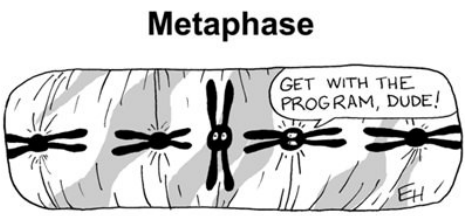
Your Pseudonym: _____

PBGG 8890 Plant Cytogenetics

Behavior & Evolution of the Plant Genome

Spring 2025 Exam I



With kinetochores starting to grow,
The chromosomes all in a row,
Are tidy and straight,
On the metaphase plate,
With a spindle above and below.

Fill in and Email this page to Kendall Erkens Kendall.Erkens@uga.edu.

Also email your completed exams to Kendall.

The email time stamp must be **before 5 pm on Monday 31st @ 5:01 pm,**

Name: _____

Pseudonym: _____

Use a nickname that is not easily associated with your name, and place it on each page. So, Meiosis Maven and Captain Chromosome would be OK, but RoyalSoy would not be as it is not anonymous enough. This pseudonym needs to be on each page of your exam. This allows me to grade the answers anonymously. Only Kendall will know which student goes with which nickname.

The exam is open **notes**. But, it must be your own work, and you cannot communicate with others about it. Since the questions are inspired from current literature, answers really will not be readily available elsewhere. *All answers should fit in the space provided.*

By submitting my answers, I acknowledge that these answers are strictly my own work, and that I did not request or receive assistance from anyone else or from any information provider, search engine, A.I., etc.

Name in lieu of a signature: _____

Date: _____

General advice-complete the exam, then review your answers the next day before submittal.

Your Pseudonym: _____

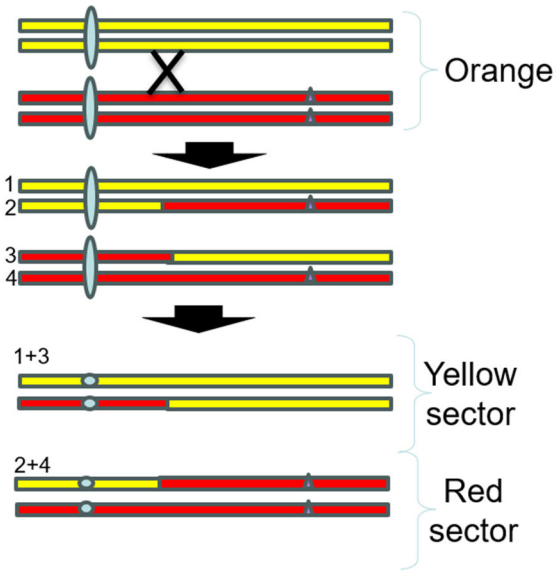
The following is from: (will be revealed after exam)

Samach A, F Mafessoni, O Gross, C Melamed-Bessudo, S Filler-Hayut, T Dahan-Meir, Z Amsellem, WP Pawlowski, & AA Levy. 2023. CRISPR/Cas9-induced DNA breaks trigger crossover, chromosomalloss, and chromothripsis-like rearrangements. Plant Cell, 35(11): 3957-3972.

Tomato flowers are normally yellow. In this work, the authors used a transgenic construct (a precursor to the RUBY marker) that produces betalein→ the purple pigment in beets.

Homozygotes for the transgenic construct are purple. Non-transgenics remain yellow. Heterozyotes are lightly colored purple, which against the yellow background, appear orange.

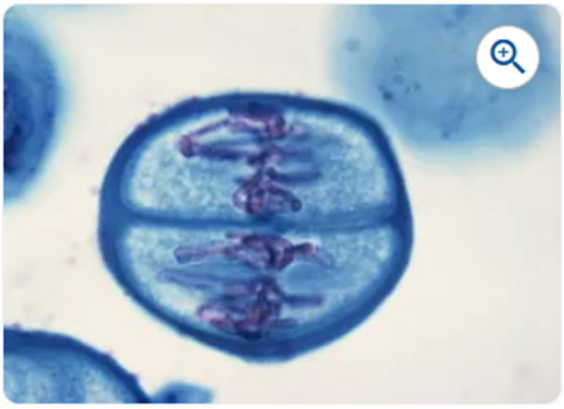
Explain the cytological event that would give rise to the flower shown at right, and provide a diagram.



