

The observed frequency of homozygous recessive gametes can be used to calculate α . With α , the following formulae can be used to answer several questions in this problem set:

The first formula can be used to calculate the remainder of the gametic frequencies in a duplex (assuming only one crossover per chromosome):

$$\frac{1+2\alpha}{6} \times AA + \frac{4-4\alpha}{6} \times Aa + \frac{1+2\alpha}{6} \times aa = 1$$

Furthermore, the second equation can be used to calculate the gametic frequencies of a triplex:

$$\frac{2+\alpha}{4} \times AA + \frac{2-2\alpha}{4} \times Aa + \frac{\alpha}{4} \times aa = 1$$

Finally, the third equation can be used to calculate the gametic frequencies in a simplex:

$$\frac{\alpha}{4} \times AA + \frac{2-2\alpha}{4} \times Aa + \frac{2+\alpha}{4} \times aa = 1$$

1. In tomato, an F₂ population from plants trisomic for chromosome 2 segregated 143 tall and 15 dwarf. The trisomic for chromosome 4 segregated 146 tall and 15 dwarf. Explain the above results.

2. In corn, chromosome 6 has a gene Y that codes for yellow endosperm. A homozygous white endosperm plant trisomic for chromosome 6 is used as female with a homozygous yellow stock. Root tip counts are used to differentiate between normal and trisomic plants in the F₁. One plant of each type is then crossed with a yy male plant. Assuming that the transmission rate for the extra chromosome is 26%, ¿what percentage of the progeny will have yellow endosperms if the female was the trisomic Can you tell the trisomic from the non trisomic based on segregation? ¿If the ♀ was not trisomic?

3. In tobacco (an allotetraploid) there are two genes that condition for the appearance of the yellow burley character, A and B. When both of these loci are homozygous recessive (i.e., aabb) the plant is yellowish in color. When any dominant allele is present (A and/or B), the plant is green.

a) A cross is made between a homozygous green strain monosomic for one of these chromosomes (ABB) and a disomic yellow strain, ¿what is the ration of phenotypes and genotypes recovered in the F1? Assume viability of the gametes is not affected by the lack of one chromosome.

b) If one monosomic and one disomic F1 are each, ¿what ratio of phenotypes would you expect in the respective F2 of each plant?

4. You've just received a potted plant for Christmas. Since you are now taking CRSS 8890, you count the chromosomes in root tip squashes. All the cells have 15 chromosomes. Possible ways of accounting for the odd rather than even count of somatic chromosomes include aneuploidy or an XXY sex determining mechanism. There are a number of other possibilities. Cite two of these and describe what you would expect to observe in meiotic cells in the two cases.

5. The following information is from:

Plewa, M.J., and D.F. Weber. (1973). The use of monosomics to detect genes conditioning lipid content in *Zea mays* L. 1973. Can. J. Genet. Cytol. 15:313-320.

In this paper, the authors used nuclear magnetic resonance to measure the lipid content of diploid and monosomic embryos of corn. Other than the monosomic condition, all embryos were genetically identical.

Sub-population	No. Kernels tested	Mean % lipid	<i>t</i> values
Monosome 2	18	4.6	5.3*
Monosome 6	11	4.8	2.6*
Monosome 7	28	5.1	2.3ns
Monosome 8	74	5.3	-0.02ns
Monosome 10	22	5.0	2.8*
Diploid control	1282	5.4	--

A) What is the interpretation/significance of these results?

B) What is the origin of the monosomic lines.

6. In the following paper:

Menken, S.B.J., E. Smit, and H. (J.) C.M. den Nijs. 1995. Genetical population structure in plants: Gene flow between diploid sexual and triploid asexual dandelions (*Taraxacum* section *ruderalia*). Evolution 49:1108-1118.

the authors found evidence of considerable gene flow between diploid ($2n=2x=16$) and triploid ($2n=3x=24$) diplosporous dandelions. One possible mechanism for this would be if the triploid dandelions would be producing haploid ($n=x=8$) pollen. What is the probability with which triploid dandelions should produce such haploid pollen? Show all your calculations.

7. Triploid watermelons have $2n=3x=33$ chromosomes. Assuming that eggs with x , $x+1$, $x+2$, $2x$, and $2x-1$ are viable, and that pollen with x and $2x$ chromosomes are viable, and that plants with $2x$, $2x+1$, $3x-1$, and $3x$ chromosomes are viable, answer the following questions:
- ¿What percent of all pollen will be viable?
 - ¿What percent of all eggs will be viable?
 - Following ... pollination, ¿what frequency of zygotes will be viable?
 - Following pollination with pollen from a $2x$ ♂, ¿what percentage of the zygotes will be viable?

8. A cross was made between a plant of genotype *aaaa* and one of genotype *AAAa*. One percent of the progeny were homozygous recessive.

a) State if this gene is most likely to be near the centromere or near the telomere, and explain (in one sentence) the rationale behind your answer.

b) Give the gametic frequencies you would expect from a plant of genotype *AAaa*.

9. A cross was made between a plant of genotype *LgLg* and one of genotype *lglg* (liguleless). Nitrous oxide was used to double the chromosome number immediately after fertilization. If a value of $\alpha = 0.2$ is observed in the gametes, ¿what is the gametic ratio of the gametes?

Alternatively, a plant of genotype *Lglg* which produces unreduced pollen was crossed onto a ♀ plant of genotype *lglglglg*. ¿What is the gametic frequency produced by the F_1 ?

10. Many years ago in Kew Gardens, London, two ornamental primrose plants were crossed, *Primula verticillata* and *P. floribunda*. This cross produced an exceedingly vigorous hybrid that was unfortunately sterile but could be propagated vegetatively by cuttings. After many propagations, the hybrid yielded a branch that bore fertile seeds and was then distributed under the name *Primula kewensis*.

- a. ¿How would you explain the origin of *P. kewensis*?
- b. If the two original parents had haploid gametes containing 9 chromosomes in each, ¿how many bivalents would you expect to find in *P. kewensis* during meiosis?

11. Clausen and Cameron, 1944 (Genetics 29:447-477), crossed stocks of green tobacco plants (genotype *AABB*) that were monosomic for various chromosomes with a disomic yellow tobacco plant (genotype *aabb*). Then they backcrossed monosomic F_1 plants to the yellow parent. Their results for BC_1 progeny plants from 9 different monosomic plants were as follows:

Monosomic plant lacking chromosome:	BC ₁ Phenotypes	
	Green	Yellow
M	36	9
N	28	8
O	19	17
P	33	9
Q	32	12
R	27	12
S	27	4
T	28	8
U	37	8

Given that the presence of either *A* or *B* will result in a green plant, which of the chromosomes most likely contains one of the genes for yellow? In one sentence, what is the basis for your answer?

12. Bananas are $2n = 3x = 33$. Assuming that pollen with x and $2x$ chromosomes is viable, and that eggs with x , $x+1$, $x+2$, $2x$, and $2x-1$ are viable, and that seeds with $2x$, $2x+1$, $3x-1$, $3x$, $4x-1$, and $4x$ chromosomes are viable. Based on this information, answer the following questions: (SHOW ALL YOUR CALCULATIONS)

A) For breeding purposes, one alternative could be to make $3x-2x$ crosses, and depend on an x egg to get $2x$ progeny. If one ovary contains 100 ovules, how many flowers must a breeder pollinate to get one $2n = 2x = 22$ seed?

B) If the plantation superintendent got lax and placed the plantation of triploid bananas next to a grove of diploid wild bananas (ie., a source of pollen), what frequency of fruits off the triploid plants would be expected to have a seed in them?

13. The following paragraph is the abstract from:

Morton, J. F. 1994. Lantana, or red sage (*Lantana camara* L., {Verbenaceae}), notorious weed and popular garden flower - some cases of poisoning in Florida. *Economic Botany* 48:259-270.

"Lantana, or red sage (*Lantana carara*, Verbenaceae) is an aromatic shrub with rough, opposite or whorled leaves and four-angled, prickly or smooth stems, showy heads of small flowers (usually pink-and-yellow changing to red-and-orange) and dark blue or blackish fruits. Native from tropical America north to lower Texas and southern Georgia, the bird-distributed plant has become naturalized in the Caribbean area, the Pacific islands, Australia, New Zealand, South Africa and southern Asia. Hundreds of cultivars are grown as ornamentals in temperate climates."

One of the lantana cultivars sold in the Athens area is "Miss Huff". This cultivar differs from other lantana cultivars in that it is winter hardy in Athens. It is also larger and much more vigorous than other lantanas. Finally, while other lantanas have almost complete seed set, Miss Huff sets seed only in extremely rare occasions.

Having taken CRSS 8890, you immediately suspect that this plant is a triploid or an aneuploid. You collect a root tip, and count the chromosome number, and arrive at $2n = 33$.

A) Describe how you would distinguish between the following possibilities for this cultivar:

- a) Lantanas are normally $2n = 2x = 32$, and this plant is a trisomic
- b) Lantanas are normally $2n = 2x = 34$, and this plant is a monosomic
- c) Lantanas are normally $2n = 2x = 22$, and this plant is a triploid
- d) Lantanas are normally $2n = 4x = 32$, and this plant is a trisomic
- e) Lantanas are normally $2n = 4x = 34$, and this plant is a monosomic

B) Assume for the time being that c) above is correct. Furthermore, assume that aneuploid gametes are all inviable. What is the probability that a cross between Miss Huff and a diploid lantana will provide:

- a) A diploid progeny
- b) A triploid progeny
- c) A tetraploid progeny

14. As a Christmas special, a seed company is advertising a special variety of an ornamental species. They claim that about 90% of the plants grown from the seed will be trisomic, and the resulting phenotype especially attractive.

- A) How they possibly produce seed lots with such a high percentage of trisomics?
- B) Why would trisomy affect the phenotype?
- C) Is this ornamental most likely to be a self pollinator or an outcrosser?

15. The following paper:

Kumar, H., V.C. Mercykutty, and D.P. Srivastava. 1993. Fertility improvement in autotetraploids of pea— selection for seed-set and disjunction index. Plant Breed. 112:81-83.

The authors selected two groups in a C_3 population ($2n=4x=28$) derived from cultivar T163. The two groups were characterized as follows:

Group	Quadrivalents	Bivalents
1	2.78	7.72
2	3.37	6.09

Seed set for one of the groups was 2.24 seeds/pod, while for the other it was 1.34 seeds/pod.

A. Which seed set belongs to which group?

B. Explain the reason behind your answer.

16. A seed catalogue of exotic bulbs offers a “Butterfly amaryllis” (*Hippeastrum papilio*) for sale.

Attracted by the caption that reads, “RARE SOUTH AMERICAN SPECIES LOOKS LIKE EXOTIC ORCHID!” you cannot resist the temptation and buy a bulb. As soon as root tips are available, you perform a root tip squash and find it has $2n = 48$ chromosomes.



- A) What are three possible ploidies for this species?
- B) List three things you would examine/do to determine if it is a diploid or polyploid,
- C) If this plant turned out to be a polyploid, list three things you would examine/do to determine if it is an allo or autopolyploid.

17. The following information is from:

Kuspira, J., and J. Unrau. 1957. Genetic analysis of certain characters in common wheat using whole chromosome substitution lines. *Can. J. Plant Sci.* 37:300-326.

Yield in grams of Chinese Spring, Thatcher, and Thatcher Substitutions into Chinese Spring.

Substitution lines and cultivars	Significantly higher than Chinese Spring	Same as or similar to Chinese Spring
1B	830.7	--
2A	--	682.0
2B	--	426.3
2D	--	590.3
3A	721.3	--
3B	704.9	--
4A	--	502.2
4B	763.5	--
4D	--	580.8
5A	--	637.8
Chinese Spring	--	523.7
Thatcher	1013.8	--

A) What is the interpretation/significance of these results?

B) Diagram a method used to obtain one of the above substitution lines.

The next two questions are based on work with sainfoin, a forage legume, by:

de Vicente, M.C., and P Arús. 1996. (...) inheritance of isozymes in sainfoin (*Onobrychis viciaefolia* Scop.). J. Hered. 87:54-62.

Sainfoin is a forage legume that is $2n=4x=28$. The authors were studying the inheritance of isozymes in an effort to determine if this plant had disomic or tetrasomic inheritance.



18 First, the authors studied the segregation of two allozymes of *Pgi-2* by selfing an individual which had 1 allele for a band of one Sainfoin size and 3 alleles for a band of another size (ie, of genotype $A_1A_2A_2A_2$). Dosage was determined by measuring band intensity. Fill in the table below (The first row has been filled in for you):

Progeny Genotype	Gametes involved	Gametic frequency for disomic	Frequency of progeny if disomic	Expected numbers of progeny if disomic	Gametic frequency for tetrasomic	Frequency of progeny if tetrasomic	Expected numbers of progeny if tetrasomic	Actually Observed
$A_1A_1A_1A_1$	$A_1A_1 + A_1A_1$	0×0	0	0	0×0	0	0	0
$A_1A_1A_1A_2$								0
$A_1A_1A_2A_2$								85
$A_1A_2A_2A_2$								156
$A_2A_2A_2A_2$								100
Totals:			1	341		1	341	341

In the above table:

- the first column has possible tetraploid genotypes.
- the second column has the gametes that would have to fertilize each other to obtain the genotype in the first column
- the third column has the expected frequency with which each gamete in the second column would be formed if disomic inheritance was involved. Notice that in this case, the original $A_1A_2A_2A_2$ is unable to produce A_1A_1 gametes as long as either disomic or random chromosome inheritance is involved. Hence, the frequency must equal 0. [Hint: the $A_1A_2A_2A_2$ genotype can be obtained from both $A_1A_2 + A_2A_2$ **and** from $A_2A_2 + A_1A_2$]
- the fourth column is the frequency with which each genotype in the first column would be obtained. It is the product of the frequencies in column 3. The frequencies in this column must add up to 1.
- the fifth column is the actual number of progeny expected of the genotype in column 1. This column must sum 341, the number of progeny evaluated.

- columns 6-8 are as columns 3-5, except for the tetrasomic situation
- column nine has the progeny genotypes which were actually recovered in this experiment.

Once you have filled in the entire table, answer the following question:

Is it possible to determine if sainfoin is showing tetrasomic or disomic inheritance based on this set of data? Explain your answer fully.

19. Next, the authors studied the segregation of two allozymes of *Pgi-2* by selfing an individual which had 2 alleles for a band of one size and 2 alleles for a band of another size (ie, of genotype $A_1A_1A_2A_2$). Dosage was determined by measuring band intensity.

Fill in the table below. The first row has been filled in for you:

Progeny Genotype	Gametes involved	Gametic frequency for disomic	Frequency of progeny if disomic	Expected numbers of progeny if disomic	Gametic frequency for tetrasomic	Frequency of progeny if tetrasomic	Expected numbers of progeny if tetrasomic	Actually Observed
$A_1A_1A_1A_1$	$A_1A_1 + A_1A_1$	0.25×0.25	0.0625	20.5	0.167×0.167	0.027889	9.15	10
$A_1A_1A_1A_2$								73
$A_1A_1A_2A_2$								150
$A_1A_2A_2A_2$								79
$A_2A_2A_2A_2$								16
Totals:			1	328*		1	328*	328

In the above table:

- the second column has the gametes that would have to fertilize each other to obtain the genotype in the first column
- the first column has possible tetraploid genotypes
- the third column has the expected frequency with which each gamete in the second column would be formed if disomic inheritance was involved. Notice that in this case, the original $A_1A_1A_2A_2$ can have two possible configurations: one set of homologues could be A_1A_1 and its homoeologues would be A_2A_2 . Alternatively, both sets of homoeologues would be A_1A_2 . Note too that one of these two possibilities can be immediately excluded based on the presence of certain genotypes in the progeny which would not be possible to obtain if that was the case.
- the - $A_1A_2A_2A_2$ genotype can be obtained from both $A_1A_2 + A_2A_2$ **and** from $A_2A_2 + A_1A_2$

- the fourth column is the frequency with which each genotype in the first column would be obtained. It is the product of the frequencies in column 3. The frequencies in this column must add up to 1.
- the fifth column is the actual number of progeny expected of the genotype in column 1. This column must sum 328 (*=within rounding error!), the number of progeny evaluated.
- columns 6-8 are as columns 3-5, except for the tetrasomic situation
- note that a **random chromosome** model is being used
- column nine has the progeny genotypes which were actually recovered in this experiment.

A) Do the data more closely fit a tetrasomic or a disomic pattern of inheritance?

B) Is there any obvious evidence double reduction is taking place?
C)

20. An off-type plant is found in a species with which you are working. How could one determine whether the variant was due to gene mutation or the presence of an extra chromosome? Give 3 criteria.
21. Triploid *Paeonia albiflora* has a chromosome number of $2n=3x=15$, and the chromosome distribution at meiosis is at random. Female gametes with x , $x+1$, $2x$, and $2x-1$ are functional. Male gametes with x , $2x$, and $2x-1$ chromosome numbers are functional. Plants with $2x$, $2x+1$, $3x$, and $3x-1$ survive.
- A) What is the expected frequency of non-viable pollen?
 - B) What percentage of the eggs are functional?
 - C) What are the expected frequencies of different chromosome numbers in the offspring obtained by selfing?

22. The following is from:

Tenkouano, A, JH Crouch, HK Crouch, and D. Vuylsteke. 1998. Ploidy determination in *Musa* germplasm using pollen and chloroplast characteristics. HortSci 33:889-890.

The authors measured the diameter of pollen produced by genotypes of different ploidies, and came up with the following data:

Ploidy Class	Observed pollen diameter (μm)	Expected pollen diameter
Diploid	100 ± 1	
Triploid	112 ± 1	
Tetraploid	135 ± 2	

In the final column of the table, fill in the pollen diameters which would have been theoretically expected.



23. The following statement was taken from:

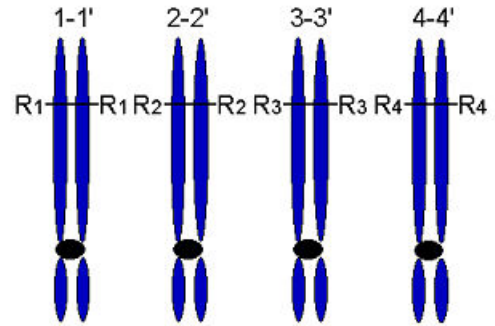
Lindstrom, E.W. 1936. Genetics of polyploidy. Bot. Rev. 2:197-215.

“Were additional proof of the gene-chromosome law of heredity necessary, the genetical behavior of polyploids affords a most potent experimental verification.”

Elaborate on that statement— why would polyploid genetics afford proof that genes are on chromosomes?

24. *Bulldogia hypothetica* is an autotetraploid species that grows profusely on campus. You have identified a particular plant that is tetraallelic at its R locus (i.e., the plant is $R_1R_2R_3R_4$), which controls the intensity of the red color in its petals. What is the probability of recovering a gamete of genotype R_1R_3 given:

- A) A random chromosome model?
- B) A random chromatid model?
- C) A maximal equational model?



For the same three models, what are the chances of recovering an R_4R_4 gamete?

25. The following article was used as the basis for a question in a previous exam:

Caetano-Anollés, G. 1998. Genetic instability of bermudagrass (*Cynodon*) cultivars 'Tifgreen' and 'Tifdwarf' detected by DAF and ASAP analysis of accessions and off-types. *Euphytica* 101:165-173

The author looked at Tiftgreen, a very popular bermudagrass cultivar released 1956. It is $2n = 3x = 27$, and derived from *Cynodon dactylon* \times *C. transvaalensis*. Being a triploid, this grass is sterile, and must be propagated vegetatively. Despite the popularity of these grasses, lawns of Tiftgreen or Tiftdwarf get patches of contrasting morphology and performance, which leads to law suits. In this article, the author concluded that off-types were not due to genotypes blown in from elsewhere, but were most likely due to somatic instability. However, the author did not consider that off-types might be due to seeds produced from rare selfing events.

A) Calculate the percent pollen that would be fertile if only x and $2x$ pollen are viable.

B) Calculate the percent eggs that would be fertile if only x , $x+1$, $2x$, and $2x-1$ eggs are viable.

26. Following is the partial abstract from:

Raina, S.N., Y. Mukai, and M. Yamamoto. 1998. In situ hybridization identifies the diploid progenitor species of *Coffea arabica* (Rubiaceae). Theor. Appl. Genet. 97:1204-1209.

Abstract The most important commercial coffee species, *Coffea arabica*, which is cultivated in about 70% of the plantations world-wide, is the only tetraploid ($2n=4x=44$) species known in the genus. Genomic in situ hybridization (GISH) and fluorescent in situ hybridization (FISH) were used to study the genome organization and evolution of this species. Labeled total genomic DNA from diploid species (*C. eugenioides*, *C. congensis*, *C. canephora*, *C. liberica*) closely related to *C. arabica* was separately used as a probe for chromosome spreads of *C. arabica*.



