).
    - Fertility is restored because the chromosomes can pair appropriately during meiosis

Types: Autopolyploids and Allopolyploids

Allopolyploidy



Animation: https://cienciascondgerman.wikispaces.com/file/view/Poliploidia.swf

Polyploidy in Plant Breeding

- Diploid crops: forages, turfgrass, sweet-corn, vegetables (tomatoes).
- Triploid crops: citrus, tufgrass, forages, trees, banana.
- Tetraploid crops: peanuts, ryegrass, alfalfa, blueberries, etc.
- Hexaploid crops: wheat, triticale, oats, flowers, etc.
- Octoploid crops: strawberry, sugarcane.

Polyploidy in Plant Breeding

- Used to produce sterile (seedless) triploid strains of plants that must be vegetatively (asexually) propagated through cuttings or by apomixis.
  - **Seedless bananas, watermelons.**
  - **Hybrid bermudagrass used for turf.**
  - Grow normally because mitosis is not interfered with.

Apomixis – progeny are identical to the mother.

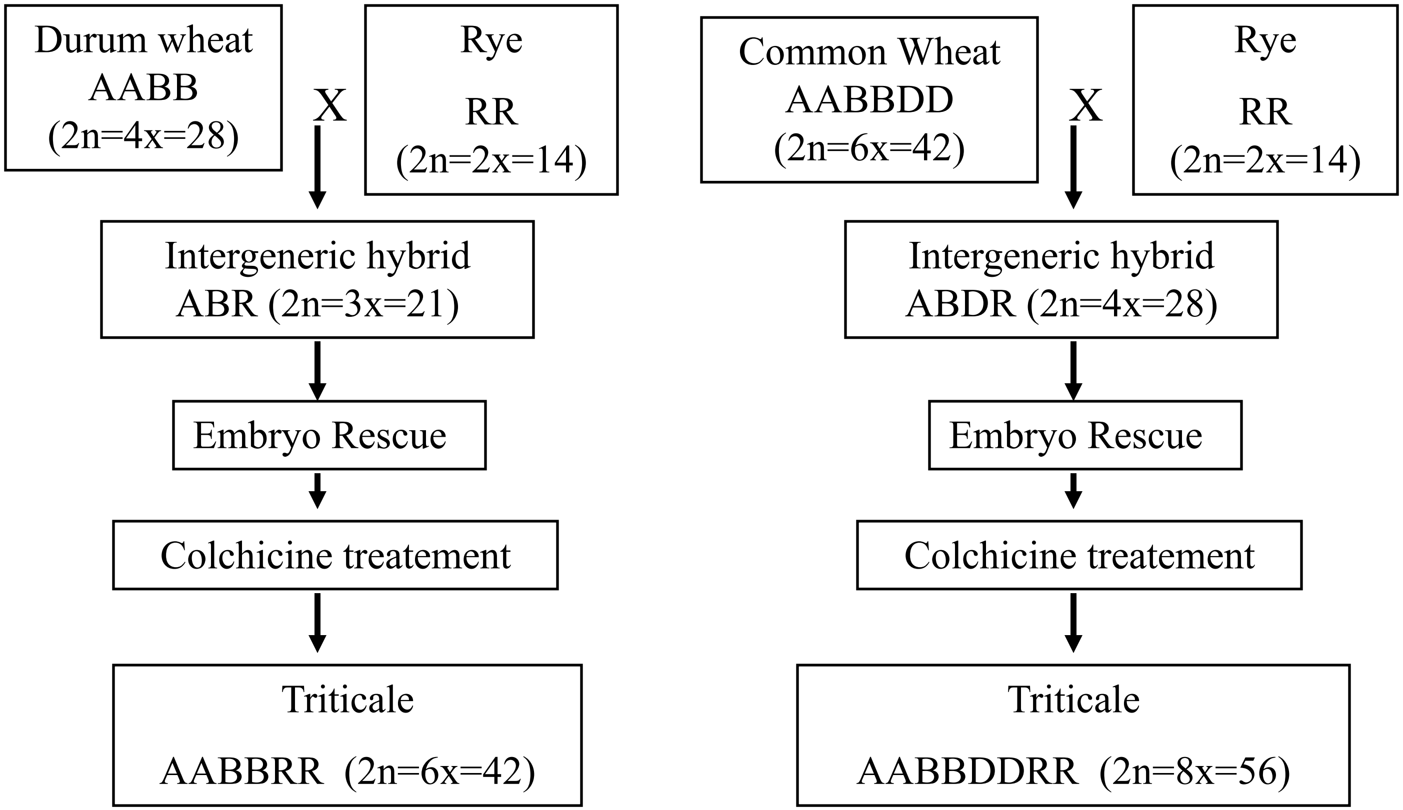
- Triticale (*Triticosecale* spp.)

  Man made crop species

  - Genomes of wheat (*Triticum* spp.) and rye (*Secale cereale*).
  - Hybrids combine features of both wheat and rye.
    - Intergeneric
    - High protein and yield of wheat.
    - High lysine (amino acid) content and stress tolerance of rye.

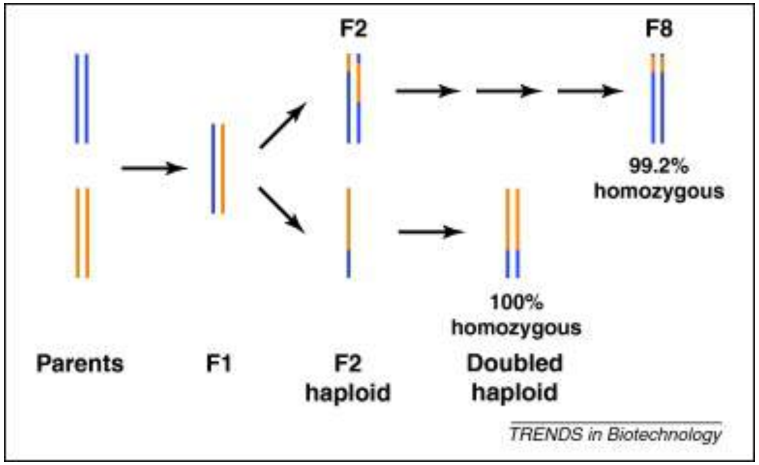
Polyploidy in Plant Breeding

Triticale



Polyploidy in Plant Breeding

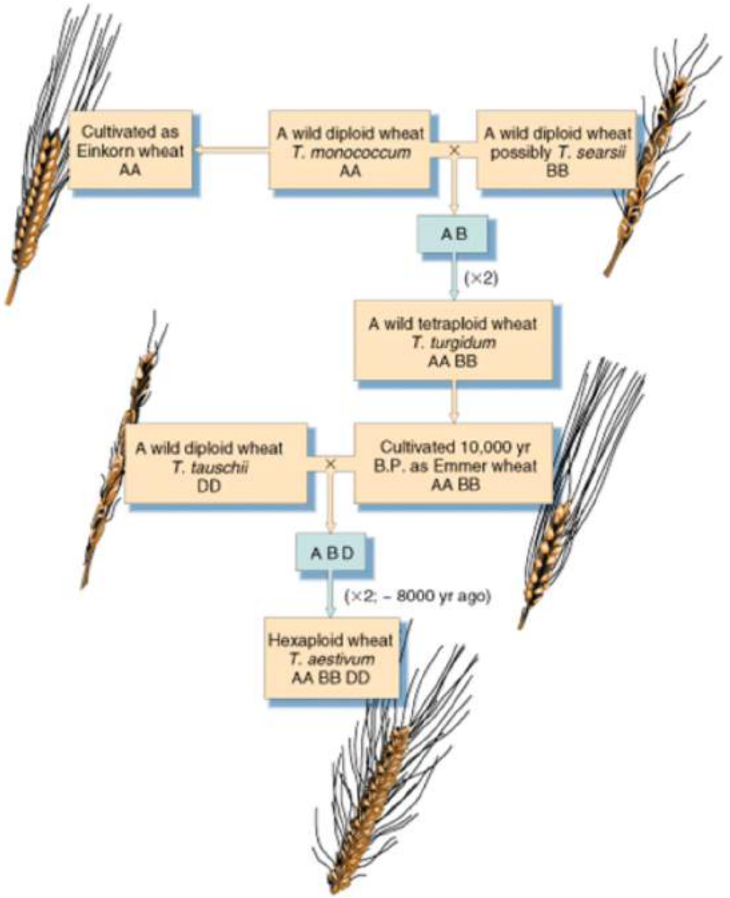
- It is possible to produce haploid plants that have only a single set of chromosomes.
- Tissue culture regeneration of somatic embryos produced from pollen grains (microspores).
  - Infertile
  - Treatment with cochicine restores fertility
  - Plants are called double haploid (identical homologs).
    - Plants are 100% homozygous and are extremely useful for plant geneticists and breeding.



Polyploidy and speciation

Key points:

Biological species concept: organisms belong to the same species if they can interbreed to produce viable, fertile offspring (in some cases, organisms of different species mate and produce healthy offspring, but the offspring are infertile).



Speciation: process by which new species form. It occurs when groups in a species become reproductively isolated and diverge.