Your Pseudonym: _____

A. **The following is from** the encyclopedia Britannica web page on meiosis:

https://www.britannica.com/science/meiosis-cytology

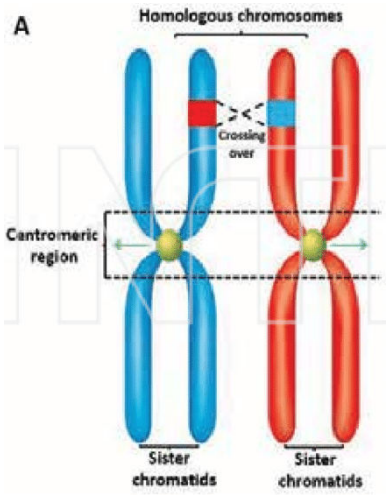


meiosis Meiosis in the anther of a lily, showing prophase II.

Do you agree with the caption in their figure? Explain why or why not.

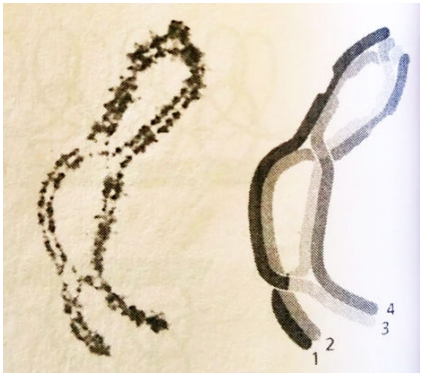
No. It is showing Met II. The chromosomes have not even separated into different nuclei yet. Meiosis II seldom has more than a rudimentary prophase.

B. The diagram at right is from *ibiologia.com*. Do you agree with their representation of crossing over?
Explain your answer and if needed, provide a corrected diagram.



No—1 chiasma does not result in a double CO

C. The diagram at right is from https://thebiologyislove.com/prophase-1/ and repsents. "***A bivalent with three chiasmata resulting from three crossover events.***" *Do you agree with their interpretation of chromatid pairing? Why or why not?*



Yes, b/c the chromatids are not switching partners

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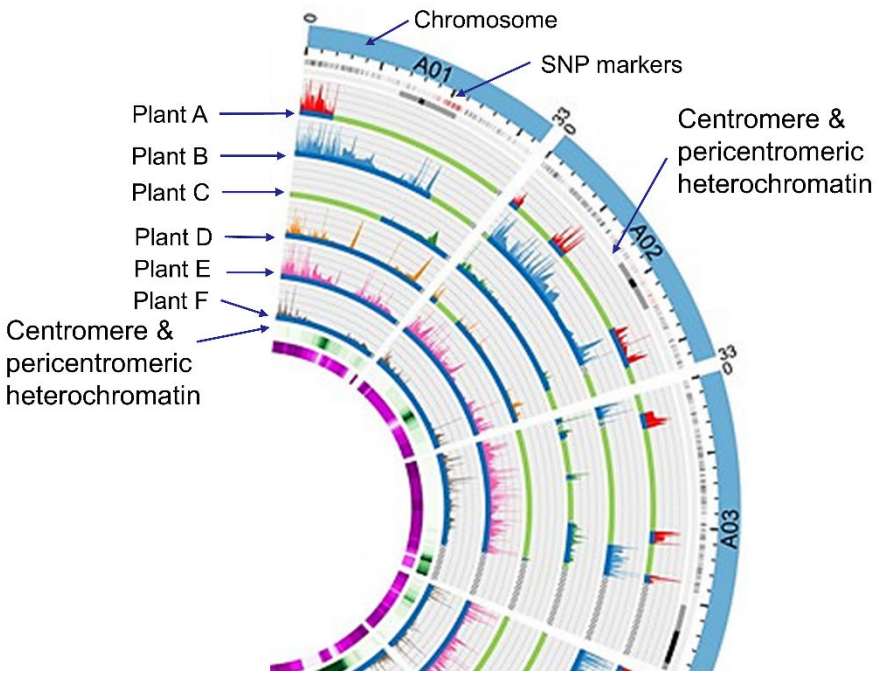
The following is from: (will be revealed after exam)