Species	2n	Status	Hybridization to <i>C. arabica</i>
<i>C. congensis</i>	22	wild	yes
<i>C. eugenioides</i>	22	wild	yes
<i>C. canephora</i>	22	cultivated	no
<i>C. liberica</i>	22	wild	no
<i>C. arabica</i>	44	cultivated	yes

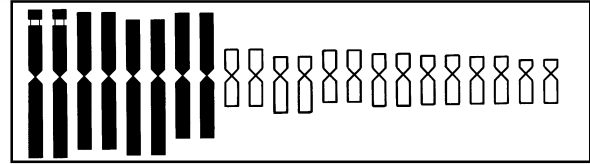
A) Is it possible to absolutely rule out an autotetraploid origin for coffee based on these results? Justify your answer

B) Describe the property of chromosomes which allows them to have species-specific DNA.

C) Compare and contrast the approach these authors used to identify the progenitors of coffee, with the classic approach used by Goodspeed and Clausen to identify the progenitors of tobacco. Be sure to point out relative advantages and disadvantages of each technique.

27. The following information is from

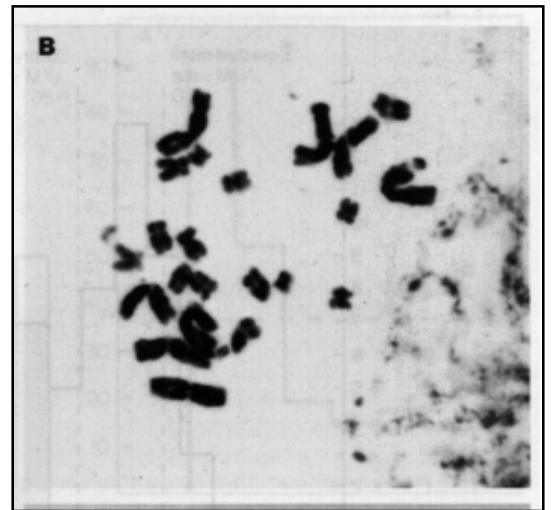
Bennett, S.T., and M.D. Bennett. 1992. Spatial separation of ancestral genomes in the wild grass *Milium montianum* Parl. Ann. Bot. 70:111-118.



This grass is found in Spain. It is a naturally occurring tetraploid, with $2n = 4x = 22$, and a genomic constitution of VVMM. The V genome came from *M. vernale* ($2n = 2x = 8$), while the donor of the M genome is unknown. An idiotype of *M. montianum* is given at right, with the V genome in black and the M genome in white.

- A) Given the photograph at right, what inferences can you draw about the nuclear architecture of this species?

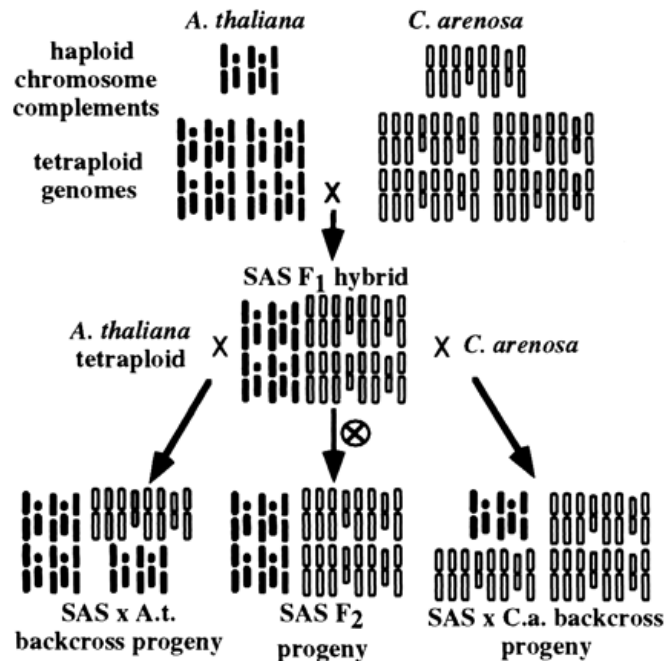
- B) Based on this, would you expect *M. montianum* to be phenotypically more similar to the V genome donor or to the M genome donor?



28. The following is from:

Chen, ZJ, L Comai, and CS Pikaard. 1998. Gene dosage and stochastic effects determine the severity and direction of uniparental ribosomal RNA gene silencing (nucleolar dominance) in *Arabidopsis* allopolyploids. *Proc. Natl. Acad. Sci. USA* 95:14891-14896.

In allopolyploids, the NOR of one of the parental species is usually silenced, in a phenomenon known as nucleolar dominance. The authors wanted to investigate the cause, so they resynthesized *Arabidopsis suecica*, an allopolyploid derived from *Arabidopsis thaliana* and *Cardaminopsis arenosa*. These allotetraploid were derived as diagrammed at left. In such hybrids, the *Arabidopsis* NOR is always suppressed.



The authors then backcrossed the amphidiploid to each parental species, also as shown in the diagram. NOR dominance was switched in some of the backcross progeny. They determined that the hypothesis that naturally dominant rRNA genes have a superior binding affinity for a limiting transcription factor is inconsistent with dominance switching. Inactivation of a species-specific transcription factor is argued against by showing that *A. thaliana* and *C. arenosa* rRNA genes can be expressed transiently in the other species. Transgenic *A. thaliana* rRNA genes are also active in *A. suecica* protoplasts in which chromosomal *A. thaliana* rRNA genes are repressed.

In the end, the authors conclude that, "Collectively, these data suggest that nucleolar dominance is a chromosomal phenomenon that results in coordinate or cooperative silencing of rRNA genes." This is a fancy sentence that means very little.

Briefly discuss one aspect of allopolyploidy which the authors neglected entirely, and which may be one of the factors associated with NOR silencing.

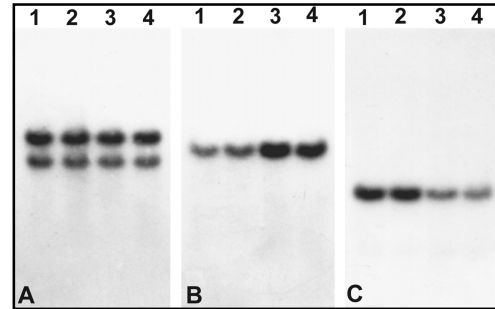
Limit your answer to 3 sentences. Use the back side of this sheet only.

29. The following question comes from:

Cheng, Z., H. Yan, H. Yu, S. Tang, J. Jiang, M. Gu, and L. Zhu. 2001. Development and applications of a complete set of rice telotrisomics. *Genetics* 157: 361-368.

In this work, the authors started with a set of 1° trisomics of rice cultivar, 'Zhongxian 2037'. They grew out 30,000 progeny each year between 1994 and 1999 (i.e., a total 270,000 plants), looked for off-types, and found telotrisomics for all 24 arms.

In the diagram, lanes 1 and 2 have DNA from $2n + 1S$ plants. Lanes 3 and 4 have DNA from $2n + 1L$ plants.



The first panel has been probed with RFLP G275. The middle panel is probed with RFLP RG350, and the third panel is probed with RFLP C749.

- A. What is the principle that allows telotrisomics to be used to place markers on specific chromosomes?

- B. What is the advantage or disadvantage of using a telotrisomic instead of a trisomic for the same purpose?

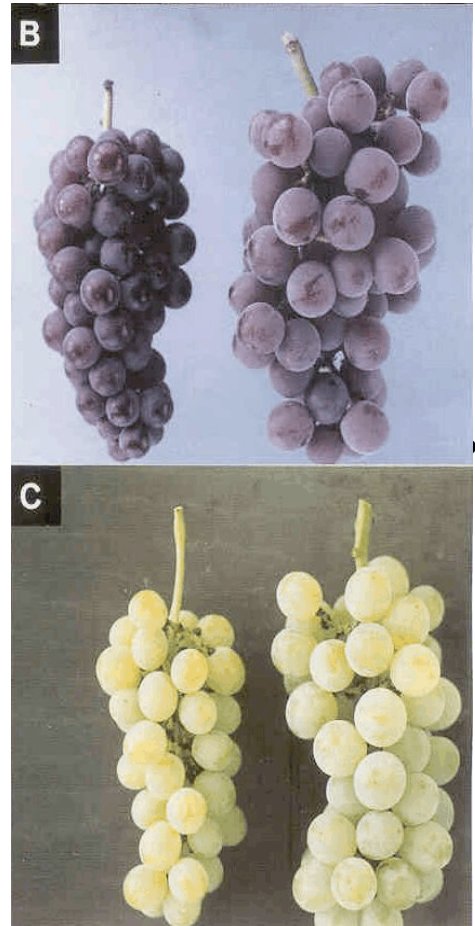
- C. What inferences can you make about the chromosomal locations of G275, RG350, and C749?

30. The following question is from:

Notsuka, K., T. Tsuru, and M. Shiraishi. 2000. Induced polyploid grapes via *in vitro* chromosome doubling. J. Japan. Soc. Hort. Sci. 69:543-551.

In this paper, doubling the chromosome number of grapevines led to production of fruit that were from 1.1 to 1.5 \times larger in diameter, with an average of 1.3.

What is the theoretical value one would expect to obtain from chromosome doubling, and how do the values they obtained compare with the theoretical value?



31. The following is from:

Guo, W.W., X.X. Deng, and H.L. Yi. 2000. Somatic hybrids between navel orange (*Citrus sinensis*) and grapefruit (*C. paradisi*) for seedless triploid breeding. *Euphytica* 116:281-285.

The ultimate goal of the authors is to obtain a triploid pummelo (a hybrid citrus fruit, much like a grapefruit), which they plan to derive by crossing diploid pummelo with a tetraploid hybrid they obtained by fusing protoplasts of orange and grapefruit. [note: the grapefruit is itself a hybrid between a sweet orange and a pummelo].

Assume an average of 10 seeds per diploid pummelo fruit. Viable female gametes can be $2x$, $2x-1$, $x+1$ or x . Viable male gametes are n or $2n$. In the absence of embryo rescue techniques, survival of triploid or near-triploid embryos is too low to matter in this case, so assume these are not viable. What is the expected frequency of fruit with a seed in it from an orchard of triploid pummelo?



**Root-tip of somatic hybrid
with $2n = 4x = 36$
chromosomes.**

32. The following is from:

Tsuchiya, T., and R.J. Singh. 1982. Chromosome mapping in barley by means of telotrisomic analyses. Theor Appl Genet 61:201-208.

The authors used 2n plants that were homozygous recessive for the gene of interest, and crossed them as males onto telotrisomes that were homozygous dominant for the gene. F₁ progeny containing the telosome were selected, and selfed to produced the F₂ generation. Segregation data are as follows:

Triplo	Marker Genes	2x progeny		2x + 1 telo progeny	
		Dominant	Recessive	Dominant	Recessive
2L	<i>f(=lg)</i>	62	6	30	11
2L	<i>gs6</i>	69	26	30	14
2L	<i>v</i>	122	50	124	0
2L	<i>gs5</i>	55	14	30	0
2S	<i>f(=lg)</i>	95	16	71	0
2S	<i>gs6</i>	50	3	41	0
2S	<i>v</i>	121	26	56	11
2S	<i>gs5</i>	56	8	19	5

Based on these data, which genes are on 2L and which are on 2S?

33. The following is from:

Niemeyer HM and JM Jerez. 1997. Chromosomal location of genes from hydroxyamic acid accumulation in *Triticum aestivum* L. (wheat) using wheat aneuploids and wheat substitution lines. *Heredity* 79:10-14.

Hydroxamic acids are defensive secondary metabolites in wheat, the most abundant aglucones being DIMBOA and DIBOA. The location of genes coding for the accumulation of hydroxamic acids by wheat seedlings was explored comparing the euploid of cultivar Chinese Spring and nullisomic/tetrasomic lines. Data are presented in the table below.

Genome	<i>n</i>	Hydroxamic acids (mmol kg ⁻¹ fresh weight)	
		DIBOA	DIMBOA
Chinese Spring	10	0.08 ± 0.023	5.9 ± 2.0
CS-N4AT4B*	5	0.39 ± 0.20	7.0 ± 3.4
CS-N4AT4D	1	0.20	0.48
CS-N5BT5A	4	0.12 ± 0.12	0.34 ± 0.60
CS-N5BT5D	4	0.06 ± 0.04	0.63 ± 0.69

* Terminology: CS- N4AT4B = a plant of Chinese Spring which is nullisomic for chromosomes 4A and tetrasomic for chromosomes 4B. In other words, it is a chromosome substitution line.

- a) Based on the data, which chromosomes contain genes for DIBOA or DIMBOA production?

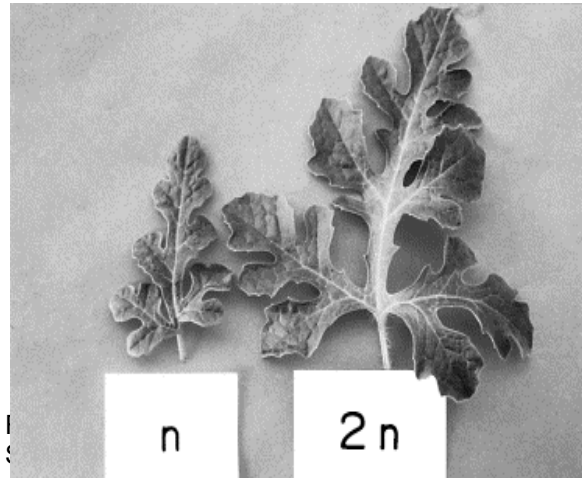
- b) Diagram the way in which a nullisomic/tetrasomic plant would have been obtained.

34. The following is from:

Sari, N., K. Abak, and M. Pitrat. 1999.
Comparison of ploidy level screening methods in
watermelon: *Citrullus lunatus* (Thunb.) Matsum.
and Nakai. *Scientia Horticulturae*, 82:265-277.

A. Correct the terminology, providing the correct
way to identify haploid and diploid plants.

B. What is one factor that might contribute to the
smaller size of the haploid?

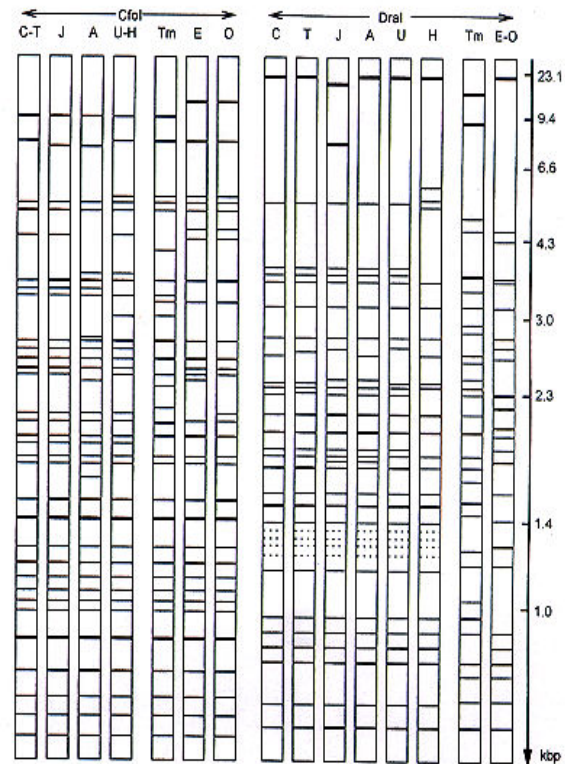


35. The following is from:

Gauthier, P, R Lumaret, and A Bedecarrats. 1997.
Chloroplast-DNA variation in the genus *Lotus* (Fabaceae) and
further evidence regarding the maternal parentage of *Lotus*
corniculatus L. Theor. Appl. Genet. 95:629-636.

Lotus corniculatus, ($2n = 4x = 24$), commonly known as birdsfoot trefoil, is a forage legume used in colder climates. Some people believe it to be an autotetraploid, others a segmental allotetraploid, and yet others an allotetraploid. These authors are operating on the latter assumption, and wanted to determine which was the maternal parent. Therefore, the authors looked at restriction patterns in chloroplast DNA, which are most commonly transmitted only through the female parent in angiosperms. As seen in the figure at right, the DNA pattern of *Lotus tenuis* was the most similar to that of *L. corniculatus*, indicating that *L. tenuis* is the maternal parent.

Indicate two additional lines of evidence you would expect to see to confirm the type of tetraploidy (ie, auto, allo) involved here.



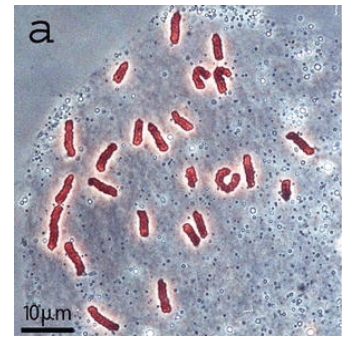
C stands for *Lotus corniculatus*. T is for *L. tenuis*. The remaining letters stand for other *Lotus* species evaluated.

36. The following is from:

Barba-Gonzalez, R., A.C. Lokker, K.-B. Lim, M.S. Ramanna, and J.M. van Tuyl. 2004. Use of $2n$ gametes for the production of sexual polyploids from sterile Oriental x Asiatic hybrids of lilies (*Lilium*). Theor. Appl. Genet. 109:1125-1132.

The genus *Lilium* is divided into six sections. Species are $2n = 2x = 24$. Species can be crossed between sections to give sterile hybrids.

At right is a photo of Metaphase I in an Oriental x Asiatic hybrid.



- A. Explain why the hybrid is sterile
- B. What can be done to restore fertility in the hybrid?
- C. Assume that only pollen that is $n = 12$, $n + 1 = 13$, $2n = 24$, and $2n - 1 = 23$ are fertile. What percent of the resulting pollen grains would be fertile?

- D. Using the same assumptions as for C, If this plant was used to pollinate another diploid lily, what percent of pollen should result in viable embryos?

37. The following is from:

De Schepper, S., L.Leus, T. Eeckhaut, E., Van Bockstael, P. Debergh, and M. De Loose. 2004. Somatic polyploid petals: regeneration offers new roads for breeding Belgian pot azaleas. *Plant Cell Tissue and Organ Culture*. 76:183-188.

The top photo is a flower of azalea cv 'Marcella' in which the outer edges of the petals are white. It also turns out the white cells are tetraploid, while the colored petal cells remain diploid.

The authors took the white pieces from the petals, placed them in tissue culture, and regenerated tetraploid azaleas. These had all-white flowers, as seen in the bottom photo.



What is the coefficient of inbreeding for the regenerated plants? Show your calculations.



38. The following is from:

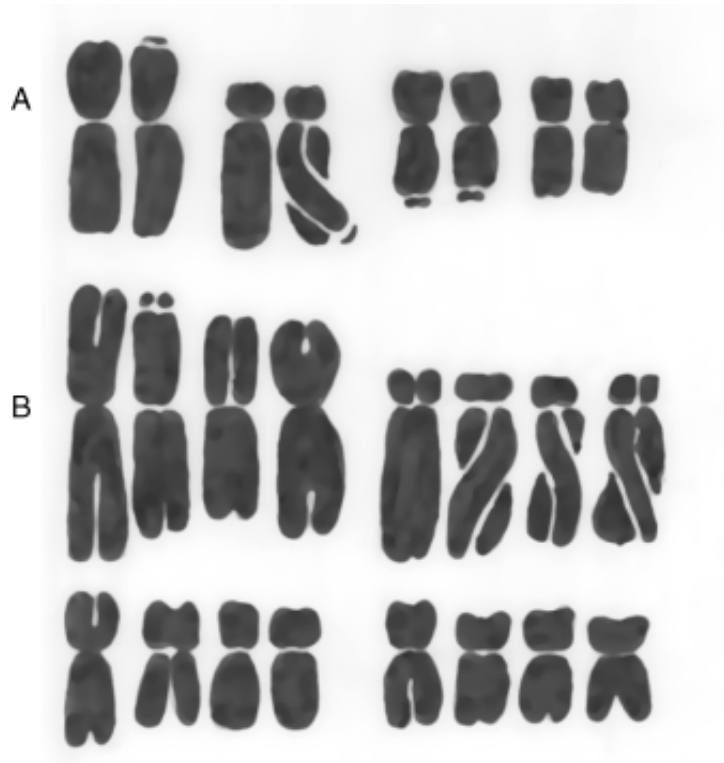
Johnson, M.A.T. 2003. Polyploidy and karyotype variation in Turkish *Bellevalia* (Hyacinthaceae). Botanical Journal of the Linnean Society 143: 87-98.

A and B represent karyotypes for two *Bellevalia* plants.

Based on the karyotype alone, is the second individual an allo tetraploid or an autotetraploid?

Explain which karyotype characteristics you used to make your determination.

Remember, not all chromosomes contract the same when placed in a pretreatment.



39. The following is from:

Rahiminejad, R., and R.J. Gornall. 2004. Flavonoid evidence for allopolyploidy in the *Chenopodium album* aggregate (Chenopodiaceae). Plant Syst. Evol. 246:77-87.

Here, the authors looked at the flavonoids present in 7 diploid species of *Chenopodium*: *glaucum*, *hybridum*, *murale*, *ficifolium*, *suecicum*, and *vulvaria*.

