Using Ploidy to Improve Cannabis

August 16 3:55 Session

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Scientist - Molecular Biology, Dark Heart Nursery

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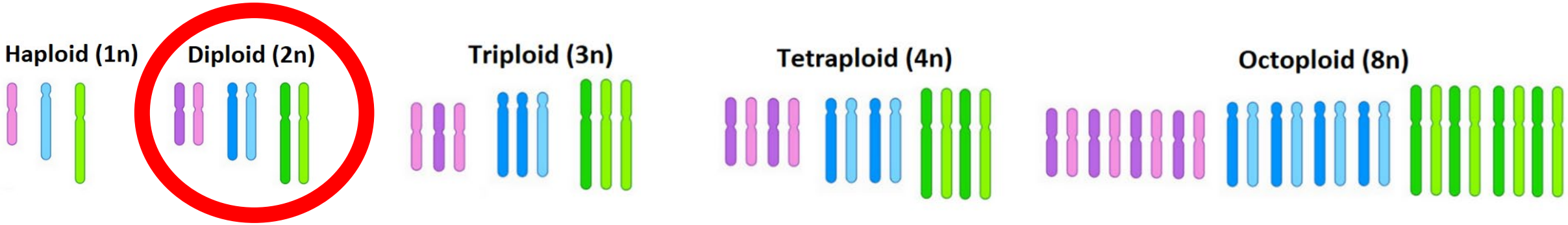
What is Ploidy?

- Ploidy refers to **number of copies of each chromosome** in an organism
- Humans, most animals are **diploid**
  - Some exceptions: amphibians, insects
  - Higher levels than diploid are referred to as “polyploid”

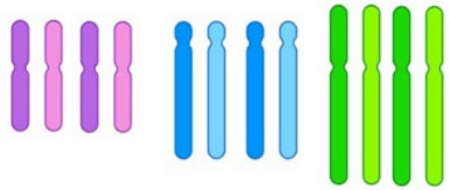
Ploidy Levels

- Ploidy is organized into “levels”
  - Xn = number of sets & number of chromosomes

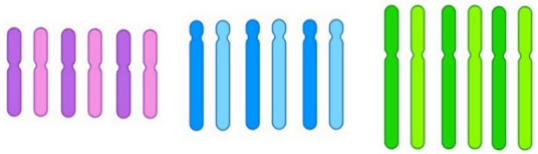


Polyploidy in Plants

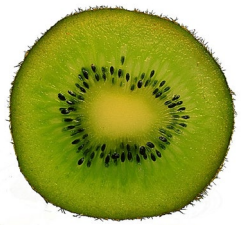
Plants can tolerate polyploidy much better than animals

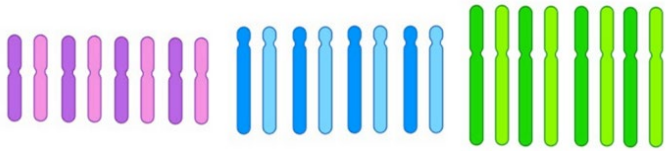


Tetraploid 4n



Hexaploid 6n





Octoploid 8n

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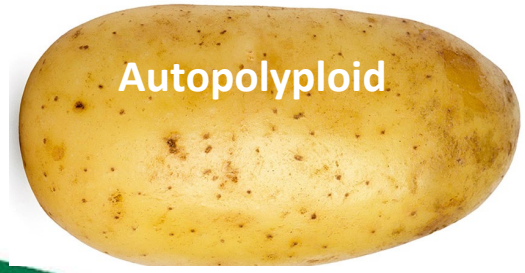
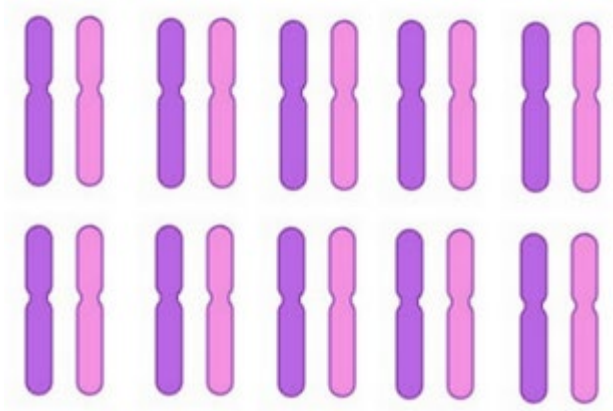
Polyploidy in Plants

- Natural polyploidy occurs frequently in plants
  - Can lead to increased yield/vigor
  - Increased genetic diversity/mutation tolerance for adaptation
- **Autopolyploid:** An organism's genome duplicated spontaneously
- **Allopolyploid:** Two organisms with different ploidy levels create viable hybrid



Manipulating Ploidy Levels

- **Cannabis is diploid**
  - Plant breeders can also create polyploids from diploid plants for crop improvement
  - Cannabis normally has two sets of ten chromosomes from each parent



10 pairs of chromosomes = 20 total

2n = 20

What is NOT Polyploidy?

- Misinformation about polyploidy in Cannabis has propagated via internet forums
  - Several developmental mutations have been misattributed as polyploid historically

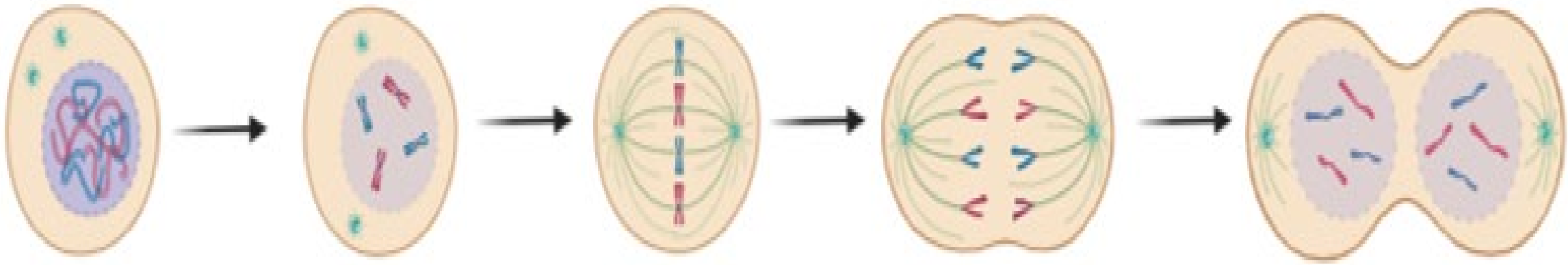
What is NOT Polyploidy?

- Fasciation aka “crested” growth
  - Mislabelled as “polyploid” online
  - Occurs in many plant species, usually as a result of genetic mutation or pathogen infection

What is NOT Polyploidy?

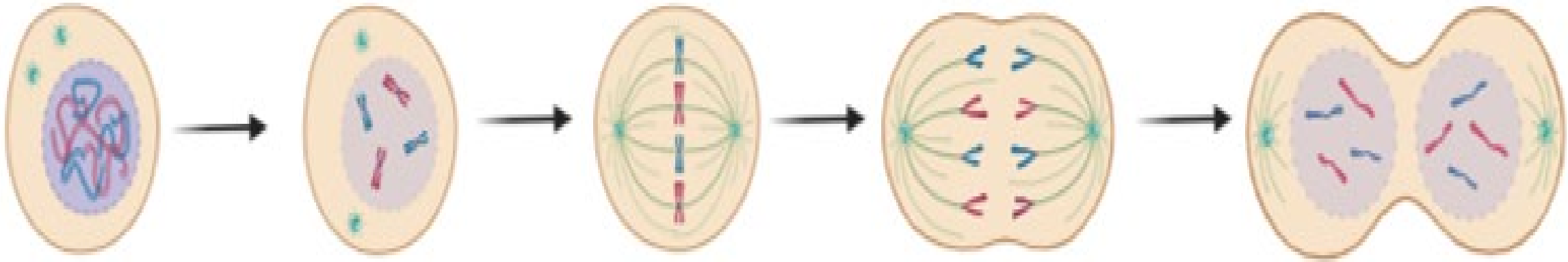
- Whorled phyllotaxy aka “tricot” mutation
  - Mislabelled as “triploid” in the past
  - Mutation in meristem development gene(s)

