Your Pseudonym: ____

PBGG 8890 Plant Cytogenetics

Behavior & Evolution of the Plant Genome

Spring 2025 ● Exam 2

Complete this sheet and mail to Kendall Erkens, Kendall.Erkens@uga.edu.

Also Email your completed exams to Kendall.

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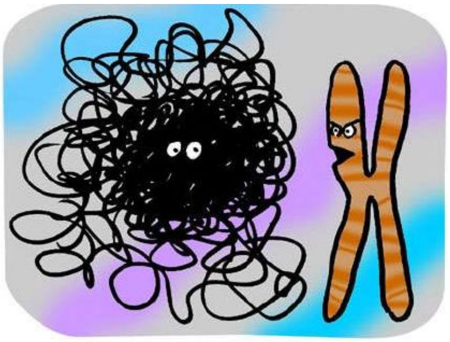
The email time stamp must be **before 5 pm on Tuesday May 6**



Dude, mitosis starts in five minutes...
I can't believe you're not condensed yet.

http://www.promega.com/

Name: ____

Pseudonym: ___________________________ Use same as for exam 1

The exam is open **notes**. Textbooks are OK as well. But, it must be your own work, and you cannot communicate with others about it- this includes AI. 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, etc.

Name in lieu of a signature: ______________________________

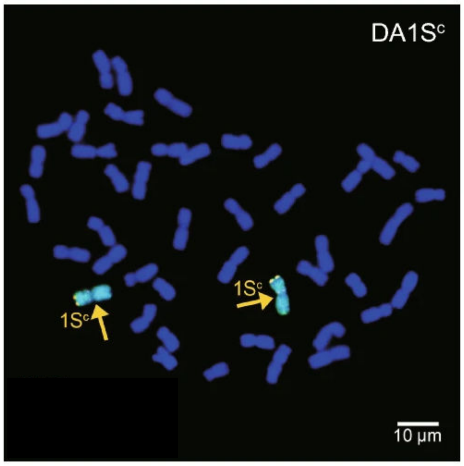
Date: ______________________________

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

Your Pseudonym: ____

The following is from *(will be revealed after the exam)*

Cheng M, H Zhang, Y Zhang, X Tang, Z Wang, X Zhang, X Song, X Li, H Cui, T Wang, R Song, J Xiao, H Wang, & X Wang. 2024. Cytological mapping of a powdery mildew resistance locus *PmRc1* based on wheat-*Roegneria ciliaris* structural rearrangement library. Theoretical and Applied Genetics, 137: 276. doi.org/10.1007/s00122-024-04768-w



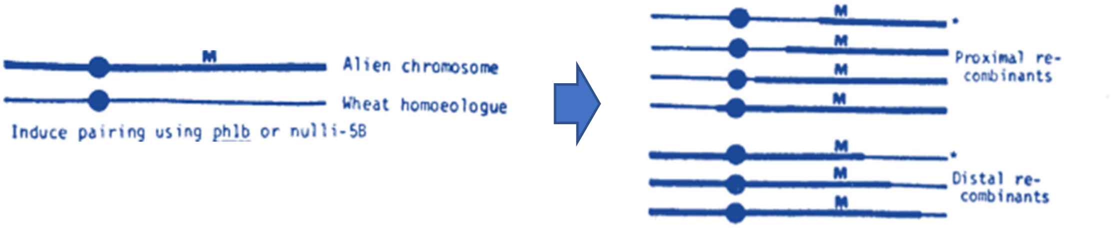
The photo shows a disomic addition line of wheat, and the alien chromosomes have a needed resistance gene on them.

a) Diagram how to make a disomic addition line

Wheat (recipient)× donor
↓
× F1
↓
× BC1
↓
× BC2 or 3
↓
wheat with one 1S from donor
↓ ⊗
wheat with two 1S from donor = disomic addition line

b) Commercializing the disomic addition line is not an option because the alien chromosomes have too many detrimental genes on them. Outline a **non-biotech** strategy to move the R gene from the alien chromosomes into the wheat genome.

They could always induce a translocation, but if they use the ph1/ph1 Chinese Spring as the recurrent parent for the disomic line, they can get homoeologous pairing between 1S and either 1A, 1B, or 1D.



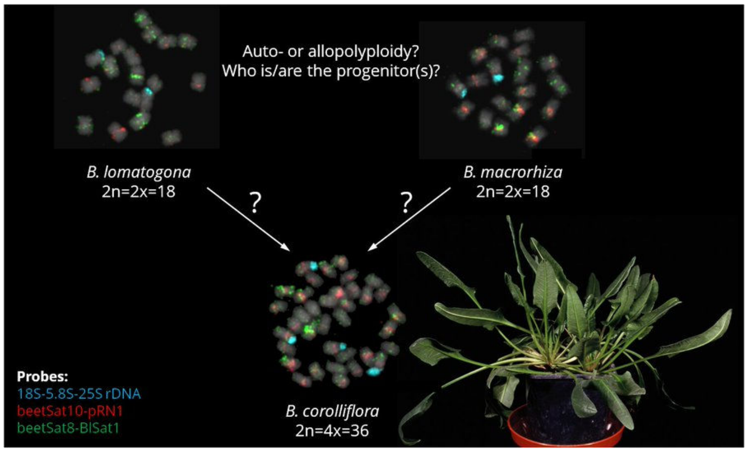
Use molecular markers to find distal and proximal recombinants that still have the trait.