The premise is that they could determine the parents of the hexaploids by seeing which of the diploid species produced the same flavonoids as the hexaploid. Data are as follows:

Table 3. Distribution of flavonoid compounds among 16 species of *Chenopodium*. Species are designated by their first three letters (Table 1)

Flavonoids ^a	Diploid species							Tetraploid species					Hexaploid species			
	gla	hyb	pol	mur	fic	sue	vul	ber	bus	nov	qui	str	alb	gig	opu	pro
Q-3-O-glu	+		+	+	+	+	+	+		+	+	+	+	+	+	+
Q-3-O-glu (acyl)	+				+	+				+			+	+	+	+
Q-3-O-xyl	+		+	+	+	+	+			+			+	+	+	+
Q-3-O-xyl (acyl)	+															
Q-3-O-ara			+		+	+	+			+			+	+	+	+
Q-3-O-rha	+		+	+	+	+	+			+			+	+	+	+
6-O-Me-Q-3-O-rha			+													
(6,7)-diO-Me-Q-3-O-rha			+													
6-O-Me-Q-3-O-gly			+													
Q-3-O-gly (acyl with cinnamic acid)		+														
Ir-3-O-glu	+				+					+		+	+	+	+	+
Ir-3-O-glu (acyl)					+											
Ir-3-O-xyl					+					+			+	+	+	+
Ir-3-O-rha	+				+					+			+	+	+	+
Ir-3-O-gal					+											
K-3-O-glu			+	+		+				+			+	+	+	+
K-3-O-glu (acyl with cinnamic acid)		+		+												
K-3-O-glu (acyl)						+										
K-3-O-ara				+												
(6,7,4')-triO-Me-K-3-O-rha			+													
K-3-O-gly								+	+		+					
K-3-O-gly (acyl with cinnamic acid)		+		+												
Q-3-O-glu-glu	+			+	+	+	+			+			+	+	+	+
Q-3-O-glu-gal	+			+	+	+				+			+	+	+	+
Q-3-O-rha-glu				+	+	+				+			+	+	+	+
Q-3-O-glu-rha					+	+				+			+	+	+	+
Q-3-O-glu-xyl	+				+	+				+			+	+	+	+
Q-3-O-xyl-glu	+			+	+	+				+			+	+	+	+
Q-3-O-(ara, glu)				+	+	+				+			+	+	+	+
6-O-Me-Q-3-O-digly I			+													

Based on Table 3 above, which diploid species are the best candidates to be the parents of the hexaploid?

40. The following is from:

Santos, J.L., D. Alfaro, E. Sanchez-Moran, S.J. Armstrong, F.C.H. Franklin, and G.H. Jones. 2003. Partial diploidization of meiosis in autotetraploid *Arabidopsis thaliana*. Genetics 165:1553-1540.

In this paper, the authors were looking at meiotic configurations in 4 lines of arabidopsis (E1-E4) that were 13 generations away from the polyploidization event, and 1 line (C) that was in the first generation of polyploidization.

A) Although the authors do not mention seed set, you should be able to look at the data and predict which of the 5 lines tested would have the highest seed set. What criteria did you use for your answer?

B) What type(s) of mutation(s) have probably taken place over time to account for differences in the frequency of multivalents/bivalents?

Multivalents (M) and bivalent pairs (IIs) observed for each of the five chromosomes in 50 pollen mother cells per line

Lines	Chromosomes					T	x
	1	2	3	4	5		
E ₁							
M	43 (86)	29 (58)	41 (82)	25 (50)	40 (80)	178 (71.2)	3.56
IIs	7 (14)	21 (42)	9 (18)	25 (50)	10 (20)	72 (28.8)	1.44
Xta	3.98	3.20	3.66	3.30	3.94		18.08
E ₂							
M	41 (82)	18 (36)	40 (80)	28 (56)	38 (76)	165 (66.0)	3.30
IIs	9 (18)	32 (64)	10 (20)	22 (44)	12 (24)	85 (34)	1.70
Xta	4.18	3.54	3.90	3.20	3.98		18.80
E ₃							
M	43 (86)	13 (26)	40 (80)	22 (44)	35 (70)	153 (61.2)	3.06
IIs	7 (14)	37 (74)	10 (20)	28 (56)	15 (30)	97 (38.8)	1.94
Xta	4.04	3.38	3.94	3.32	3.80		18.48
E ₄							
M	40 (80)	12 (24)	26 (52)	22 (44)	32 (64)	132 (52.8)	2.64
IIs	10 (20)	38 (76)	24 (48)	28 (56)	18 (36)	118 (47.2)	2.36
Xta	4.10	3.12	3.68	3.40	3.64		17.94
C							
M	40 (80)	38 (76)	41 (82)	37 (74)	41 (82)	197 (79.0)	3.94
IIs	10 (20)	12 (24)	9 (18)	13 (26)	9 (18)	53 (21.0)	1.06
Xta	4.04	3.74	3.82	3.64	3.98		19.22

Numbers in parentheses are percentages of multivalents and bivalent pairs. T, totals; x, means per cell; Xta, mean chiasma frequencies per chromosome and per cell.

41. The following is from:

Budzianowski, G. and H. Woś. 2004. The effect of single D-genome chromosomes on aluminum tolerance of triticales. *Euphytica*. 137:165-172.

Triticale is an amphiploid crop obtained by crossing tetraploid wheat (AABB) \times rye (RR) to get a hexaploid of genomic constitution AABBRR. In this case, the authors substituted several triticales chromosomes for their D genome homoeologues, and looked at aluminum tolerance of the resulting seedlings.

In the table below, lines with a “+” are significantly ($p < 0.01$) more resistant than “Rhino”; those with a “-” are significantly ($p < 0.01$) less resistant. **See reverse side for questions.**

Table 1. Chromosomal constitution and mean values of percentage of Al-tolerant seedlings across two replications of 30 entries of Set 1 tested at different Al concentrations

Entry number	Chromosomal constitution	Root regrowth after Al-treatment (% of tolerant seedlings)		
		10 mg Al·L ⁻¹	20 mg Al·L ⁻¹	mean
Substitution lines of cv. Presto				
1	1D(1A)	20.0 ^{n.s.}	10.0 ^{n.s.}	15.00 ^{n.s.}
2	1D(1B)	34.0 ⁺	26.0 ⁺	30.00 ⁺
3	1D(1R)	25.0 ^{n.s.}	7.5 ^{n.s.}	16.25 ^{n.s.}
4	2D(2A)	18.5 ^{n.s.}	7.0 ^{n.s.}	12.75 ⁻
5	2D(2B)	25.0 ^{n.s.}	11.0 ^{n.s.}	18.00 ^{n.s.}
6	2D(2R)	14.5 ⁻	12.5 ^{n.s.}	13.50 ^{n.s.}
7	3D(3A)	53.5 ⁺	23.5 ⁺	38.50 ⁺
8	3D(3B)	24.0 ^{n.s.}	19.0 ⁺	21.50 ⁺
9	3D(3R)	0.0 ⁻	0.0 ⁻	0.00 ⁻
10	4D(4A)	37.5 ⁺	19.0 ⁺	28.25 ⁺
11	4D(4B)	51.5 ⁺	26.0 ⁺	38.75 ⁺
12	4D(4R)	8.5 ⁻	0.0 ⁻	4.25 ⁻
13	5D(5A)	27.0 ^{n.s.}	12.5 ^{n.s.}	19.75 ^{n.s.}
14	5D(5B)	27.0 ^{n.s.}	5.5 ⁻	16.25 ^{n.s.}
15	5D(5R)	16.0 ⁻	7.5 ^{n.s.}	11.75 ⁻
16	6D(6A)	27.0 ^{n.s.}	4.0 ⁻	15.50 ^{n.s.}
17	6D(6B)	37.5 ⁺	21.0 ⁺	29.25 ⁺
18	6D(6R)	11.0 ⁻	6.0 ⁻	8.50 ⁻
19	7D(7A)	15.5 ⁻	6.5 ^{n.s.}	11.00 ⁻

A) Place an 'x' in each of the appropriate boxes according to what the chromosome in the first column does to aluminum tolerance, based on the data in column labeled 'mean' on the table.

	Increases it	Decreases it	No effect	Insufficient data
1A				
1B				
1D				
1R				
5A				
5B				
5D				
5R				
7A				
7D				

B) Diagram the most likely method the authors used to get the substitution lines of "Presto".

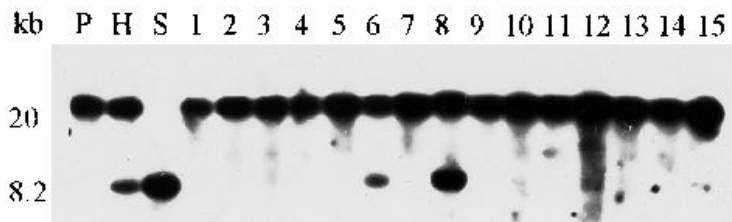
42. The following is from:

Suen DF, CK Wang, RF Lin, YY Kao, FM Lee, and CC Chen. 1997. Assignment of DNA markers to *Nicotiana sylvestris* chromosomes using monosomic alien addition lines. Theor Appl Genet. 94:331-337.

The authors took a sesquidiploid hybrid (H, $2n = 3x = 32$)* between *Nicotiana plumbaginifolia* (P, $2n=20$) and *N. sylvestris* (S, $2n = 24$) was used to produce monosomic alien addition lines. A total of 89 alien addition lines ($2n = 2x+1 = 21$) plants, each containing a single *N. sylvestris* chromosome, were obtained.” These plants are labeled 1-15 in the figure, and were probed with a DNA segment from *N. sylvestris*.

* I.e., an allotriploid, containing 2 sets of chromosomes from *Nicotiana plumbaginifolia* and one set from *N. sylvestris*

A) Given that only one probe was used with plants 1-15 in the figure, which alien addition lines have the same chromosome?



B) Beginning with the parents used by the authors, diagram a set of crosses which would give an alien addition line, as in this example.

43. The following is from:

Hayashi M, J Kato, Y Ichikawa, N Matsubara, H Ohashi, and M Mii. 2007. Inter-sectional hybrids with various ploidy levels between *Primula denticulata* and three varieties of *P. modesta*. Breed Sci 57:165-173.¹

A = *Primula denticulata* ($4x = 44$)

B = *P. modesta* var. *fauriae* ($2x = 18$)

D, E, and F are hybrid progeny obtained from the cross of A x B, as follows:

C = $3x = 31$

D = $4x = 40$

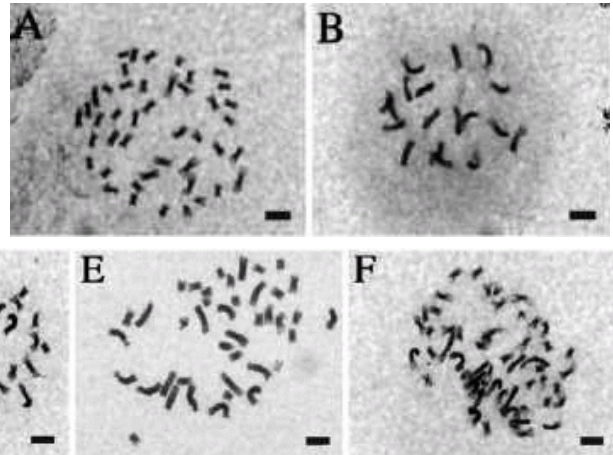
E = $5x = 53$

Give the chromosome number of each gamete that gave origin to plants D, E, and F



Primula denticulata

P. modesta var *fauriae*



¹Photo Credits:

http://em.ca/garden/per_primula_denticulata_rubin_selection.html

<http://www.dkimages.com/discover/Home/Plants/Ornamental-Groups/Perennials/Primulales/Primulaceae/Primula/Unspecified-Forms/Primula-modesta-var-fauriae/Primula-modesta-var-faur-1.html>

44. *The following is from:*

Liu G, Z Li, and M Bao. 2007. Colchicine-induced doubling in *Platanus acerifolia* and its effect on morphology. *Euphytica*. DOI 10.1007/s10681-007-9406-6

The authors were working with London Planetree (a type of sycamore) with the intent purpose “of the production of tetraploid plants of *Platanus acerifolia* with the ultimate aim of improving the ornamental qualities of this important urban landscaping tree.”

A) Why would the authors believe that a 4x plant would have better qualities than the 2x version?

B) Why are the guard cells larger in the 4x plant? How much larger (volume-wise) would you expect them to be than their 2x counterparts? Diameter-wise?

C) Based on photo H, why would the 4x plant be growing more slowly than the 2x plant? What should the authors do to get more vigorous 4x trees?



Three year old 2x (L) and 4x (R) plants. Bar = 20 cm.

45. In the following paper:

Akutsu M, S Kitamura, R Toda, I Miyajima and K Okazaki. 2007. Production of 2n pollen of Asiatic hybrid lilies by nitrous oxide treatment. *Euphytica*. 155:143-152.

The authors wanted to induce 2n pollen formation in a monocot species, namely diploid Asiatic lily, cv 'Mona' so that they could cross it with 4x lilies.

They thus exposed lily buds to nitrous oxide (a C-mitotic agent) at various stages of meiosis. The results are as follows:



http://www.stratsplace.com/othergardens/ra_mona.jpg

S tage	Control	Pro I	Pro-Met I	Met I	Met II	Ana II
% plants with high 2n pollen	0	0	100	60	33	0

A) What mode & mechanism should result from application of N₂O at the metaphase I (or right before it at pro-metaphase I)? Explain your answer.

B) What about Met II?

C) Had they gotten any 2n pollen at Ana II, what would the mode be? Why?

46. The following is from:

Du MC and MS Mendioro. 2001. Cytogenetics of three *Canna* cultivars. The Philippine Agricultural Scientist. 84:147-154.

'Salmon Pink' is a diploid genotype ($2n=18$). The authors studied meiosis and observed

1. 9 II 89.80%
2. 8 II + 2 I 4.08%
3. 6 II + 6 I 2.04%
4. 1 V + 4 II 1.02%
5. 1 III + 6 II 2.04%



'Salmon pink' canna

A. Indicate the type of pairing abnormality or aneuploidy indicated by #s 2 to 5.

B. In each case, the multivalents were present as chains. Draw the pairing configuration that would result in a chain of III. Be sure to show the crossovers.

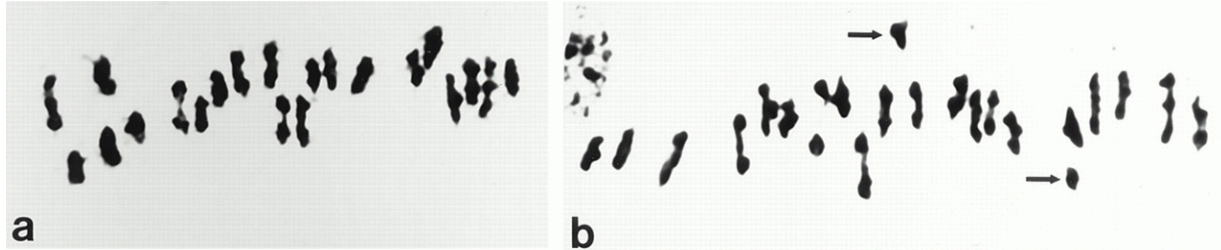
Photo credit:

[Http://www.agrigoldinc.com/canna%20images%5CSalmon%20Pink%2002.jpg](http://www.agrigoldinc.com/canna%20images%5CSalmon%20Pink%2002.jpg)

47. The following is from:

Xu SJ, RJ Singh, KP Kollipara, and T Hymowitz. 2000. [] trisomics in soybean: origin, identification, breeding behavior, and use in linkage mapping. *Crop Science* 40:1543-1551.

Trisomics have been developed for soybean ($2n = 40$) that exhibits disomic genetics. Given the following photos of metaphase I:



A) What type of trisomics are shown in photos a and b, respectively? In b), the arrows point to 2 unpaired monosomes)

B) Briefly explain how a trisomic is useful for assigning genes to chromosomes.

48. The following is from:

Górny AG. 1999. Effects of D-genome substitutions on the water use efficiency and response of the 'Langdon' durum wheat (*Triticum turgidum* L. var *durum*) to reduced nitrogen content. Cereal Res. Comm. 27:83-90.

Langdon is a 4x pasta wheat (AB genome). Langdon has low Water use efficiency (WUE) in general, and under low N conditions in particular.

In an effort to look for genes that contribute to low WUE or to high WUE, the authors substituted D genome chromosomes from the 6x 'Chinese Spring' for their A or B homoeologues.

A. Diagram how the chromosome substitution lines would have been derived.

B. Based on the results in the adjacent table, which chromosomes affect WUE, and is their effect positive or negative?

Line	Water use efficiency under low N, measured as mg per mol at vegetative stage
1D(1A)	46.5
1D(1B)	48.9
2D(2A)	55.7*
2D(2B)	52.1
3D(3A)	51.0
3D(3B)	48.1
4D(4A)	50.9
4D(4B)	52.3*
5D(5A)	44.7
5D(5B)	49.6
6D(6A)	51.0
6D(6B)	49.1
7D(7A)	53.7*
7D(7B)	57.3*
Langdon	47.3
Chinese Spring	57.4*
* significantly different at the P<0.05 from the recipient cv Langdon	

49. The following is from:

Prieto P, C Ramírez, A. Cabrera, J Ballesteros and A Martín. 2006. Development and cytogenetic characterization of a double goat-grass-barley chromosome line in tritordeum. *Euphytica* 147-342.

Tritordeum is a new grain that is an amphiploid between durum wheat and *Hordeum chilense* (also known as goat-grass-barley). Its genomic constitution is AABBH^{ch}H^{ch}, $2n = 6x = 42$.

Tritordeum has many desirable traits, such as good agronomic quality, high protein content, and good quality flour. However, it has brittle rachis, which means the seeds fall off before it can be harvested. They developed substitution lines, and found that the 2D for 2H^{ch} and the 3H^v for 3H^{ch} substitution lines had non-brittle rachis, where H^v indicates the *Hordeum vulgare* (barley) genome.

Design a non-genetic engineering strategy to move the non-brittle rachis trait from 2D into 2H^{ch} of tritordeum, *while eliminating linkage drag as much as possible*. To avoid having to induce translocations, make use of the *Ph* locus.

50. The following is from

Du MC and MS Mendioro. 2001. Cytogenetics of three *Canna* cultivars. The Philippine Agricultural Scientist. 84:147-154.

'President' is a triploid genotype ($2n=27$), as is 'Richard Wallace'. The authors studied meiosis and found only 2 normal cells that survived to Telophase II. for President, and 3 for Richard Wallace.

What percent of pollen would be expected to be fertile (either n , $n+1$, or $2n$)? Show your work.

% n pollen = _____

% $n+1$ pollen = _____

% $2n$ pollen = _____

Total = _____



'President' canna



'Richard Wallace' canna

51. The following is from

Photo credit:

<http://www.dungevalley.co.uk/Rhododendron%20'Cunningham's%20White'.jpg>

Väinölä, A. Polyploidization and early screening in *Rhododendron* hybrids. *Euphytica*. 112:239-244.

In this paper, the author used oryzalin or colchicine to induce tetraploids in tissue culture. According to the author, “Immediately after the treatment the polyploids grew very slowly, whereas most of the unaffected diploids were vigorous from the very beginning.”



Cunningham's White, one of the rhododendrons used for this work.

- A. How do oryzalin and other c-mitotic agents induce polyploidy?
- B. Why would polyploids from a somatically doubled out-crossing species be slower-growing than the diploids?
- C. List at least 2 things you could do to verify that the slow-growing plants were indeed tetraploids.

52. The following table is from:

Barone A, J Li, A Sebastiano, T Cardi and L Frusciante. 2002. [...] Theor. Appl Genet. 104:539-546.

In this paper, the authors used protoplast fusion to obtain a tetraploid hybrid from *Solanum commersonii* and *S. tuberosum* (the common potato). They were curious to know if the hybrid would behave most like an autotetraploid or an allotetraploid, so they selfed the hybrid and monitored the segregation of AFLP markers in 90 progeny. Data are in the adjacent table.

Each row represents an AFLP set of primers, and the number in the first column indicates the number of bands whose segregation was fit to various disomic or tetrasomic ratios. The next columns indicate which of the bands from the first column fit a given ratio, based on χ^2 analysis at $P>0.05$.

cmm-specific AFLPs (no.)									
Total Segregating									
	1:0	3:1	20:8:1	35:1/ 20:8:1	3:1	2.5:1	3:1/ 2.5:1	Other	
8	0	0	1	3	0	1	1	2	
14	0	0	1	11	0	1	1	0	
10	0	0	0	6	0	0	3	1	
10	0	0	5	3	0	0	1	1	
12	1	0	1	4	0	4	2	0	
11	2	0	3	2	1	0	1	2	
1	0	0	0	0	0	0	0	1	
13	0	0	2	6	0	0	1	4	
6	1	0	0	2	1	0	2	0	
85	4	0	13	37	2	6	12	11	
100	4.7	0.0	15.3	43.5	2.4	7.1	14.1	12.9	

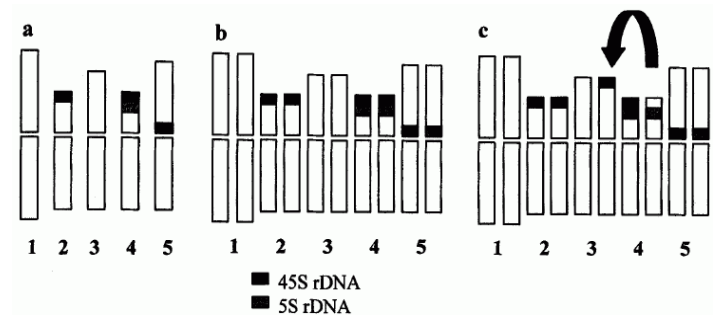
A) Based on these data, is the hybrid most like an auto or allotetraploid? Explain your answer.