- Allopatric: ancestral population evolve into separate species due to a period of geographical separation.
- Sympatric: ancestral population evolve into separate species without any geographical separation.

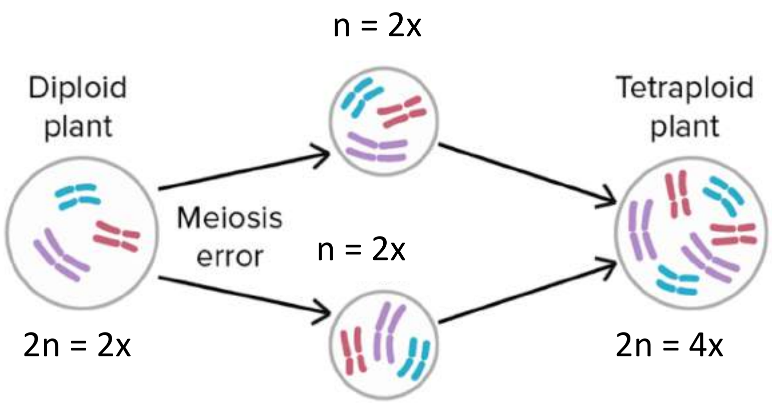
Polyploidy and speciation

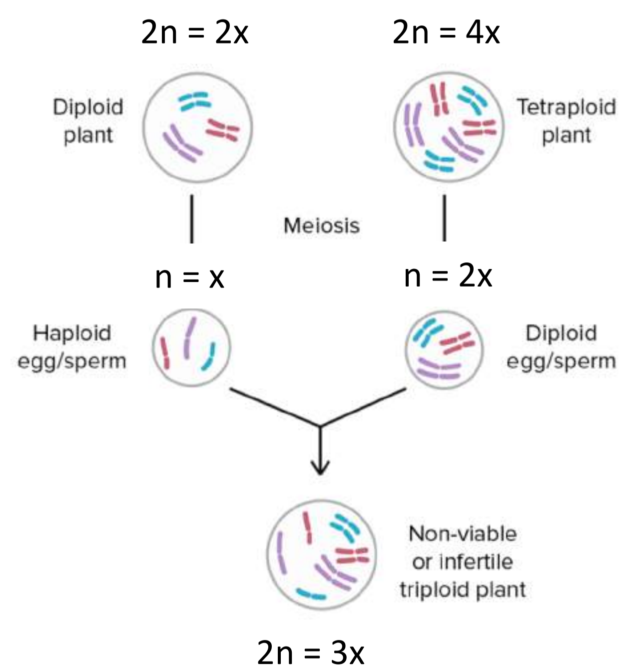
Polyploidy typically results in instant speciation—the new polyploid may be immediately isolated reproductively from its parent or parents. This process greatly:

- increases biodiversity
- provides new genetic material for evolution.

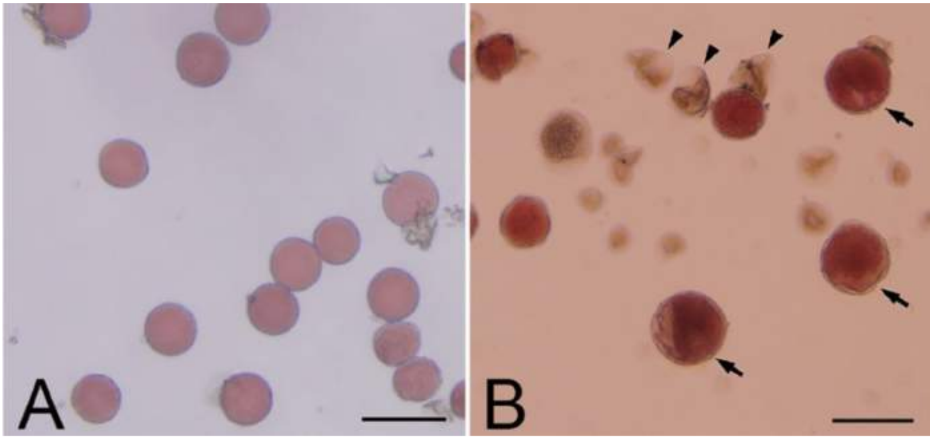
Unlike humans and other animals, plants are often tolerant of changes in their number of chromosome sets, and polyploidy can be an instant recipe for plant sympatric speciation.

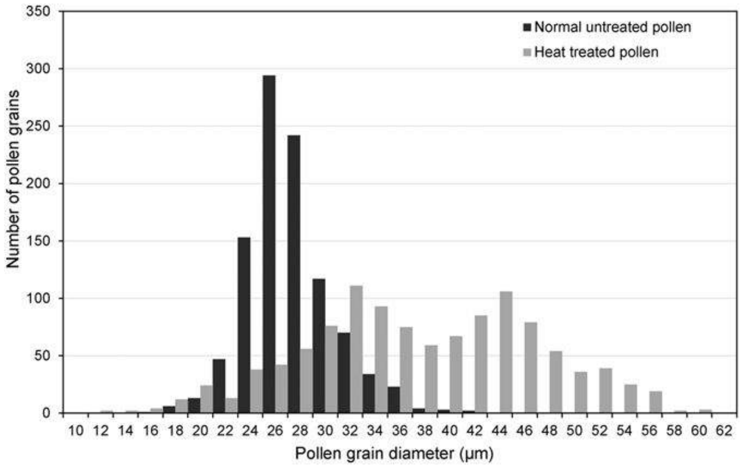
How could polyploidy lead to plant speciation?