Genetics 101



Interphase Prophase Metaphase Anaphase Telophase

Genetics 101



Interphase

Prophase

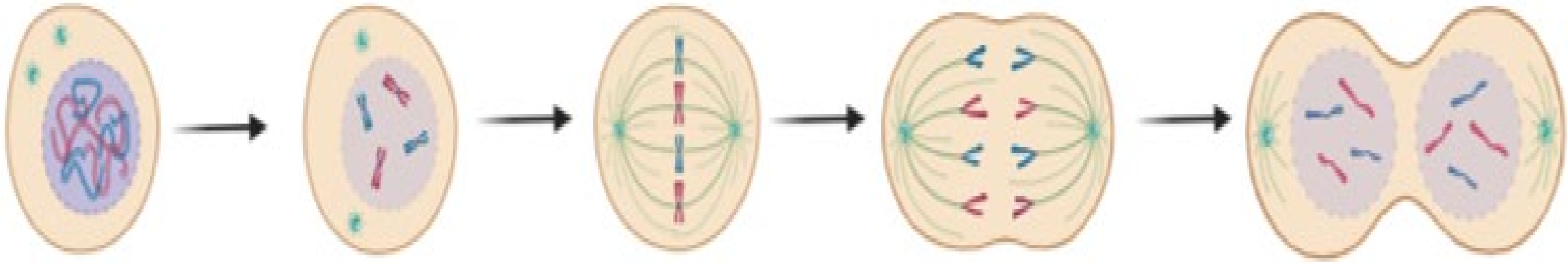
Metaphase

Anaphase

Spindle pulls chromosomes apart, cell begins to divide

Telophase

Genetics 101



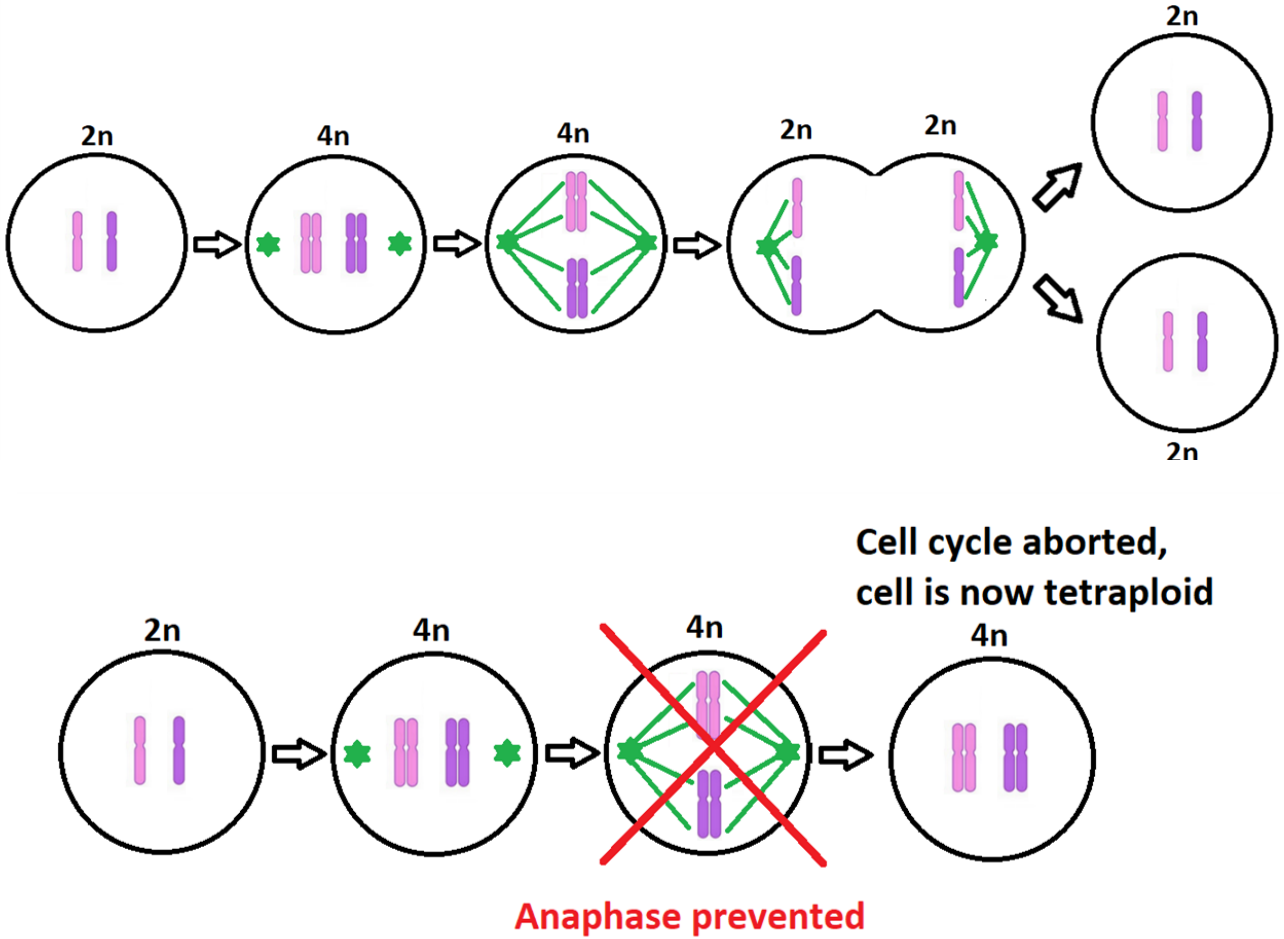
Telophase

Interphase **Prophase** **Metaphase** **Anaphase**

Cells finish dividing, 2 identical daughter cells

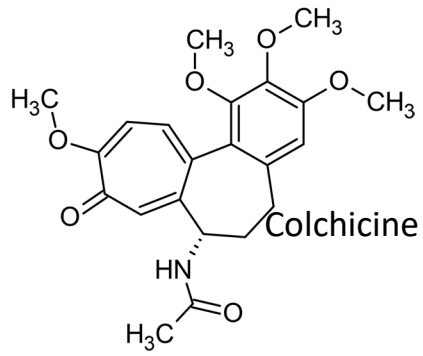
Creating Tetraploids

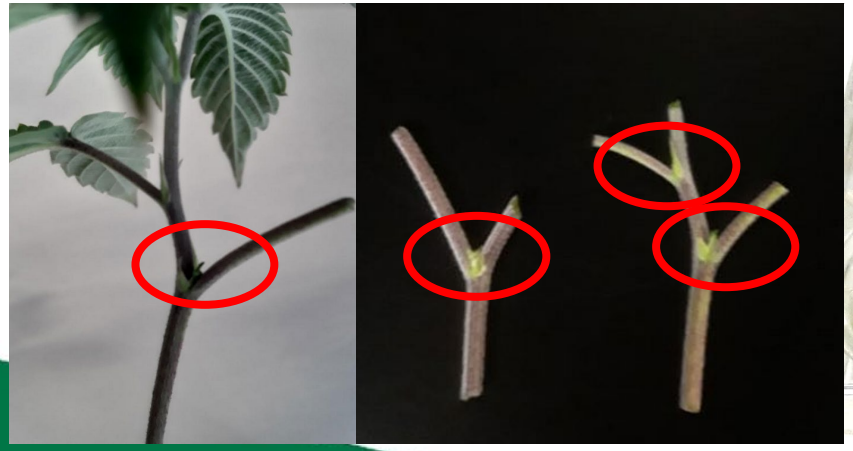
- Microtubule inhibitors disrupt cell division during mitosis
- Cell duplicates genome but cannot split apart
- Oryzalin and colchicine inhibit microtubule function

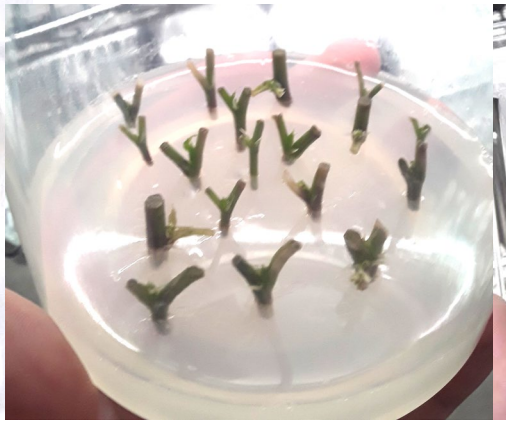


Creating Tetraploids

- Must target meristematic tissue
  - Areas of cell division ∴ undergoing mitosis
  - Incubate in oryzalin/colchicine for 24 hours (one cell cycle)

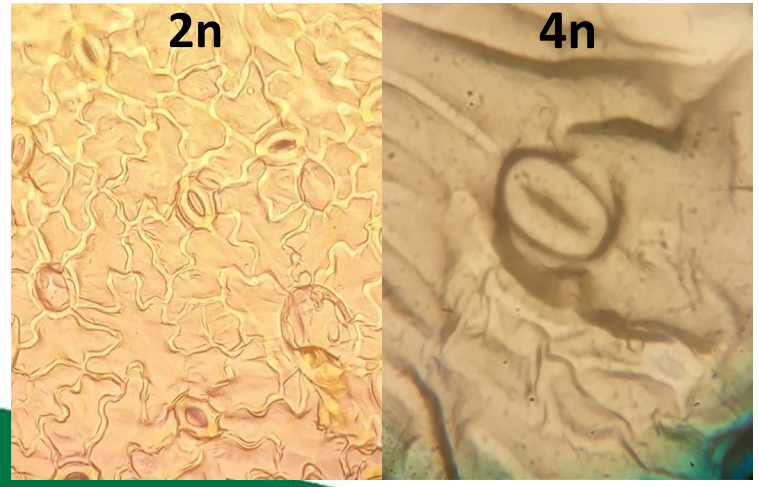


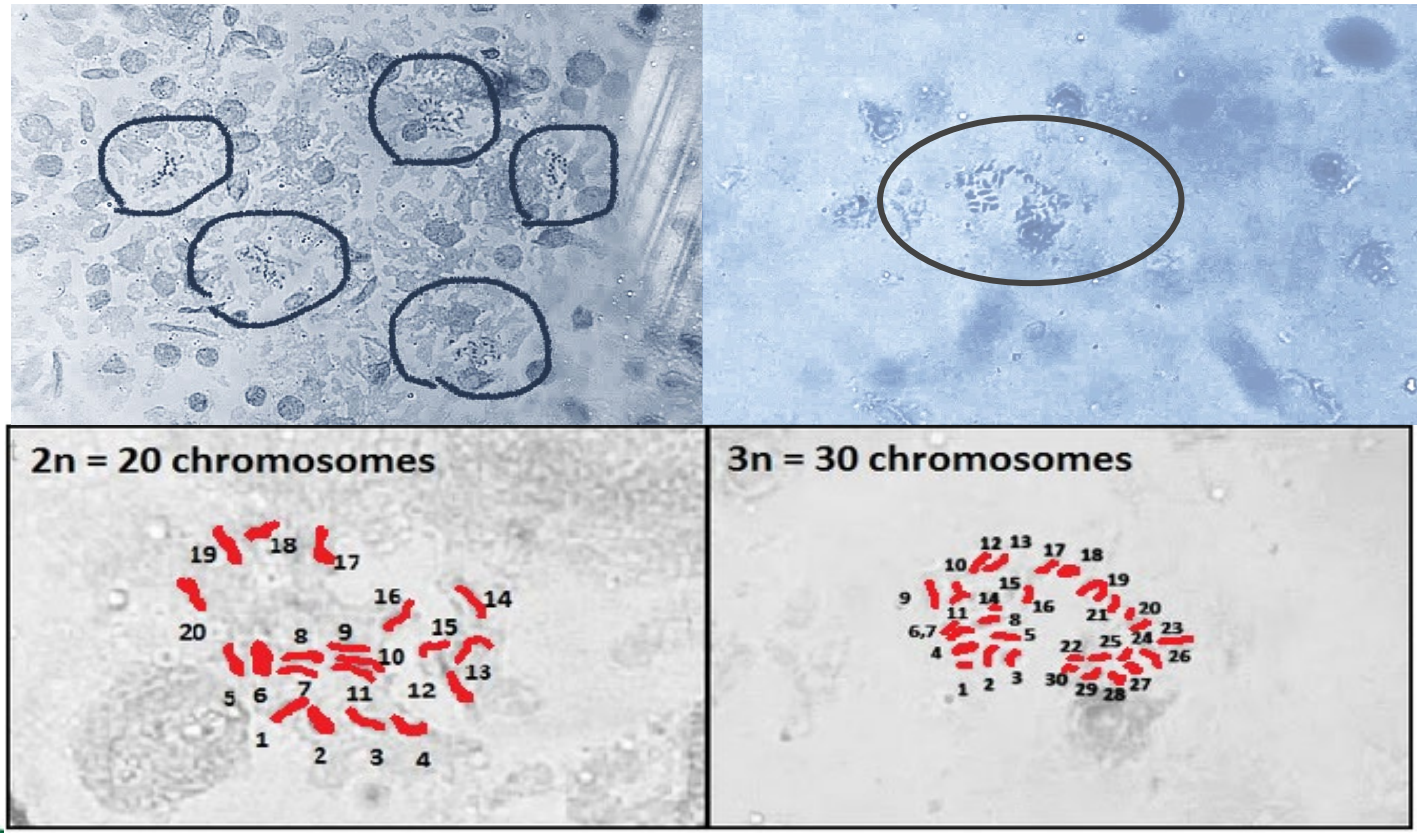




Measuring Ploidy

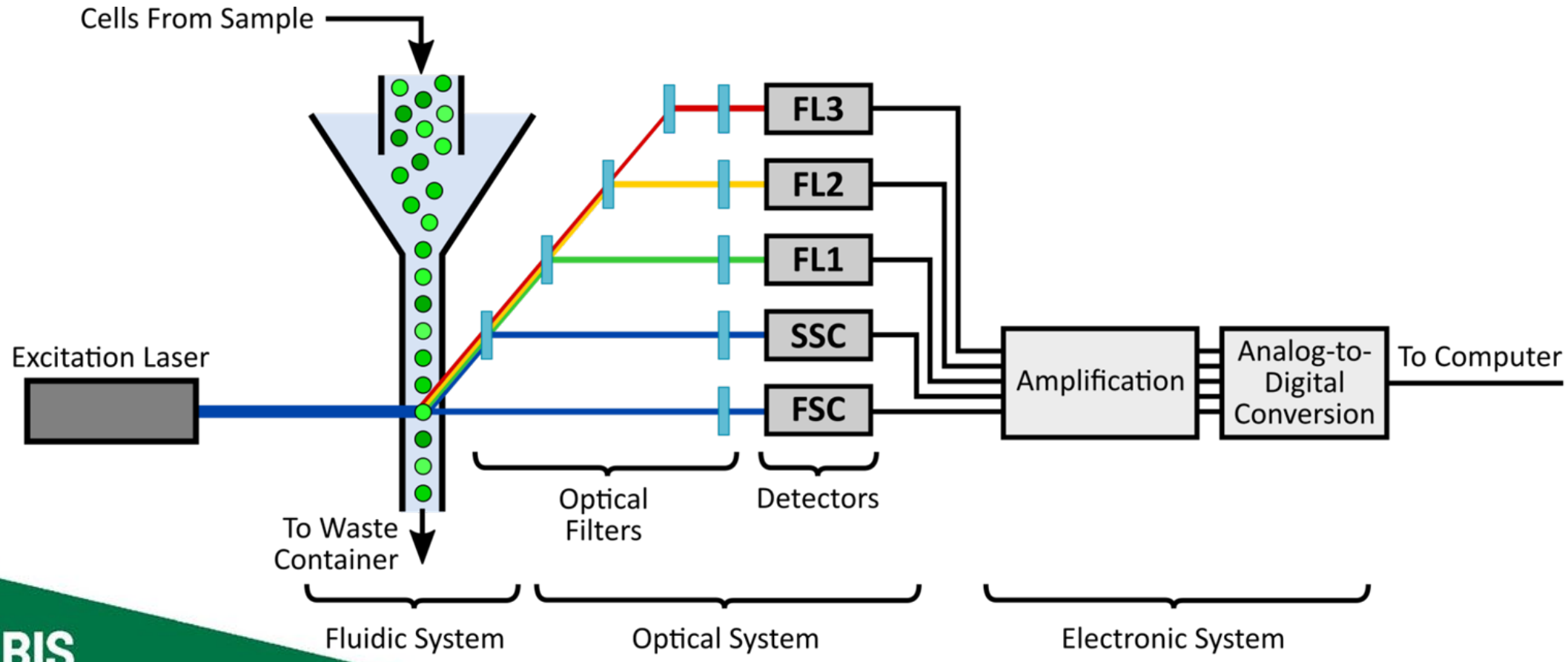
- Classic methods of measuring ploidy use microscopy
  - Labor intensive, not reliable for determining chimerism