B) Based on these data, where on the chromosome are most of the AFLP loci located? Explain your answer.

53. The following is from:

Weiss H and J Maluszynska. 2000. Chromosomal rearrangement in autotetraploid plants of *Arabidopsis thaliana*. Hereditas 133:255-261.

In this paper, the authors found that the chromosomes autotetraploid arabidopsis paired as bivalents. Upon further examination, they found a translocation in one pair of chromosomes, as depicted in the figure.



a) karyotype for 2x arabidopsis; b) expected karyotype for auto4x arabidopsis; c) actual karyotype for auto4x arabidopsis.

Their premise is that such micro changes in chromosome structure play a role in the diploidization of autotetraploids (ie, make the transition from auto to allotetraploids), and are responsible for its chromosomes pairing as bivalents.

Is bivalent pairing in autotetraploids necessarily a sign of diploidization? Justify your answer by reviewing the supporting evidence covered in class. Answer should not exceed 1 or 2 short paragraphs. The answer must not exceed the remainder of this page.

54. The following is from:

Wise-Schneeweiss H, GM Schneeweiss, TF Suessy, T Mabuchi, J-M Park, C-G Jang, and B-Y Sun. 2007. Chromosomal stasis in diploids contrasts with genome restructuring in auto- and allopolyploid taxa of *Hepatica* (Ranunculaceae). *New Phytologist* 174:669-682.

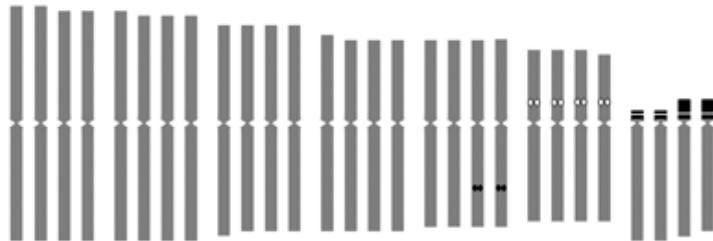
The authors were trying to determine if the polyploids were allo or auto. In the karyotype below for *H. nobilis*, black = 45S rDNA, and white circles = 5S rDNA genes.



Hepatica nobilis.

http://www.senecahillperennials.com/uploads/images/plant_h-k/hepatica_nobilis_blue1.jpg

nobilis var. *pubescens*



A) What about this karyotype might suggest an autotetraploid origin?

B) What about this karyotype might suggest an allotetraploid origin?

C) List at least 2 additional pieces of information you would like to have to that could help distinguish between allo and autotetraploidy in this case.

55. In the following paper:

Stebbins GL. 1940. The significance of polyploidy in plant evolution. Amer. Nat. 74:54-66.

Stebbins, who lacked currently available information on genetics and genomics, made the following statement:

“The evidence from the plant kingdom as a whole, therefore, suggests that polyploidy has been most important in developing large, complex, and wide-spread genera; but in respect to the major lineages of evolution, it has been more important in preserving relics of old genera and families than in

producing new ones.”



G. Ledyard Stebbins

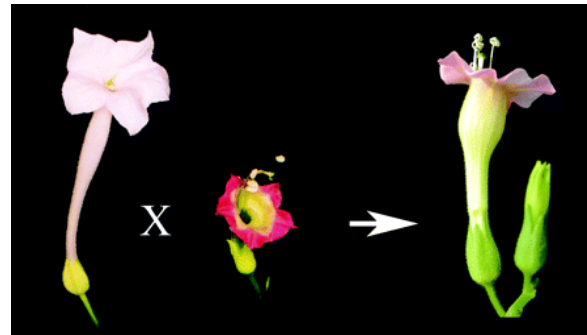
How has Stebbin's view point held up over time? Give an analysis of why his statement has or has not withstood the test of time, discussing at least 2 key evolutionary features of polyploidy covered in class to support your argument. Do not exceed a paragraph

56. The following is from:

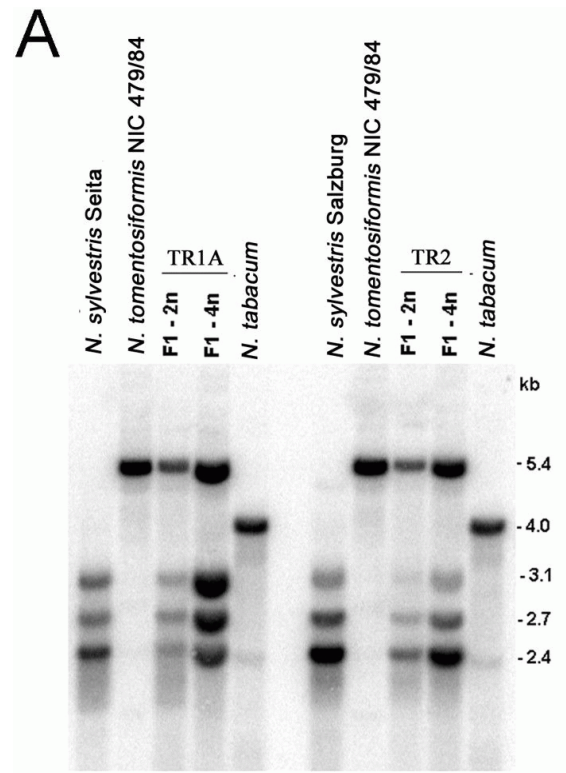
Lim KY, K Souckova-Skalicka, V Sarasan, JJ Clarkson, MW Chase, A Kovarik, and AR Leitch. 2006. A genetic appraisal of a new synthetic *Nicotiana tabacum* (Solanaceae) and the Kostoff synthetic tobacco. *Am J. Bot.* 93:875-883.

Tobacco (*Nicotiana tabacum*) has long been considered to be an allotetraploid derived from a cross between *N. sylvestris* and *N. tomentosiformis*. In this paper, the authors remade the cross, and use oryzalin to create a tetraploid. Then they probed with an rDNA probe, and got the following pattern:

Give two possible reasons why the banding pattern of the synthetic 4x plant does not match that of *N. tabacum*.



Flowers of *N. sylvestris* x *N. tomentosiformis* → 4x synthetic tobacco



57. The following is from:

Aline DB, LF Viccini, and SM Recco-Pimentel. 2005. Meiotic analysis of two putative polyploid species of Verbenaceae from Brazil. *Caryologia* 58:315-319.

Lippia alba is either a diploid of $2n = 2x = 30$ or an allotetraploid of $2n = 4x = 30$

The authors studied meiosis to see if there was anything that would give a clue as to which of the two possibilities is the more likely. The photos are at right.

A) What is the stage shown in photos:

c =

f =

g =

j =

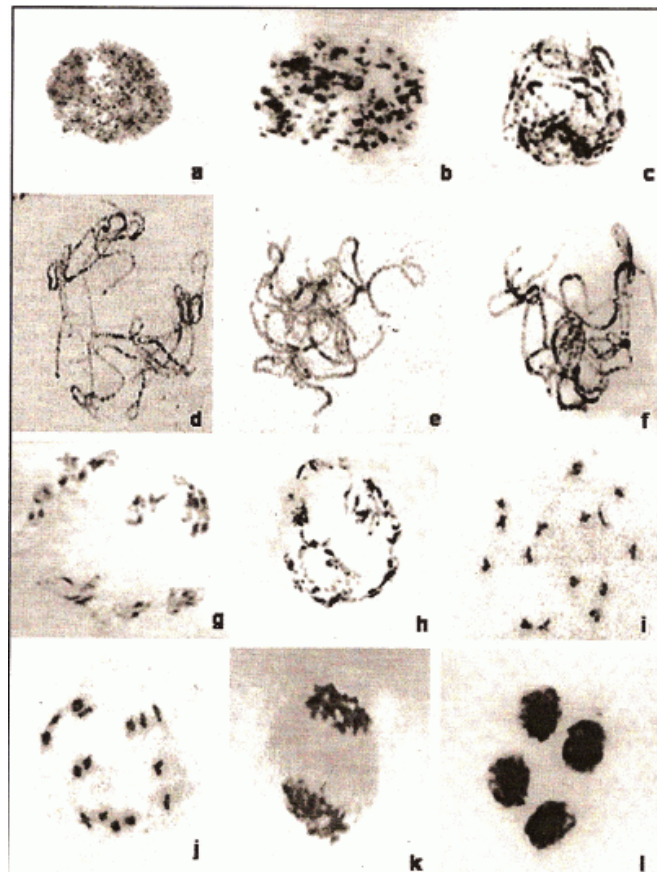
k =

l =

B) 4 cells will be formed after stage L is over. For one of these cells, fill in the blanks below, keeping in mind that they will differ depending on if the original plant is $2x$ or $4x$:

___ n = ___ x = ___ c = 15 *or* ___ n = ___ x = ___ c = 15

C) Is there anything in the photos that give a clue as to whether the plant is $2x$ or $4x$? Explain your answer, which should not exceed one well thought out paragraph or two.



58. The following is from:

Shan F, G Yan, and JA Plummer. 2003. Cytoevolution of *Boronia* genomes revealed by fluorescent in situ hybridization with rDNA probes. Genome 46:507-513.

In this paper, the authors came up with ideograms for:

- a) *Boronia clavata*
- b) *Boronia megastigma*
- c) *Boronia heterophylla* cv 'Red'
- d) *Boronia heterophylla* cv 'Almost White'
- e) *Boronia molloyae*
- f) *Boronia denticulata*

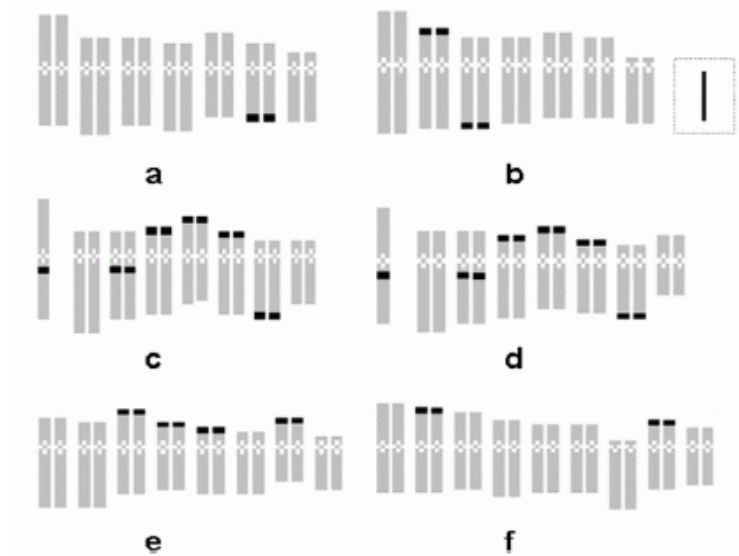


Boronia heterophylla, cv 'Red'
<http://www.plantsafari.com/Catalog2/Detail/00169.html>

Note that the two ornamental types (c & d) are $2n = 15$, a rather unusual number for a plant.

Three possibilities come to mind:

- 1) The plant is a triploid, with base number of $x = 5$
- 2) The extra chromosome is a B chromosome
- 3) The real number is $2n = 16$, and the plants are monosomic, missing a chromosome



Boronia karyotypes. Black = NOR sites. Bar = 2 μ m

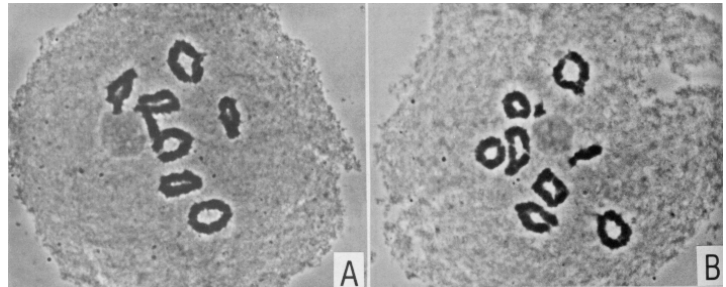
- 4) The real number is $2n = 14$, and the plants are trisomic, with an extra chromosome.

Explain how you would distinguish among these four possibilities, and pick the most likely option. Be sure to explain why you consider it the most likely option.

59. The following is from:

Friedt W, B Foroughi-Wehr, G Mix and HM Wilson. 1977. Anther culture of autotetraploid *Hordeum vulgare* varieties and the production of trisomic individuals. Barley Genet Newsl 7:29-33.

Barley is normally $2n = 2x = 14$



- A) Identify the meiotic configurations (ie, number of I's, II's and/or III's) in A and B.
- B) To preserve these genetic stocks, would one use them as a male or female? Why?
- C) For 6 pts extra credit, describe the cross overs needed to give you the unusual configuration in figure A

60. The following label is from a seedless ($2n = 3x = 33$) watermelon. Notice the warning, “May contain occasional seeds” is needed.

A) Assume that a diploid watermelon can have 1000 seeds, and that only $2x$, $3x$ and $4x$ seeds are viable. How many watermelons would you have to go through to find one seed?

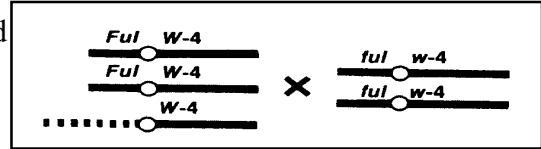


B) For 5 extra credit points, Dulcinea is a character in a very famous novel. Which novel?

61. The following is from:

Khush, G.S. and C.M. Rick. 1967. Tomato tertiary trisomics: Origin, identification, morphology and use in determining position of the centromeres and location of markers. *Can. J. Gen. Cytol.* 9:610-631.

The diagram is from class, and shows the setup up used by Khush & Rick, 1967, whereby they used tertiary trisomics to place genes onto chromosome arms. Modify their system to permit gene-centromere mapping of the W-4 locus.



Extra Credit (5 pts)

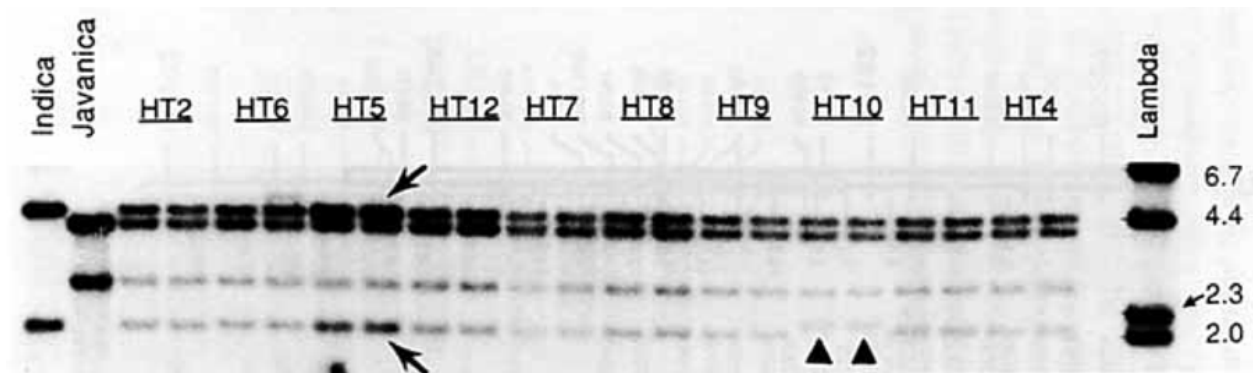
Describe a scheme to produce tertiary trisomics in high frequency

62. The following is from:

Yu ZH, GS Khush, and SD Tanksley. 1994. Assignment of RFLP linkage groups to rice chromosomes using primary trisomics. Rice Genetics Newsletter 11:149-154.

<http://www.shigen.nig.ac.jp/rice/rgn/vol11/v11p149.html>

The authors were trying to assign RFLP probes to their respective chromosomes. The blot below shows their results with probe RG182 using a series of trisomics (trisomics 1 and 3 were not represented) derived from a *japonica* x *indica* cross. The use of a hybrid provides a built-in control to ensure even DNA loading within a lane.



A. Which chromosome is RG182 located on?

B. Give a brief overview of the principles that allow this technique to be used to assign genes/probes to chromosomes.

C. How would you modify the technique to get down to the level of an arm, instead of a whole chromosome?

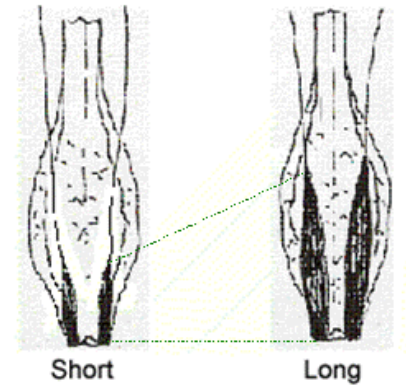
Describe a scheme to produce tertiary trisomics in high frequency

63. The following is from:

Watanabe N, T Sekiya, K Sugiyama, Y Yamagishi and I Imamura. 2002. Telosomic mapping of the homoeologous genes for long glume phenotype in tetraploid wheat. *Euphytica* 128: 129-134.

The authors looked at glume length in progeny plants with and without the telosome, and got these data:

	$2n$	$2n + t$
Long:Normal	68:9	14:72



A) Long glumes are conditioned by the recessive P1 gene. The dominant allele gives normal glumes. Diagram the 3 chromosomes involved, including the location of the P1 locus, whether the alleles are dominant or recessive, and the expected location of the crossover.

B) What is the P1-centromere distance?

Note: In the class example, only the $2n$ category was recombinant. In this case, both the $2n$ and the $2n + t$ categories are recombinant. Therefore it is not necessary to multiply the recombination frequency as we did in class.

C) Would the gene-centromere distance be shorter, longer or the same if they had used half-tetrad analysis instead? Explain the reason for your answer

64. The following table is from:

Rubiales D, SM Reader and A Martin. 2000. Chromosomal location of resistance to *Septoria tritici* in *Hordeum chilense* determined by the study of chromosomal addition and substitution lines in 'Chinese Spring' wheat. Euphytica 115: 221-224.

H. chilense is a wild barley species that is resistant to septoria blotch of wheat. Based on Table 1:

A) How were the addition lines derived?

B) Which *H. chilense* chromosomes have resistance genes for *Septoria* (measured as a low % of pycnidia coverage), and for each chromosome with a resistance gene, which arm is it on?

Table 1. Percentages of pycnidia coverage in seedlings and adult plants of wheat 'Chinese Spring' (CS), *Hordeum chilense* and CS/*Hordeum chilense* single chromosome addition lines inoculated with *Septoria tritici*

Line	% pynidia coverage	
	Seedlings	Field
Chinese Spring	40 a	40 a
<i>H. chilense</i> H1	0 d	0 c
CS/4H ^{ch} disomic	1 d	0.3 c
CS/5H ^{ch} disomic	14 bcd	25 ab
CS/6H ^{ch} disomic	15 bcd	17b
CS/7H ^{ch} disomic	9 cd	20 b
CS/1H ^{ch} S monosomic 1H ^{ch} + monotelosomic 1H ^{ch} S	32 ab	23 ab
CS/1H ^{ch} S ditelosomic	26 abc	30 ab
CS/2H ^{ch} α ditelosomic	24 abc	— ¹
CS/5H ^{ch} L ditelosomic	14 bcd	23 ab
CS/6H ^{ch} S ditelosomic	4 d	17 b
CSS/7H ^{ch} α ditelosomic	14 bcd	20 ab
CS/7H ^{ch} β ditelosomic	17 abcd	27 ab

¹ Not tested.

² Letters in common within a column indicate that differences are not statistically significant at $p = 0.05$.

C) Diagram a breeding scheme to transfer the major resistance gene from *H. chilense* to wheat. Make use of *ph* stocks.

65. The following is from:

Guo Y-P C Vogl, M van Loo, and F Ehrendorfer. 2006.
Hybrid origin and differentiation of two tetraploid *Achillea*
species in East Asia: molecular, morphological and
ecogeographical evidence. *Molecular Ecology* 15: 133-144.

The authors observe that “The allopolyploid *A. alpina*.
exhibits considerable morphological variation and ecological
flexibility, and has expanded throughout eastern Asia and to
northern North America, far beyond the ranges of their
presumed 2x-ancestors.”

A) Why would an allotetraploid be adapted to a wider range
than its 2x ancestors? Give 2 reasons.



Morphological differentiation of the series
Achillea acuminata-2x (a, b, h), *Achillea
alpina*-4x (c, d, i), *Achillea wilsoniana*-4x
(e, f, j) and *Achillea asiatica*-2x (g, k):
lower stem leaves (a–g; natural size) and
inflorescences (h–k; h–j $\approx 3/4$, k $\approx 1/2$
natural size).