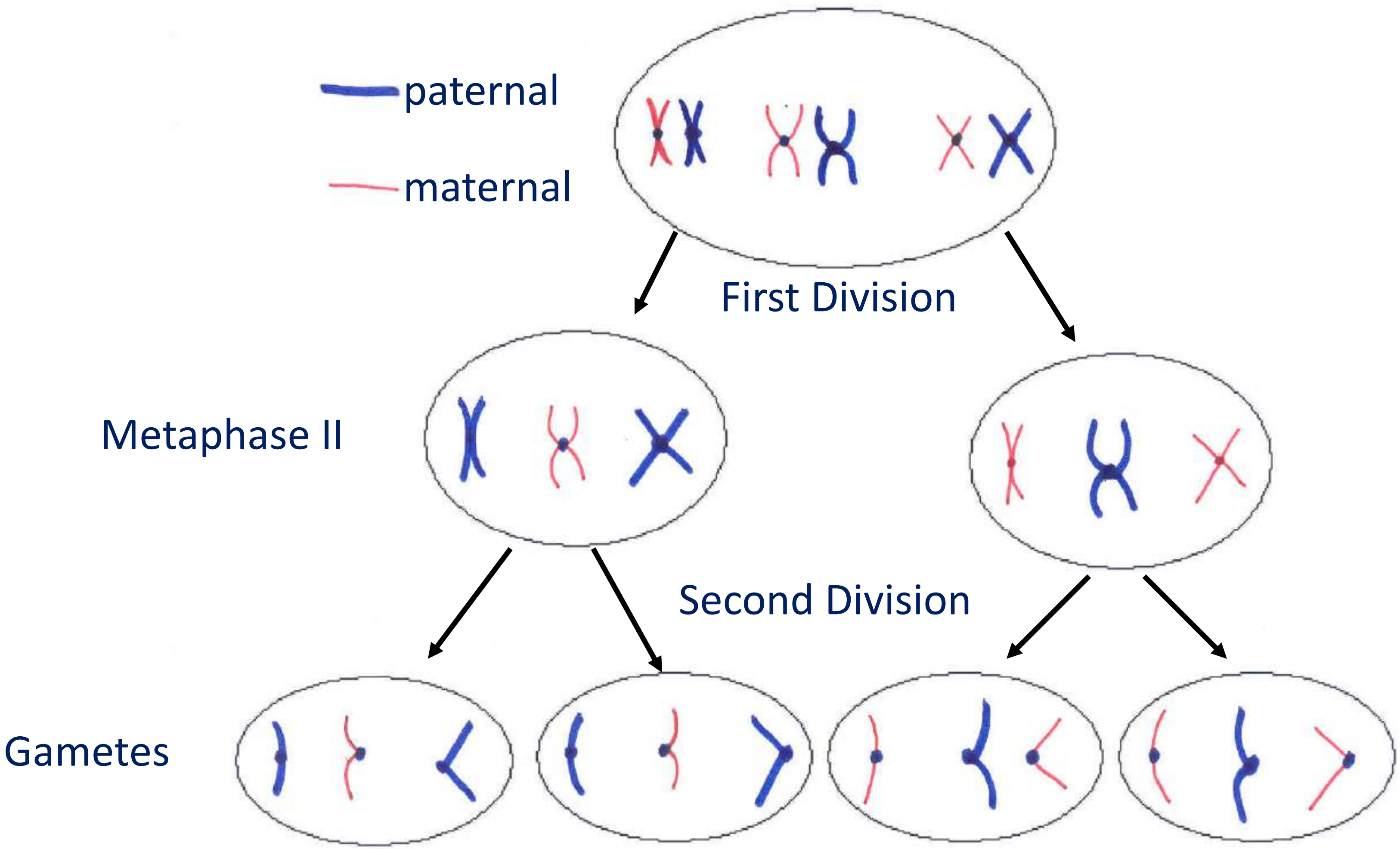


2n gametes and Autopolyploid Genetics

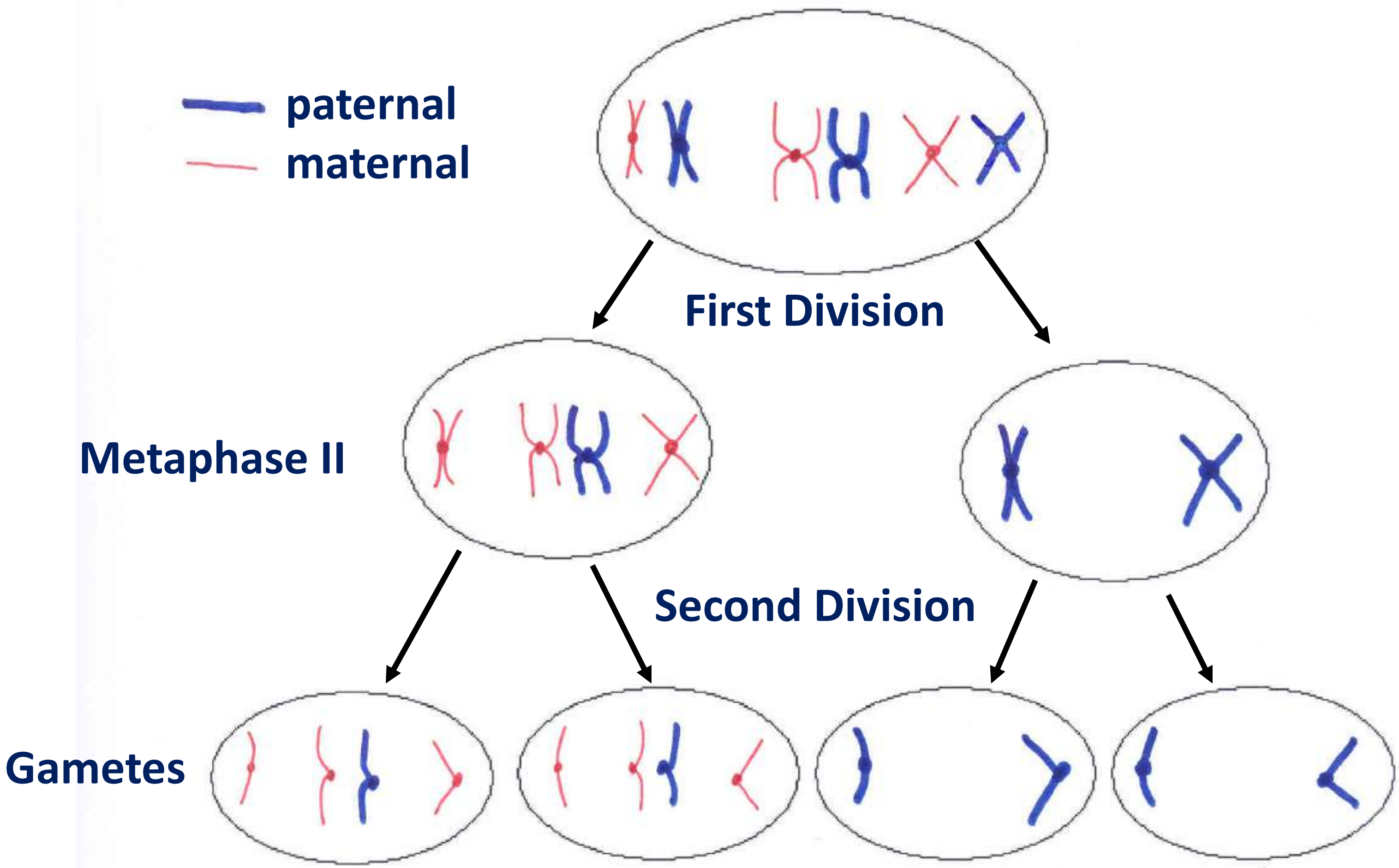




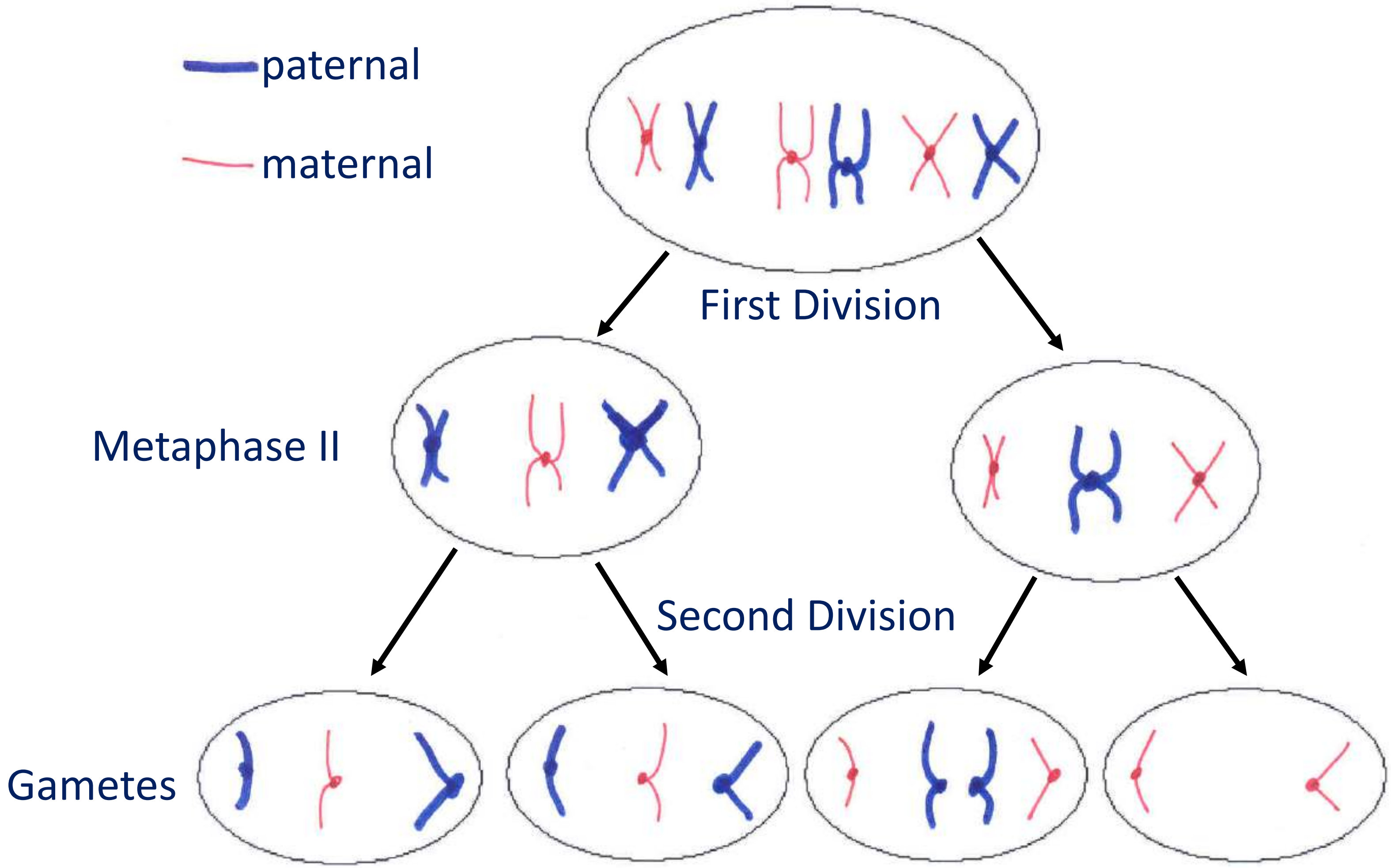
Normal Disjunction/Meiotic Division



Nondisjunction – First Division (Meiosis I)



Nondisjunction – First Division (Meiosis II)