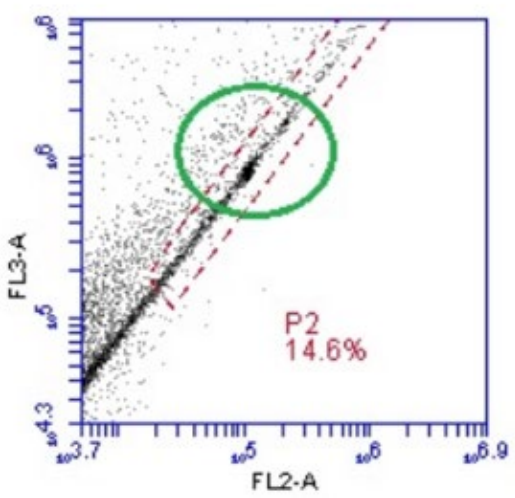
Measuring Ploidy

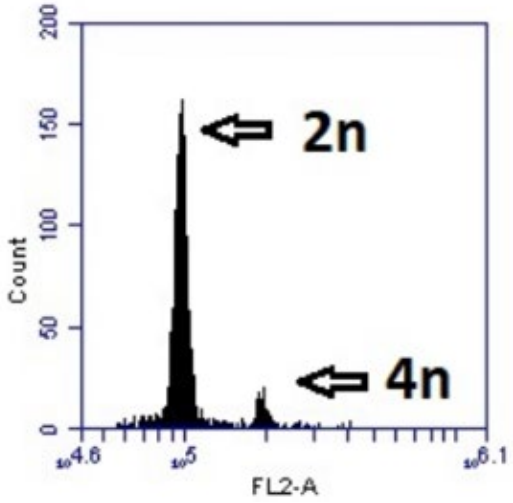
- Easiest and most reliable method is flow cytometry



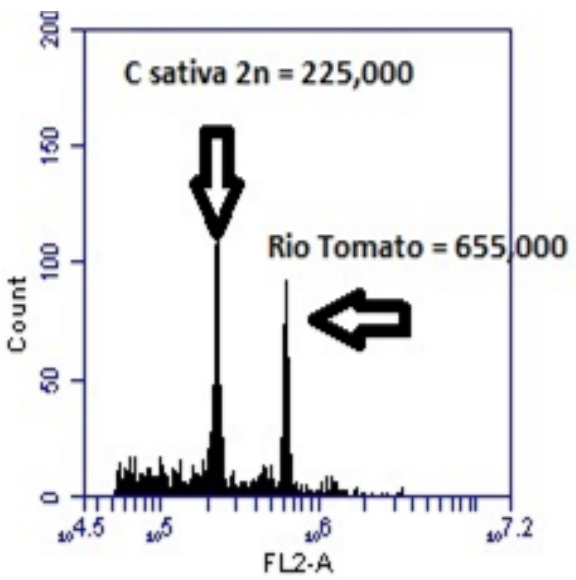
Flow Cytometry

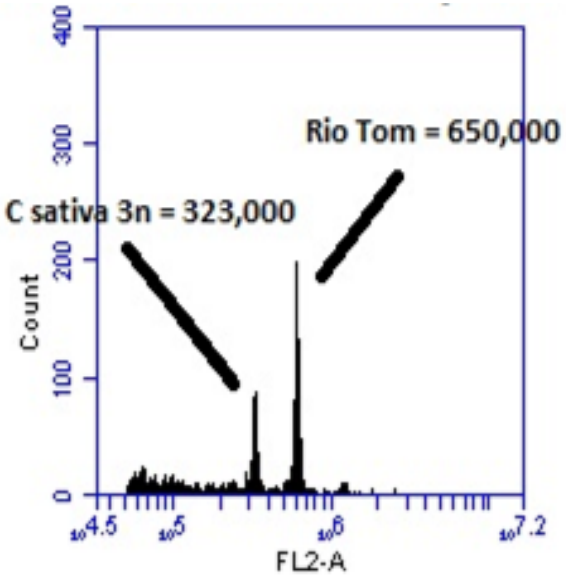
- Nuclei stained with DNA-binding fluorescent dye pass through a laser, intensity of signal is measured
  - Nuclei with larger genomes will produce more signal

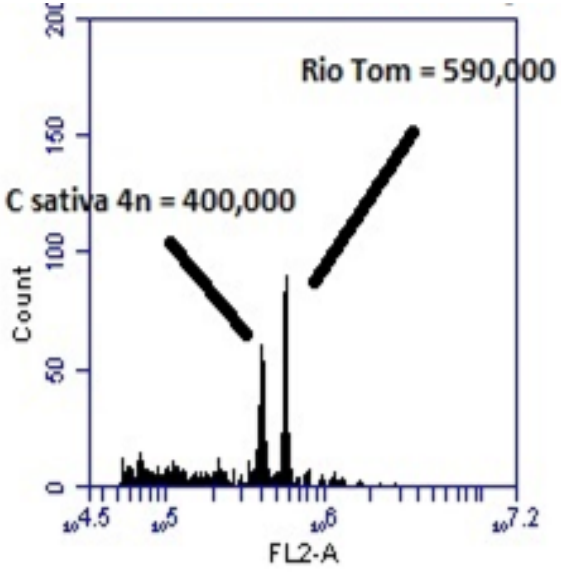


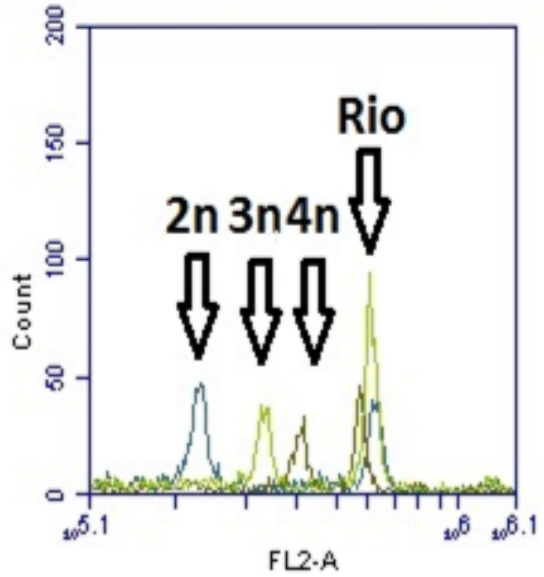


Flow Cytometry









Internal tomatillo control used to measure unknown Cannabis samples
Tomatillo ~ 3x genome size of Cannabis

Applications for Ploidy in Cannabis

- Are polyploids universally better than diploids?
  No
- Some strains do better as 2n, some do better as 4n
  - Ploidy above 4n is severely stunted
- Just because a plant is polyploid, does not mean it will perform better. Genetics/selection matters!
  - Will not replace conventional breeding, but harnessing ploidy can greatly boost it in specific applications

Applications for Ploidy in Cannabis

- **Triploid sterility**
- Disrupting mendelian genetics
- Stacking traits/novel allelic combinations
- Double Haploids

Triploid Sterility

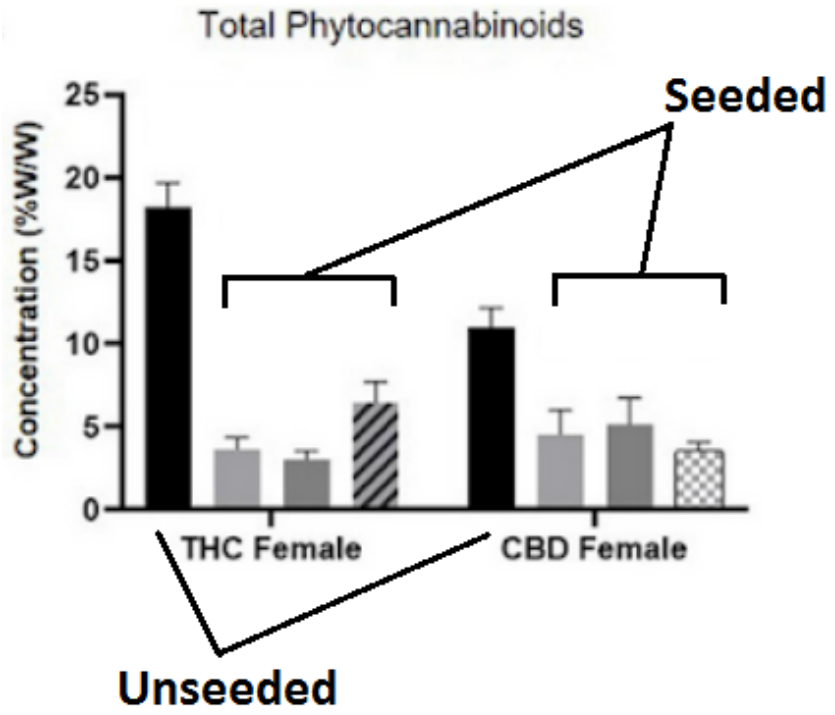
- Trichomes
- High in cannabinoids
- XX Sex Chromosomes
- Develops seeds

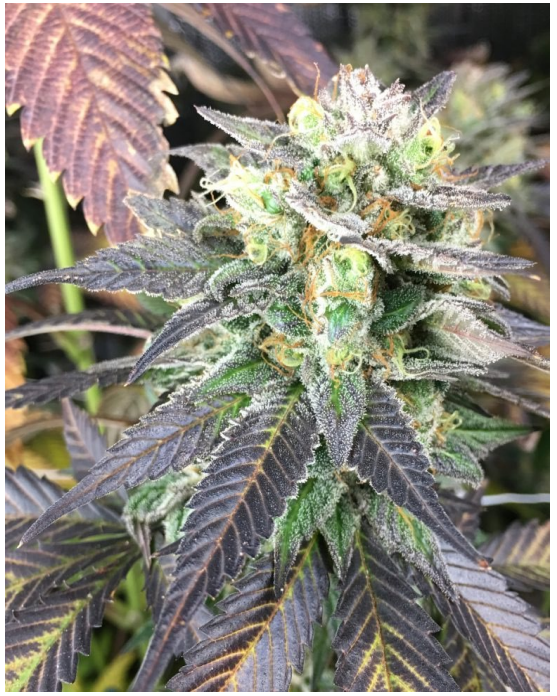
- Few trichomes
- Low cannabinoids
- XY Sex Chromosomes
- Develops pollen

- Male and female flowers
- Can be XX or XY
  Both pollen and seed

Triploid Sterility

- Seed set can cause yield/crop loss





VS

Triploid Sterility

- Current methods of preventing seed set are laborious, inefficient or not effective enough

Female Only Clones

Feminized Seeds

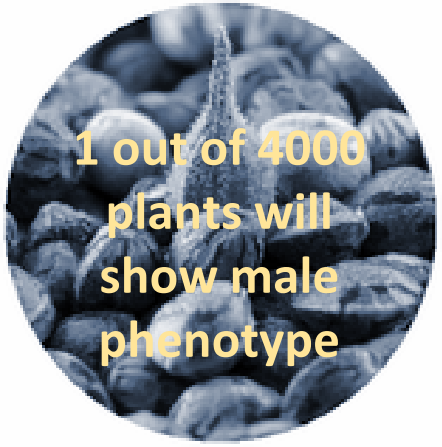
Scouting for Anthers/Removing Males

Triploid Sterility

- Current methods of preventing seed set are laborious, inefficient or not effective enough

Female Only Clones

Feminized Seeds



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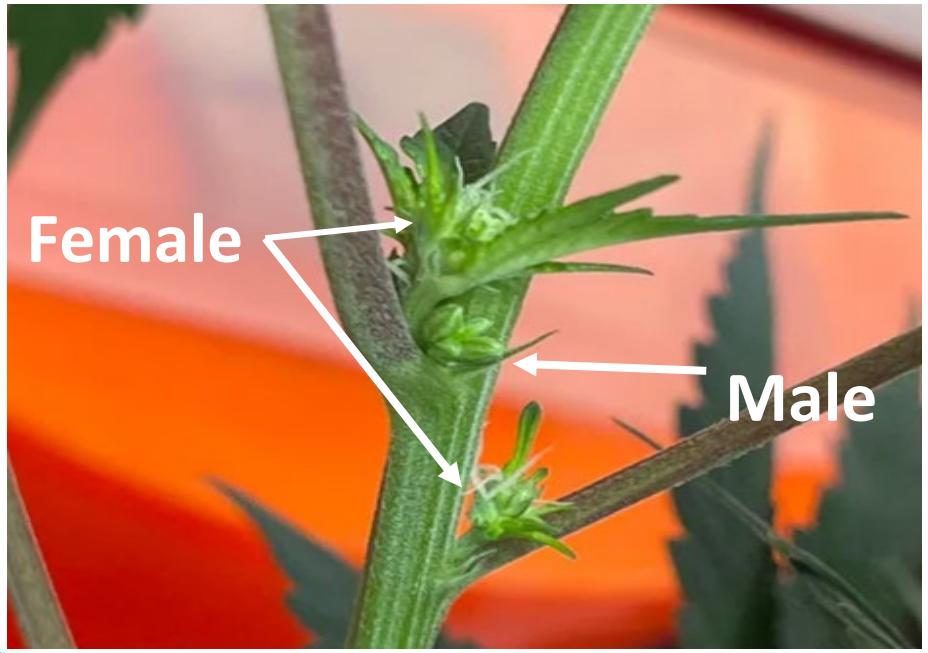


Triploid Sterility

- No method of seed prevention in outdoor grows prevents cross-pollination from nearby males
  - Hemp cultivation often includes monoecious or male plants
  - Stokes et al 2000: 36% of pollen sampled was C. sativa