Boideau F, V Hueau, L Maillet, A Brunet, O Coriton, G Denoit, G Trotoux, M Taburel-Lodé, F Eber, M Gilet, C Baron, J Boutte, G Richard, J-M Aury, C Belser, K Labadie, J Morice, C Falentin, O Martin, M Falque, A-M Chèvre & M Rousseau-Gueutin. 2024. The Plant Cell, 36(10): 4472–4490. https://doi.org/10.1093/plcell/koae208

In the Circos plot below, the first ring is the chromosome. The 3rd circle shows the centromere and the pericentric heterochromatin as gray/black sectors. Between these are the SNP markers used in the study, with the ones in the pericentromeric heterochromatin in red. Rings A-F represent recombination (i.e., crossovers) in different allotriploids or allotetraploids.

The authors studied Chromosomes A01 and A02. Which plants are the allotriploids? What is the diagnostic feature you used?

The allo3x plants are the ones with recombination in the pericentromeric heterochromatin: B, D, & E.



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The following is from: (will be revealed after exam)

Clot CR, L Vexler, M de La O Leyva-Perez, PM Bourke, CJM Engelen, RCB Hutton, J van de Pelt, E Wijnker, D Milbourne, RGF Visser, M Juranić & HJ vanEck. 2024. Identification of two mutant *JASON-RELATED* genes associated with unreduced pollen production in potato. Theoretical and Applied Genetics, 137: 79. https://doi.org/10.1007/s00122-024-04563-7

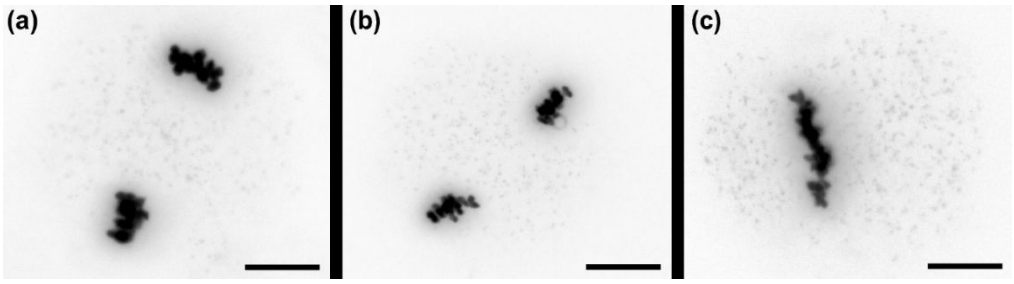
In this paper, the authors were trying to find the genes responsible for 2n gamete formation in diploid potato. Cytologically, they found 3 types of events.

a) The stage of meiosis shown is ____Met II_____

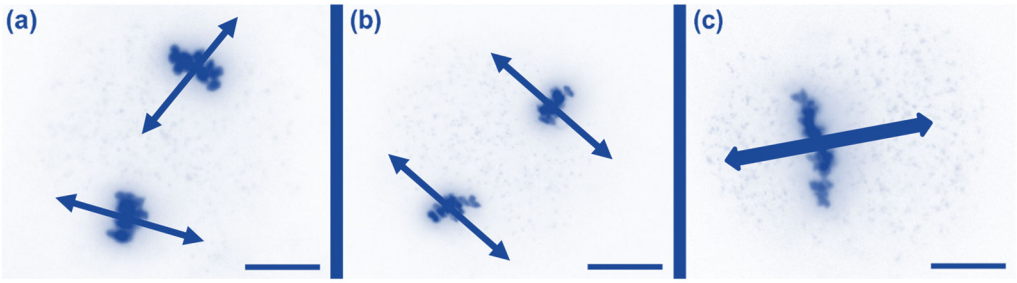
b) The mechanism of 2n gamete formation is __parallel/fused_____

c) The mode of 2n gamete formation is _____FDR_____

d) The plant shown is a monocot, is a monocot, a dicot, or a eudicot? _____eudicot_____