Cross the distal and proximal recombinants that have the perennial trait and the largest amount of wheat DNA

The following is from *(will be revealed after the exam)*

Your Pseudonym: ____

Sieleman K, N Schmidt, J Guzik, N Kalina, B Pucker, P Viehöver, S Breitenbach, B Weisshaar, T Heitkam & D Holtgräwe. 2023. Pangenome of cultivated beet and crop wild relatives reveals parental relationships of a tetraploid wild beet. BioRxiv, doi: https://doi.org/10.1101/2023.06.28.546919



Wild beet species are of great interest, as they have a lot of traits useful to breed sugar beets. One species of interest is *Beta corolliflora* (2n=4x=36). Its origin is unknown—there are 3 hypotheses:

- It is an allotetraploid between *B. lomatogona* and *B. macrorhiza*, each of which is 2n=2x=18
- It is an autotetraploid derived from *B. lomatogona*
- It is an autotetraploid derived from *B. macrorhiza*

The authors first tried to use FISH on the landmarks, but the results were inconclusive. They then selected unique sequences from each parent, and they found that *B. corolliflora* share most of the *B. macrorhiza* sequences. The conclusion is that *B. corolliflora* is an autotetraploid of *B. macrorhiza*.

However, as explained in class, assigning type of ploidy often boils down to the weight of the evidence. Describe/explain 3 other lines of additional evidence you would like to see before reaching any conclusions.

- Calculate a t_1 or Tau statistic to determine if pairing is disomic, tetrasomic, or something in between
- Is it an outcrosser or a selfer?
- Extent to which quadrivalents are present
- Resynthesize the tetraploid
- Enzymology
- Look for presence of nuclear domains

We already know chromosome # is divisible by 4, so that is not informative. As seen from the photo, karyotype symmetry or whether the chromosomes occur in pairs or groups of four will not be helpful due to their tiny size.

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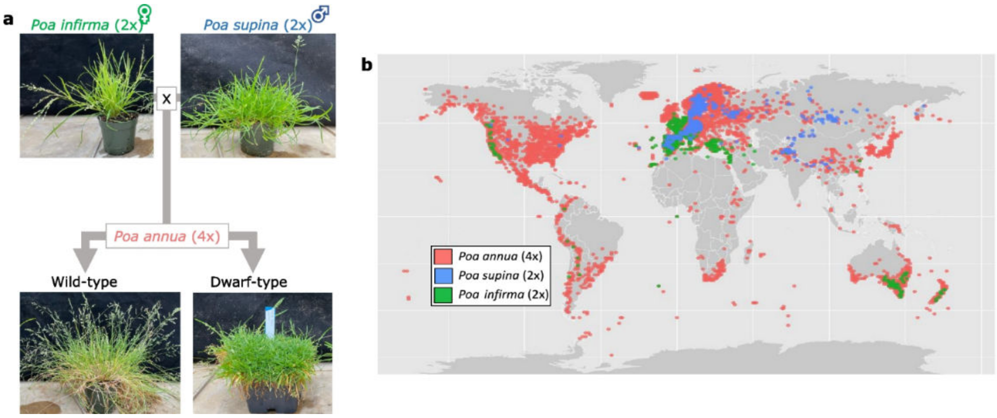
The following is from *(will be revealed after the exam)*

Benson CW, MR Sheltra, PJ Maughan, EN Jellen, MD Robbins, BS Bushman, EL Patterson, ND Hall & DR Huff. 2023. Homoeologous evolution of the allotetraploid genome of *Poa annua* L. *BMC Genomics 24: 350* . *https://doi.org/10.1186/s12864-023-09456-5*

Annual bluegrass (*Poa annua*) is an allotetraploid derived from *P. infirma* x *P supina*. As shown in the map, *P. annua* is considered to be one of the most successful weeds on earth.



Briefly explain/describe 3 attributes of alloploids that can explain this widespread success.

- If the parents of an alloploid are adapted to different environmental niches, the resulting hybrid can inherit both parents' adaptations. These may be based on
    - Multiple alleles at a locus permit phenotypic plasticity
    - Metabolic richness can be adaptive (eg, diversity of defense compounds)
    - Enzymatic and biochemical diversity also leads to adaptability

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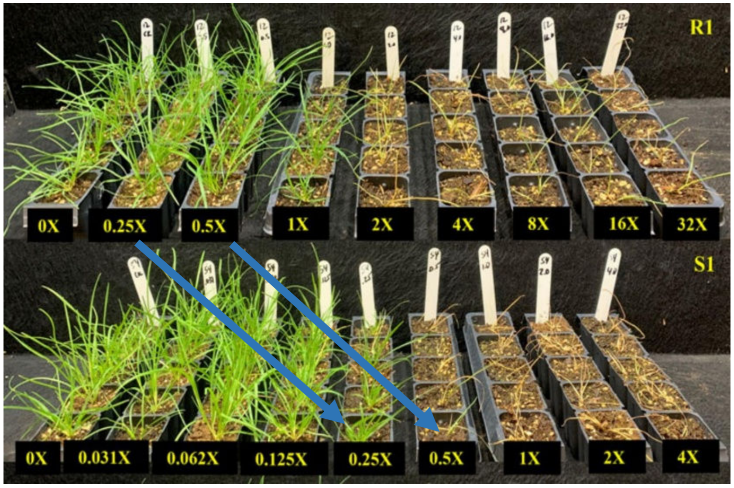
The following is from *(will be revealed after the exam)*

Sudhakar S, JK Norsworthy, T Avent, F González0Torralva, S McElroy & TR Butts. 2025. Confirmation of glyphosate resistance in annual bluegrass (*Poa annua*) via *EPSPS* duplication in a soybean and rice rotation. Weed Science 73(1): 1-9. doi.org/10.1017/wsc.2024.86.