BOARD OF SUPERVISORS, COUNTY OF HUMBOLDT, STATE OF CALIFORNIA
Certified copy of portion of proceedings, Meeting of December 10, 2019

AN UNCODIFIED ORDINANCE EXTENDING FOR 1 YEAR A TEMPORARY MORATORIUM ON CULTIVATION OF INDUSTRIAL HEMP

ORDINANCE NO. 2637

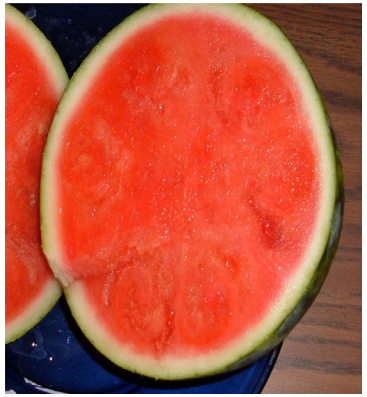
The Board of Supervisors of the County of Humboldt ordains as follows:

Triploid Sterility

- The problem with pollen:
  - Wind-borne pollen can travel miles
  - A single male flower can produce thousands of seeds
  - Seed set diverts energy from cannabinoid production and produces sub-par flower
  - Current prevention methods are still lacking
- For cannabis to compete as a large-scale outdoor ag commodity, the pollen problem must be solved

Triploid Sterility

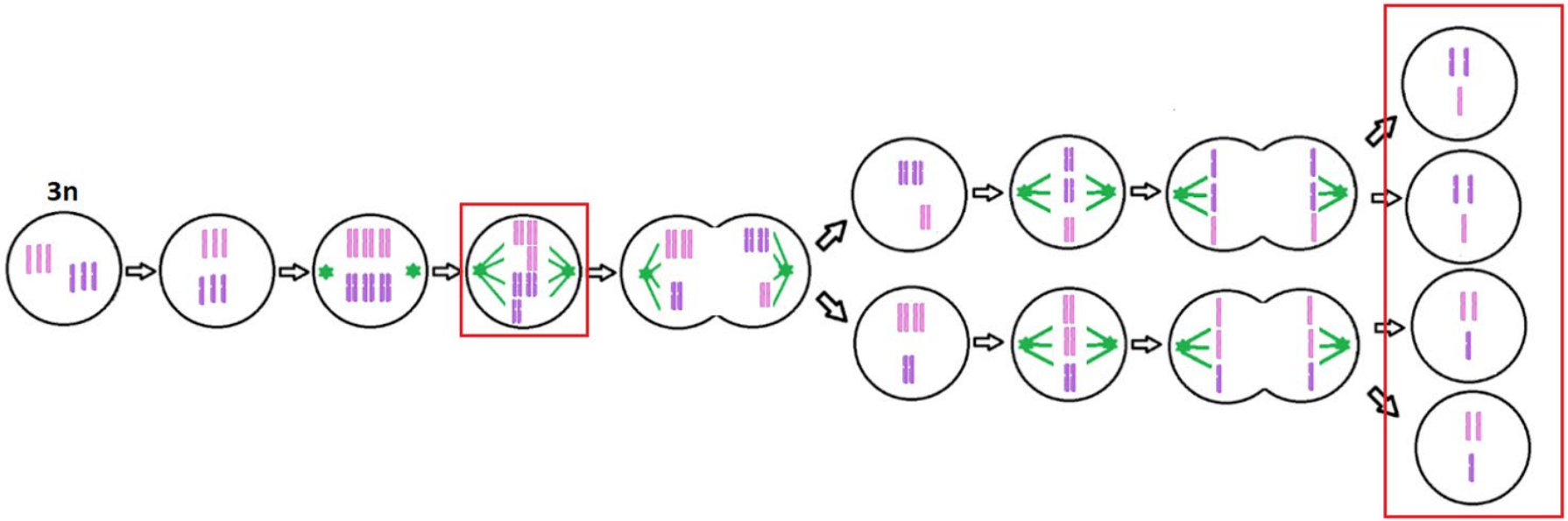
- There are established methods of producing “seedless” or sterile organisms




- What do they have in common? <u>Triploidy</u>

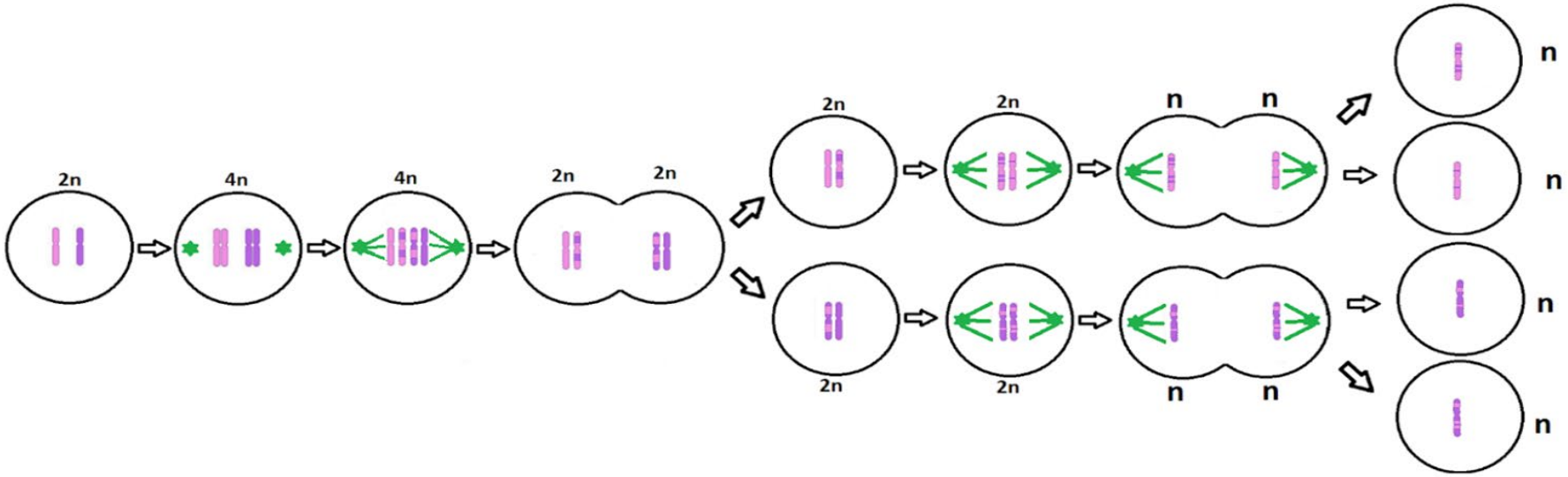
Why are Triploids Sterile?

- Odd set of chromosomes do not pair properly during meiosis, abnormal gene dosage causes gamete/embryo abortion



How to create triploids

- Must first create a tetraploid, then cross it with diploid
  - Meiosis in diploid results in 4 haploid gametes



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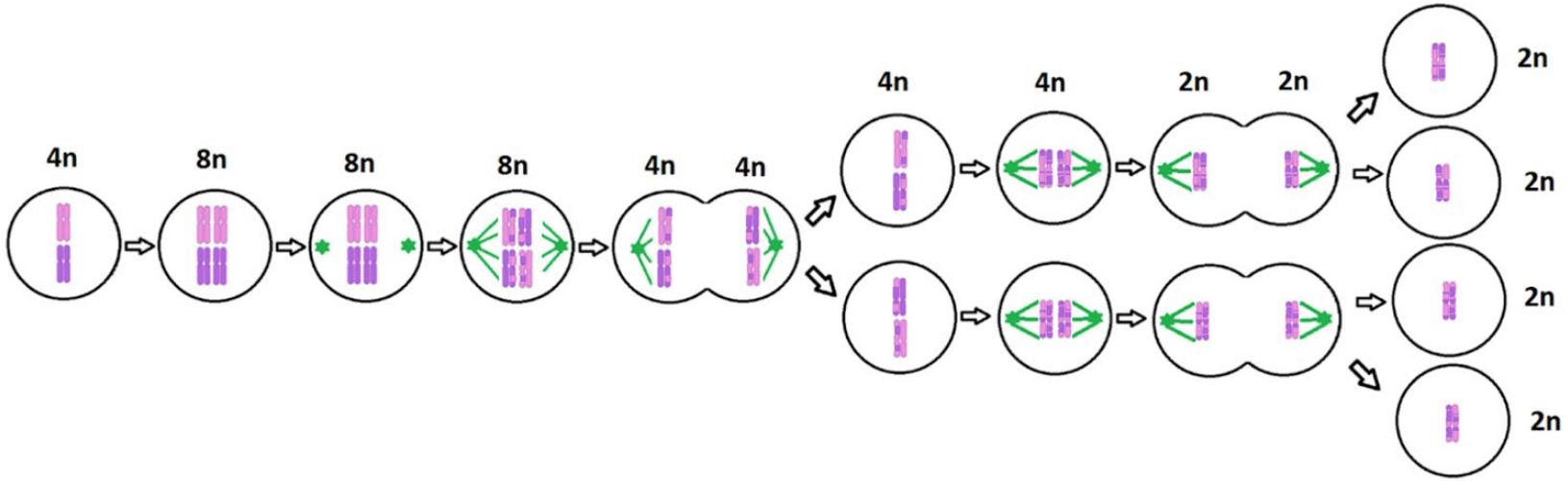
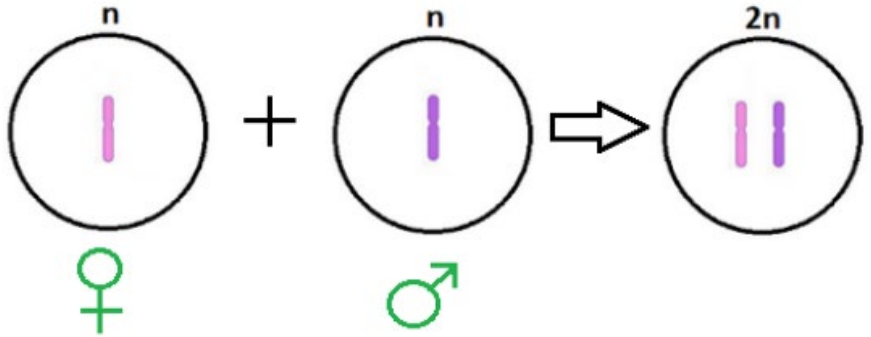
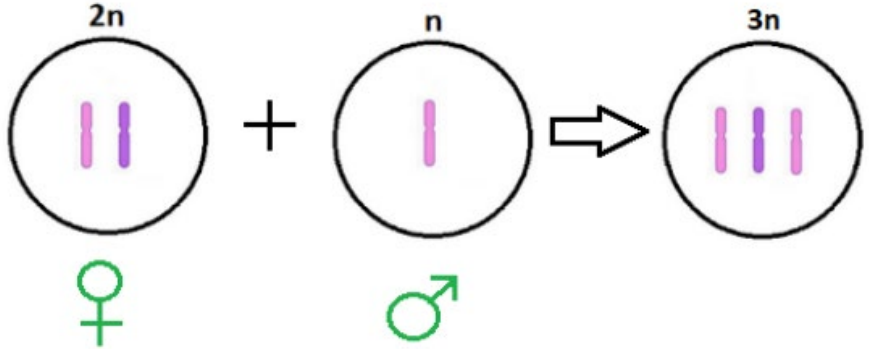