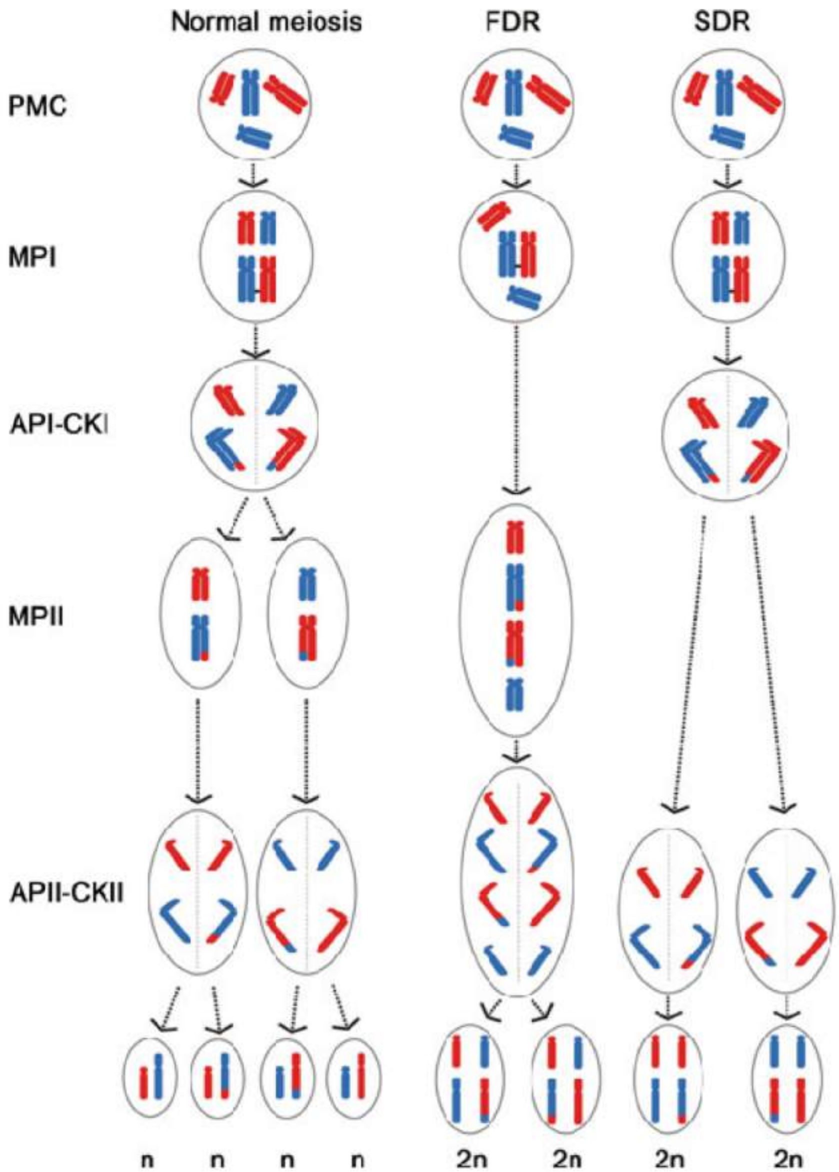


2n Gametes

Polyploidy plays a major role in plant evolution and diversification, and polyploids can be induced by two mechanisms.

- Somatic polyploidization: somatic chromosome doubling could happen spontaneously or by the application of mitosis spindle inhibitors (colchicine, etc.).
- Sexual polyploidization: gametes with somatic chromosome numbers (2n gametes) are considered to be the driving force behind the formation of polyploids in nature. 2n gametes arise from abnormal meiotic processes (it is genetically controlled), resulting in different formation mechanisms:
    1. First Division Restitution (FDR)
    2. Second Division Restitution (SDR)
    3. Indeterminate Meiotic Restitution (IMR)
    4. Post Meiotic Restitution (PMR)

2n Gametes – Formation mechanism



a. First Division Restitution (FDR)
Incomplete 1st meiotic division. FDR 2n-gametes comprise the non-sister chromatids of each homologous pair of chromosomes and are genetically identical to parent.

b. Second Division Restitution (SDR)
Incomplete 2nd meiotic division. SDR 2n gametes include the sister chromatids. Spores are completely homozygous but genetically different.

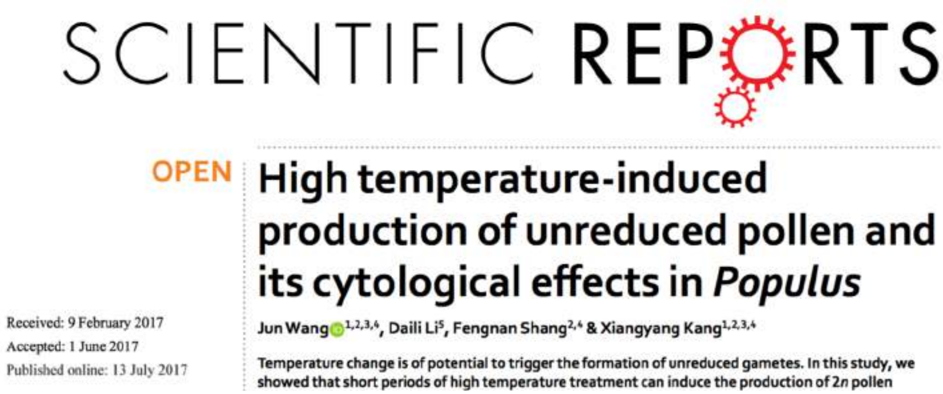
Their formation mechanism, i.e., FDR or SDR, greatly impacts the gametic and population structure and, therefore, breeding efficiency.

Younis et al., 2013

2n Gametes – Environmental Factors

The production of 2n gametes is not only governed by genetic factors, but also affected by environmental factors:

- Stress: temperature (cold and hot*), water, nutrient, radiation, etc.
- Artificial induction: colchocine, N_2O, oryzalin, caffeine, etc.



SCIENTIFIC REPORTS

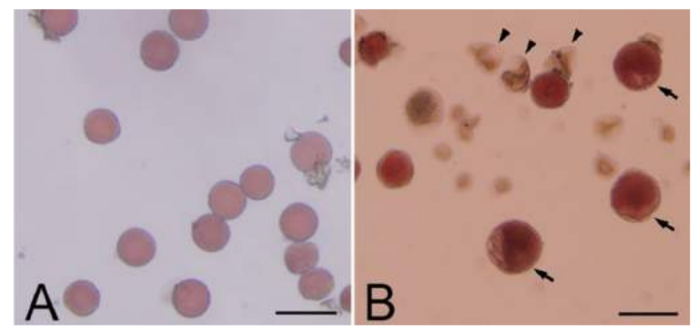
OPEN

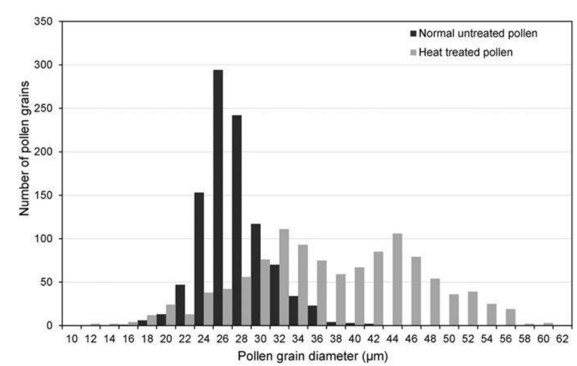
High temperature-induced production of unreduced pollen and its cytological effects in *Populus*

Received: 9 February 2017
Accepted: 1 June 2017
Published online: 13 July 2017

Jun Wang[1,2,3,4], Daili Li[5], Fengnan Shang[2,4] & Xiangyang Kang[1,2,3,4]

Temperature change is of potential to trigger the formation of unreduced gametes. In this study, we showed that short periods of high temperature treatment can induce the production of 2*n* pollen





Sources:
Wang et al., 2017: https://www.nature.com/articles/s41598-017-05661-x.pdf
Younis et al., 2013: https://link.springer.com/article/10.1007%2Fs00299-013-1534-y

2n Gametes – Environmental Factors

Table 1 Chemicals that have been used to create polyploids in various plants species

Polyploiding agent	Plant/species	References
Nitrous Oxide	*Elymus* spp.	Berdahl and Barker (1991)
	Zea mays	Kato (2002)
	Tulipa spp.	Okazaki et al. (2005)
	Triticum spp.	Kihara and Tsunewaki (1960)
	Lilium spp.	Nukui et al. (2011), Akutsu et al. (2007), Sato et al. (2010), Barba-Gonzalez et al. (2006), Kitamura et al. (2009)
	Begonia spp.	Dewitte et al. (2010)
	Phalaenopsis spp.	Wongprichachan et al. (2013)
Colchicine	*Cymbidium* spp.	Kim et al. (1997)
	Brachiaria spp.	Mendes-Bonato et al. (2009)
	Vaccinium spp.	1.Chavez and Lyrene (2009)
	Citrus spp.	2.Xiaoling et al. (2006)
	Solanum spp.	Sree Ramulu et al. (1991)
	Brassica spp.	Kumar and Dwivedi (2013)
	Alocasia spp.	Thao et al. (2003), (2004)
	Colophospermum spp.	Rubuluza et al. (2007)
	Strelitzia spp.	Yaqiong et al. (2007)
	Populus spp.	Quanjun et al. (2002), Kang et al. (2004), Li et al. (2008), Wang et al. (2010)
	Allium cepa	Fei et al. (2011)
	Brassica rapa	Cheng et al. (2010), Zhong et al. (2010)
	Lily	Wu et al. (2007)
Oryzalin	*Lilium* spp.	Van Tuyl et al. (1992)
	Nerine spp.	Van Tuyl et al. (1992)
	Rosa spp.	Kermani et al. (2003), Allum et al. (2007)
	Alocasia amazonica	Thao et al. (2003), (2004)
	Triticum spp.	Jing-Qiu and Zhuo (2008)
	Musa spp.	Ganga and Chezhiyan (2002)
	Watsonia lepida	1.Erwin (2008)
Trifluralin	*Begonia* spp.	Dewitte et al. (2010)
	Rhododendron spp.	Eeckhaut et al. (2006)
	Zea mays	Kato (1999)
	Rosa chinensis	Zlesak et al. (2005)
Caffeine	*Lilium*	Lim et al. (2005)

Source: Younis et al., 2013: https://link.springer.com/article/10.1007%2Fs00299-013-1534-y

2n Gametes – Application in Plant Breeding

- Facilitating crosses of diploid wild relatives with tetraploid cultivated species (potato, alfalfa, blueberry [especially if there is a triploid block]): introgression of genes from wild species.
- Create new cultivars at higher ploidy levels (triploid-sterile crops): one-step triploid generation (Citrus).
- A method to produce autotetraploids of diploid species (assuming the 2n gamete production can be found in both male and female gametogenesis).
- Breeding of tetraploid species at the diploid level (using haploids of the tetraploid) and then crossing back up to the tetraploid level (alfalfa).