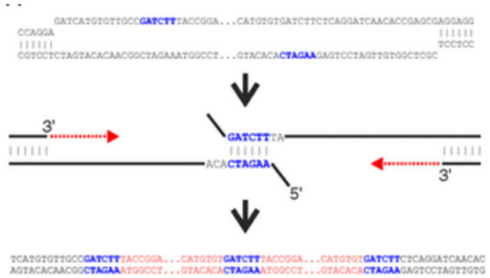
This study is also about annual bluegrass. A glyphosate-resistant population was identified in Arkansas and found to have 15x more EPSPS copies than susceptible genotypes.

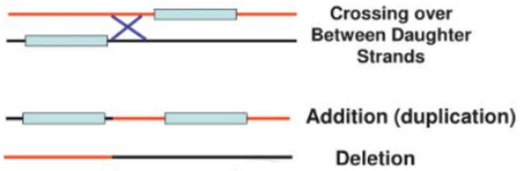


Response of resistant (top) and susceptible (bottom) plants to applications of glyphosate. 1x is the normal rate applied in the field. Blue arrows point out corresponding rates applied.

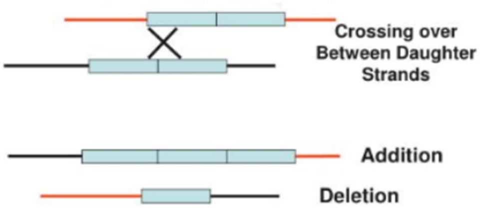
Diagram 2 methods that can result in paralog formation and 2 methods that will then amplify the paralogs to numbers consistent with the ones observed in this study.

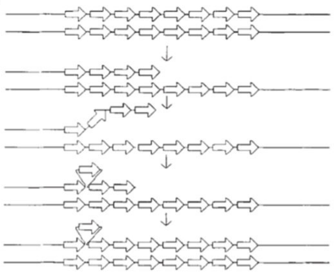
Start by creating a duplication via double strand break repair or unequal crossing over:





Then, additional unequal COs or replication slippage can increase copy number





The relatively "low" copy number probably means extrachromosomal circles are not involved or the copy number would be much higher. Transposon capture also not known to lead to high copy #s.

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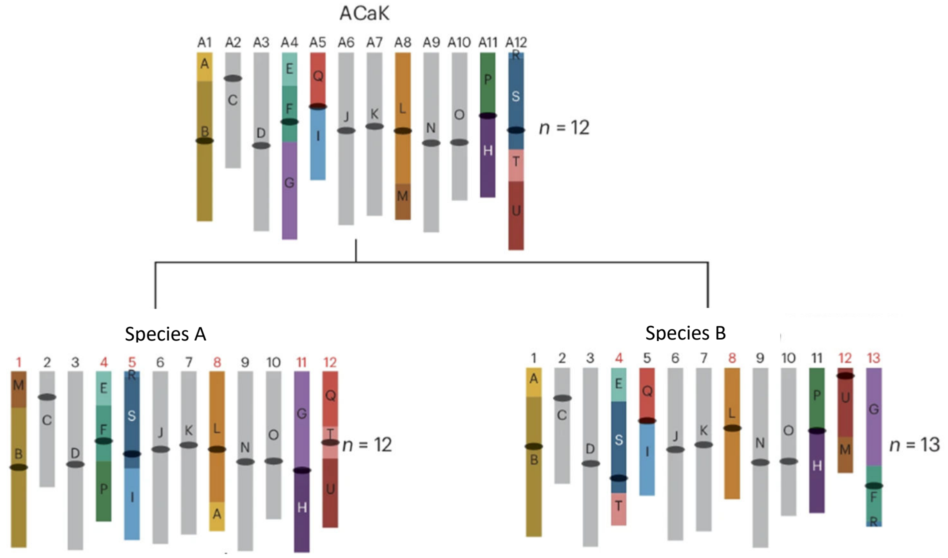
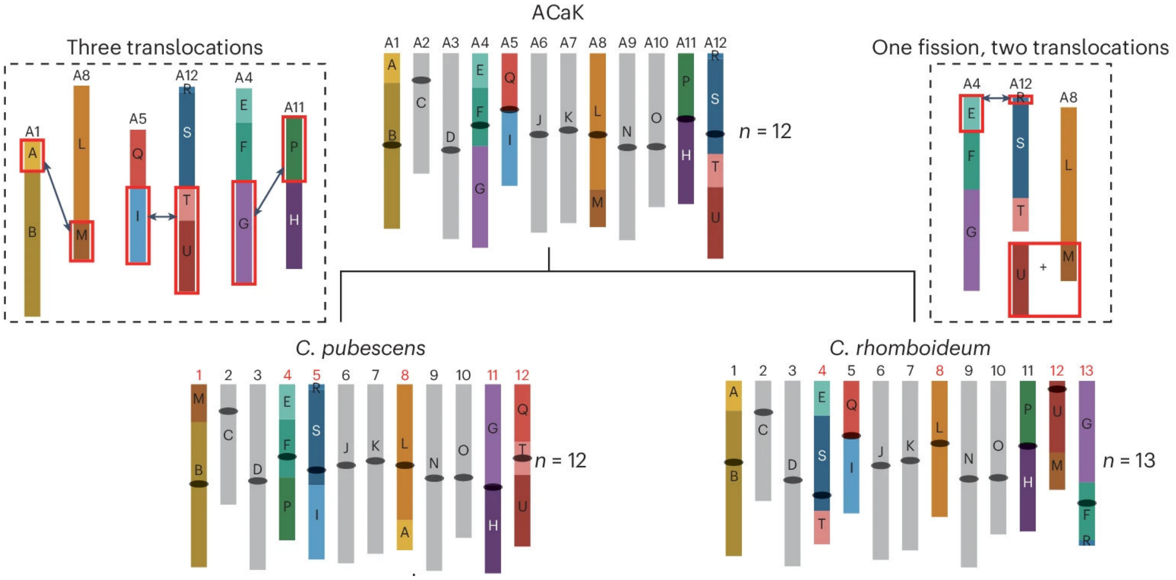
***The following is from** (will be revealed after the exam)*