The authors then used AFLPs to try and determine the diploid
ancestors of the allotetraploids. Their data are as follows:

	Number of bands			
	total	In 4x <i>A. alpina</i>	In 4x <i>A. wilsoniana</i>	In both 4x spp.
2x <i>A. acuminata</i>	36	3	4	19
2x <i>A. salicifolia</i>	10	1		
2x <i>A. impatiens</i>	4			
2x <i>A. ptarnica</i>	11			1
2x <i>A. asiatica</i>	74	8	2	45

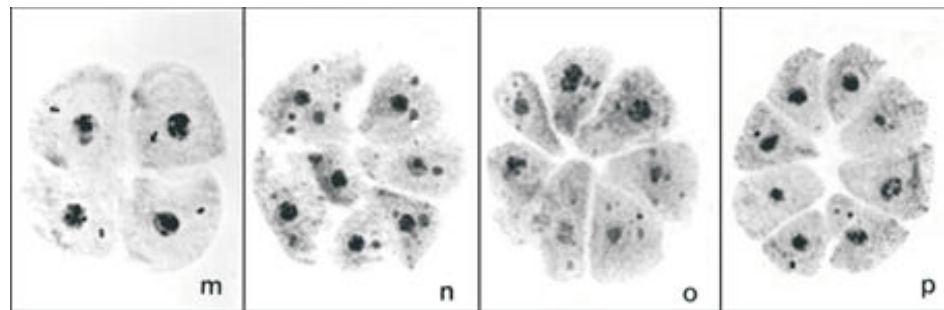
B) **Extra credit.** Based on these data, it looks like *A. acuminata* and *A. asiatica* gave rise to
both tetraploid species. Why would two parents lead to different allotetraploid species?

66. The following is from:

Mendes Bonato AB, M Ferrari Felisimo, AM Souza Kaneshima, C Pessim, V Calisto, M Suely Pagliarini, and C Borges do Valle. 2009. Abnormal meiosis in tetraploid genotypes of *Brachiaria brizantha* (Poaceae) induced by colchicine: its implications for breeding. J Appl Genet 50:83-87.



Cattle grazing brachiaria in Costa Rica.



Brachiaria is a grass used as a forage in the tropics. The photo depicts the results of normal (m) and abnormal (n-p) meiosis in colchicine-derived autotetraploids.

- A) What is the term used to describe what is pictured in n-p?
- B) What will happen to the pollen grains derived from n-p?
- C) Why would the auto4x be more prone to this type of problem than the 2x? What 3 traits or characteristics can one select for in order to overcome this problem?

67. The following is from:

Pandey RM 2008. Chromosome behaviour and fertility in induced polyploids of grain amaranths. *Caryologia* 61:199-205.

Amaranth is a grain traditionally grown in the Andes. In this paper, the author doubled the chromosome number with colchicine, and then measured several parameters. Some of the data are below:

Based on these data, the author concludes, “The induced tetraploids in the present material were definitely ‘inferior’ to their respective diploids and it is amply clear that optimum threshold limits for all the species studied is at the 2x level, beyond which polyploidy has an adverse effect.”

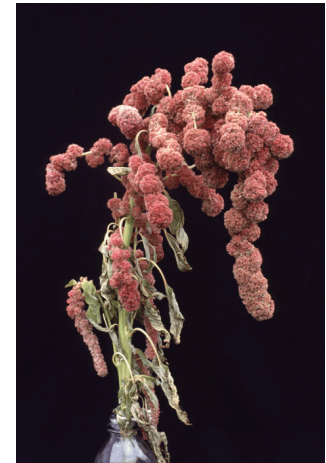


Photo credit: CIP

		Days to flower	Height (cm)	Grain yield per plant (g)
<i>A. cruentus</i>	2x	48	180	156
	4x	59	92	45
<i>A. flavus</i>	2x	38	120	98
	4x	49	58	38

A) There are at least 3 factors that explain the results, namely days to flower (ie, time for mitosis), height (ie, vigor) and grain yield (ie, fertility). Discuss why these 3 traits would be affected in the raw autotetraploid.

B) Do you agree with Pandey’s conclusion? Why or why not. Can anything be done (and if so, what) to overcome the problems Pandey observed with days to flower and height?

68. The following is from:

Celebi-Toprak, F., B. Behnam, G. Serrano, M. Kasuga, K. Yamaguchi-Shinozaki, H. Naka, J.A. Watanabe, S. Yamanaka and K. N. Watanabe. 2005. Tolerance to salt stress of the transgenic tetrasomic tetraploid potato, *Solanum tuberosum* cv. Desiree appears to be induced by the *DREB1A* gene and *rd29A* promoter of *Arabidopsis thaliana*. *Breeding Science* 55:311-319.

In this paper, the authors engineered potato with a gene from *Arabidopsis* that, when expressed in potato, made the potato salt-tolerant. They next wanted to know if the transgene had inserted itself into one locus in the genome, or two different loci in the genome. They took a transgenic plant, pollinated it with non-transgenic pollen, planted the seed, and then tested the seedlings for salt resistance. The transgene is dominant, not recessive.

The assumptions were 1) the transgene was in Simplex mode, and that 2) random chromosome segregation was taking place in the tetraploid. Following is from their materials and methods

The expected tolerance ratio in the progeny was 1:1 for tolerant : susceptible, and the observed phenotype in the true seed progeny was 32 tolerant and 44 susceptible. ... Therefore, a Duplex mode was rejected. Thus it is likely that the genotype of the parental [potato plant] shows a Simplex mode, namely that a single transgenic allele is present. That is, there 76 progeny, and for a random chromosome model they expected 38 tolerant : 38 susceptible.

A) Explain why “Duplex” is the wrong term for them to have used.

B) What are the expected numbers (not ratio) of tolerant : susceptible progeny plants if they had used a random chromatid or a maximal equational model instead of a random chromosome segregation model?

The table below gives the formulas for the gametic frequencies produced by a Simplex ($T t t t$) genotype. In this case, T= transgene; t = no transgene

Gametes	Formulae
$t t$	$\frac{1}{2} + \frac{1}{4} \alpha$
$T t$	$\frac{1}{2} - \frac{1}{4} \alpha$
TT	$+\frac{1}{4} \alpha$

C) Based on these results, is the transgene most likely near the centromere or the telomere? Explain your answer.

69. The following is from:

Ahanchede, Poirier-Hamon & Darmency. 2004. Why no tetraploid cultivar of foxtail millet? Genetic Resources and Crop Evolution 51: 227-230.

Photo of foxtail millet: jgi.doe.gov

In this paper, the authors used colchicine to double the chromosome number from an unspecified number of seedlings of cultivar 'Yellow Sand' (YS). Data are below:



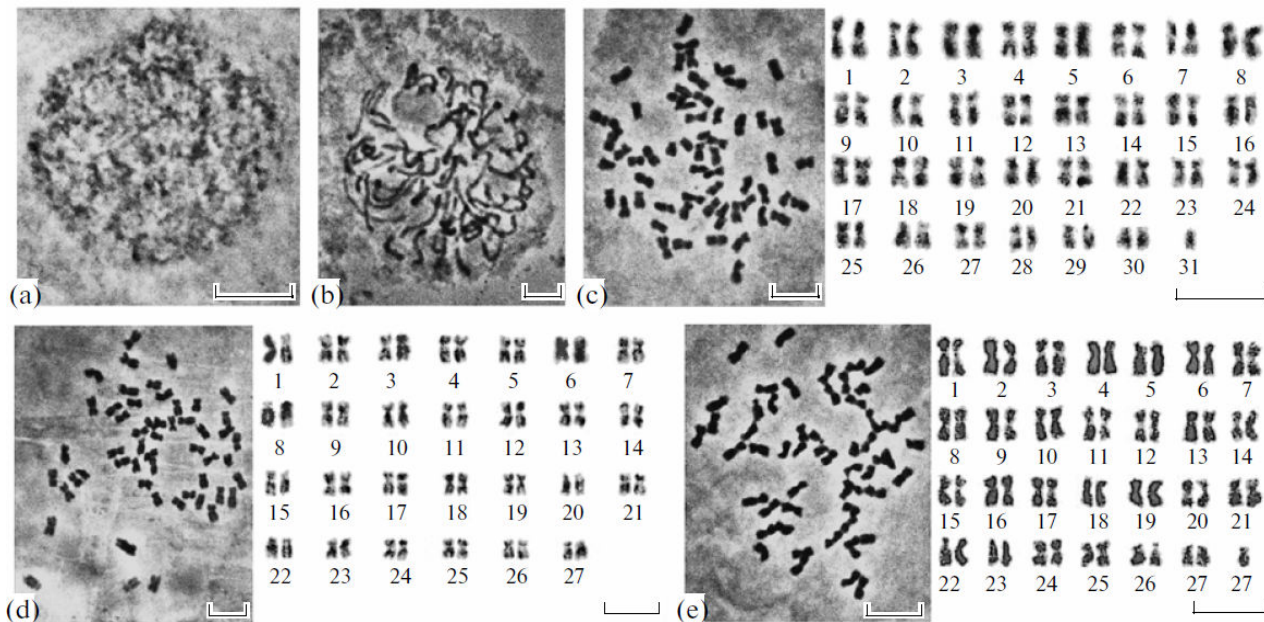
	Flowering date	Plant height	Spike length	Grain weight on main spike	Grain yield per plant	1,000 grain weight
	day	cm		g		
1990*						
YS diploid	72.6 b§	73.7 a	16.5 a	8.2 a	18.5 a	2.06 b
YS tetraploid	78.8 a	62.8 b	14.8 a	5.2 b	10.7 a	2.52 a
1991						
YS diploid	–	98.8 b	19.0 a	11.0 a	41.4 a	2.40 b
YS tetraploid	–	76.9 d	17.5 b	5.6 b	18.7 b	2.97 a

A) Explain their results

B) Propose a solution if they really wanted a tetraploid variety

70. The following is from:

Li C, S Chen, F Chen, J Li and W Fang. 2011. Cytogenetic study of three edible chrysanthemum cultivars. Russian J of Genetics 47:176-181.



Metaphase + karyotype of

(c) “Baohuatangyijin” with $2n = 61$

(d) “Zifengmudan” with $2n = 54$

(e) “Jingxingxiying” with $2n = 55$

Scale bar = 10 μm

A) For Baohuatangyijin, the authors claim it is a monosomic. Assuming they are correct, are they dealing with a diploid, allopolyploid, or autopolyploid? Explain your choice.

B) For Zifengmudan, the authors claim it is a euploid. Assuming they are correct, are they dealing with a diploid, allopolyploid, or autopolyploid? Explain your choice.

C) For Jingxingxiying, the authors claim it is a trisomic. Assuming they are correct, are they dealing with a diploid, allopolyploid, or autopolyploid? Explain your choice.

Extra Credit) Describe up to 4 lines of evidence you would want to see to confirm the monosomic or trisomic nature of these cultivars.

71. The following is from:

Winterfeld et al., 2009. Allopolyploid origin of Mediterranean species of *Helictotrichon* (Poaceae) and its consequences for karyotype repatterning and homogenisation of rDNA repeats. *Systematics and Biodiversity* 7: 277-295.

Taxon and sequence clone	Alignment position																			
	45	54	93	107	114	117	174	198	217	385	401	402	420	434-441	451	494	536	544	564	
<i>H. bromoides</i> (2x) ²	G	G	T	G	G	T	G	A	T	C	C	C	G	GCTCCCCG	T	C	A	G	G	
<i>H. cincinnatum</i> (4x)																				
clone_CIN1	G	G	T	G	G	T	G	C	T	C	A	–	G	GCCCCCG	C	C	G	G	G	
clone_CIN2	G	G	T	G	G	T	G	C	T	C	A	–	G	GCCCCCG	C	C	G	G	G	
clone_CIN3	G	G	T	G	G	T	G	C	T	C	C	–	G	GCCCCCG	T	C	A	G	G	
clone_CIN4	G	G	T	G	G	T	G	C	T	C	A	–	G	GCCCCCG	C	C	G	G	G	
clone_CIN5	G	G	T	G	G	T	G	C	T	C	A	–	G	GCCCCCG	C	C	G	G	G	
clone_CIN6	G	G	T	G	G	T	G	C	T	C	A	–	G	GCCCCCG	C	C	G	G	G	
clone_CIN8	G	G	T	G	G	T	G	C	T	C	A	–	G	GCCCCCG	C	C	G	G	G	
clone_CIN9	G	G	T	G	G	T	G	C	T	C	A	–	G	GCCCCCG	C	C	G	G	G	
clone_CIN10	G	G	T	G	G	T	G	C	T	C	A	–	G	GCCCCCG	C	C	G	G	G	

In this case, *H. cincinnatum* is an allotetraploid between *H. bromoides* and an unknown species. When the authors sequenced the interspace region between repeats of the 5.8S rDNA repeats from *H. cincinnatum*, they found little evidence for the presence of DNA from *H. bromoides*, so they infer that only the 5.8S sequence from the unknown parent survived.

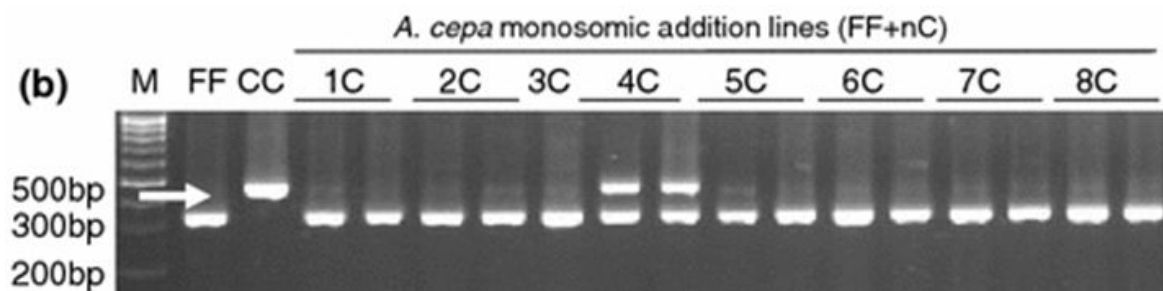
A) The name for this phenomenon is:

B) Diagram the most likely way that this happened.

72. The following is from:

Tsukazaki et al., 2011. Direct determination of the chromosomal location of bunching onion and bulb onion markers using bunching onion-shallot monosomic additions and allotriploid-bunching onion single alien additions. Theor Appl Genet 122:501-502.

In this work, the authors were assigning molecular markers to their chromosomes, so they derived monosomic addition lines of onion (*Allium cepa*) in shallot (*A. fistulosum*). 1C thru 8C are the individual onion chromosomes in the shallot background. FF stands for shallot DNA, and CC stands for onion DNA.



A) Diagram how the monosomic addition lines are derived

B) In the above figure, which onion chromosome contains the molecular marker?

73. The following question is from Yu, Kim, Rayburn, Widholm and Juvik. 2011. Bioenergy 1:404-412.

Miscanthus (*Miscanthus x giganteus*, $2n = 3x = 57$) is promising bioenergy crop due to its potential to produce large amounts of biomass. However, only one genotype is known to exist, and it is sterile, as it is an allotriploid derived by *M. sinensis* ($2x = 38$) and *M. sacchariflorus* ($4x = 76$).

Describe how you would go about getting a fertile population of *Miscanthus x giganteus* so that breeding could be done.



Miscanthus x giganteus. Photo credit: Bluestem.ca

74. The following is from:

Trueblood, Ranney, Lynch, Neal & Olsen. 2010.
Evaluating fertility of triploid clones of *Hypericum androsaemum* L. For use as non-invasive landscape plant.
HortScience 45:1026-1028.

This is a species native to Europe and western Asia. The premise of the paper is that this species is attractive as an ornamental, but came invasive when introduced in Australia. Thus, a highly sterile genotype is needed in order to use safely as a genotype.



Photo credit: Wikipedia

A) A) This species is $2n = 40$. If x , $2x$, $1x + 1$, and $2x - 1$ gametes are all fertile, what percent of pollen will be viable?

B) Suppose 1% fertile haploid gametes was used as a threshold whereby triploidy could be used as a containment measure. What is the minimum number of x that is necessary to comply with the threshold?

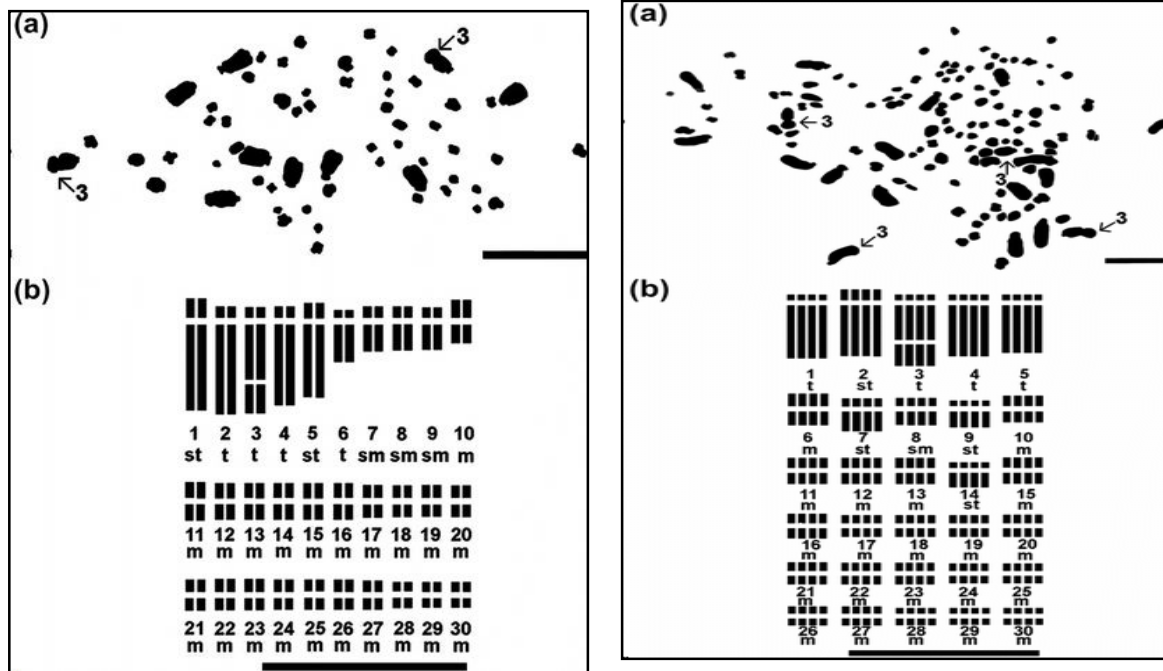
75. A. What is the significance of the increased frequency of multivalents in nullisomic 5B wheat plants?

B. What is the practical application of such plants?

76. Tetraploid bahiagrass is apomictic. When crossed with an induced tetraploid of 2x Pensacola bahiagrass, the F_2 included 9 apomictic and 267 sexual offspring. If the *aaaa* genotype is the only one that is apomictic, what ratios might be expected from the various genotypes among the sexual offspring?

77. The following is from:

Palomino et al. 2012. Nuclear genome size and cytotype analysis in *Agave cupreata* Trel. & Berger (Agavaceae). *Caryologia* 65: 281-294.



Left: "Chromosomes of diploid *Agave cupreata* (2n = 2x = 60) from Ayahualco 574: (a) mitotic metaphase; (b) idiogram."

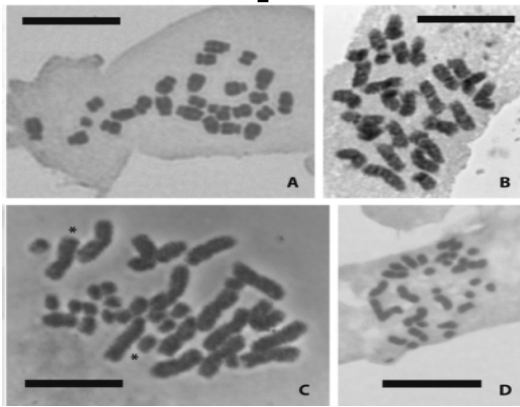
Right: "Chromosomes of tetraploid *Agave cupreata* (2n = 4x = 120) from Xochapa 571: (a) mitotic metaphase; (b) Idiogram"

1. Discuss the concept of 'diploid' as used to describe the cytotype on the left. Limit answer to no more than 2 sentences.

- B) According to the authors, "The diploid and polyploid populations of *Agave* showed highly conserved bimodal karyotypes, which is a result of an evolutionary process known as orthoselection. This process consists of amplifications of non-coding DNA sequences that are distributed between regions where the crossing over of the chromosomes of the complement takes place." Orthoselection is invoked to explain why certain asymmetrical karyotypes are maintained in different families

Discuss whether you agree or do not agree with the explanation. Be sure to give all your reasons. If you do not agree with the answer, what is your explanation for the asymmetrical karyotype? Limit your answer to no more than 2 well-constructed paragraphs.

78. Zepeda-Cornejo et al., 2012. Intersexual comparison of DNA content by flow cytometry, and chromosome number in four dioecious *Chamaedorea* palms from Mexico. *Caryologia* 65:263-270.



"Somatic chromosomes of (A) *C. ernesti-augusti* $2n = 2x = 26$; (B) *C. pinnatifrons* $2n = 2x = 26$; (C) *C. alternans* $2n = 2x = 32$; and (D) *C. tepejilote* $2n = 2x = 32$. The bar on each panel represents the scale of the photo ($= 10 \mu\text{m}$)"

- A. Although the authors refer to these as 2x species, there are indications that they might be 4x. What is the main indication?
- B. Of these which is the best candidate to be an auto4x? Explain your answer.
- C. What observations would you want to make to corroborate your answer to B)? Give 2.

B.) *C. tepejilote* appears to have less DNA than the other species? What type of selection pressure could favor less DNA in a 4x?