Sources:
Dewitte et al., 2012: http://cdn.intechopen.com/pdfs/25553/InTech-Use_of_2n_gametes_in_plant_breeding.pdf
Wang et al., 2017: https://www.nature.com/articles/s41598-017-05661-x.pdf
Younis et al., 2013: https://link.springer.com/article/10.1007%2Fs00299-013-1534-y

Genetics of Autopolyploids

Types: Autopolyploids and Allopolyploids

- Autopolyploids
  - Each additional set of chromosomes is identical to the parent species.
  - If **A** represents a haploid set of chromosomes, then a normal diploid would be **AA**.
  - **Autotriploids – AAA**
  - **Autotetraploids - AAAA**

Importantly, autopolyploids should exhibit tetrasomic or higher level inheritance, rather than disomic inheritance.

Although it is now clear that autopolyploids are much more common than traditionally maintained, they are probably not as common as allopolyploids (we still have no firm estimate of the relative abundance of autopolyploids). There are numerous examples of single taxonomic species with multiple cytotypes, but most of these have not been critically investigated (many of these polyploid series likely represent autopolyploids or perhaps segmental autopolyploids).

Source: Soltis et al., 2003: http://onlinelibrary.wiley.com/doi/10.1046/j.1469-8137.2003.00948.x/full

Grant V. 1981. *Plant speciation*. New York, USA: Columbia University Press.

Autopolyploids - Characteristics

- More frequent in perennials.
- Higher frequency in self-pollinating than out-crossing species.
- Infrequent in animals.
- Evolutionarily important.
- Large chromosome number results in increased nuclear and cell volume.
- General large vegetative parts.
- Counteracting to chromosome reducing mechanisms.
- Often have become diploidized.

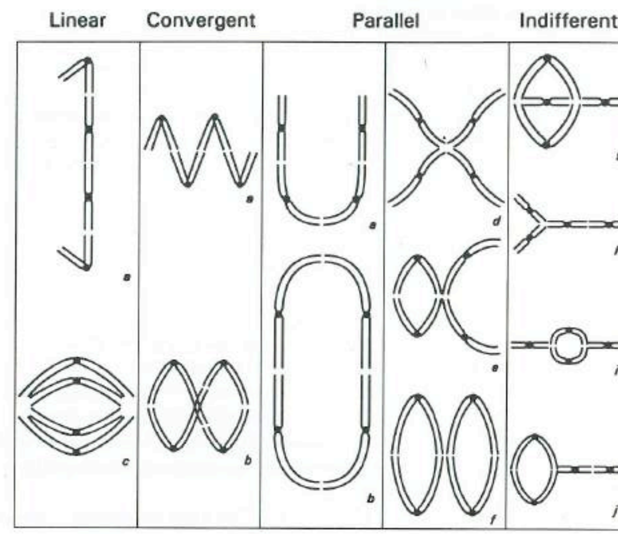
Autopolyploids - Sources

Chromosome doubling – non-disjunction during meiosis or mitosis

1. Natural - Low frequency in nature
   a. Cell regeneration - cut ends, callus
   b. 2n gamete formation
2. Physical agents
   a. Temperature
      1) Cold treatment (Datura)
      2) Hot treatment (Corn)
   b. Colchicine (0.5 to 1%)
      1) Blakeslee (1937) first use. All major agronomic crops have been doubled with little value. Ex: Red clover and rye grass.
      2) Important for synthetic amphidiploids.
      3) Techniques for using colchicine
         a) emerging seed, b) young seedlings, c) buds, d) root crowns
   c. Other chemicals – Oryzalin, trifluralin, or N_2O (laughing gas)

Autopolyploids – Meiotic behavior

Quadrivalent co-orientations at metaphase-I



- Autotetraploid
  a. Quadravalent (IV)
  b. Trivalent and univalent (III and I)
  c. Two bivalents (II)
- Reduced fertility
  - ➢ Selection for high fertility
  - ➢ % ~ 80s vs 90s in diploid

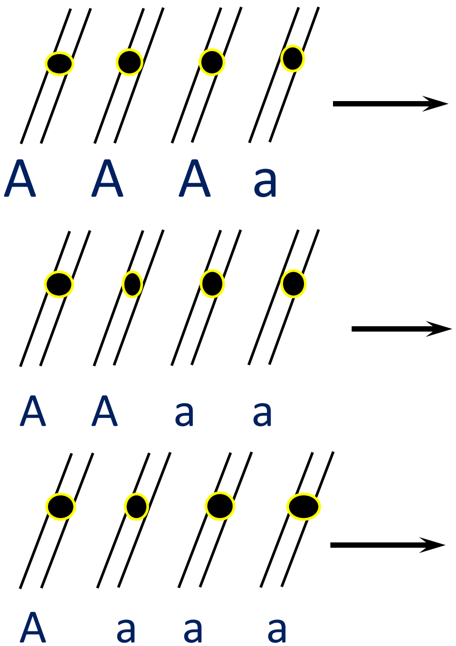
TABLE 6.21. Mean Number of Chromosome Configurations Observed per Microsporocyte at Metaphase-I in Tetraploids of Nine Species with $2n = 4x = 28$

Species	Univalents	Bivalents	Trivalents	Quadrivalents	Ref.
Arrhenatherum elatius	0.0	4.3	0.0	4.8	Morrison and Rajhathy 1960a
Avena strigosa	0.1	5.0	0.1	4.4	Morrison and Rajhathy, 1960a
Hordeum bulbosum	0.1	5.6	0.2	4.0	Morrison and Rajhathy, 1960a
Hordeum vulgare	0.3	5.7	0.1	3.9	Morrison and Rajhathy, 1960a
Pennisetum americanum[a]	2.64	8.97	0.38	1.49	Hanna et al., 1976
Petunia hybrida	0.1	4.6	0.1	4.6	Morrison and Rajhathy, 1960b
Pisum sativum	0.3	5.21	0.16	4.2	Mercy kutty and Kumar, 1983
Secale cereale	0.5	6.1	0.2	3.7	Müntzing, 1951
Triticum monococcum	0.2	3.5	0.1	5.1	Morrison and Rajhathy, 1960a
	0.62	9.86	0.23	1.74	Kuspira et al., 1985

[a] Pennisetum glaucum.

Autopolyploids – Chromosome segregation

Eg. Autotetraploid, heterozygote



3AA 3Aa

$C_4 2=6$

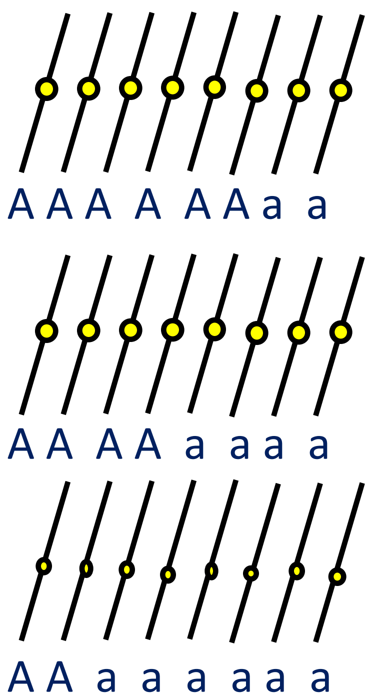
1AA 4Aa 1aa

3Aa 3aa

- ➢ The chromatids from the same chromosome go to different gamete derived from a multivalent

Autopolyploids – Chromosome segregation

- Random chromatid segregation
  - In mulitvalents, if an infinite number of CO's occur between the gene and centromere, any one chromatid may be combined with any other chromatid randomly.