How to create triploids

- Combining diploid gamete with haploid gamete results in triploid embryo



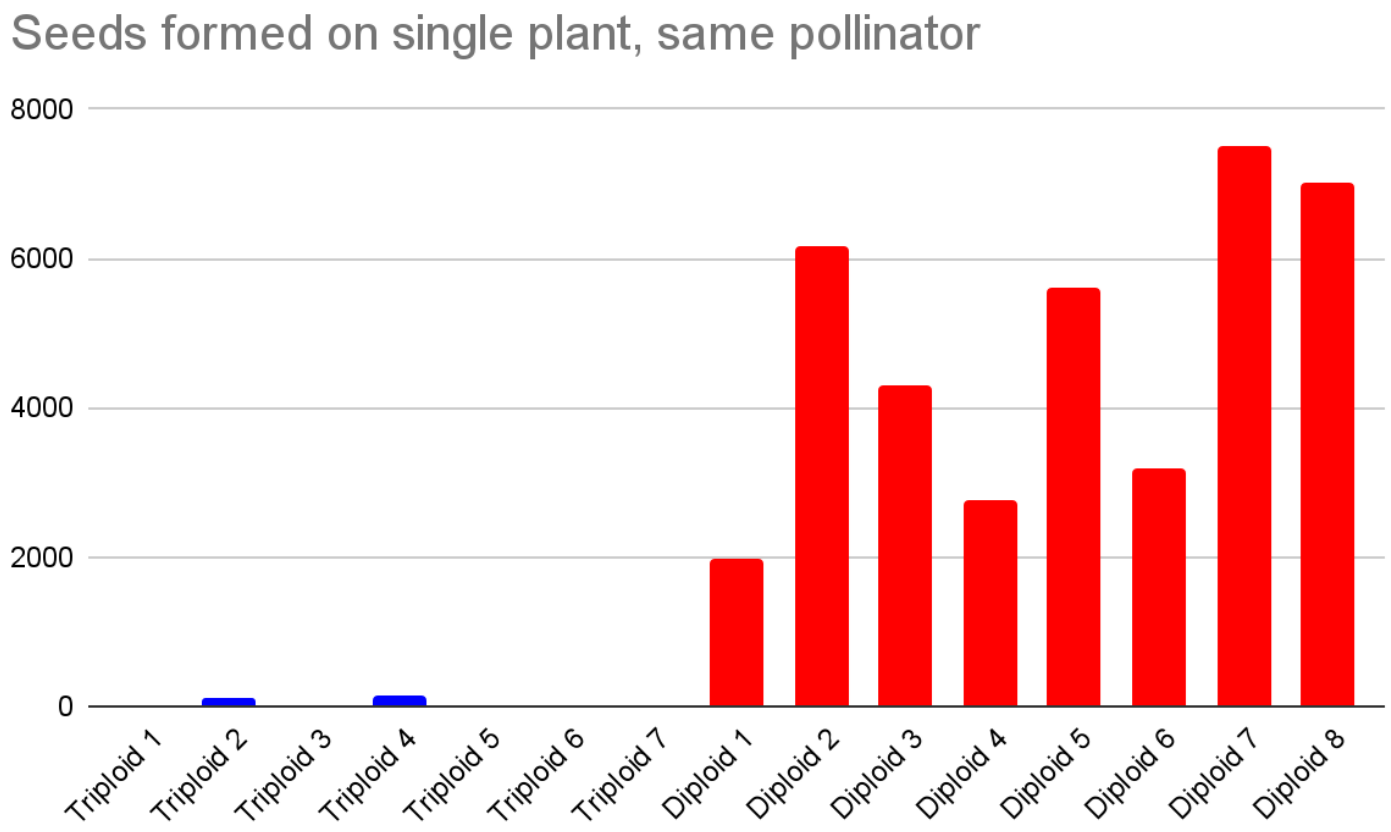
Haploid gametes combine to form diploid embryo



Diploid gamete and haploid gamete combine to form triploid embryo

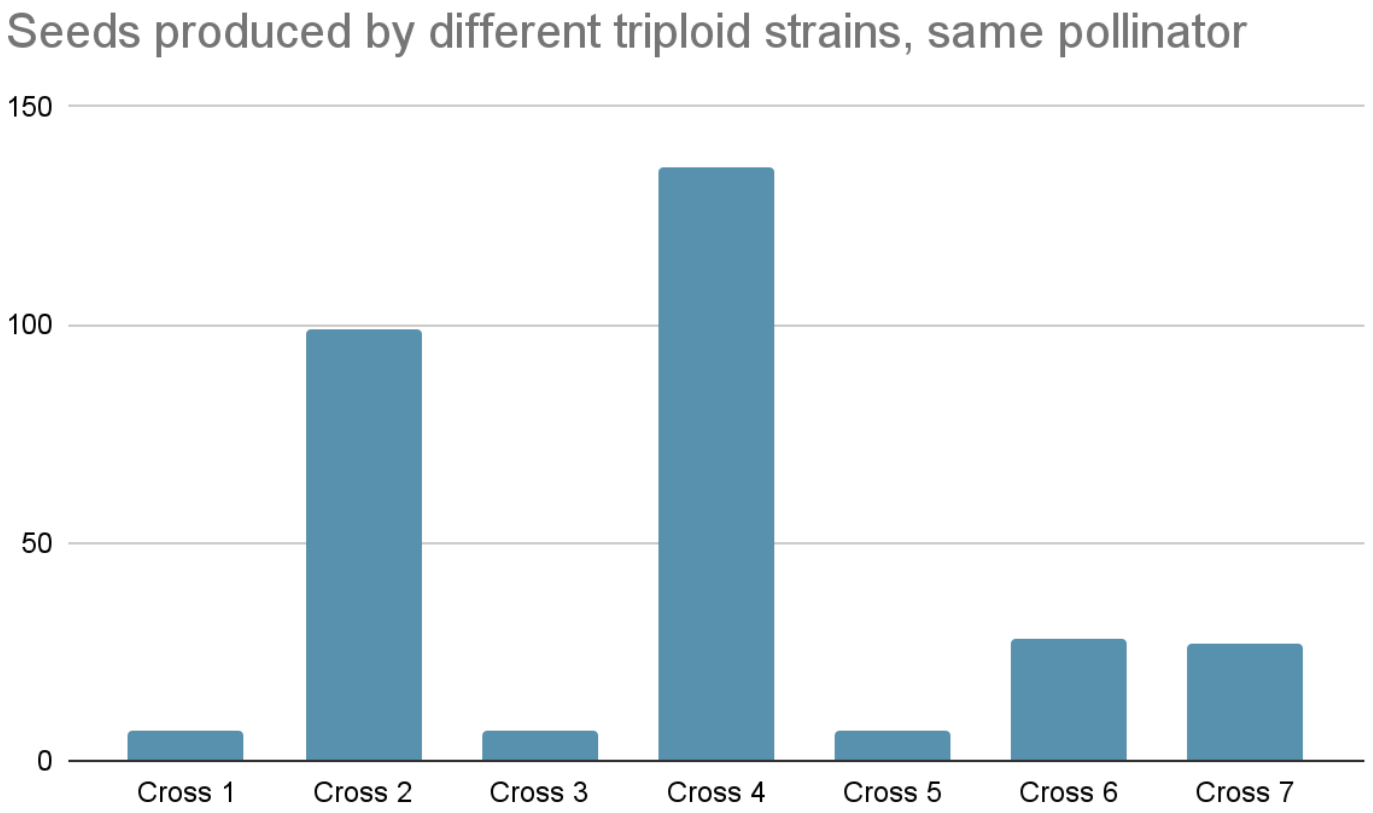
Sterility Data

- Greatly reduced seed set, but will produce some seeds when intentionally pollinated



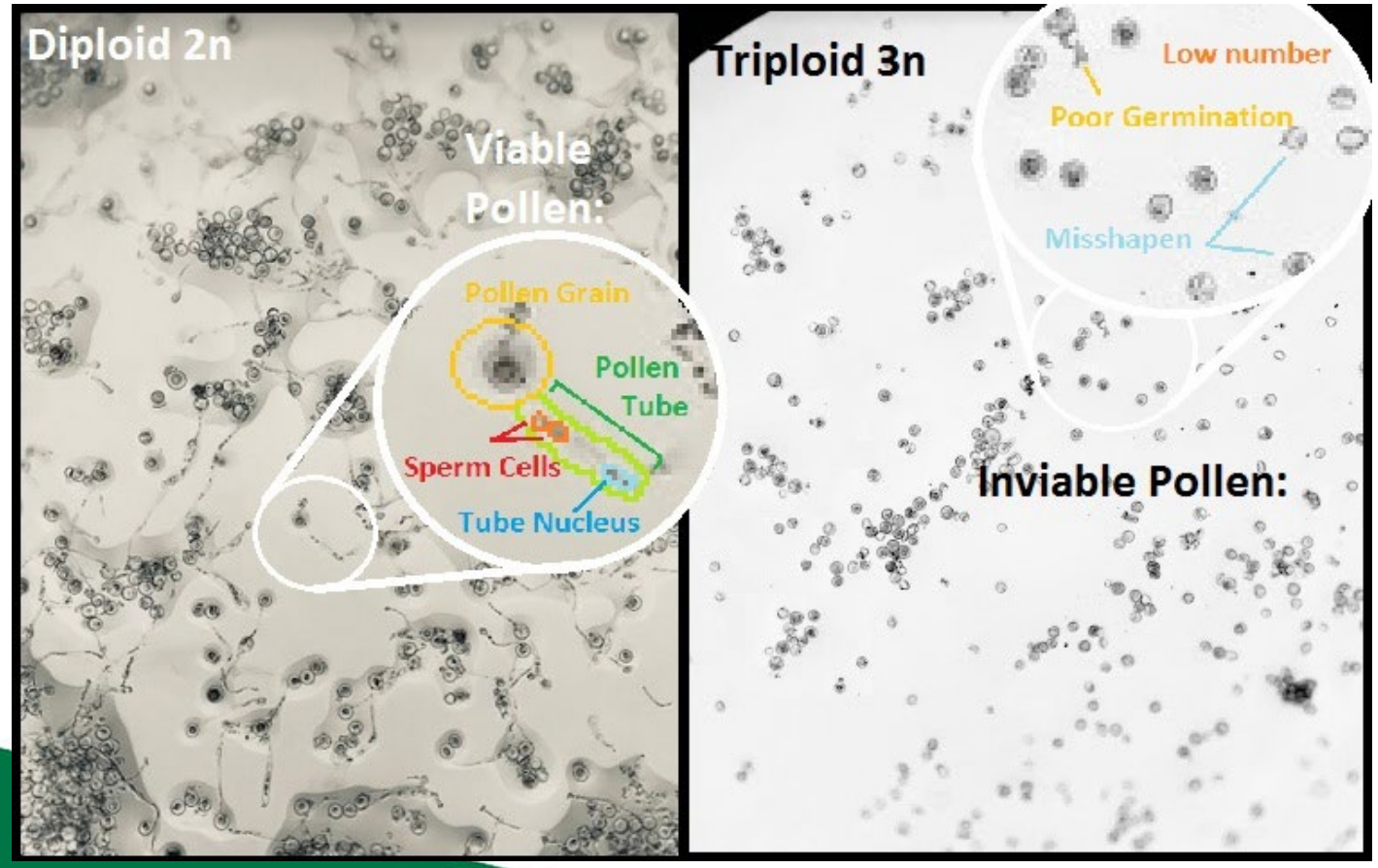
Sterility Data

- There appears to be a genetic component to sterility
  - QTL mapping could reveal mechanism



Sterility Data

- Pollen from triploids is particularly sterile
  - Triploids that herm much less likely to pollinate crop



Behavior of Triploids

- No noticeable detriment to extra set of chromosomes
  - Some evidence that triploids have higher yields

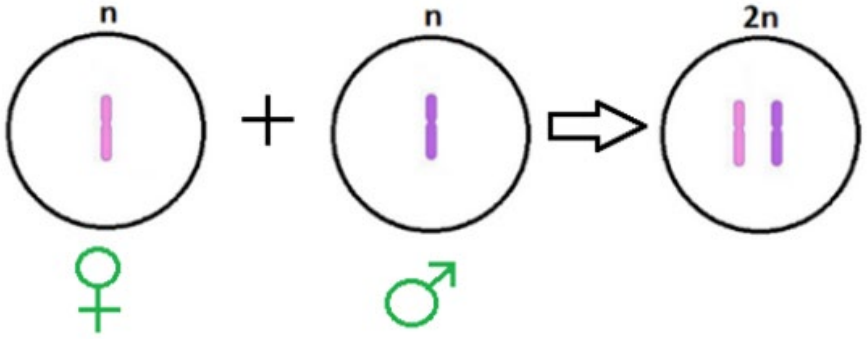


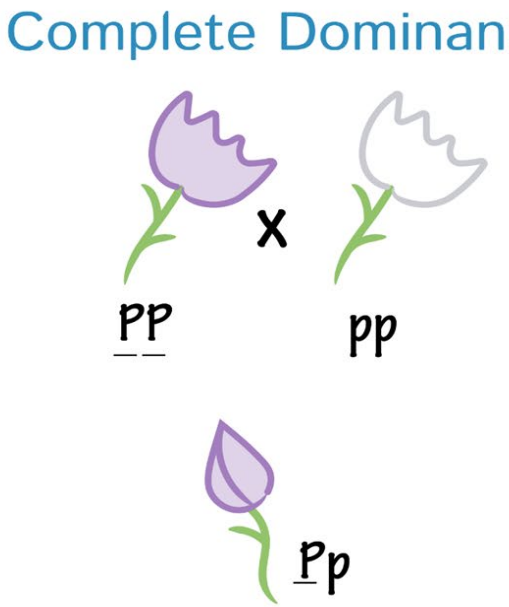
Applications for Ploidy in Cannabis

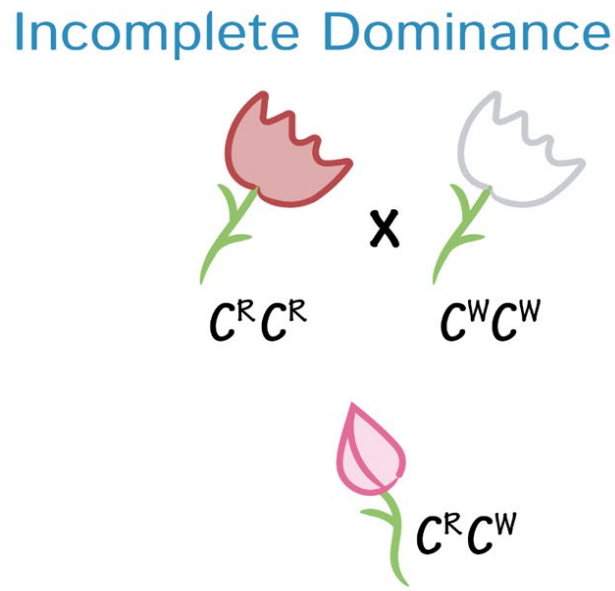
- Triploid sterility
- **Disrupting mendelian genetics**
- Stacking traits/novel allelic combinations
- Double Haploids