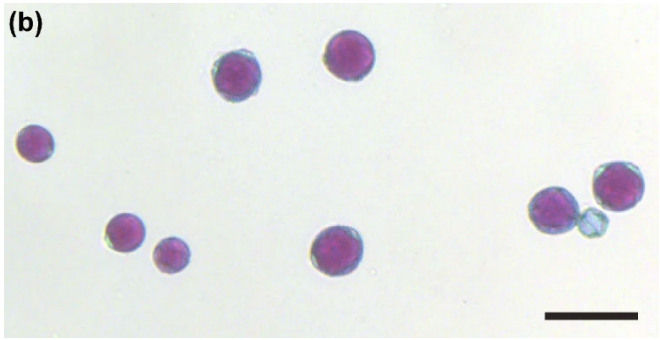
e) Redraw these figures, add the spindle and the spindle axes to each figure, scan and upload in the space provided below:



Your Pseudonym: _____

The following question is from the same paper as the previous question.

The authors used pollen diameter to identify 2n pollen.



a) Why is it possible to use pollen diameter to ID 2n pollen, and what are the expectations in terms of diameter and volume?

DNA amount correlates with cell volume. Doubling the DNA content doubles the cell volume, which in turn increases the cell diameter by 1.26x.

b) List 3 additional methods that could be used to identify 2n pollen production.

- Flow cytometry
- 2x-4x cross that result in 4x progeny
- Cytologically
- Count germination pores
- Shape of dry pollen

Your Pseudonym: _____

The following is from (will be revealed after exam):

Liu Y, B Jiao, J Champer & W Qian. 2024. Overriding Mendelian inheritance in arabidopsis with a CRISPR toxin–antidote gene drive that impairs pollen germination. Nature Plants, 10: 910-922. doi.org/10.1038/s41477-024-01692-1

In this paper, the authors devised a synthetic toxin antidote drive they call CRISPR-Assisted Inheritance utilizing NPG1 (CAIN). NPBG1 is an essential gene, *No Pollen Germination 1*, which prevents pollen germination upon loss of function.

a) Provide a diagram and a description of how this system would work; What part is the toxin and what part is the antidote? Give the general characteristics of a gene that can be used as a toxin gene.

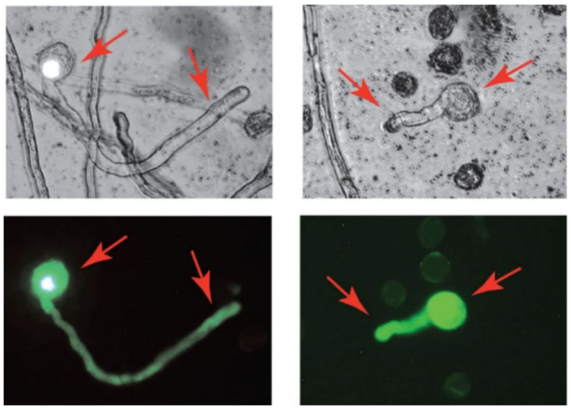


Figure 1. Comparison of WT and NPG1 pollen. Source = doi.org/10.1038/srep05263

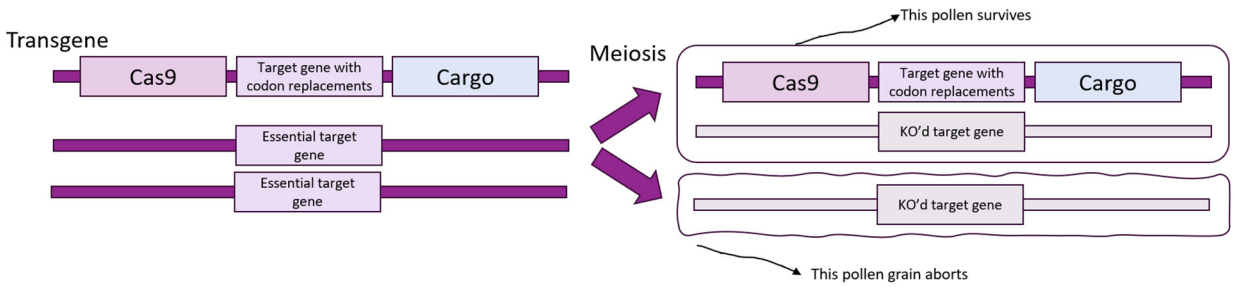
The toxin is a Cas9 construct that will knock out a gene that is essential for pollen function or survival.