Zhang K, H Yu, L Zhang, Y Cao, X Li, Y Mei, X Wang, Z Zhang, T Li, Y Jin, W Fan, C Guan, Y Wang, D Zhou, S Chen, H Wu, L Wang & F Cheng. 2025. Transposon proliferation drives genome architecture and regulatory evolution in wild and domesticated peppers. Nature Plants, 11: 359-275. https: //doi.org/10.1038/s41477-025-01905-1

This paper looks at the genomic evolution of 2 species of pepper from a common ancestor:



Describe or diagram the specific chromosomal rearrangements needed to go from the ancestral genome shown to the derived genomes. Hint – one requires 3 translocations & the other requires 1 fission and 2 translocations

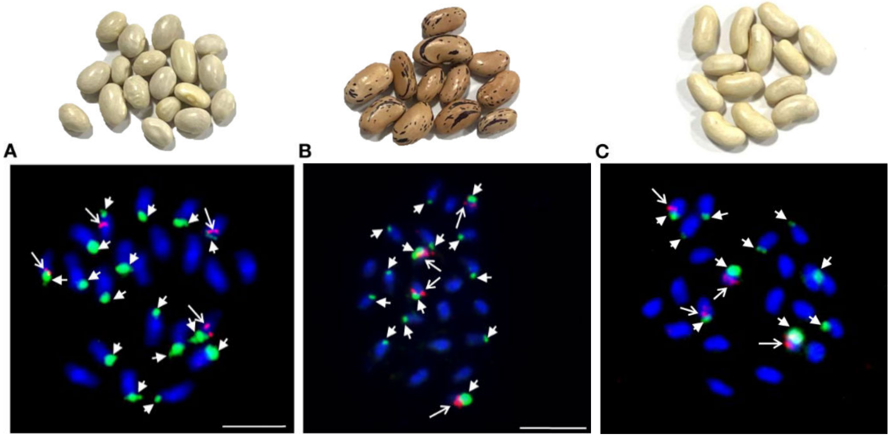


Your Pseudonym: ____

The following is from *(will be revealed after the exam)*

Tomlekova N, D Idziak-Helmcke, P Franke, M Rojek-Jelonek & J Kwasniewska. 2024. *Phaseolus vulgaris* mutants reveal variation in the nuclear genome. 14: https://doi.org/10.3389/fpls.2023.1308830

In this paper, the authors looked at 3 Bulgarian bean genotypes- A) BM2; B) BM4, and C) Evros.



	2n	5S rDNA sites	35S rDNA sites
BM2	22	4	16
BM4	22	4	14
Evros	22	4	10

While all these genotypes have the same number of 5S rDNA sites (red FISH with arrows), they differ in their number of 35S (usually called 45S) rDNA sites (green FISH with arrow heads). Not only do these latter differ in number, but they also differ in intensity/brightness.

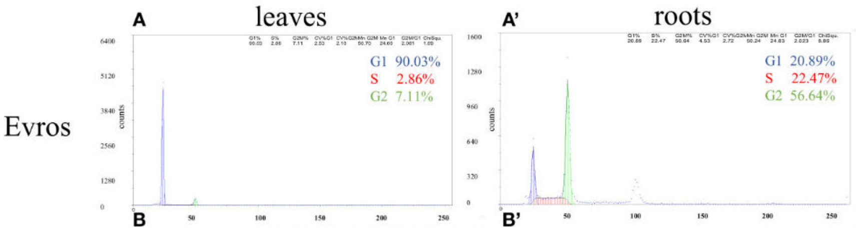
a) Explain why the different 35S rDNA sites would differ in intensity/brightness

Differences probably due to changes in repeat copy number at each locus, brought about by unequal CO or replication slippage

b) Explain how the 35S rDNA sites can change in number or location

Intrastrand recombination will excise tandem repeats, which can then insert elsewhere in the genome. These might undergo rolling circle replication prior to insertion.

c) Explain why there is so much tetraploidy in the root tissues



Beans are legumes, and their nitrogen-fixing nodules on the roots are known to have double the chromosome number.