79. The following is from:

Beyaz et al. 2013. Sugar beet (*Beta vulgaris* L.) growth []. Caryologia. 66:90 -

The authors conclude that "From the results of the present study, the lower levels of parameters recorded in tetraploid genotypes confirmed that the effects of increased ploidy level could not be anticipated all the time. Diploid genotypes were found to be superior to tetraploids in generative characteristics such as total chlorophyll and protein content, sugar content, root and sugar yields."



Table 2. Germination and seedling growth in diploid and tetraploid sugar beet genotypes.

Genotype	Day 4			Day 14	
	Germination (%)	Root length (cm)	Seedling height (cm)	Root length (cm)	Seedling height (cm)
Agnessa (2X)	75.10 a	7.40 a	6.34 a	12.67 a	9.05 a
Felicita (2X)	89.10 a	8.45 a	7.00 a	13.96 a	9.52 a
Mean	82.10	7.93	6.67	13.32	9.29
AD 440 (4X)	60.10 b	5.05 b	5.74 a	7.50 b	6.21 b
CBM 315 (4X)	60.10 b	5.86 b	5.49 a	7.52 b	7.38 b
Mean	60.10	5.46	5.62	7.51	6.80

Values followed by different letters in a column are significantly different at the 0.01 level.

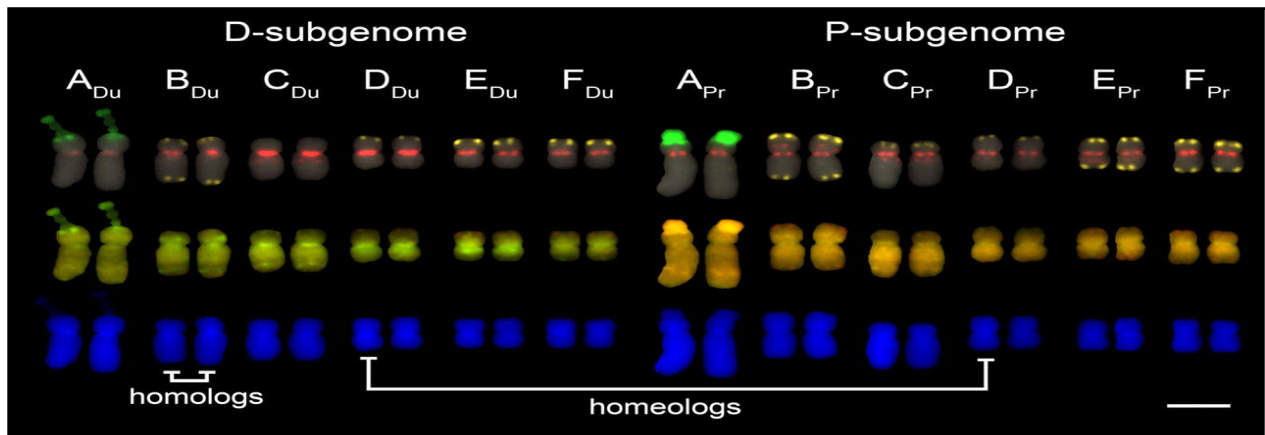
A) What is the most likely reason that 2x is > than 4x?

B) If you were a breeder geneticist taking over the project, what 2 approaches could you do to reverse the situation?

80. The following is from:

Chester et al. 2012. Extensive chromosomal variation in a recently formed natural allopolyploid species, *Tragopogon miscellus* (Asteraceae). PNAS 109 :1176–1181

T. miscellus is a new allotetraploid that formed some 80 years ago after the introduction of *T. dubius* (dyed green) and *T. pratensis* (orange) to the western USA.



The above karyotypes were obtained with GISH and FISH

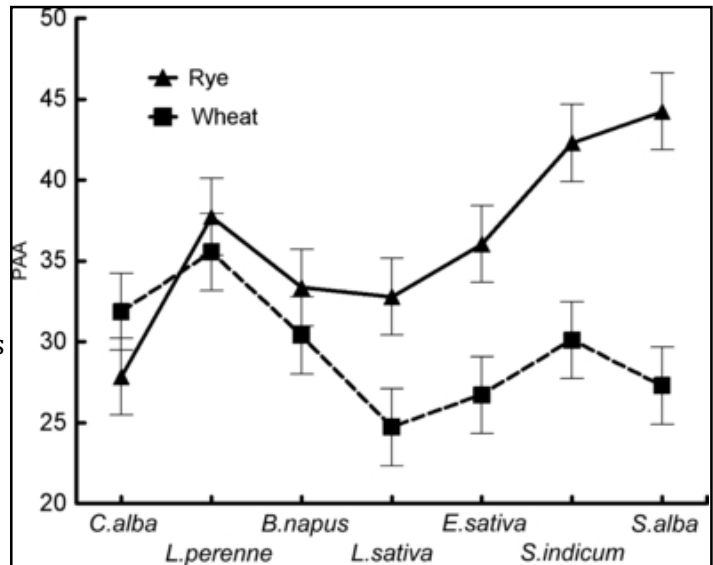
- A) A) Using the GISH karyotype, choose a pair of homologs and put a star below each one
- B) B) Using the GISH karyotype, choose a pair of homeologs and put an asterisk below each one

Explain what FISH is and how it can be used to paint chromosomes. List the major steps in conducting FISH

81. The following is from:

Bertholdsson et al. 2012. Allelopathic potential of *Triticum* spp., *Secale* spp. and *Triticosecale* spp. and use of chromosome substitutions and translocations to improve weed suppression ability in winter wheat. Plant Breeding 131:75-80.

The premise is that some species secrete more allelopathic chemicals (chemicals that kill other species); in essence, these are naturally occurring herbicides that kill competing plants. In theory, chromosome substitution could be used to further characterize and move allelopathy.



Extra Credit: The graph shows the ability of rye and wheat to control different weed species. Why is the graph wrong?

A.) Show or describe how a chromosome substitution series could be derived for the proposed experiment

B.) Once in hand, explain how the substitution series could be used to characterize if any given rye chromosome is responsible for allelopathy

82. The following is from:

Gar et al., 2011. (...)tetraploid linkage map of rose (*Rosa hybrida*) validated using the strawberry (*Fragaria vesca*) genome sequence



A segregating population of hybrid tea roses ($2n=4x=28$) was derived from the cross of 'Golden Gate' by 'Fragrant Cloud' seen in the photos.

	Bi-parental markers		
Parent map	FC & GG		
Scoring method	Dominant	Dominant	Dominant
Parents genotype (FC X GG)	Aaaa X Aaaa	Aaaa X AAaa / AAaa X AAaa	AAaa X AAaa
Segregation ratio	01:03	01:11	01:35
Segregation group type	5	6	7

$$\frac{1+2\alpha}{6} \times AA + \frac{4-4\alpha}{6} \times Aa + \frac{1+2\alpha}{6} \times aa = 1$$

$$\frac{2+\alpha}{4} \times AA + \frac{2-2\alpha}{4} \times Aa + \frac{\alpha}{4} \times aa = 1$$

$$\frac{\alpha}{4} \times AA + \frac{2-2\alpha}{4} \times Aa + \frac{2+\alpha}{4} \times aa = 1$$

- A.) Based on the data in the table, are hybrid roses auto or allotetraploid? Explain your answer.
- B.) Which of the 3 crosses (there is 1 cross per column) is the informative one, and why?
- C.) Is the marker used near a telomere or centromere? Name the type of 4x segregation it represents. Explain your answer
- D.) The above formulae are for duplex, triplex, and simplex, respectively. What would be the expected **gametic** ratio for the triplex if $\alpha = 0.1$? Based on these ratios, what is the frequency of nulliplex individuals that could come from crossing two triplexes together?

83. The following is from:

<http://io9.com/5791035/mutation-breeding-creates-the-worlds-most-perfect-orange>

Mutation breeding creates the world's most perfect orange

The photo at left is of the Kinnow mandarin orange. After mutagenesis they got the KinnowLS variety (LS for low seeded).



1. What is the advantage that the mutagenesis approach has over the triploid approach?
2. What is the advantage that the triploid approach has over the mutagenesis approach?

Answer both questions in terms of biology and breeding objectives, not consumer perception of safety. **These are extra credit questions**

84. The following is from:

Del Bosco et al., 2007. Production and characterization of new triploid seedless progenies for mandarin improvement. *Scientia Horticulturae* 114:258-262.

In their efforts to get seedless tangerines, the authors crossed 'Dancy' ($2n=4x=36$) with 2 diploid tangerines, 'Wilking' and 'Fortune' mandarines, and 'Monreal' clementines. [Botanically speaking, tangerines and clementines are all varieties of mandarins, *Citrus reticulata*].

The photo at right is of <http://www.citrusvariety.ucr.edu/citrus/fortune.html> Fortune mandarin

Assuming that x , $2x-1$ and $2x$ gametes are viable, and $x+1$ eggs are viable, calculate the probability of getting a seed in a $3x$ tangerine. Assume 12 sections per fruit, 6 ovules per section. Show all your work.



Photo from <http://verdego.com/07/dancy-tangerine/>, which notes that it "may have a quite a few seeds inside of it, but a delicious fruit none the less."

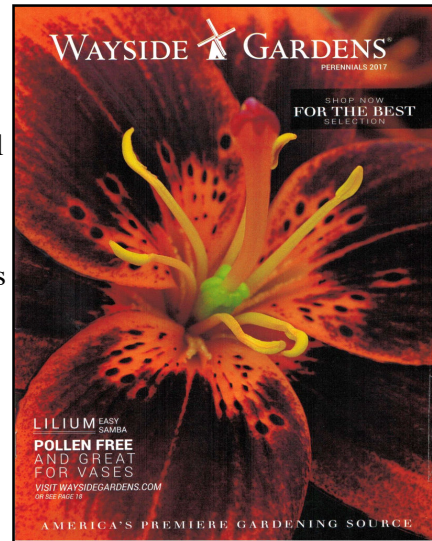


85. The following is from: the Wayside Gardens 2017 Perennials Catalog

Its cover features the Easy Samba lily, which does not shed pollen.

Cut flowers in vases wilt once they are pollinated. Hence, it is common practice to remove the anthers of lilies used in floral arrangements to prevent pollination. A pollen-free lily is desirable as removal of anthers is not necessary.

Having taken PBGG 8890, you suspect that the developers of this lily used a cytogenetical approach to obtain a pollen-free flower.



- A) What cytological phenomenon is most likely to give pollen-free plants?
- B) Describe two types of observations you could try to make to prove your premise. In both cases, describe your basis for comparison.

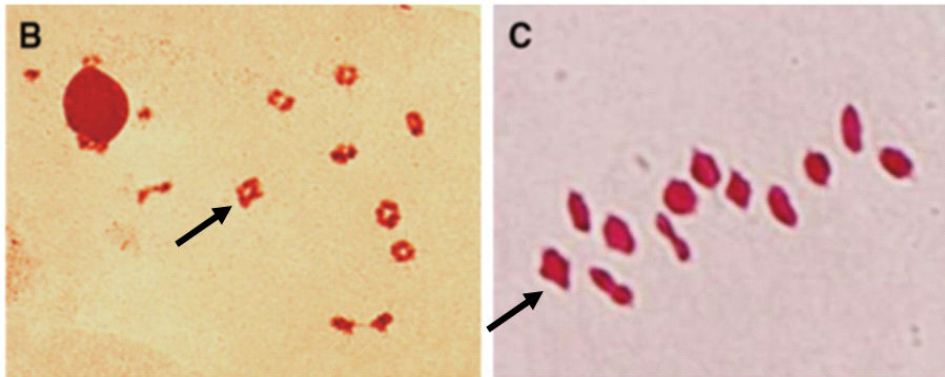
86. The following is from: Jena, Ballesfin & Vinarao. 2016. Development of *Oryza sativa* L. by *Oryza punctata* Kotschy ex Steud. monosomic addition lines with high value traits by interspecific hybridization. Theoretical and Applied Genetics. 129: 1861-1871.

In this paper, the authors to use chromosome addition lines to bring in traits from a wild rice species. To that end, they first crossed an autotetraploid cultivated variety (AAAA) by a wild diploid (BB).

A.

the

the



Looking at below pictures, which picture belongs with which parent in cross?

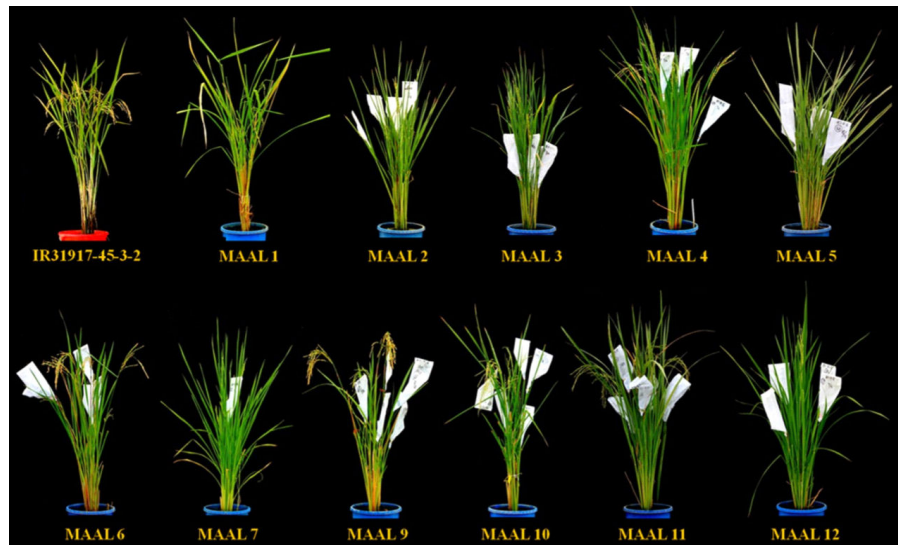
B. Diagram out the chromosomes that have been singled out by the black arrow in figures b and c (above).

C. When 95 PMC's of the AAAA plant were analyzed cytogenetically, they found a variety of chromosome configurations. The table at right gives the configuration and the number (and %) of observations. Name two characteristics that lend themselves towards an increased amount of IV formation relative to II.

Chromosome configurations	IR31917-45-3-2 (4n) ^a
0IV + 24II	1 (1.06)
2IV + 20II	2 (2.11)
3IV + 18II	8 (8.42)
4IV + 16II	17 (17.89)
5IV + 14II	28 (29.47)
6IV + 12II	16 (16.84)
7IV + 10II	5 (5.26)
8IV + 8II	8 (10.52)
9IV + 5II	6 (6.32)
10IV + 4II	2 (2.11)

D. Diagram a likely crossing scheme that would give the anticipated monoplids ($2x + 1$) plants

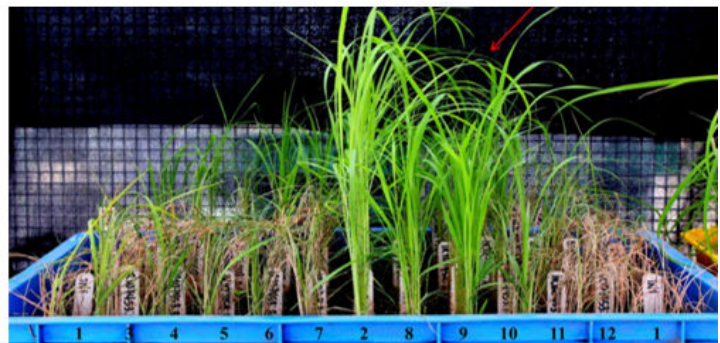
- E. Here is a photo of the parental rice genotype, and the 12 disomic addition lines (obtained by selfing the monophloids). Other than chromosome-specific trait introgression, describe two additional uses for these lines:



- F. In the photo at the bottom of the page, line 1 is the susceptible check, line 2 is a resistant check, and 3-12 are the disomic addition lines. All were exposed to the brown line hopper.

Which addition lines contain a resistance gene?:

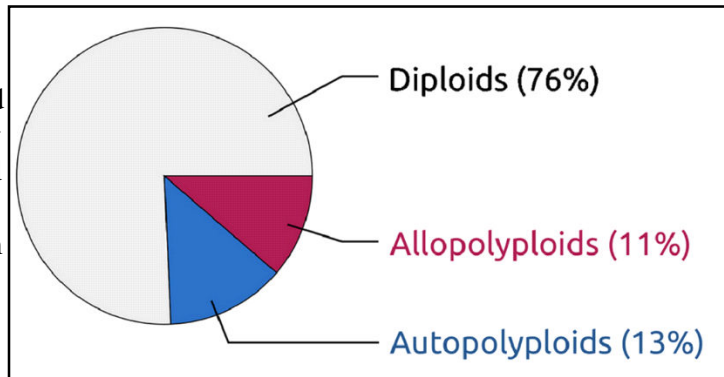
- G. Extra Credit: How would one move the resistance gene into rice, while minimizing linkage drag? Rice does not have known pairing genes like wheat does; nevertheless, rice chromosomes (A genome) rarely pair with the B genome of the wild species parent), although the A and B genomes are the rice genomes most closely related to each other.



87. The following is from: Barker, Arrigo, Baniag, Li and Levin. 2015. On the relative abundance of autopolyploids and allopolyploids. New Phytologist. 1-7.

1.

A- One of premises behind his forum letter is that successfully established autopolyploid species in nature may be undercounted because the 2x and 4x individuals are too similar to each other. Based on what has been discussed on auto4x species, assess the validity of the premise.



B.-This authors determined that the numbers of allo and autopolyploid plant species are the same; yet they hypothesize that autopolloid individuals are much more likely to form, given they arise from intraspecific crosses. In contrast, formation of allopolyploids requires 2 closely related species to be growing in close proximity for them to cross. 2n gametes are involved in the formation of both auto and allo species.

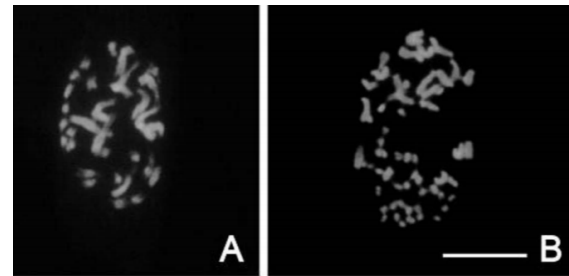
The bottom line is that auto4x spp are less common than expected, leading the authors to conclude that allopolyploids have an evolutionary advantage. No mention is made of any disadvantages that autopolyploids can have. List and briefly described 2 advantages of allopolyploids discussed in class, and 2 disadvantages that autopoloids have that could limit their establishment as successful species.

88. The following is from: Tungkajiwangkoon, Shirakawa, Azutamani and Hoshi. 2016. Breeding and cytogenetic characterizations of new hexaploid *Drosera* strains colchicine-Induced from triploid hybrid of *D. rotundifolia* and *D. spatulata*. *Cytologia* 81:263-269.

Droseras are sundews, carnivorous plants. The authors had previously crossed *Drosera rotundifolia* ($2n = 2x = 20$) with *D. spatulata* ($2n = 4x = 40$) to get a triploid with $2n = 3x = 30$. They then derived a hexaploid with colchicine. This $6x$ is then compared to a natural $6x$, *D. tokaiensis*.

The chromosomes of the $3x$ and $6x$ plants are in figures A and B, respectively.

A. If you knew absolutely nothing about the history of this plant, and could only see photo B, would you assume the plant was an auto or allopolyploid? Explain the reason for your answer.



2http://www.drosophyllum.com/Bilder/Dr_rotundifolia_Julia.jpg

The flow cytometry results confirm what is seen in the pictures A and B.

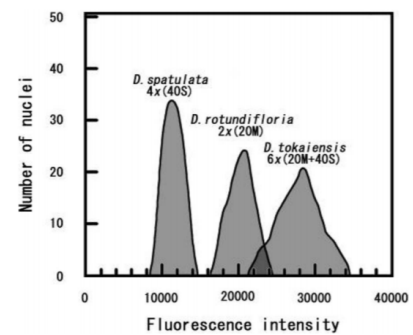
B. Based on this 1 run of the flow cytometer, can the authors make any inferences on the absolute size of the genomes they are studying? Why or why not?

C. Briefly describe/diagram 2 processes that will decrease the size of the tetraploid genome over the size of the diploid genome

D. One of the parental species is about 4 cm in diameter, while the other is 5. Invoke nucleotypic effects and explain which of the parental species is most likely to be the smaller of the two.

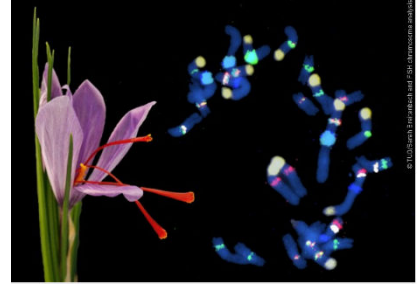
E. Extra Credit: The best colchicine treatment had a success rate of 5%. An alternative to the use of colchicine is to count on the formation of $2n$ eggs and pollen. With what frequency can selfing the $3x$ plant be expected to give a $6x$ seed? Explain your calculations.

F. Extra Credit: Despite the obvious advantage of colchicine in terms of frequency, would there be any advantages to trying to get a $6x$ via $2n$ gametes? Explain your answer.