The antidote is a copy of the KO'd gene that did not get KO'd because it has an altered sequence that is not recognized by the Cas9 gRNA. It is supplied as a transgene.

b) Give a couple of examples of traits that can be addressed by the cargo gene.

It could be a resistance/quality trait that one wants to drive through a population; It could also be a trait removal gene—eg, a silencing construct for an essential gene, eg, silencing the resistant version of an herbicide resistance gene.

Upload your diagram in the space below.

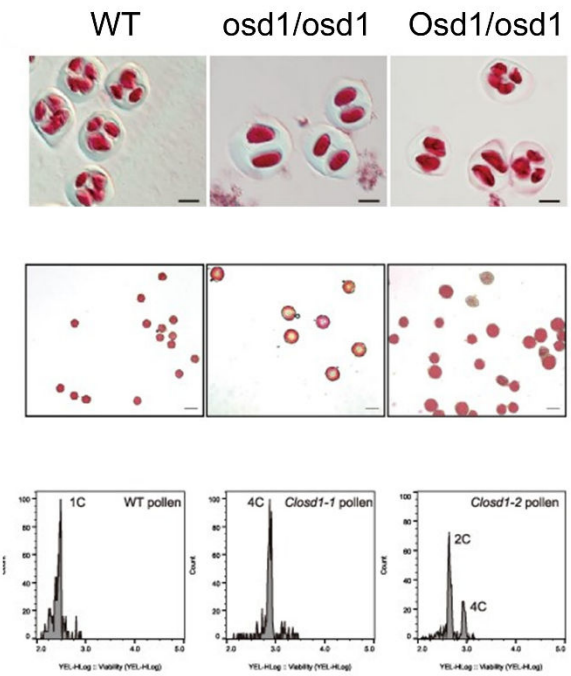


Your Pseudonym: _____

The following is based on: (will be revealed after exam)

Pang W, W He, J Liang, Q Wang, S Hou, X Luo, J Li, K Wang, S Tian & L Yuan. 2025. Disruption of *ClOSD1* leads to both somatic and gametic ploidy doubling in watermelon. *Horticulture Research*, 12(1): uhae288. doi.org/10.1093/hr/uhae288

In this paper, the authors used CRISPR/Cas9 to knock out the OSD1 (omission of second division) gene in watermelon. In arabidopsis and rice, OSD negatively regulates the anaphase-promoting complex, so meiosis stops after Telo I.



a) Looking at the microspores and resulting pollen grains, does OSD1 appear to work in watermelon the way it does in rice and arabidopsis? Explain the basis for your answer, using 3 lines of evidence shown here.

The presence of dyads at Telo II shows 2n pollen formation. The resulting pollen is also larger than the haploid pollen. The heterozygote has a mixture of tetrads and dyads, which is reflected in the pollen size. DNA content measurements confirm the results.

b) In this case, is the resulting pollen FDR or SDR? Explain your answer.

It is SDR, because the resulting spores will have sister chromatids in them.

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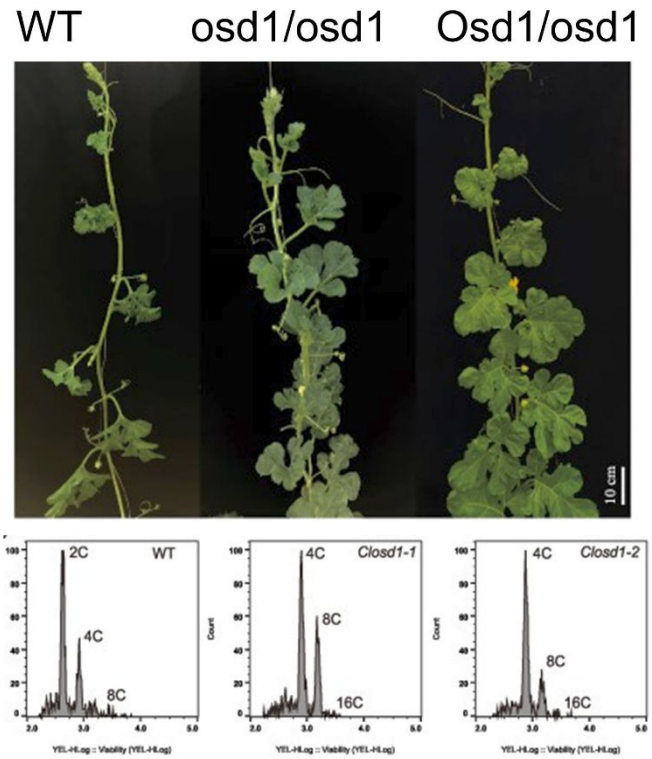
The following question is based on the same paper as the previous one.