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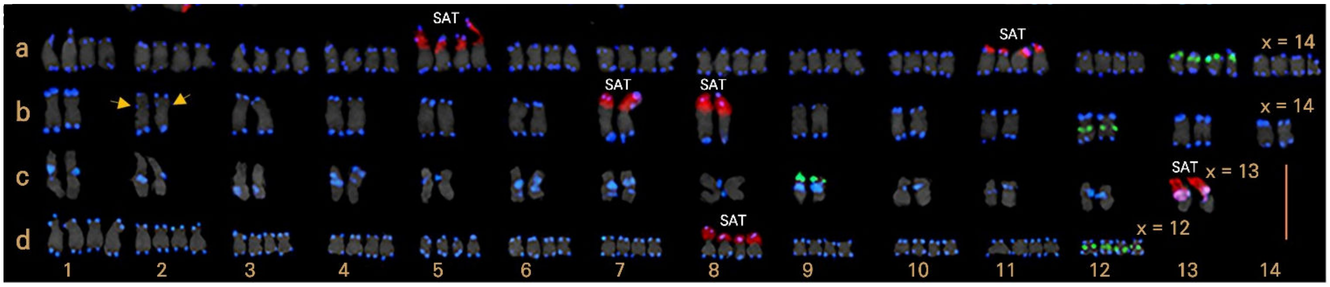
The following is from *(will be revealed after the exam)*

Dinh TMP, BY Kang, HH Kim. 2024. FISH Mapping of four Senna species revealing genome dynamics during species diversification of the genus. Horticultural Science and Technology. 42(2): 180-187. https://doi.org/10.7235/HORT.20240015

https://apps.lucidcentral.org/ppp_v9/text/web_full/entities/sicklepods_465.htm

Sickle pod (*Senna obtusifolia*) is a common weed in Georgia. It is 2n = 2x = 26, while related species have different x numbers as seen below for four *Senna* species: *S. artemisioides* (a), *S. pallida* (b), *S. obtusifolia* (c), and *S. aphylla* (d).



a) Based on their karyotypes, what type of tetraploids are *S. artemisioides* and S. *aphylla*? Explain the specific traits used for your diagnosis.

Both appear to be autotetraploids:

- *S. artemisioides* – 2n number is a multiple of 4, chromosomes are in groups of 4.
- *S. aphylla* – 2n number is a multiple of 4, chromosomes are in groups of 4.

b) How would the chromosomes of *S. aphylla* become smaller than the ones of *S. artemisioides?* Describe two mechanisms that could be responsible.

- Intra-strand recombination between repeats excising the DNA between the repeats
- Unequal crossing over

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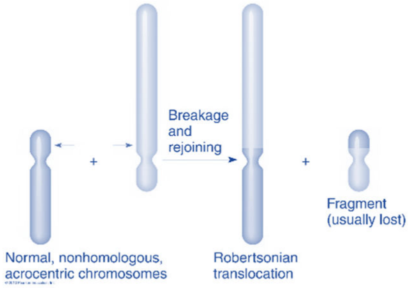
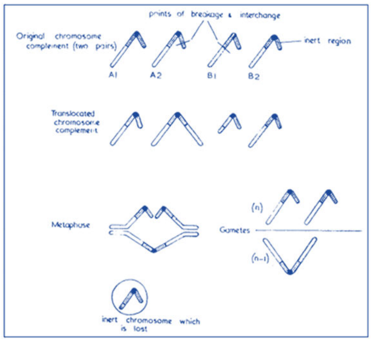
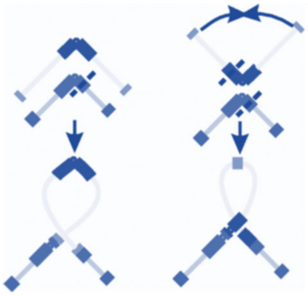
Continuing from the same paper as the previous question, *Senna* has gone from x = 14 to x = 13 and x = 12. Diagram 2 methods that could account for reduction in chromosome number.

- Robertsonian fusion
- Progressive reduction
- Chromosomal insertion into centromere



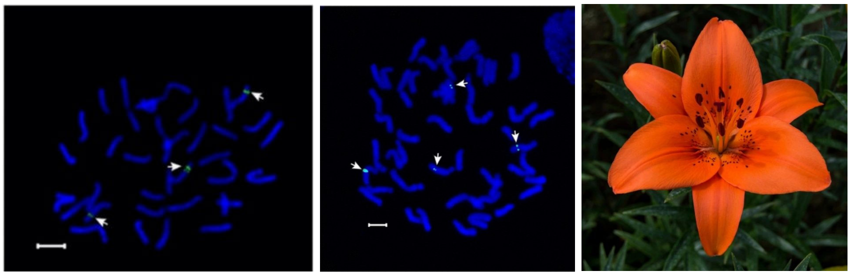




Your Pseudonym: ____

The following is from *(will be revealed after the exam)*

Liu S, J Chen, Y Sun & S Zhou. 2025. Fluorescence in situ hybridization confirms a theory–Triploid *Lilium* produce variable aneuploidy eggs and invariable hexaploidy secondary nuclei. Euphytica, 221: 45 doi.org/10.1007/s10681-025-03497-5