89. The following is from:

Schmidt et al, 2019. Adding color to a century-old enigma: multicolor chromosome identification unravels the ----- nature of saffron (*Crocus sativus*) as a ----- . New Phytologist doi: 10.1111/nph.15715

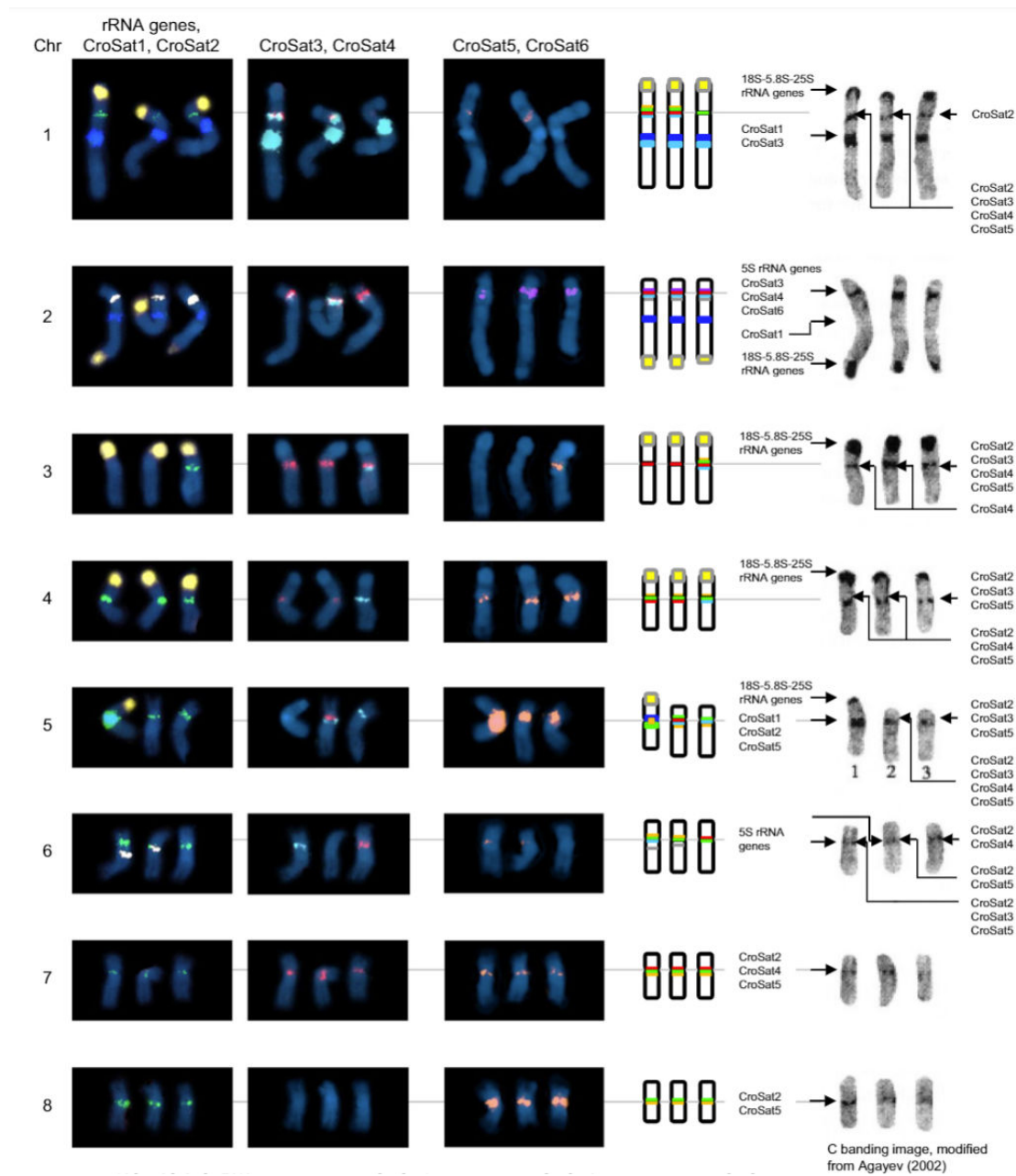


Saffron is the world's most expensive spice, selling for over \$15,000 per lb. It comes from the stigma/styles of a sterile clone ($2n = 3x = 24$) that has been vegetatively propagated for some 3500 years. The authors used FISH to determine if saffron is an auto triploid or an allo triploid

- A) Assuming that n , $n+1$, $2n$, and $2n-1$ eggs are fertile, what percent of eggs can be expected to be fertile?
- B) If pollinated by a diploid plant, what is the expected amount of $2x$ seed formation?

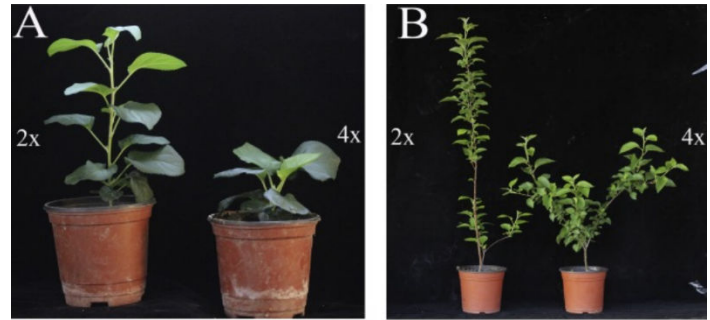
Show all your calculations

- C) Below are the FISH results. Based on the karyotype below, is saffron an auto triploid, an allo triploid, or something else?



90. The following is from:

Xue et al., 2017. Comparison of the morphology, growth and development of diploid and autotetraploid 'Hanfu' apple trees. *Scientia Horticulturae*, 18: 227-285.



2x and 4x plants at 1 and 2 years of age

According to the authors, “Before flowering, the autotetraploids showed retarded growth and significant dwarfing characteristics. The autotetraploid plants delayed flowering until 5 years of age, which was 2 years older than the age at which the diploids first flowered, ...” The differences are evident in the photos.

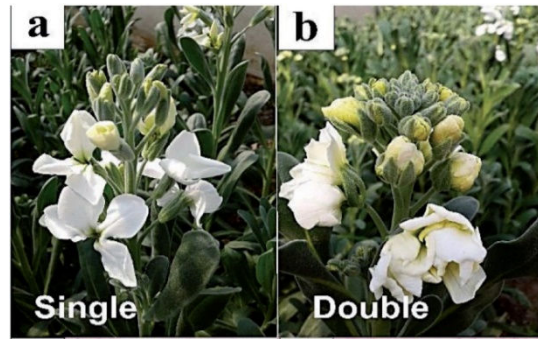
Though fruit number or yield data are not provided, the authors do note that “However, the fruit drop rate was significantly higher in the autotetraploid than in the diploid.” The authors go on to conclude that “the results will make a meaningful contribution to the breeding of polyploid apples.”

- A) What most likely explains the retarded growth seen in young seedlings?
- B) What advice would you give the authors as a way to create better performing autotetraploid plants than is possible with colchicine?
- C) Fruit drop reflects poor seed set. Why would auto4x apples have poor seed set?

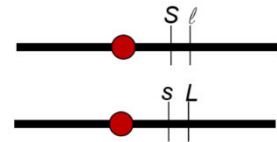
91. The following is based on:

Frost HB & MM Lesley. 1954. High-double stock varieties: extra chromosome in trisomic stock responsible for higher ratio in production of double flowers. California Agriculture, 8(2): 11-12. Photo from Irani & Arab, 2017.

Matthiola is an ornamental flower. In some genotypes, the stamens and carpels are converted into petals. Such double-flowered types are valued by the industry, but they are sterile. Thus, seed for doubled flowered plants must be obtained from single-flowered types.



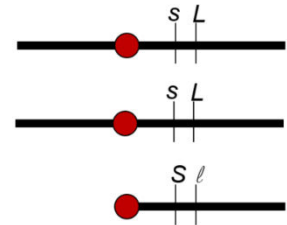
The doubled flowered phenotype comes from a recessive mutation in the agamous gene, known as the S gene in matthiola. Thus, the homozygous recessive (ss) gives double-flowers.



The S locus is very tightly linked to the L locus, which gives pollen lethality when recessive. Thus, in heterozygous plants, Sl pollen aborts, so $\frac{1}{2}$ the progeny will be single flowered and the other half will be double flowered. The limitation to the system is that the grower must wait for the plants to flower before knowing if they will be single or double-flowered.

Male → Female ↓	S ℓ (pollen lethal)	sL	
S ℓ		SsL ℓ	Normal single
sL		ssLL	Normal double

There is a phenotype that appears, called slender, that is due to a telotrisomic with the SL loci on it. A particularly useful method of seed production can be obtained when the Sl alleles are carried on the telosome, as shown at left. The telosome has very poor male transmission, and bad female transmission. Regardless, its transmission will result in a single-flowered type, but with the slender phenotype. Lack of transmission of the telosome results in doubled flower plants with normal phenotype.



F/M → ↓	SsL ℓ (poor pollen transmission)	sL	
SsL ℓ		SssLL ℓ	Slender single
sL		ssLL	Normal double

Because the telosome transmission is low, doubled-types will predominate in the seed. Furthermore, seedlings with the slender phenotype can be eliminated, leaving only doubles behind.

Every now and then, however, slender doubles and normal singles are obtained. **Explain the basis for their appearance.**

92 The following is from:

Irani SF & M Arabl, 2017. Meiotic behaviour and morpho-phenological variation in cut stock (*Matthiola incana* L.) flower. Folia Horticulturae 29(1): 51-61. Photo credit = jackseeds.com

Matthiola is an ornamental plant, $2n = 2x = 14$. In this study, the authors looked at meiotic pairing in 12 different cultivars. If you woke up one morning and decided you needed to breed an autotetraploid *matthiola* variety, which cultivar would you choose to chromosome-double as having the highest probability of being a fertile auto4x plant? Explain the basis for your selection.

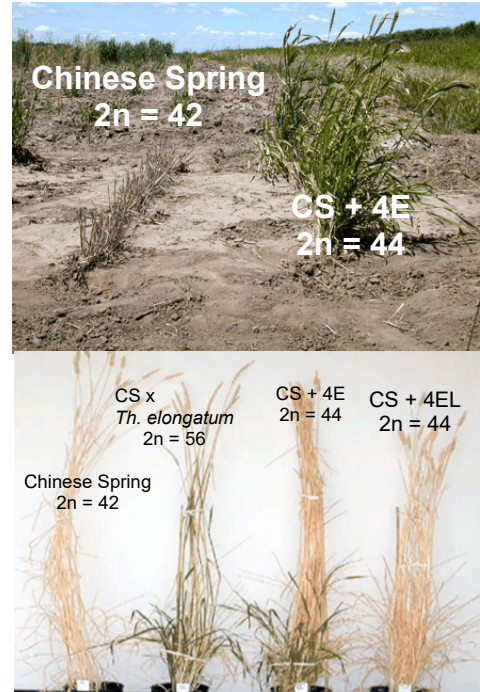


CV ^a	Chiasma ^b			II ^c			IV ^d	
	Terminal	Middle	Total	Ring	Rod	Total	Range	Mean
CR	10.20	1.53	11.73	4.40	2.33	6.73	0-1	0.10
NB	9.33	1.60	10.93	3.23	1.40	4.63	0-3	1.16
GD	9.20	1.83	11.03	3.10	2.53	5.63	0-3	0.70
PC	8.56	1.40	9.96	2.93	3.66	6.60	0-1	0.20
RP	10.03	1.40	11.43	4.00	2.26	6.26	0-2	0.36
LL	11.96	0.80	12.76	5.73	0.96	6.70	0-1	0.13
MB	10.03	1.36	11.40	4.30	2.10	6.40	0-1	0.30
AV	10.03	1.53	11.56	4.33	1.66	6.00	0-2	0.53
HP	10.53	1.43	11.96	4.93	1.73	6.66	0-1	0.16
WS	10.33	1.33	11.66	4.43	1.56	6.00	0-2	0.50
DB	10.26	1.46	11.73	4.56	1.63	6.20	0-2	0.40
ES	11.46	0.80	12.26	5.10	1.43	6.53	0-1	0.23

93. The following is from:

Lammer et al, 2004. A single chromosome addition from *Thinopyrum elongatum* confers a polycarpic, perennial habit to annual wheat. Journal of Experimental Botany, 55(403): 1715–1720.

- A) Diagram how a disomic addition line would be created

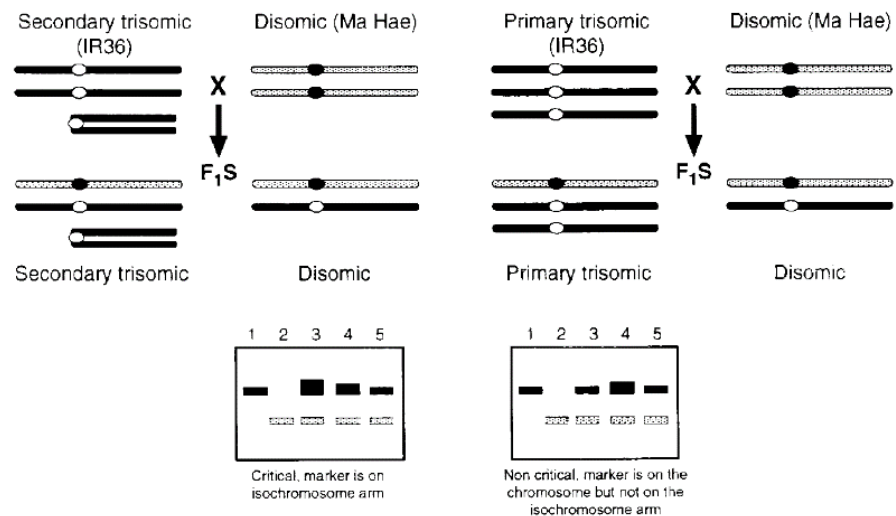


- B) Why are disomic addition lines not released commercially?
- C) The authors did further work to narrow down the location of the perennial trait, and located it down to 1 arm. Devise and diagram a breeding strategy that does not involve translocations to introgress perenniality into wheat.

94. The following is from:

Singh et al., 1996. Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa* L.). PNAS 93: 6163-6168.

This paper made ingenious use of primary and secondary trisomics to determine which chromosomal arm contained the RFLP marker, using an approach as diagrammed at right. If the marker was on the long arm—which gave rise to the isosome—it would give a darker band.



When primary trisomics were used, a darker band indicates the marker is on the trisome, but will not narrow down its location to one arm or the other. Thus, a marker on the trisome but not on the isosome must be on the short arm.

Basically, they went down their genetic map, evaluating each marker in turn as described in the diagram, and placing it either on the short arm or the long arm.

Given that the RFLP maps were a linear sequence of markers along the length of a chromosome, how does this procedure identify the flanking markers for the centromere? In other words, explain the observation that needs to be made.

95. The following is based on: Heo JY & SM Park. 2017. 'Sujeong' a green seedless table grape cultivar. HortSci 52: 462-464 **and** Royo C, R Torres-Pérez, N Mauri et al., 2018. A major origin of seedless grapes is associated with a missense mutation in the MADS-box gene VviAGL11. Plant Physiol. 177: 1234-1253. Seedless grapes ($2n=2x=38$) are due to a dominant regulatory locus that affects three recessive mutations that collectively cause the seeds to abort, while the fruit develops via parthenocarpy. The condition is termed stenopermocarpy. Barring a few grape varieties that are female, the rest hermaphrodites that still produce pollen.



Figure 1. Sujeong triploid grapes

Due to the complications of breeding for stenopermocarpy, the development of seedless varieties has emerged as an alternative. 'Sujeona' is one such variety.

Your plan is to plant 1 ha of Sujeona grapes, noting that all your neighbors plant 2x Thompson Seedless, which are stenopermocarpic. To promote the new variety, you are thinking of offering \$100 to anyone who finds a seed.

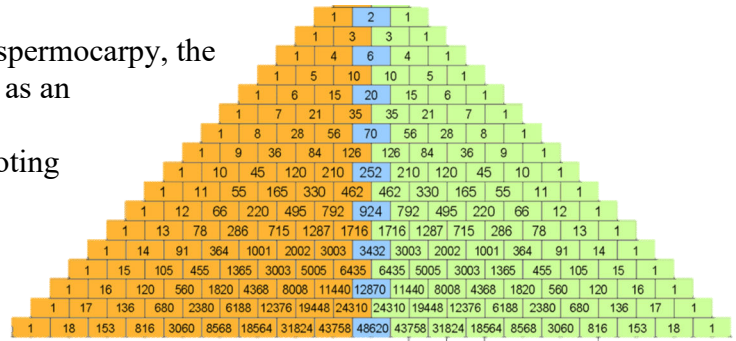
First, though you calculate the odds of a consumer finding a seed so that you can calculate your potential payout.

Assuming that n , $n+1$, and $n+2$ eggs give viable seeds, and that each grape can have 2 seeds, what percent of Sujeona fruits could be expected to have a seed? Show your math, not just the answer

- % n seeds =
- % $n+1$ seeds =
- % $n+2$ seeds =
- Total % seeds =
- % berries with a seed =

What is your potential payout per ha? On average, Sujeon yields 14 tonnes/ha and average berry weight is 5.6 g. A tonne is 1000 kg.

- Thus expected # of grapes per ha =
- Payout you would have to make =



95. The following is from: Khun LH, S Miyaji, K Motomura K, S Murayama, S Adaniya & A Nose. 2006. Trisomic analysis of new gene for late heading in rice, *Oryza sativa* L. *Euphytica* 151: 235–241.

Rice has several genes that determine whether the plant will head (send up a flower stalk) early or late. Here the authors found a late-heading line. They ran allelism tests with the other known late-heading genes, and invariably recovered a digenic ratio, showing they had a new gene. Then, to find which chromosome this new gene is on, they crossed to trisomics and looked at F₂ ratios, shown in Table 4. The distorted segregation showed it is on chromosome 7:

Table 4 Segregations for heading times caused by *eff(t)* locus in F₂ populations of the crosses between Triplo lines and T65-LH7

Cross	Type of F ₂ plant	Number of plant			χ^2 value for			
		Early	Late	Total	3:1	8:1	35:1	44:1
Triplo-2/T65-LH7	Disomic	133	39	172	0.496			
	Trisomic	93	33	126	0.095			
Triplo-4/T65-LH7	Disomic	147	41	188	1.021			
	Trisomic	85	20	105	1.984			
Triplo-5/T65-LH7	Disomic	119	34	153	0.630			
	Trisomic	99	28	127	0.591			
Triplo-7/T65-LH7	Disomic	144	29	173	6.260**	5.595*		
	Trisomic	121	4	125	31.683**		0.083	0.550
Triplo-9/T65-LH7	Disomic	154	39	193	2.364			
	Trisomic	80	23	103	0.392			
Triplo-10/T65-LH7	Disomic	119	30	149	1.881			
	Trisomic	83	25	108	0.198			
Triplo-12/T65-LH7	Disomic	123	50	173	1.405			
	Trisomic	82	31	113	0.357			

*, **Significant at 5% and 1% levels, respectively.

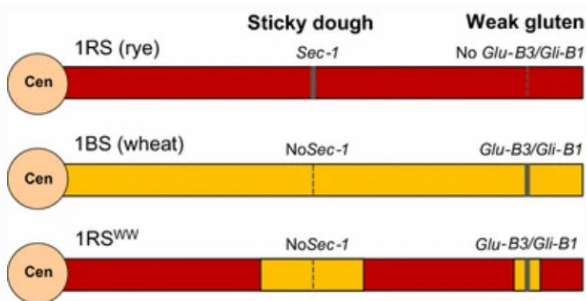
The 8:1 ratio was covered in class. 35:1 and 44:1 are reported by the authors to be trisomic ratios under the Maximal Equational and Random Chromosome models. [they are not, but has no bearing on the answer].

- What is the frequency of crossover between the centromere and the late heading locus? Explain your answer. A diagram might help sort your thoughts.
- How does the gene centromere-distance obtained with trisomics compare with one obtained by half-tetrad analysis?

96. The following is derived from:

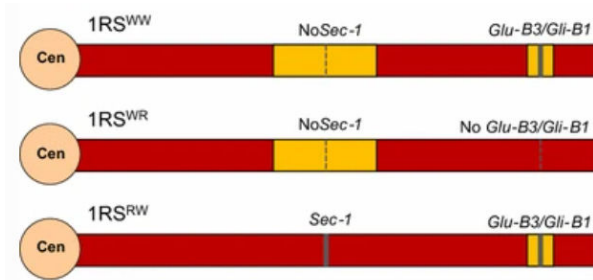
Lukaszewski AJ. 2000. Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. *Crop Science* 40: 216-226 **and** Howell T, I Hale, L Jankuloski, M Bonafede, M Gilbert & J Dubcovsky. Mapping a region within the 1RS.1BL translocation in common wheat affecting grain yield and canopy water status. *Theoretical & Applied Genetics* 127: 2695-2709

There is a 1BL.1RS translocation that is used frequently in wheat breeding because the rye segment conditions for high yield, drought stress, and multiple disease resistances. But, rye secalins from the *Sec1* locus cause dough stickiness, while loss of wheat glutenins (GluB3) and gliadins (GliB1) causes weak dough (cannot be stretched). Thus the goal was to replace the rye segment containing *Sec1* with the equivalent wheat segment, and the distal rye segment with the equivalent wheat segment containing GluB3 and GliB1 as diagramed:



- a. This translocation was introduced in cv 'Veery.' The first step was to move the 1RS.1BL chromosome into 'Pavon' using a 1B monosomic line of Pavon. Describe 1 method that could have been used to obtain a Pavon 1B monosomic line (it need not be the method used in the paper).