The authors also noticed that OSD1 knockout watermelon plants were larger, showing symptoms of polyploidy. So, they measured DNA levels.

a) Explain why the WT plant would have 4C and 8C peaks?

Endopolyploidy and/or endoreduplication takes place as tissues get older and differentiate.



b) The author hypothesized that the higher DNA content in the knockouts had to be from endoreduplication or from endomitosis. So they counted chromosomes. Watermelon is normally 2n = 2x = 22. The knockout plants had ~37 chromosomes in the counts. Based on this, is it polyploidy from endomitosis, or is it endoreduplication? That is happening. Explain the basis for your answer.

Endoreduplication would not increase chromosome number, so it must be polyploidy coming from endomitosis.

c) The wild-type plant will be ___2_n = ___2__x = ___2__C = ___2_Cx = __22__

d) Most cells in the plant in the middle plant would be __2_n = __4__x = ___4__C = __2__Cx = __22_

e) Based on these results, what inference can you make about the way OSD1 works in watermelon compared to rice and arabidopsis?

In arabidopsis and in rice, OSD1 works at Ana II. In watermelon, it also works at Ana II, but it also appears to prevent Ana of mitosis.

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The following is from the reference below:

Emerson RA, GW Beadle & AC Fraser. 1935. A summary of linkage studies in maize. Cornell University Agricultural Experiment Station Memoir 180.

The data below comes from a 3-point test cross whereby

- po = polymitotic = erratic chromosome distribution during gametogenesis → male sterile, partial female sterile
- Y1 = yellow endosperm-1 (recessive = white)
- pl = purple plants color (recessive = green)

Parental cross: *Po Po-y1 y1-pl pl* × *po po – Y1 Y1 – Pl Pl*

Fertile – white endosperm – green plants × polymitotic – yellow endosperm – purple plants

Test cross progeny:

Phenotype	Number observed
Fertile – white endosperm – green plants	306
Polymitotic – yellow endosperm – purple plants	239
Fertile – yellow endosperm – purple plants	46
Polymitotic – white endosperm – green plants	52
Fertile – white endosperm – purple plants	124
Polymitotic – yellow endosperm – green plants	124
Fertile – yellow endosperm – green plants	18
Polymitotic – white endosperm – purple plants	9
	918

a) The Po – y1 distance = 13.61 cM

b) The y1 – pl distance = 29.96 cM

c) The Po – pl measured map distance = 37.69 cM

d) The Po– pl best estimate of true map distance = 43.57 cM

e) The interference observed for the DCOs = 27.8 %

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Parents: *Po Po-y1 y1-pl pl* × *po po – Y1 Y1 – Pl Pl*

(Fertile – white endosperm – green plants) x (polymitotic – yellow endosperm – purple plants)

Phenotype	Number
Fertile – white endosperm – green plants	306
Polymitotic – yellow endosperm – purple plants	239
Fertile – yellow endosperm – purple plants	46
Polymitotic – white endosperm – green plants	52
Fertile – white endosperm – purple plants	124
Polymitotic – yellow endosperm – green plants	124
Fertile – yellow endosperm – green plants	18
Polymitotic – white endosperm – purple plants	9
Total	**918**