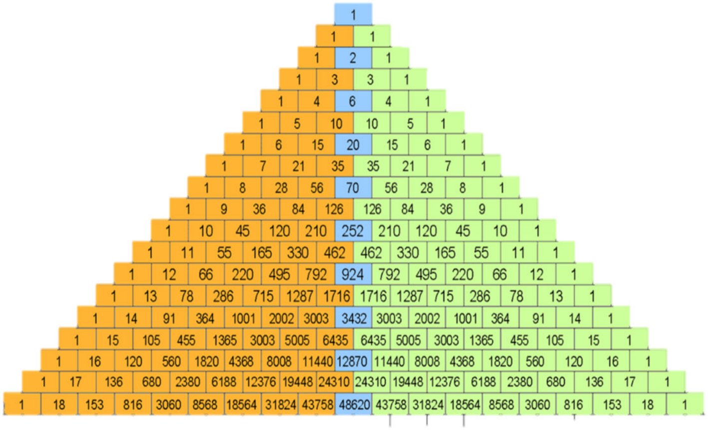


Chromosomes of 'Ningmeng' (3x =36), and of 'Tresor' (4x=48) & flower of Tresor, a common daylily variety.

In this work, the authors crossed Ningmeng x Tresor. They obtained 7 seeds, the chromosome number of which ranged from 43 to 60, meaning that Ningmeng was producing eggs with chromosome numbers ranging from 19 (= n=x+1) to 36 (= 2n).

a) Fill in the appropriate binomial terms and calculate the expected frequency of the egg chromosome numbers shown below from Ningmeng:

Egg chromosome number	18 n	19 n+1	20 n+2		35 2n-2	34 2n-1	36 2n
Binomial terms	a^{18}	$18ab^{17}$	$153a^2b^{16}$		$153a^{16}b^2$	$18a^{17}b$	b^{18}
Frequency	0.000038	0.000068	0.0005836		0.0005836	0.000068	0.000038



1
1 1
1 2 1
1 3 3 1
1 4 6 4 1
1 5 10 10 5 1
1 6 15 20 15 6 1
1 7 21 35 35 21 7 1
1 8 28 56 70 56 28 8 1
1 9 36 84 126 126 84 36 9 1
1 10 45 120 210 252 210 120 45 10 1
1 11 55 165 330 462 462 330 165 55 11 1
1 12 66 220 495 792 924 792 495 220 66 12 1
1 13 78 286 715 1287 1716 1716 1287 715 286 78 13 1
1 14 91 364 1001 2002 3003 3432 3003 2002 1001 364 91 14 1
1 15 105 455 1365 3003 5005 6435 6435 5005 3003 1365 455 105 15 1
1 16 120 560 1820 4368 8008 11440 12870 11440 8008 4368 1820 560 120 16 1
1 17 136 680 2380 6188 12376 19448 24310 24310 19448 12376 6188 2380 680 136 17 1
1 18 153 816 3060 8568 18564 31824 43758 48620 43758 31824 18564 8568 3060 816 153 18 1

b) Without knowing the number of crosses the authors made, it is impossible to determine the frequency of viable eggs. But based on your calculations from a), is the frequency of viable eggs higher than expected.

Higher.

B) One of the progeny plants was 2n = 61, leading the authors to conclude that the egg was 2n+1. What is another, perhaps more likely source of this extra chromosome?

Not sure where the extra chromosome could have come from for the egg. But the pollen could have been n+1, the result of non-disjunction.

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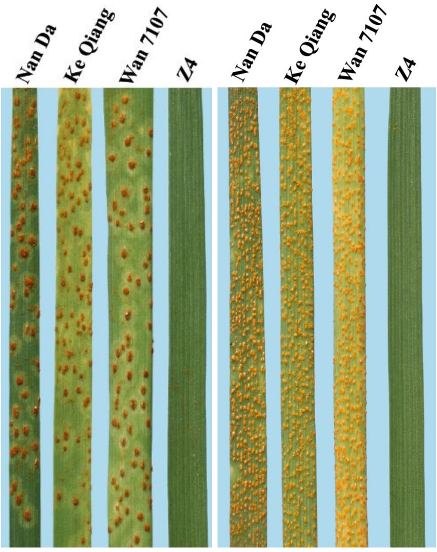
The following is from *(will be revealed after the exam)*

Li J, M Ryan, C Dong, KL Forrest, MJ Hayden, S Singh, Y Wang, N Ahmed, RA McIntosh & P Zhang. 2025. Pseudo-linkage or real-linkage of rust resistance genes in a wheat-*Thinopyrum intermedium* translocation line. Theoretical and Applied Genetics. 138: 15. https://doi.org/10.1007/s00122-024-04807-6