- b. Next, Pavon had to be converted from Ph1b to ph1b. This was done by substituting the Pavon 5B chromosome with that of 'Chinese Spring'. Chinese Spring has the original ph1 mutant developed by Sears. Describe one method that could have been used to make this substitution.



To get the desired result (top chromosome), they first had to independently get wheat segments to replace the two undesired rye segments (middle and bottom).

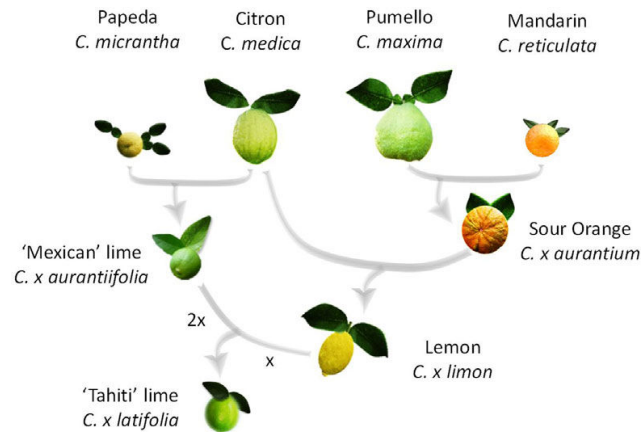
C. Describe a procedure that could have been used to produce the middle and bottom chromosomes in the above diagram. Note that the example in class looked at chromosome

pairing as a way to measure the amount of wheat chromosome present, but think of more modern ways to accomplish the same the objective.

- D) Once the middle and bottom chromosomes are on hand, what is done to get the top chromosome in the diagram?

97. The following is from:

Ahmed D, F Curk, JC Evrard, Y Froelicher & P Ollitrault. 2020. Preferential disomic segregation and *C. micrantha*/*C. medica* interspecific recombination in tetraploid 'Giant Key' lime; outlook for triploid lime breeding. *Frontiers in Plant Science*, 11: 309



'Mexican' lime is a hybrid between *Citrus micrantha* x *C. medica*, with the two parent species being highly related, which permits some chromosome pairing.

There is another lime variety, 'Giant Key' lime, that is a doubled version of 'Mexican' lime.

There is interest in using Giant Key as a parent to generate more 3x varieties.

First, the question is whether Giant Key behaves most like autopolyploid or an allopolyploid.

It has recently become possible to assess allosyndesis and autosyndesis frequencies by

looking at molecular markers in the pollen. Each marker has alleles that came from one or the other of the Mexican lime parents. Thus the pollen can have a marker from each parent species, or be homozygous

- a) Thus, does pollen heterozygosity indicate allosyndesis or autosyndesis?

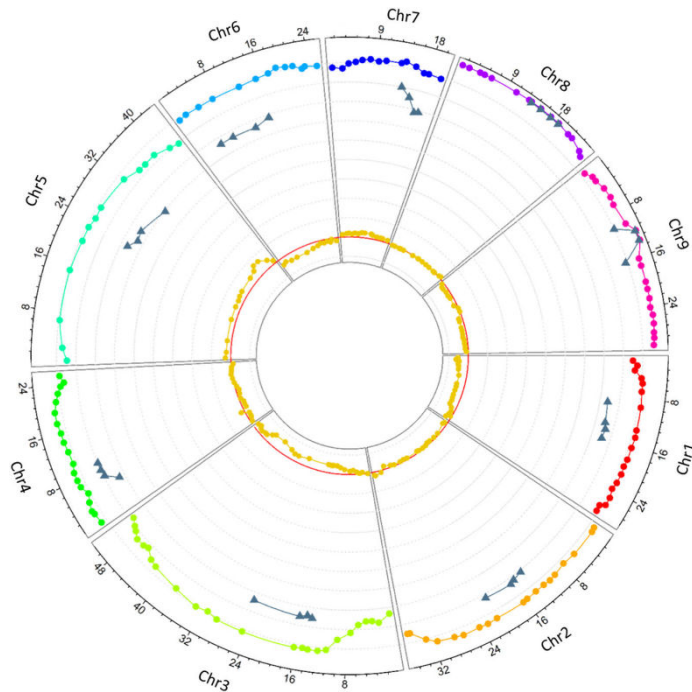
Tau (τ) = proportion of gametes that come from auto or allosyndesis. $\tau = 1$ = full tetrasomic inheritance (random pairing); $\tau = 0$ = full autosyndetic pairing. τ is calculated using markers near the centromere.

- b) What is the purpose of using pericentromeric rather than telomeric markers to measure allo/auto syndesis?

Next, the chromosomal preferential pairing rate (PP) is calculated. PP = the proportion of gametes resulting from autosyndesis and ranges from 0 – 1. $PP = 1 - \tau$.

- c) For Giant Key, PP is chromosome-specific, and ranges from 0.633 to 0.995. Is it surprising that a plant is not a full allo or a full autopolyploid? Explain your answer.

This graph shows the average gametic heterozygosity along the length of each chromosome of Giant Key. Pericentric diversity is shown in gray for each chromosome. The Y axis shows PP, and ranges from -0.1 to 1, with the red line at 0. Gray lines are 0.1 intervals.



- a) Based on this graph, which chromosome(s) is experiencing the greatest amount of preferential pairing? Explain your answer.
- b) Which chromosome has the least amount of preferential pairing? Hint: how often do intra-genomic pairings happen in a completely random model, hence leading to homozygosity?
- c) Finally, 3x 'Tahiti' lime is hypothesized to have come from an SDR egg from Mexican line and a haploid pollen from lemon. Yet, ~90% of the markers inherited from Mexican lime are heterozygous. Does this call the hypothesis of Tahiti's origin into question? Why or why not?

98. The following is from:

Shimizu-Inatsugi, A Terada, K Hirose, H Kudoh, J Sese & KK Shimizu. 2016. Plant adaptive radiation mediated by polyploid plasticity in transcriptomes. *Molecular Ecology* 26:193-207
 “The habitats of polyploid species are generally distinct from their parental species. Stebbins described polyploids as ‘general purpose genotypes’, which can tolerate a wide range of environmental conditions.”

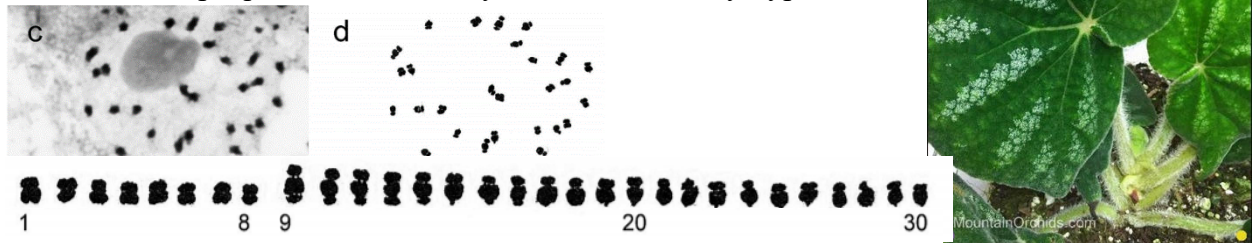
Explain why allopolyploids can be more adaptable than their parents.

99. The following is from:

Kono Y, CI Peng, K Ogunma, Y Liu, WB Xu, HA Yang & KF Chung. 2020. Cytological study of *Begonia* sect. *Coelocentrum* (Begoniaceae). *Cytologia* 85: 333-340.

You have been gifted a specimen of *Begonia filiformis*, pictured at right.

Having just completed PBGG 8890, you wonder about its genome, and do a root tip squash, from which you construct a karyotype.



Not knowing anything else about it, you have 3 hypotheses:

- It is a diploid, $2n = 2x = 30$
- It is a triploid, $2n = 3x = 30$
- It is an allotetraploid, $2n = 4x = 30$

Describe a series of observations/measurements that can be made to determine which is the right answer. In each case, give the expected observation. In other words, do not just say something like ‘look at the karyotype’ but give what you expect to see in the karyotype that is informative.

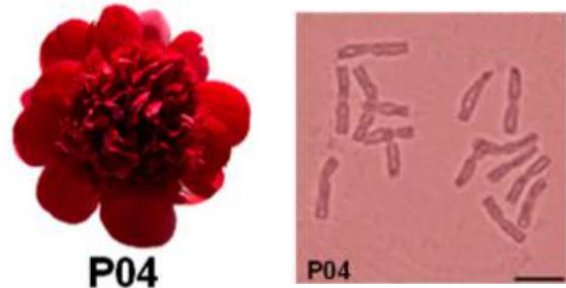
100. The following figure is from: (to be revealed after the exam)

Cui L, T Chen, X Zhao, S Wang, X Ren, J Xue & X Zhang. 2022.

Karyotype analysis, Genomic and Fluorescence In Situ Hybridization (GISH and FISH) reveal the ploidy and parental origin of chromosomes in *Paeonia* Itoh Hybrids. International Journal of Molecular Science, 23(19): 11406.

In this paper, the authors were investigating a variety called 'Red Charm' of an ornamental. A root-tip squash is shown.

You come up with several hypothesis to explain this chromosome number



- i) The plant is normally $2x=14$, but has a trisome
- ii) The plant is normally $2x=14$, but has a B chromosome
- iii) The plant is $3x=15$
- iv) The plant is normally $2x=16$, but is monosomic for a chromosome

You have been given a year to determine the answer. Describe at least 4 observations you would want to make to help answer the question.

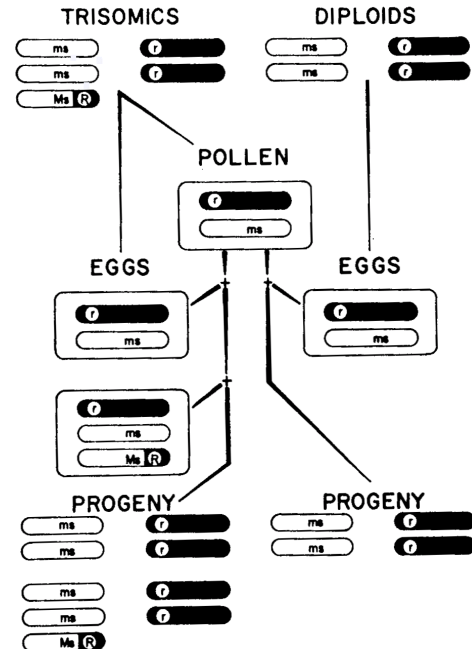
101. The following is from:

Ramage RT. 1965. Balanced tertiary trisomics for use in hybrid seed production. *Crop Science* 5(2): 177-178.

Almost 60 years ago, a method was proposed as a way to get male-sterile females for use in barley hybrid seed production.

It was based on a balanced tertiary trisomic with

- Dominant allele of a marker gene (red color) that is near the break point
- The recessive allele on the two normal chromosomes
- The extra chromosome of the trisomic is not transmitted through the pollen
- The extra chromosome is transmitted through the eggs, so eggs with and without the trisome are produced by the trisomic plants
- All gametes formed must have one complete copy of each chromosome.
 - If they only have one of the normal chromosomes plus the 3rd chromosome, they will be deficient and will abort
- The diploid parent is male sterile, so it only produces one type of egg
- All trisomic progeny will be red and male fertile.
 - These can be ⊗ for propagation or eliminated from the field
- All disomic progeny will be green and male sterile, and can be used as females in a seed production field



Bottleneck: Getting the right translocation with the correct markers proved difficult, so the system was never implemented. *However, we now finally have the necessary biotech tools to make the system functional.*

- a) Describe how you can use editing and transgenics to get the needed translocation, male sterile, and marker genes. Hint: it may be more practical to use herbicide resistance instead of leaf color
- b) How would you create the trisomic in first place?

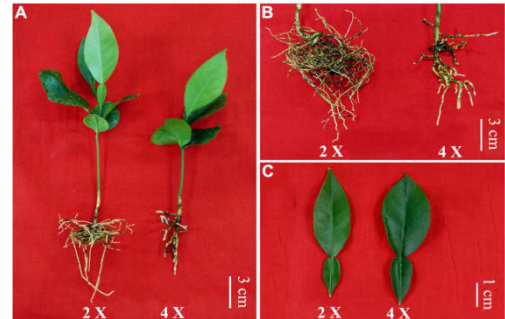
Use the next page for your answers

102. The following is from:

Jiang J, et al. 2022. Tetraploidy in *Citrus wilsonii* enhances drought tolerance via synergistic regulation of photosynthesis, phosphorylation, and hormonal changes. *Frontiers in Plant Science*, 13: 875011

In this paper, the authors obtained autotetraploids of a citrus species normally used as a rootstock. As seen in photo, the auto4x plants grew more slowly. These arise from endopolyploidy in the nucellus prior to the formation of adventitious embryos.

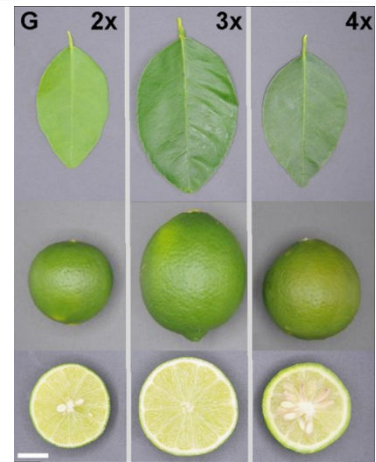
Explain the most likely reason(s) for the slower growth of the auto4x.



103. The following is based on:

Ruiz M, J Oustric, J Santini & R Morillon. 2020. Synthetic polyploidy in grafted crops. *Frontiers in Plant Science*, 11: 540894.

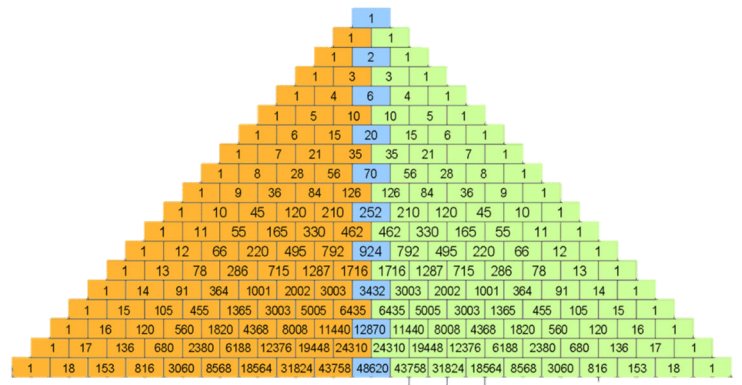
Seedlessness is a trait appreciated in lime ($2n = 2x = 18$). Assuming zygotes with 18, 19, 35, and 36 chromosomes are viable, and that the average fruit has 10 seeds, what % of fruit would be expected to have a seed in them? Show your math, not just the answer.



- % n seeds =
- % n+1 seeds =
- % 2n seeds =
- % 2n-1 seeds =

Total % seeds =

- % berries with a seed =



104. The following is from:

Sanamyan MF, SU Bobohujayev, SS Abdugarimov, AK Makamov & OG Silkova. 2022. Features of chromosome introgression from *Gossypium barbadense* L. into *G. hirsutum* L. during the development of alien substitution lines. *Plants*, 11(4): 542.

In this work, the authors obtained lines of cotton (*G. hirsutum*, $2n = 52$) in which pairs of chromosomes from cotton had been replaced with chromosomes of Pima cotton (*G. barbadense*, $2n = 52$).



G. gossypium, *G. barbadense*, and *gossypium* lines with substitutions for chromosomes 4, 6, 7, 12 & 18

Describe/diagram the chromosome substitution process.

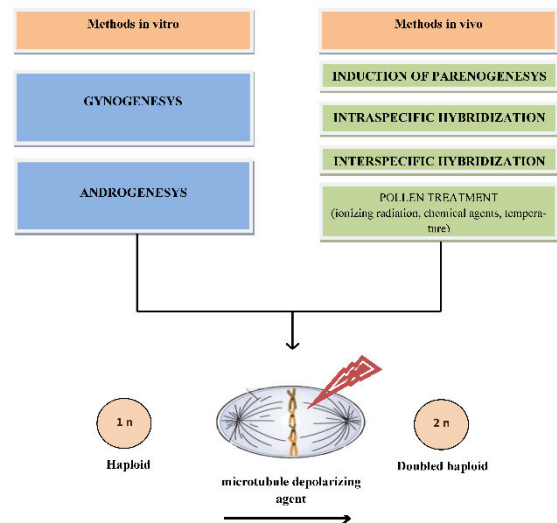
To get the process started, monosomic lines of cotton ($2n = 51$) are needed. Explain/diagram 2 ways on how such lines could be obtained.

Why would the authors want chromosome substitution lines?

105. The following is from:

Zargar M, T Zavarykina, S Voronov, I Pornia and M Bayat. 2022. The recent development in technologies for attaining doubled haploid plants in vivo. Agriculture, 12(20): 1595.

In this review, the authors appear to have used a stock image for meiosis. Aside from the translation errors in the terminology, is the diagram correct? Explain why or why not.



Though discussed in the review, the diagram leaves out more modern methods of haploid induction. Describe two of these.

107. The following is taken from:

Docket No. APHIS-2022-0076, “Request for Information: Identifying Ambiguities, Gaps, Inefficiencies, and Uncertainties in the Coordinated Framework for the Regulation of Biotechnology”

Recently, the US government’s Office of Science and Technology Policy (OSTP) requested public comment on the current regulatory framework for ag biotechnology. Comments were due Feb. 3

The USDA policy on CRISPR/Cas was frequent target for comments. To recap, CRISPR/Cas makes a double-stranded break in the DNA. When the DNA is repaired, either a new allele is created, or the gene is knocked out. Both fall under the term ‘gene edit’.

Under current regulations, only one CRISPR/Cas edit at one locus is allowed. If homoeologous loci in a polyploid are edited, or if multiple genes are edited, the plant must be regulated as a transgenic.

Provide public comment to support or not support the USDA policy on editing.

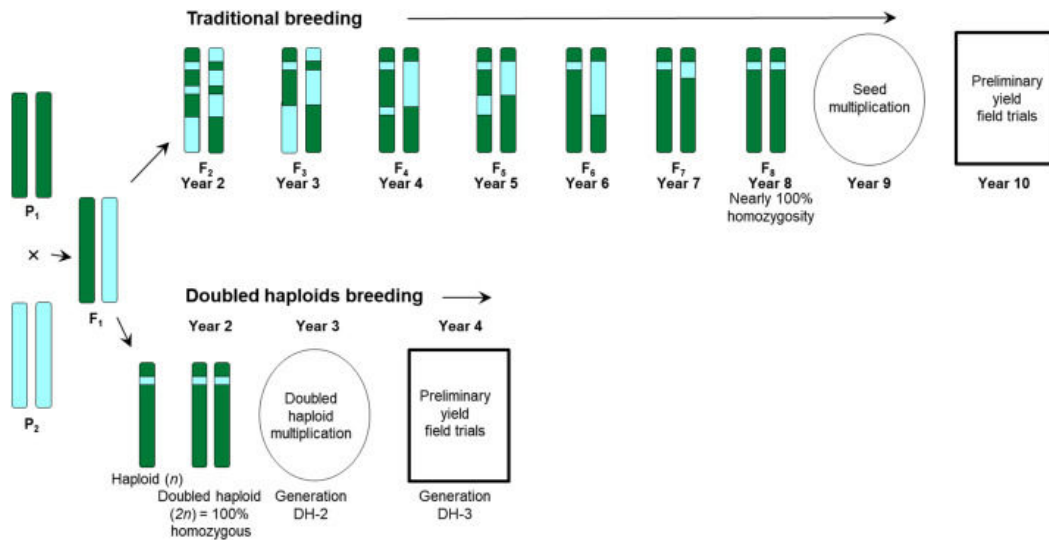
You will need to find papers in the refereed literature that you can use to justify your support or lack thereof for the proposed rule on multiple edits. Thus, the review should focus on the historical uses and the ensuing safety (or lack thereof) issues of knockouts and new allele formation in domestication and breeding. Concise arguments and clear points are helpful. As a reminder Federal rule-making is not a popularity contest. Likewise, it is not a vote. Thus, comments like ‘I support’ or ‘I am against’ do not count. Likewise, comments along the lines of ‘I believe’ or ‘in my opinion’ do not count either. *Instead, comments are supposed to be literature reviews, citing papers from the refereed literature that explain how the science on the topic supports or does not support the proposed rule.*

Limit your comments to a **maximum** of 1 page + references. Use *Plant Physiology* format for the references.

108. The following is based on:

Eliby S, S Bekkuzhina, O Kishchenko, G Iskakova, G Kylyshbayeva, S Jatayev, K Soole, P Langridge, N Borisjuk & Y Shavurkov. *Biotechnology Advances*, 60: 108007

In this paper, the authors try to show the advantages of doubled haploids over traditional selfing in a breeding program:



Explain the likelihood they will obtain genotypes shown for Y2 and Y8 conventional and Y1 and Y2 in the doubled haploid method. Think in terms of the crossovers needed to get those genotypes.