545 Parental types

98 **SCO**: Po – Y1 $\frac{98+27}{918} x\ 100 = 13.6\%$

248 **SCO**: Y1 - Pl $\frac{248+27}{918} x\ 100 = 29.96\%$

27 **DCO**: Po - Pl $\frac{98+248}{918} x\ 100 = 37.7\%$

$\frac{27}{918} x\ 100 = 2.9\%$

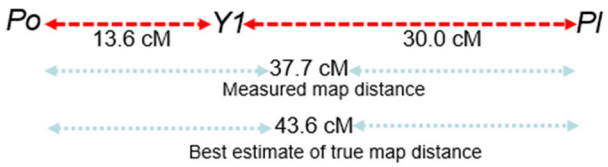
Map distances w/o DCO & with DCO

Po – Y1 $\frac{98}{918} x\ 100 = 10.67\%$ 10.67+ 2.9 = 13.6

Y1 - Pl $\frac{248}{918} x\ 100 = 27.0\%$ 27.0 + 2.9 = 29.9

Po - Pl $\frac{98+248}{918} x\ 100 = 37.7\%$ 13.6 + 29.9 =43.6

DCOs $\frac{27}{918} x\ 100 = 2.9\%$



Interference

- Observed DCOs = 27
- Expected DCOs = 0.136 x 0.30 = 0.041
  - Hence, 0.041 x 918 = 37.45

$$\frac{Observed}{Expected} = \frac{27}{37.45} = 0.72 = coefficient\ of\ coincidence$$

Interference = 1 - CC = 1 - 0.72 = 0.28 = 28%

Your Pseudonym: _____

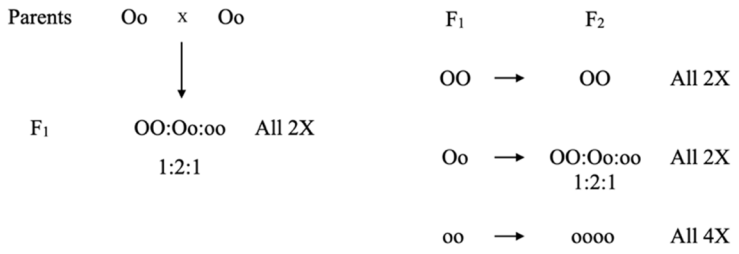
The following is from (will be revealed after exam):

d'Erfurth I, Jolivet S, Froger N, Catrice O, Novatchkova M, Mercier R. Turning Meiosis into Mitosis. PLOS Biology. 2009;7(6):e1000124-e.

OSD1 represents *Omission of Second Division1* gene. Nooseen (No-0), Landsberg (Ler), and Columbia (Col) are various ecotypes of arabidopsis.

a) *osd1* mutation in arabidopsis is completely recessive to wild type *OSD1*. Authors crossed a *OSD1*/*osd1* No-0 plant with a *OSD1*/*osd1* Ler plant to obtain F_1 progeny. What will be various genotypes (with respect to *OSD1* gene; ignore the ecotype) and their ploidies observed in F_1 progeny. If each of these F_1 genotypes is selfed, what will be various genotypes and their corresponding ploidies in the F_2 progeny.

As *osd1* is recessive to *OSD1*, all the gametes from *OSD1/osd1* plant will be normal (haploid), which after fertilization will produce diploid F_1 progeny. *OSD1/OSD1* and *OSD1/osd1* F_1 plants will produce diploid F_2 progeny on selfing, while *osd1/osd1* F_1 plants will produce diploid gametes and hence tetraploid F_2 progeny.



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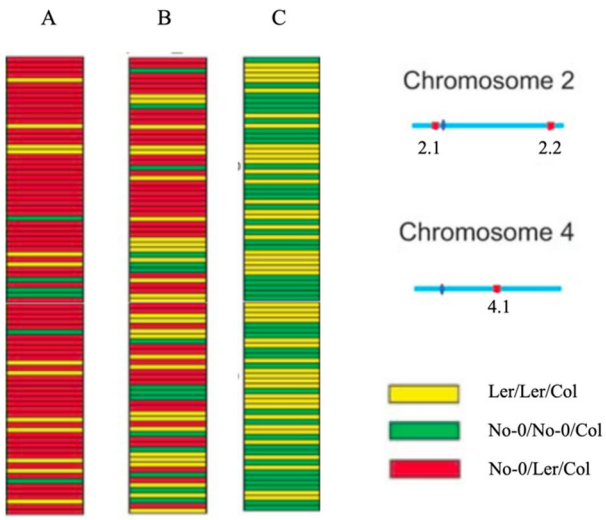
b) Authors crossed an *osd1*/*osd1* No-0/Ler F_1 plant obtained above, with a WT Col plant. The resulting triploid progeny was genotyped for markers spread across the genome. Genotyping results for three markers are shown below, where each horizontal line represents an individual F_2 plant. Plants carrying the No-0 alleles are in green, plants carrying the Ler alleles are in yellow, and plants with both the No-0 and Ler alleles are in red (Col allele should be present in all the plants). Two of these three markers (2.1 and 2.2) belong to chromosome 2 (shown as the red dots on blue chromosomes in the image) and one (4.1) belongs to chromosome 4. Violet colored line represents centromeres. Based on the genotypic profile of the progenies, deduce the corresponding matching of A, B and C markers with the 2.1, 2.2 and 4.1 markers. Explain your results and use space on next page to answer.