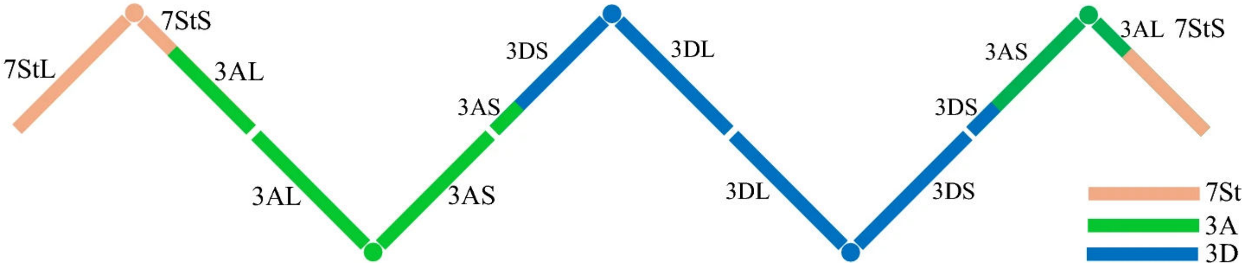


Z4, a genotype with resistance to leaf rust (left) and stripe rust (right).

In this study, the authors found that S_t genome alien-species-derived genes for leaf rust resistance are on a translocation of chromosome 3A containing a translocation of 3DS on its short arm and of 7StS on its long arm (T3DS-3AS.3AL-T7S_tS). Stripe rust resistance is on a translocation of 3AL onto chromosome 7S (T3AL-7S_tS.7SL).

At meiosis, these chromosomes form a chain pentavalent (V) with the translocation chromosomes at the ends of the chain and the wheat chromosomes in the middle.

a) Draw the chain V labeling all the arms and the segments involved in the translocation.



T7SL.7S_tS-3AL: 3A: T3AS-3DS.3DL: 3D: T3DS-3AS.3AL-7StS

b) Although the leaf rust resistance and the stripe rust resistance are not linked in the S genome donor parent, they are linked in this wheat line. Explain why.

Pseudolinkage, whereby only non-recombinant chromosomes are recovered together, as recombinant chromosomes will be duplicate-deficient, leading to abortion of the gametes that contain them.

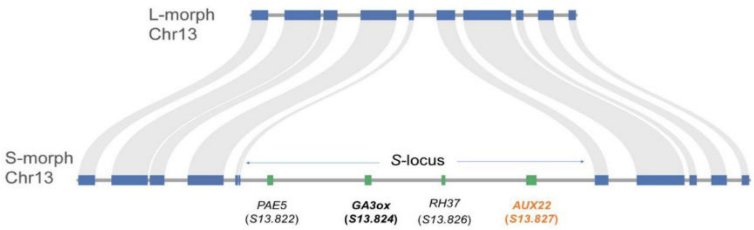
Your Pseudonym: ____

The following is from *(will be revealed after the exam)*

Luo Z, SCH Barrett, T Tu, Z Zhao, S Jia, S Gu, T Duan, Y Zhang, B Xu, L G, X Deng, L Jiang, M Shi & D Zhang. 2025. Genetic architecture of the S-locus supergene revealed in a tetraploid distylous species.
BioRxiv, https://doi.org/10.1101/2025.04.01.646584.

Supergenes are a group of genes that together give a desired phenotype. Examples of traits conditioned by supergenes include apomixis, sex determination, and distyly. The latter refers to a condition whereby plants can have short or long styles combined with long or short anthers as a way to prevent self pollination. A key feature of supergenes is that their recombination is suppressed, thus keeping all the necessary component alleles together.

In this study, the authors determined that distyly was conditioned by a supergene. Its recombination is suppressed by maintaining the supergene as a hemizygote, as shown below. As long as the plant does not self, it cannot become homozygous.



This figure is from another paper and is here just to illustrate what distyly is. doi:10.1093/jxb/erx387

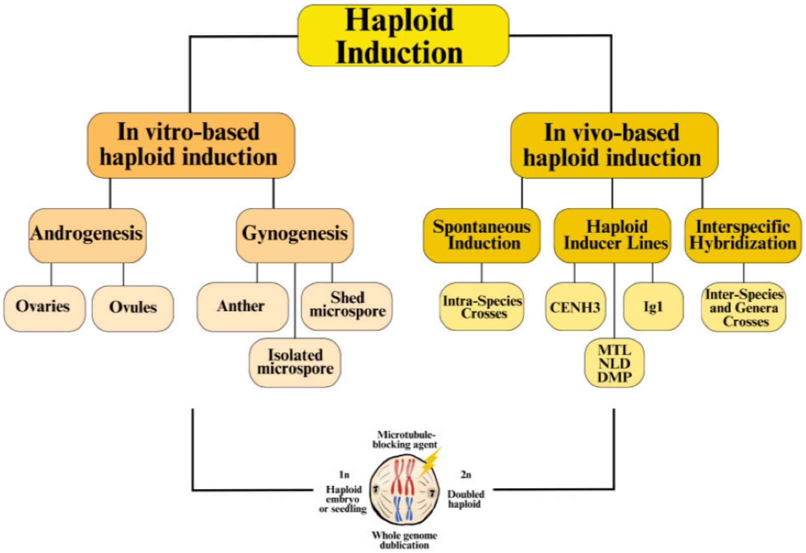
Your goal is to breed or engineer a supergene in a self-pollinated crop. Describe two strategies that you could use to achieve your goal.