15 AA 12 Aa 1 aa $C_8 2=28$

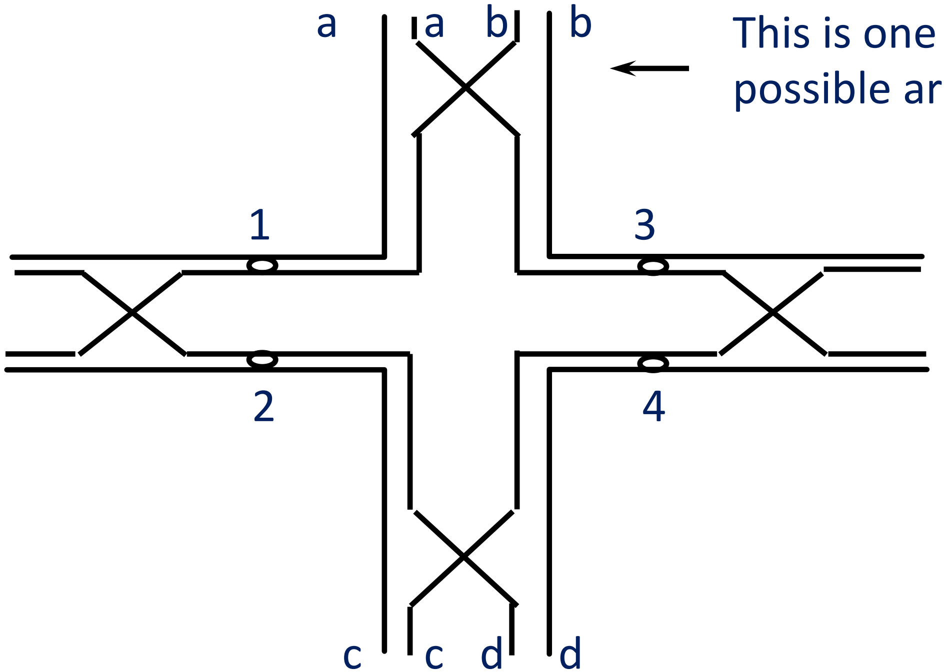
6 AA 16 Aa 6 aa

1 AA 12 Aa 15 aa

  - The chromatids from the same chromosome can go to the same gamete derived from a multivalent

Autopolyploids – Chromosome segregation

- Maximum equational segregation. Occurs if:
  a. Quadrivalents are always formed.
  b. There is at least one CO between gene and centromere.
  c. There is random segregation of chromatids at A II.



This is one of three possible arrangements

Second possible arrangement
aa cc
bb dd

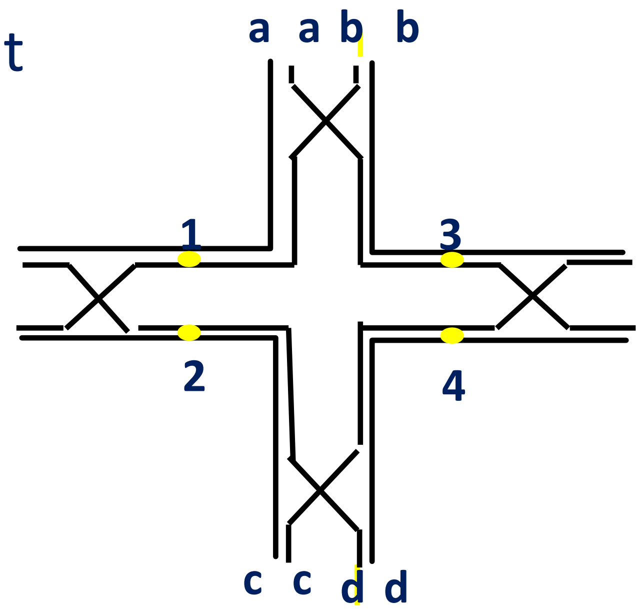
Third possible arrangement
aa dd
bb cc

Autopolyploids – Chromosome segregation

- Possible segregation types from quadrivalent

At end of Div. I	Segregation Types		
	Alt	Adj	Adj
Centromere disjunction	1+4 2+3	1+3 2+4	1+2 3+4
Chromatid pairs at the poles at end 1st Div.	ab, cd cd, ab	ab, ab cd cd	ab, cd ab, cd
at end of 2nd Div. gametes or spores	2(ac+bd+ ad+bc)	$\boxed{\text{aa + bb + cc + dd +}}$ + 2ab + 2cd	2(ac+bd+ ad+bc)

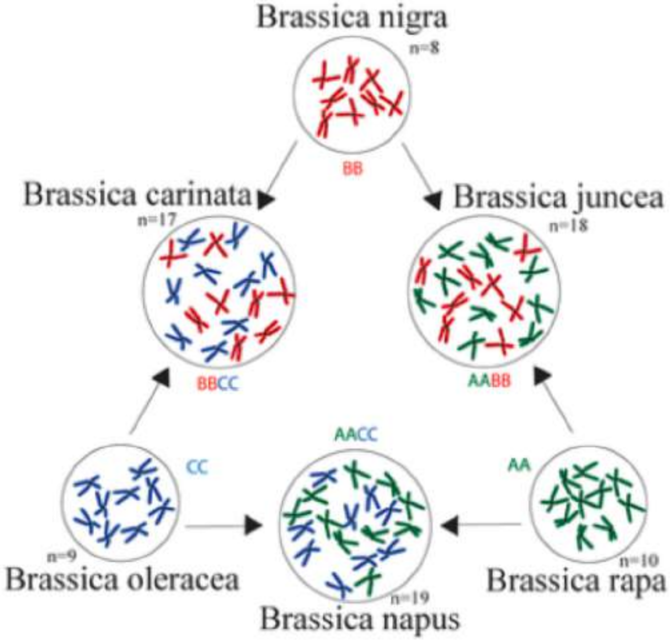
Double reduction gametes



With 3 possible arrangements: Total = 10 ab + 10 ac + 10 ad + 10 bc + 10 bd + 10 cd + 3 aa + 3 bb + 3 cc + 3 dd

Substitute AAAa for abcd = 39 AA + 30 Aa + 3 aa = 13:10:1

Interspecific cross compatibility and Allopolyploid Genetics



Interspecific cross compatibility

Barriers to interspecific/intergeneric hybridization during sexual reproduction can be divided between those that operate before and after fertilization (Stebbins, 1958):

- <u>Pre-fertilization barriers:</u> prevent mating and fertilization. Examples: lack of stigma receptivity or failure of pollen germination, pollen tube growth, or pollen tube penetration of the ovule, etc.

- <u>Post-fertilization barriers:</u> degeneration of the hybrid embryo, male and female sterility in the hybrid plants, lethality in the hybrid progeny (meiotic failures), or sterility of hybrids.

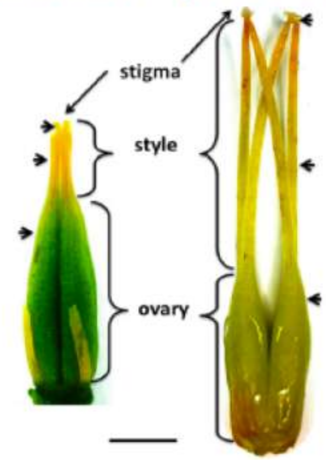


Figure 2 The morphology of pistils. Arrows indicate places in which pollen tubes were examined. *Scale bar:* 2.0 mm.

Figure 6

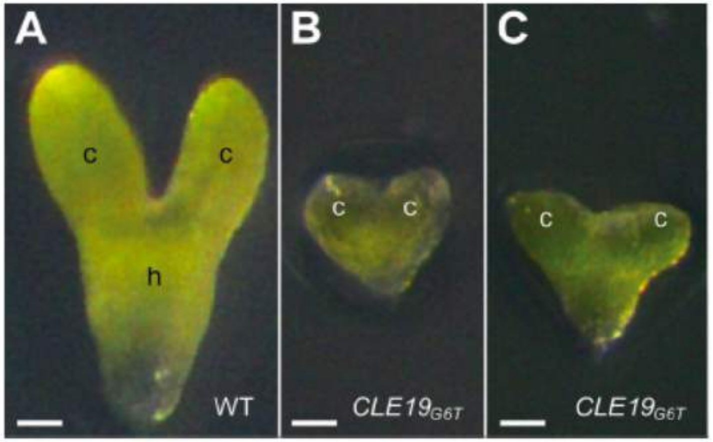
Three types of seed morphology observed after intra- and interspecific crosses in *Kalanchoë*.
Category 1: normal seeds containing fully developed endosperm and embryo, germination; category 2: wrinkled seeds containing not fully developed endosperm, no germination; category 3: seed-like structure with no sign of endosperm and embryo, no germination. *Scale bar:* 1 mm.

Kuligowska et al. BMC Plant Biology (2015) 15:15

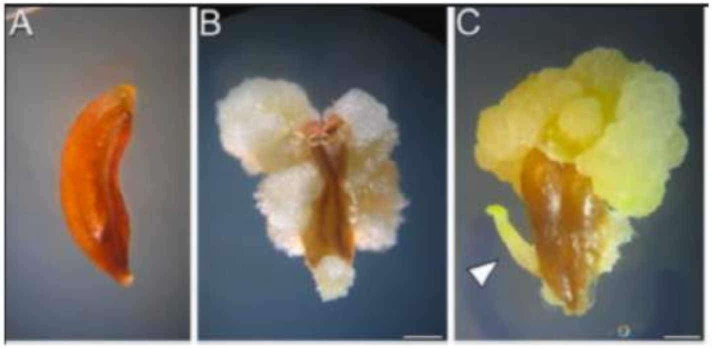
Interspecific cross compatibility

Biotechnology approaches are used to overcome cross sexual incompatibility:

- In vitro embryo rescue: tissue culture to enable a fertilized immature embryo, resulting from an interspecific cross, to avoid abortion caused by unbalanced endosperms and to continue growth and development.



- Anther culture: aseptic culture of immature anthers (within which pollen develops and matures) to generate haploid plants.



The chromosome number of interspecific hybrids or haploid plants are then doubled (colchicine).