osd1/*osd1* No-0/Ler F_1 plant will produce 2n gametes. If the CO happens between a given marker and centromere, it will produce heterozygous (No-0/Ler) 2n gametes, and the resulting progeny is indicated by red color. However, if there is no CO between the marker and centromere, the resulting 2n gametes will be homozygous either for No-0 or Ler, and resulting progeny is indicated by green and yellow colors respectively. If a marker is close to centromere (like 2.1), there are less chances of a CO happening between this marker and centromere, leading to homozygous gametes, while if a maker is away from centromere (like 2.2), there are good chances that a CO will happen between marker and centromere, leading to heterozygous gametes. A gene at an intermediate distance (like 4.1) will have both homozygous and heterozygous gametes with good representation. So, A is likely 2.2, B is 4.1 and C is 2.1.

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Extra credit:

c) Now that you have identified that which markers from 2.1, 2.2 and 4.1 are represented by A, B, and C, estimate the distance of each of the marker (2.1, 2.2 and 4.1) from its centromere. The F_2 genotyping data is summarized here.

Marker → Progeny genotyping category ↓	A	B	C
Red	70	45	0
Yellow	13	29	45
Green	6	15	44
Total	89	89	89

Meiosis in *osd1/osd1* resembles SDR, so recombination frequency is represented by

Recombination frequency = frequency(heterozygotes)/2 = f(red)/2

Recombination frequency b/w A (2.2) and centromere 2 = 70/89/2 = 0.39
∴ Distance b/w A and centromere 2 = 39 cM

Recombination frequency b/w B (4.1) and centromere 4 = 45/89/2 = 0.25
∴ Distance b/w B and centromere 4 = 25 cM

Recombination frequency b/w C (2.1) and centromere 2 = 0/89/2 = 0
∴ Distance b/w C and centromere 2 = 0 cM

c) Suppose that you want to engineer MiMe (mitosis instead of meiosis) in arabidopsis. What two additional genes, you would need to knock-out in that *osd1*/*osd1* No-0/Ler F_1 plant, in order to achieve that? Explain the effect of knocking-out of each gene.

Spo11 and *Rec8*. Knocking-out *Spo11* will disrupt pairing of homologous chromosomes at Metaphase I, while knocking-out *Rec8* will lead to separation of sister chromatids during Anaphase I instead of at Anaphase II.

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d) Let's say you were successfully able to engineer MiMe in *osd1*/*osd1* No-0/Ler plant, and you have T_0 plants with homozygous recessive mutations for all three required genes. In the T_1 progeny obtained by selfing, what will be ploidy of the plants? If you run same markers as from part b) (2.1, 2.2, and 4.1), which color (yellow, green, or red) for each marker would you assign to these T_1 plants? Explain your choice.

As MiMe plants produce diploid gametes, the resulting T_1 progeny will be tetraploid. Because of the mitosis like division instead of meiosis, the resulting gametes produced by T_0 plants will be heterozygous (No-0/Ler), and hence all the markers in the T_1 tetraploid (No-0/No-0/Ler/Ler) progenies will be indicated by red color.

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Question	Points
1	5
2a	2
2b	2
2c	2
3	5
4a	3
4b	3
4c	3
4d	3
4e	3
5a	3
5b	3
6	5
6b	4
7a	4
7b	5
8a	4
8b	4
8c	4
8d	4
8e	4
9a	3
9b	3
9c	3
9d	3
9e	3
10	5
11	5
XC1	5
XC2	5
XC3	5
Total	115