- Induce an inversion of the segment having the supergene
- Insert the supergene into the pericentric heterochromatin, or even the centromere
- Ensure the supergene is devoid of nucleosome exclusion sites

Your Pseudonym: ____

The following is from *(will be revealed after the exam)*

Liu Y, M Elshan, G Li, X Han, X Chen & X Feng. 2025. Perspectives of genome editing mediated haploid inducer systems in legumes. International Journal of Molecular Sciences 26(3): 1154. https://doi.org/10.3390/ijms26031154



a) List the 2 errors in the figure

- Androgenesis & Gynogenesis labels are flipped
- Figure shows animal meiosis, not plant meiosis

b) List an advantages and disadvantages for each technique in the 3rd tier of techniques shown in the figure. You can consider isolated & shed micropores as 1 technique. Note: many of these have the same or similar advantages/disadvantages.

- In vitro based – all require specialized facilities, are very species-dependent.
- Ovule culture can provide more normal plants than anther culture.
- Ovary & anther culture can result in plants from somatic cells- difficult to tell apart from doubled haploids
- Spontaneous – frequency too low to be useful
- CENH3 – works well for species that are transformable and have low chromosome number
- MTL, NLD – MTL and NLD work in maize- have not been implemented in other spp.
- DMP works in dicots, albeit a low frequency & has not been implemented beyond arabidopsis
- Ig1 – only works in maize & barley- other plants might could be edited for it
- Interspecific hybrids – most widely used method; depends on finding the right species to cross.

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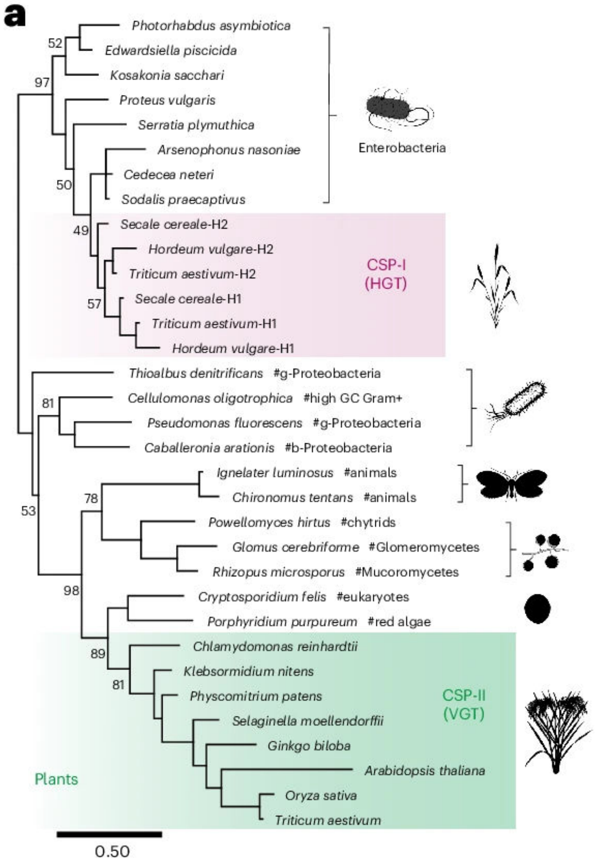
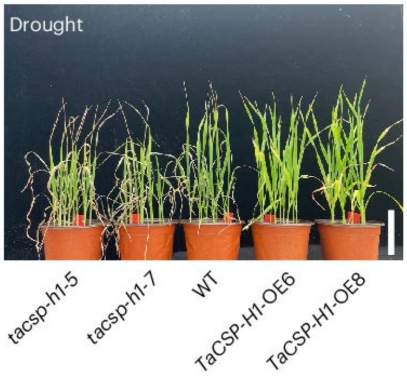


The following is from *(will be revealed after the exam)*

Song C-P. 2025. Horizontal gene transfer of cold shock protein genes boosted wheat adaptation and expansion. *Nature Plants* https://doi.org/10.1038/s41477-025-01985-z

Wheat and related grasses have two groups of Cold Shock Proteins. One is shared with other plants, and the other shared with bacteria. Both the bacterial group and the plant group are derived from a common ancestor (i.e., vertically derived from a common ancestor, making them orthologs).

Overexpression of the type I CSP gene in transgenic plants enhances drought tolerance:



The CSP-I genes are more closely related to bacterial CSP genes than to plant CSP genes, meaning an ancestral grass acquired them via horizontal gene transfer. Explain i) how genes can move from bacteria to plant cells, and ii) how these genes eventually are able to be seed-transmissible. *You might need to do additional research to answer these questions.*

How gene transfer happens between bacteria and plants is not clear, but may involve the transfer of chromosomal DNA by bacteria such as agrobacteria. Once in the nucleus, it must land behind a promoter, or get moved behind a promoter during a genomic rearrangement.

Entering a somatic cell does not make the gene seed-transmissible. It needs to get into the L2 (germline) cell layer. This could happen by wound callus regenerating into a shoot, or by cells diving in the wrong plane. Some plants can produce de novo shoots from roots or other tissues in the absence of a bud.