Interspecific hybrids

Crossing plants belonging to two different species that are not normally sexually compatible.

<u>Uses:</u>

- Increase genetic variability (nature and breeding populations)
- Transfer useful traits from wild relatives to cultivated species
- Combine favorable traits of two different species

*Disadvantage: significant amounts of time and scientific expertise need to be invested (tissue culture, colchicine treatments, etc.).

Interspecific hybridization has been carried out in many cultivated plants: UF Breeding Programs: Peaches, Citrus, Blueberry, Forages (many examples), Turfgrass, etc.

Interspecific hybrids

The offspring from this cross could develop into adults but may not develop functional gametes. Occasionally sterile interspecific hybrids can undergo a doubling of their chromosome set and become fertile tetraploids (four sets of chromosomes).

Sterility is often attributed to the different number of chromosomes the two parental species have or to the lack of homology among chromosomes (pairing).

Closely related diploids are less likely to form a polyploid than are more divergent congeneric diploid species.

Artificial interspecific hybridization and allopolyploids have been primarily synthesized in the laboratory with the goal of expanding genetic diversity of crops.

Interspecific hybrids - Examples

Evolution of cultivated wheat

Triticum monococcum
Eikorn wheat
(AA, 2n = 2x = 14)

X

Triticum searsii
Wild Grass
(BB, 2n = 2x = 14)

↓

Chromosome doubling

↓

Triticum turgidum
Emmer wheat
(AABB, 2n = 4x = 28)

X

Triticum tauschi
Wild Grass
(DD, 2n = 2x = 14)

↓

Chromosome doubling

↓

Triticum aestivum (AABBDD, 2n = 6x = 42)

Allopolyploids - Terminology

- Allopolyploid: individuals with more than 2 sets of chromosomes and the additional sets are partially homologous to the original sets. AABB
  - ✓ Homoeologous chromosomes - Those that are only "partially" homologous. A and B genomes are homoeologous
  - ✓ Derived from hybridization between related diploid species followed by chromosome doubling.
- Genome: when used in discussion of allopolyploids, generally means the set of chromosomes that traces to some progenitor species. Thus often like A, B, D, etc. genome.

Allopolyploids – Chromosome pairing

Chromosome pairing during meiosis may reveal the amount of genome homology.

1. Autosyndetic pairing or autosyndesis = also homogenetic.
   Pairing of chromosomes from same genome.

   $A_1A_1\ A_2A_2\ B_1B_1\ B_2B_2$ = All II (bivalents)

2. Allosyndetic pairing or allosyndesis = also heterogenetic.
   Involves pairing of chromosomes from different genomes.

   $A_1B_1\ A_1B_1\ A_2A_2\ B_2B_2$ = II & IV (quadravalents)

Allopolyploids – Chromosome pairing

Chromosome pairing during meiosis

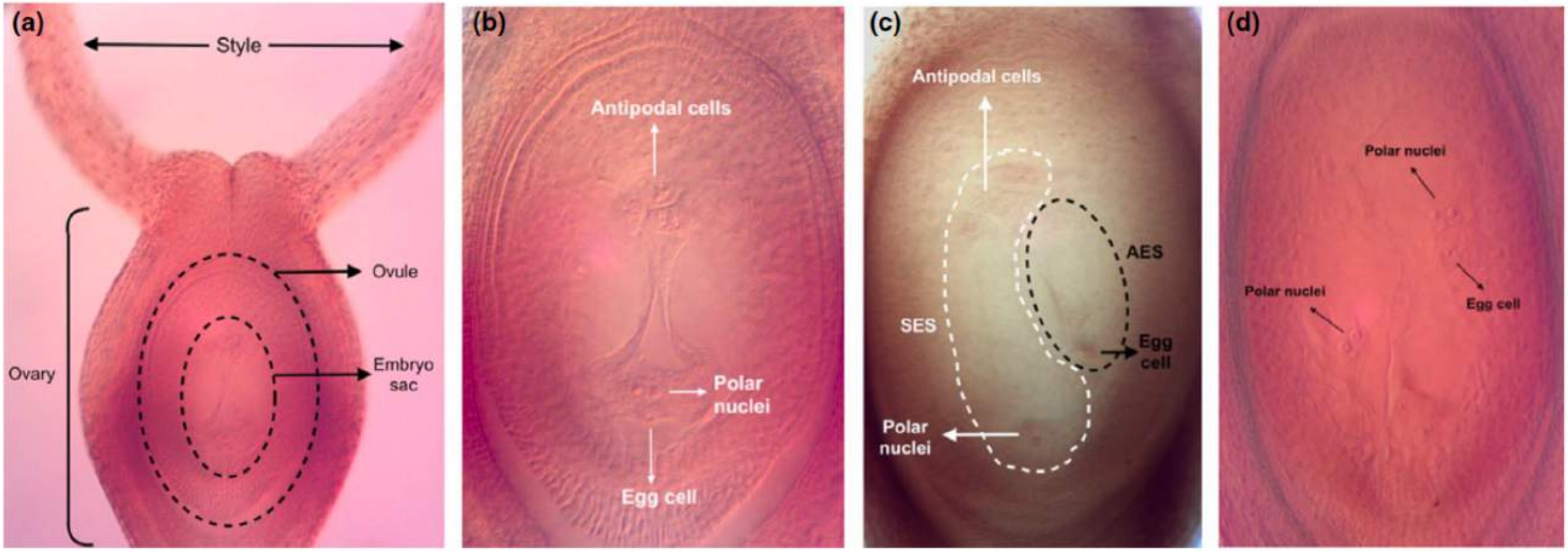
Examples from the genus *Trifolium.*

T. pratense	X	*T. diffusum*
(1) 2n=2x=14		(2) 2n=2x=16
(3) 2n=4x=28		(4) 2n=4x=32

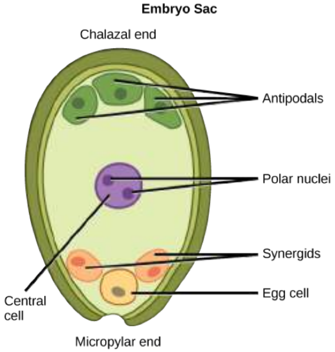
Chromosome Pairing Configuration

Cross	I	II	III	IV	V	VI	>VII
1 X 2	1.59	1.30	0.51	0.38	0.18	0.14	0.23
3 X 4	0.89	9.01	0.70	1.05	0.25	0.18	Trace
Self-3	0.50	4.69	0.23	4.36			
Self-4	0.54	5.18	0.25	5.09			

Apomixis

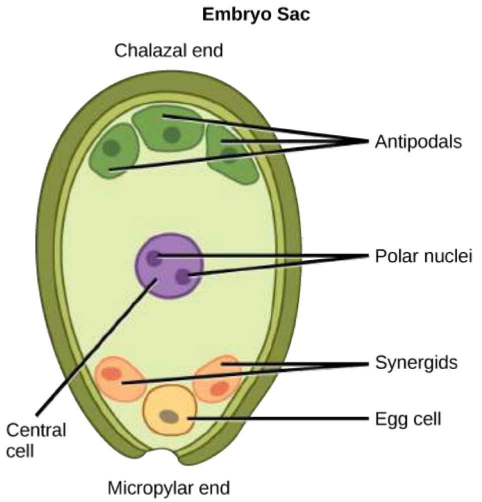

(a)
Style
Ovule
Embryo sac
Ovary
(b)
Antipodal cells
Polar nuclei
Egg cell
(c)
Antipodal cells
AES
SES
Egg cell
Polar nuclei
(d)
Polar nuclei
Egg cell
Polar nuclei

Key Concepts



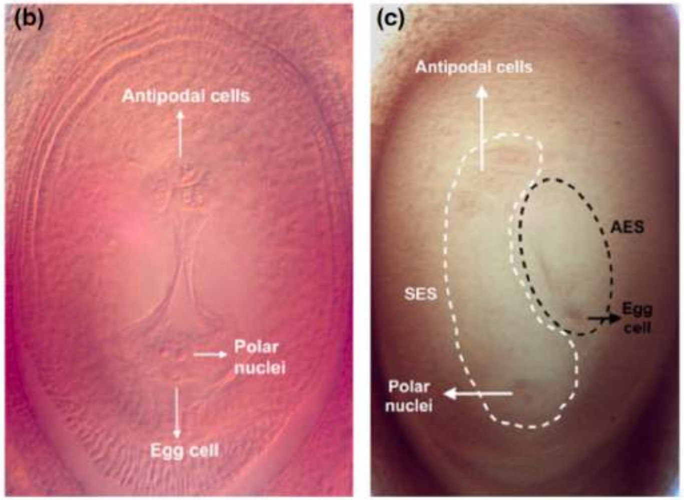
- ✓ Apomixis is an asexual mode of seed formation (clonal progeny with a maternal genotype).
- ✓ It primarily influences reproductive events in the ovule of the flower.
- ✓ Apomixis is absent in major crops (some advances were made, see later).
- ✓ Deployment of apomixis would maintain hybrid vigor that is currently lost in successive seed generations (some advances in recent years, see later).
- ✓ Apomixis comprises three developmental components that deviate from the normal sexual pattern:
  1. Avoidance of meiosis during egg cell development
  2. Fertilization-independent embryo formation
  3. Generation of viable endosperm with or without fertilization

Key Concepts



- ✓ Angiosperms exhibit two mechanistically different types of apomixis:
  - Sporophytic apomixis involves direct formation of an embryo from a diploid somatic cell within the ovule (nonsexual), and a viable seed forms when the adjacent sexual gametophyte is fertilized and forms endosperm (polyembryony).
  - Gametophytic apomixis involves formation of a meiotically unreduced (i.e. diploid) female gametophyte, followed by embryo formation from the diploid egg by parthenogenesis (i.e. without fertilization).
- ✓ In gametophytic apomixis, endosperm formation may occur autonomously (i.e. without fertilization) or pseudogamously (i.e. in response to fertilization of the central cell).