Trait Stacking Using Ploidy

Conventional Mendelian Genetics:

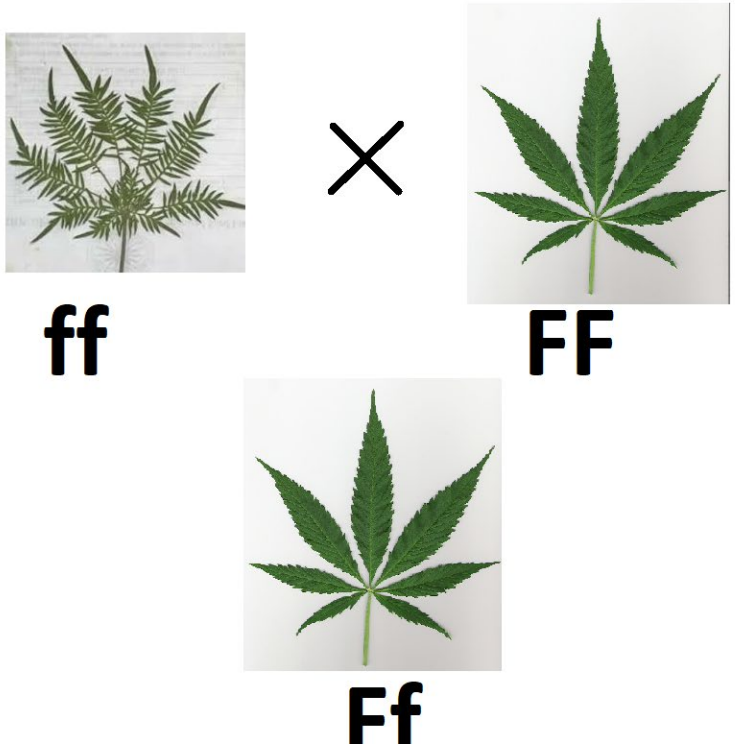






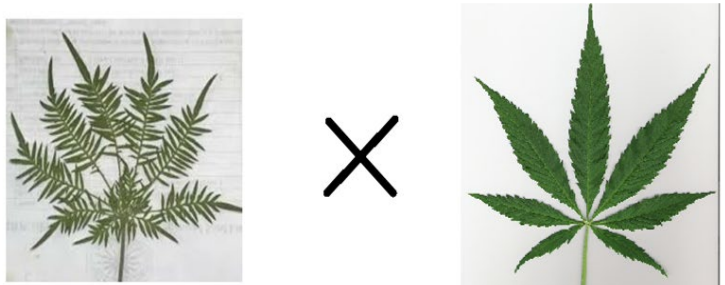
Disrupting Mendelian Dominance

- Freakshow: Classic recessive gene
  - F1 results in wild-type pheno



Disrupting Mendelian Dominance

- 2:1 f to F results in novel pheno
  - Trait no longer behaves fully recessive when stacked

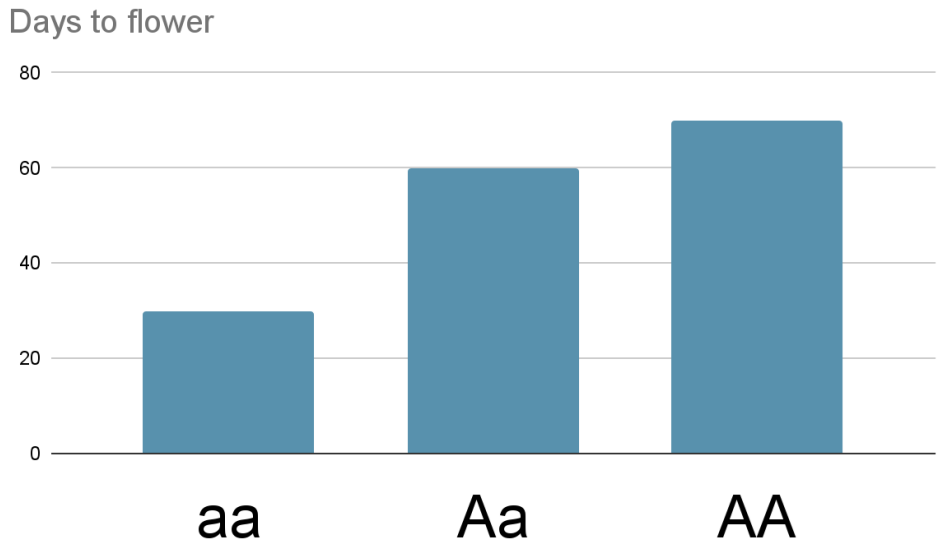


ffff × FF

Fff

Disrupting Mendelian Dominance

- Autoflower: Recessive trait that shows intermediate phenotype as F1
  - F1's referred to as "Fast flowering", will initiate flowering faster than photoperiod, but not still require light change to trigger flowering

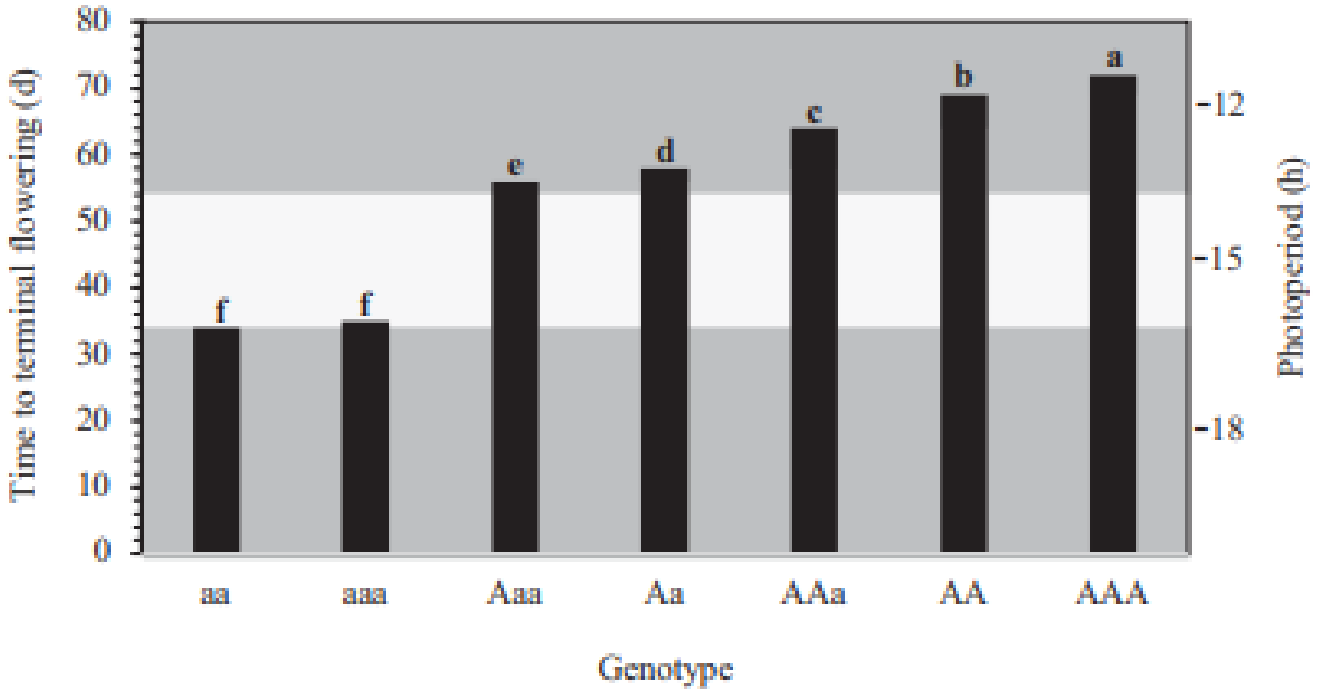


AA = Autoflower
Aa = "fast flower"
aa = Autoflower

Disrupting Mendelian Dominance

Gene Dosage at the Autoflowering Locus Effects Flowering Timing and Plant Height in Triploid Cannabis

Lauren E. Kurtz, Mark H. Brand, and Jessica D. Lubell-Brand
Department of Plant Science and Landscape Architecture, University of Connecticut, Storrs, CT 06269-4067, USA



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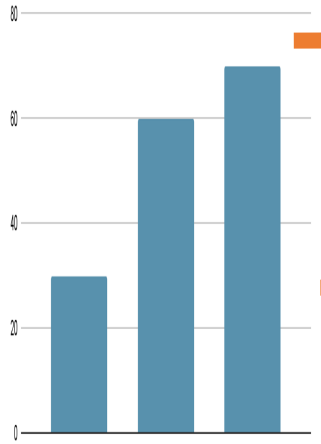
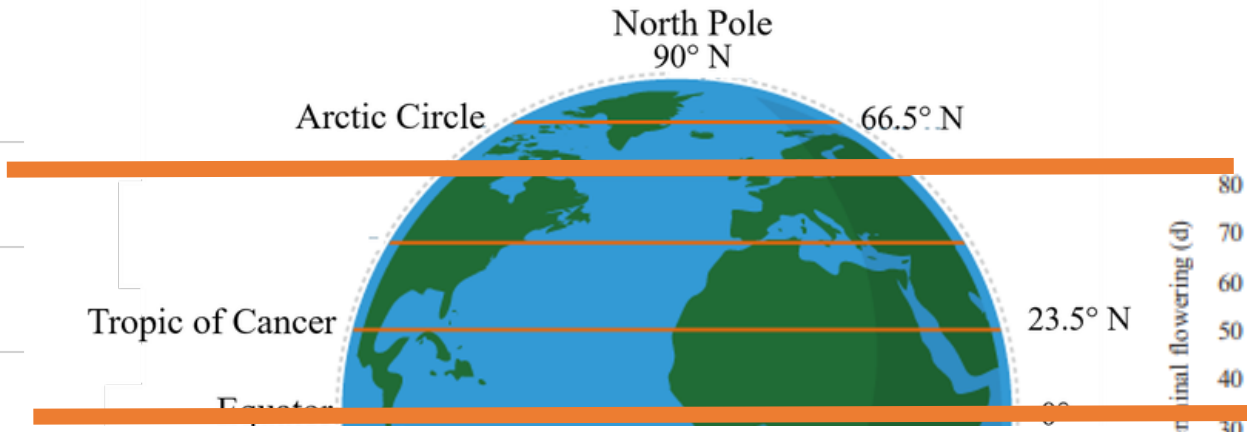
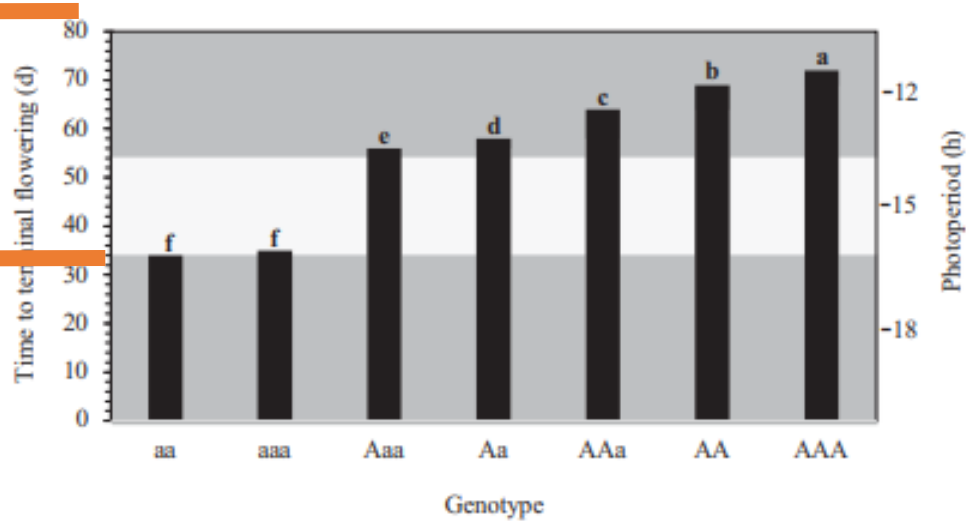
Disrupting Mendelian Dominance







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Applications for Ploidy in Cannabis

- Triploid sterility
- Disrupting mendelian genetics
- **Stacking traits/novel allelic combinations**
- Double Haploids

New Allelic Combinations

- Why stop at simple mendelian traits?
  - Most traits controlled by multiple genes and have large range of phenotypes
    - Cannabinoids
    - Terpenes

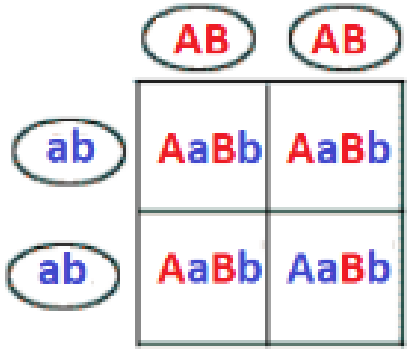
4n CBD x 2n THC = ???