Key Concepts



- ✓ Most apomicts retain the ability to produce some seed via sexual reproduction (facultative apomicts) and studies in some apomicts indicate sexual reproduction is the default reproductive mode on which apomixis is superimposed.
- ✓ Apomixis is controlled by dominant loci and those controlling the avoidance of meiosis and fertilization-independent embryo development are genetically separable in some species and tightly linked in others (segregation ratios are different).
- ✓ Apomixis loci are proposed to recruit or hijack the sexual machinery in ovule cells undergoing apomixis, modifying the timing of the sexual programe so that meiosis and fertilization are avoided leading to asexual seed formation.

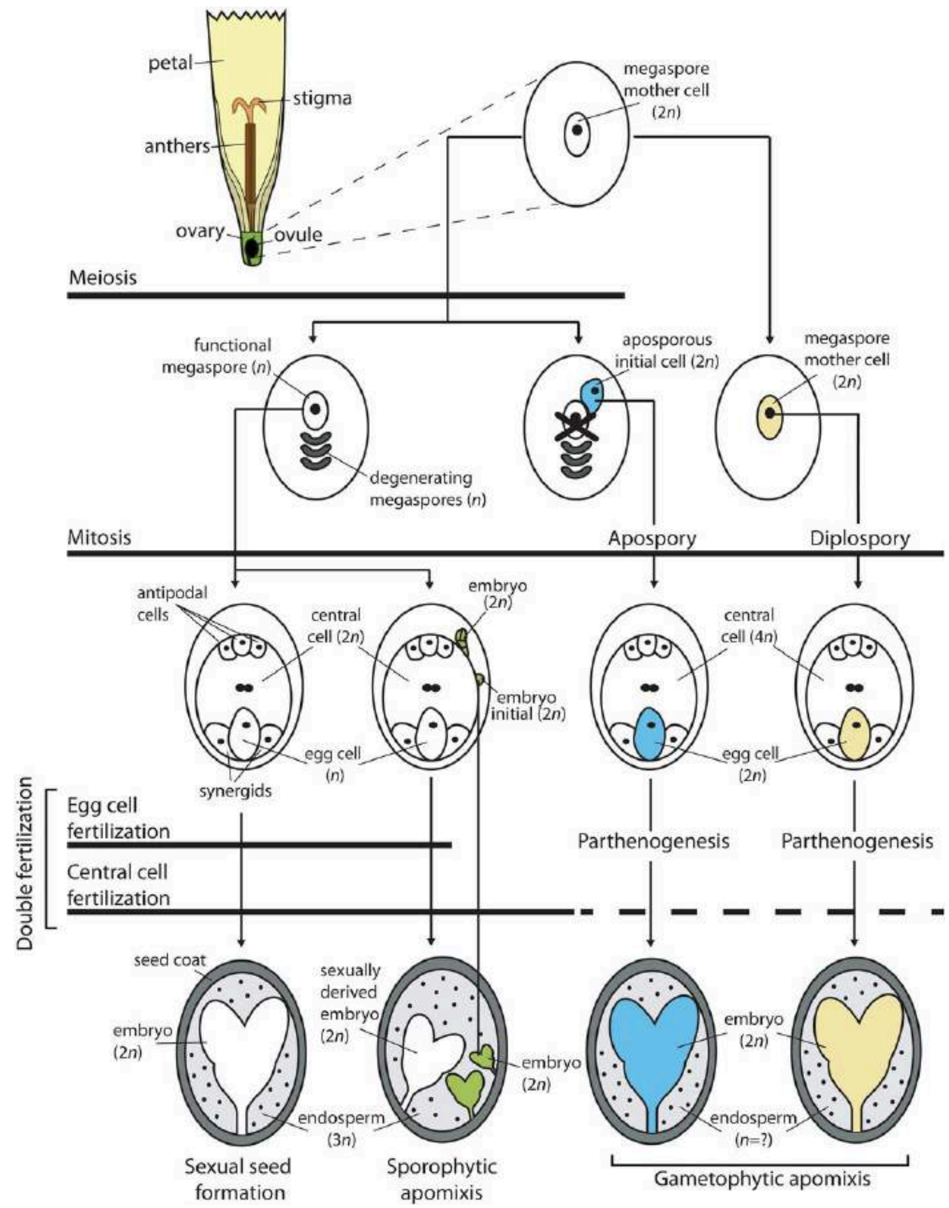
Terminology

Mode of Reproduction

- Sexual
- Asexual
  - Vegetative
  - Apomixis
    - Gametophytic
      (Megagametophyte is produced)
      - ✓ Apospory
      - ✓ Diplospory
    - Sporophytic
      (Megagametophyte is not produced)

Terminology

Mechanisms of sexual and apomictic seed development.



Terminology

- Apomixis:
  - The replacement of sexual reproduction by various types of asexual reproduction.
  - Asexual seed production, or seed production without fertilization (modern definition).
- Parthenogenesis:

  The egg develops into an apomictic embryo without fertilization. May occur also in sexual species.
- Endosperm development:
  1. Autonomous: spontaneous endosperm development - Hieracium.
  2. Pseudogamous: pollination and fertilization of the polar nuclei are required for endosperm and embryo development but no fertilization of the egg occurs. *Poa, Paspalum, Allium, Pennisetum*.

Bicknell and Koltunow 2004. The Plant Cell 16:S228

Prevalence of Apomixis

- Present in more than 400 species mainly Angiosperm.
- Representatives of monocotyledonous and dicotyledonous plants of more than 40 families.
- 75% of apomictic species in Asteraceae, Rosaceae, and Poaceae.
- Absent in major food crops (Citrus, Mango, forage grasses are exceptions). A single accession in Cassava and Sorghum.

Value in Agriculture

- Mediates formation of large genetically uniform populations.
- Perpetuates hybrid vigor though successive seed generations.
- Agronomic advantages:
  - Rapid generation and multiplication of novel genotypes.
  - Reduction in cost and time of breeding.
  - Avoidance of complications related with sexual reproduction such as pollinators, and cross-compatibility.
  - Avoidance of viral transfer (typical of vegetative propagation).

Identification of Apomixis

- Progeny test
  - Phenotypic evaluation of the progeny
- Cytohistological techniques
  - Embryo sac observations
- Flow cytometric seed screen
- Molecular techniques
  - Molecular markers linked to apomixis

Identification of Apomixis

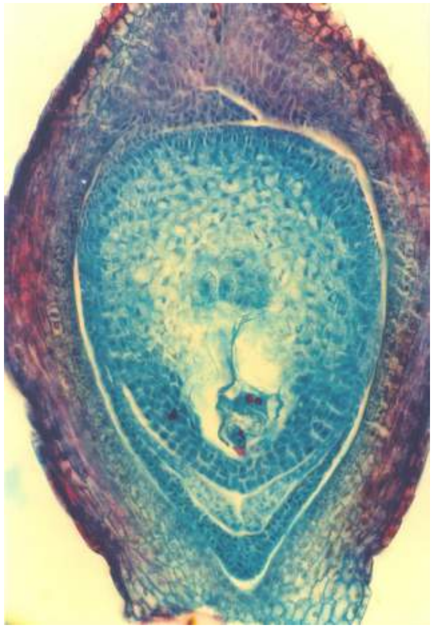
Field Progeny Test

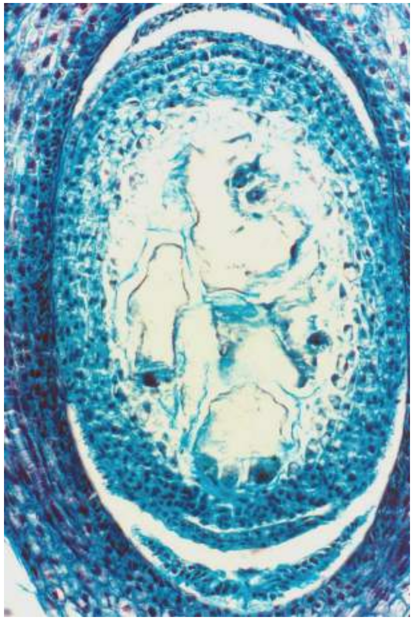
Identification of Apomixis

Embryo Sac Observations

b. Known embryo sac characteristics

Sexual vs. Apomictic





Mature embryo sacs of *Paspalum notatum* (bahiagrass)

Use of Apomixis in Plant Breeding

a. Apomictic Species