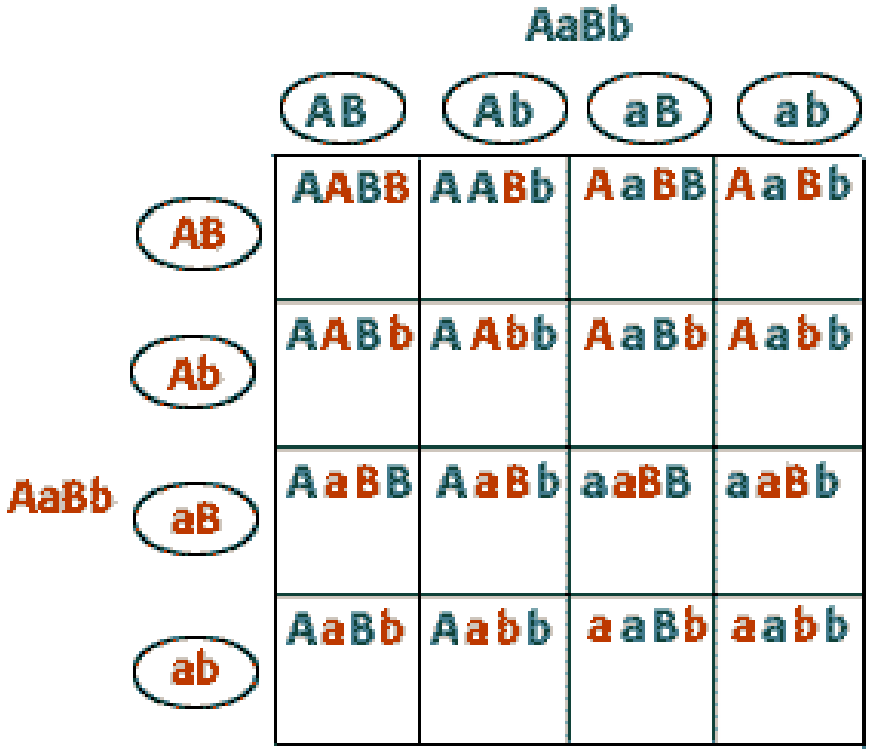
4n THCV x 2n THC = ???

4n Limonene x 2n Myrcene = ???

New Allelic Combinations

- Breeding in tetraploid background
  - Novel combinations of traits

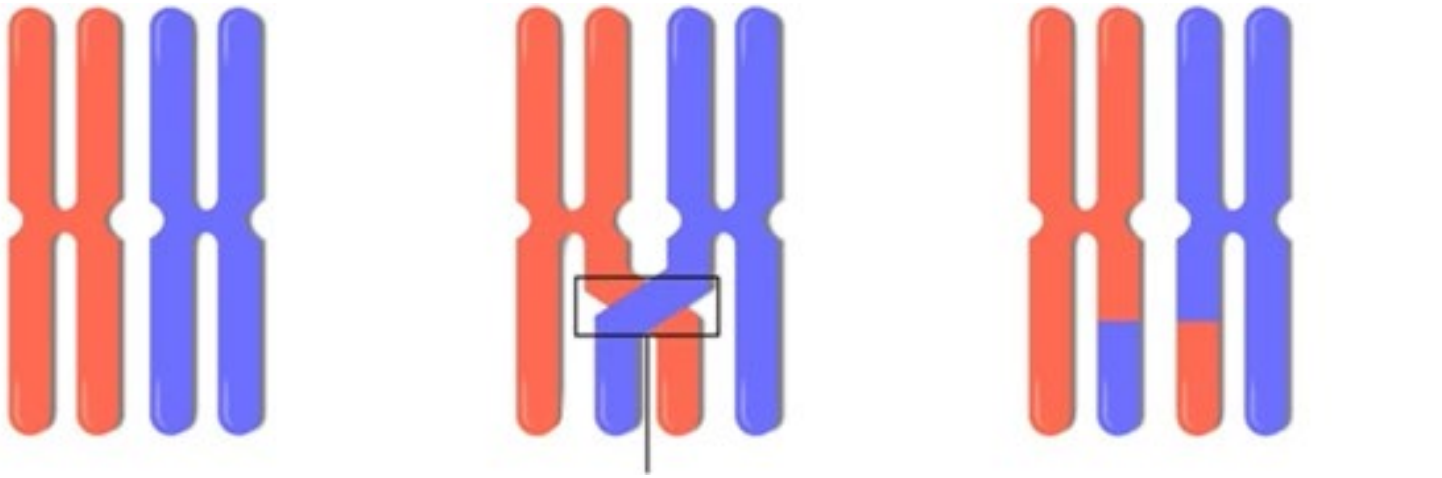




CANNABIS CONFERENCE

New Allelic Combinations

- Increased diversity
  - More chromosomes = more crossover events during meiosis, more random assortment combinations



Crossing over **←Random Assortment →**

Applications for Ploidy in Hemp

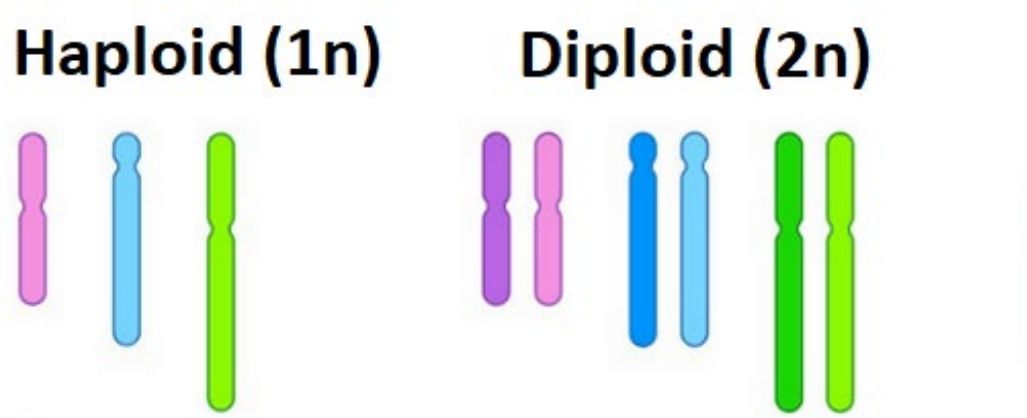
- Grain size increases in polyploids
- Increased fiber content

Applications for Ploidy in Cannabis

- Triploid sterility
- Disrupting mendelian genetics
- Stacking traits/novel allelic combinations
- **Double Haploids**

Double Haploids in Plant Breeding

- Haploids contain only one set of chromosomes
- Used in plant breeding to create “F1 Hybrid” seed

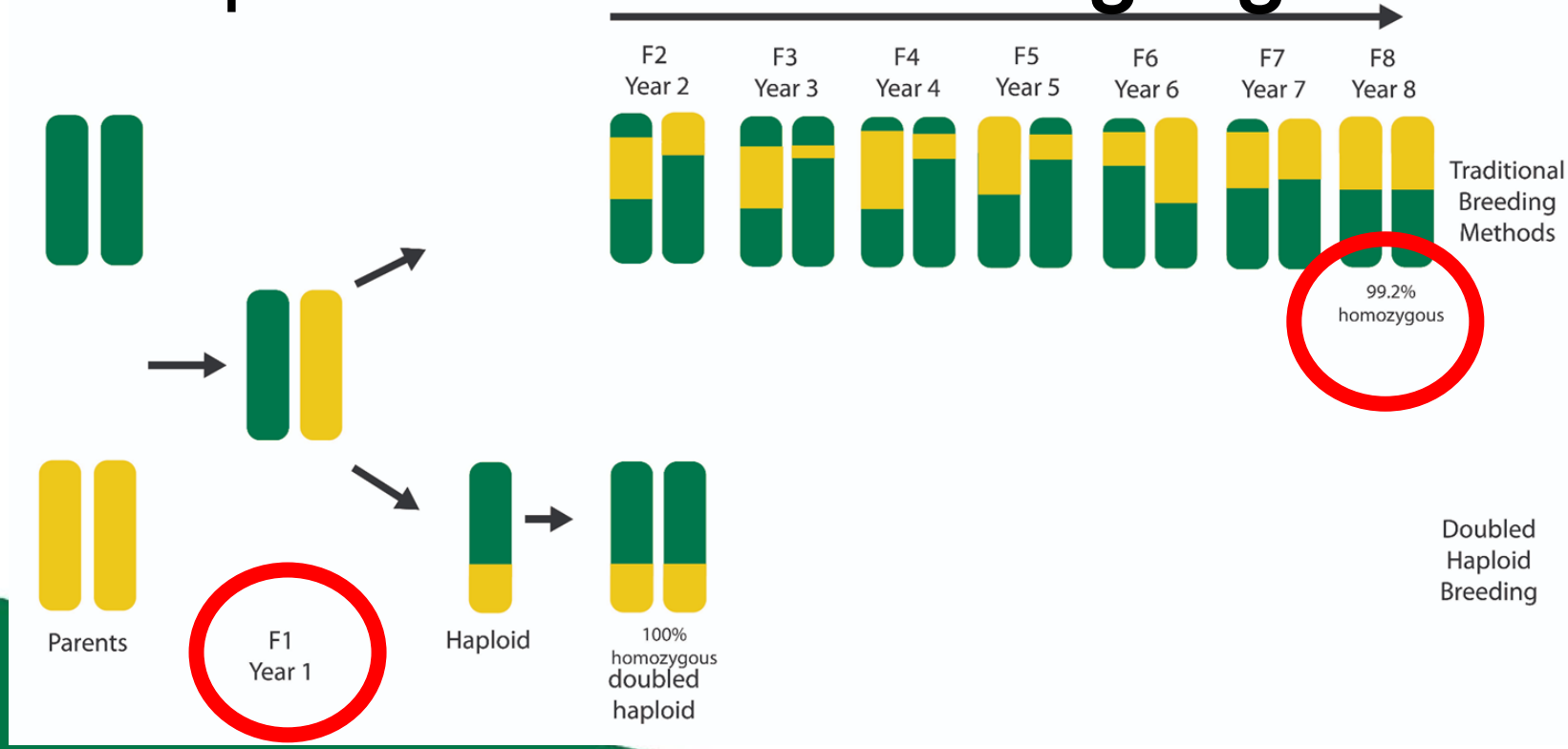


Double Haploids in Plant Breeding

- Seed is a much more preferable starting source material than cutting
  - No mom room, clone room, propagation labor
  - Easier to prevent disease transmission
  - Strong taproot, opposite leaf growth pattern
  - Much easier to transport (across borders)
- Current seeds are not reliable enough for large scale grows
  - Too much diversity in populations
  - Must phenohunt, then clone out

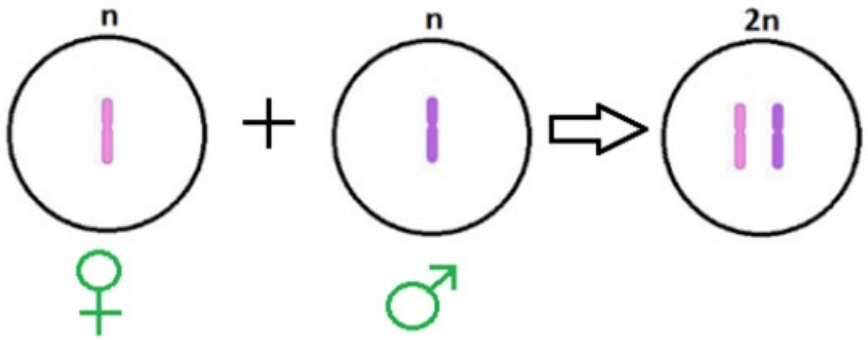
Double Haploids in Plant Breeding

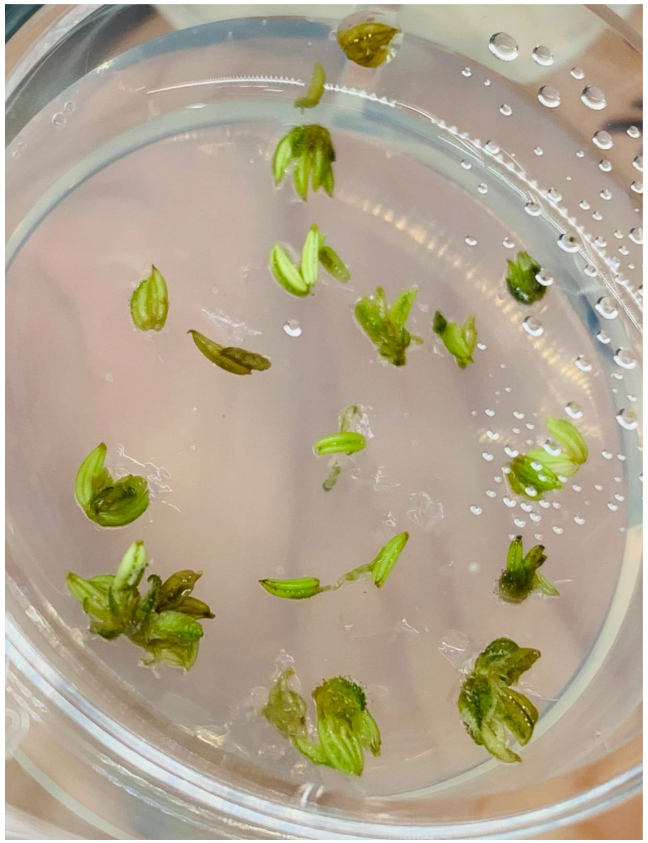
- Many generations of inbreeding can results in “true breeding” cultivars, “Clonal seed”
- Double haploids do this in a **single generation**



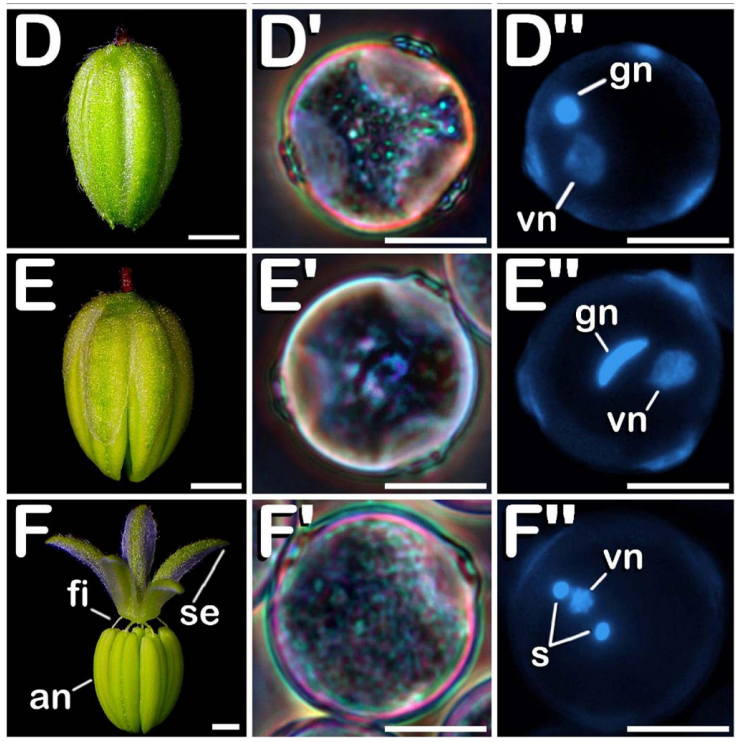
Double Haploid Limitations

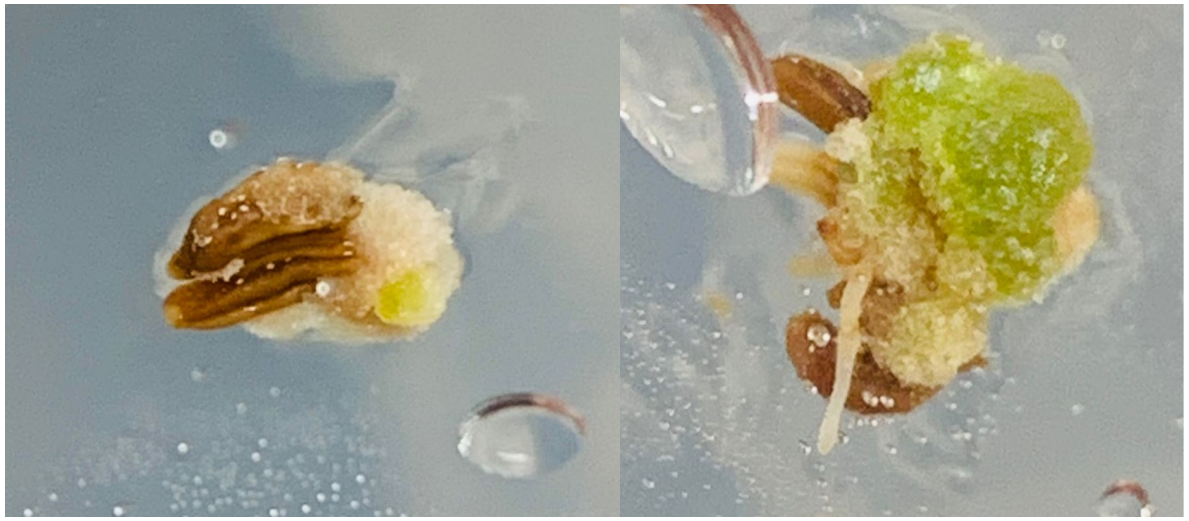
- Can be very difficult!
- Cannabis is particularly recalcitrant





Double Haploid Limitations



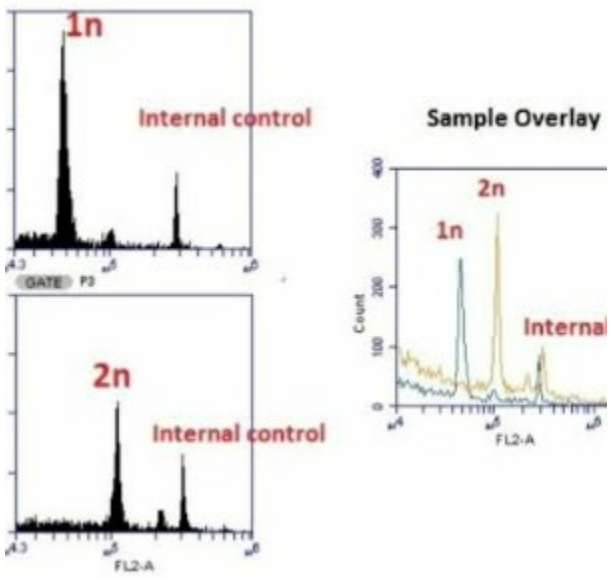


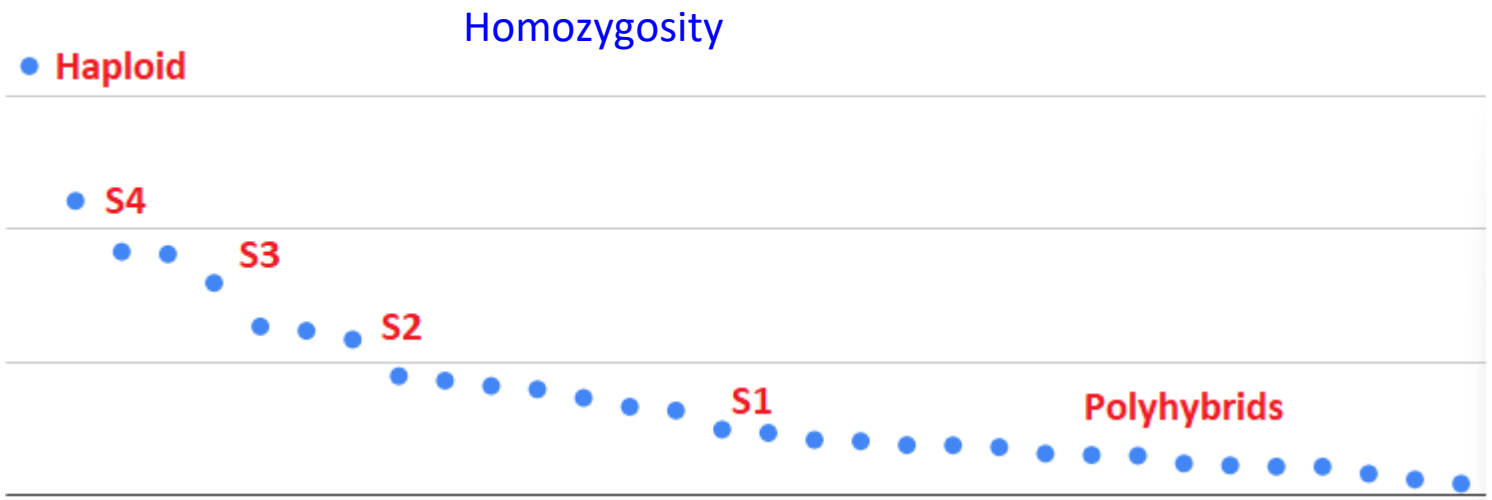
Are Haploids Viable?

- Yes!
  - Show some decrease in vigor: thin stems & branches, susceptible to disease
  - More vigorous once genome is duplicated

Confirming Ploidy/Homozygosity

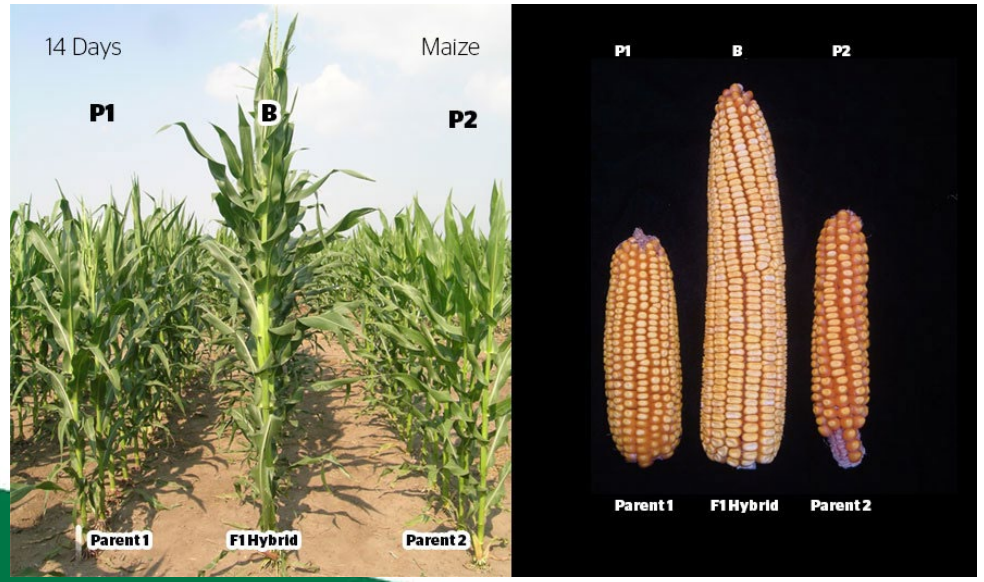
Haploid confirmed via flow cytometry and SNP genotyping





F1 Hybrid Seed

- Crossing two homozygous genetically distinct lines produces **F1 Hybrids**
  - Extremely uniform
  - Display heterosis aka “hybrid vigor”



Anticipated Questions/Criticisms

- Will this destroy small scale breeders/hobbyists?
  - Ploidy alone does not make a great plant
  - Triploids focused for specific applications, not end-all be-all
- Is this GMO/Unnatural?

Natural Polyploidy in Cannabis

- MAC1
  - Very popular clone only strain
  - Rumors of difficulty making seed when outcrossing
  - **Flow cytometry revealed it to be triploid**

Natural Polyploidy in Cannabis

- Further testing of germplasm revealed additional 2 “hidden” triploids:
  - Hades OG, Mirage
  - 3 strains out of 215: ~1.5%
- These clones selected via pheno hunts. Are triploids better?

Natural Polyploidy in Cannabis

Cultivar/Cross	Source	Fem Pollen?	Seeds Tested	3n Found
Bigfoot Glue	Humboldt Seed Co	yes	180	1
Jelly Rancher	Humboldt Seed Co	yes	190	0
CxP	Dark Heart	yes	1265	5
Gazzurple	Humboldt Seed Co	no	80	0
WMxF	Dark Heart	yes	263	2
WMxP 98	Dark Heart	yes	200	1
VxF	Dark Heart	yes	200	1
MxF	Dark Heart	yes	200	1
BxS	Dark Heart	yes	1000	3
PxP	Dark Heart	yes	128	3
CHxP	Dark Heart	yes	768	2

19/4474
= 0.004%

Natural Polyploidy in Cannabis

Abnormal Meiosis in Tetraploid (4x) *Cannabis sativa* (L.) from Lahaul-Spiti (Cold Desert Higher Altitude Himalayas)-A Neglected But Important Herb

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Conclusion

Polyploids and haploids will play an integral part of the next generation of cannabis genetics. The creativity of breeders, cultivators, operators will maximize the opportunity ploidy provides.

Thank You

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