PROBLEM SET 4 – LINKAGE AND RECOMBINATION
(covering lectures 8 & 9 and QS 5 from week 5)

1. What is wrong with your conclusion in each scenario?

1a. You are examining two loci, A and B, in a haploid yeast strain. The yeast strain has genotypes A and b at the two loci. You conclude that the strain is heterozygous.

1b. A genetic map shows that loci A and B are on the same chromosome and are 60cM away from each other. You conclude that A and B are linked.

1c. Loci A and B each code for a protein involved in adenine biosythesis. If a haploid yeast strain has alleles A and B, then it will grow on –ade media, but if it has genotypes a/B or A/b, then it will fail to grow on –ade media. You conclude from this that Gene A is epistatic to gene B.

1d. You give up working on yeast and decide to examine a tooth development phenotype in humans. You find a family with a high rate of a hereditary tooth development problem, and all of the affected individuals have a mutation in the AMELY gene on the Y chromosome. Because everyone who has one copy of the mutation is affected, you conclude that the mutation is dominant.

2. While at the Skagit Valley Tulip festival, you were chatting with a kindly farmer about some of his tulip varieties. He tells you of two true-breeding varieties he grows, one gives red flowers, and the other gives yellow flowers. He tells you that when he crosses a true-breeding red with a true breeding yellow, he only gets red flowers. However, if he then self-fertilizes these monohybrids, he observes 286 red flowers, and 108 yellow flowers.

2a. What do you think are the genotypes of the P, F1 and F2 plants? Define and use your own symbols.

2b. Does the observed phenotypic ratio fit with a 1:1 segregation, or with a 3:1 segregation? Test each possibility using a χ^2 test, and state the genetic hypothesis you will propose to the farmer.

2c. Impressed with your knowledge, the farmer then tells you of a different variety he is growing. The petals of these flowers have serrated, fringed edges. He tells you of another cross he performed, using a red, serrated parent and a yellow, normal parent. All of the offspring had red, normal-shaped petals. He then self-crossed these dihybrids, and observed the following progeny: 56 red, plain; 20 red, serrated; 24 yellow, plain; and 12 yellow, serrated. Propose a genetic hypothesis and test it with a χ^2 test, defining your genetic symbols.

3. If the a and b loci are 20 map units apart in humans, and an AB/ab woman marries an ab/ab man, what is the probability that their first child will be Ab/ab?

4. Leaf color and stem length in a certain species of diploid flowers are controlled separately by the R and S locus. The <u>R</u> allele (dominant) results in red leaves while r results in yellow leaves. <u>S</u> (dominant) results in short stems and s results in long stems.

A cross is performed between a true breeding long-stemmed red plant and a true breeding short-stemmed yellow plant.

4a. What is the genotype and phenotype of the resulting F1 plants?

The resulting F1 plants are crossed with long-stemmed plants with yellow leaves:

F1 plants   Long-stemmed plants with yellow leaves
(parent 'A')   X   (parent 'B')

100 offspring are collected from this cross, and the following phenotype counts are observed.

phenotype	count
long red	43
short red	8
long yellow	9
short yellow	40

4b. What are the corresponding genotypes for each of the four classes of the offspring from this cross?

4c. Using the information above, determine the genotype of the gametes contributed by "parent A" to each of the resulting offspring. Label the gamete types as parental or nonparental.

phenotype	count	genotype of gametes contributed by 'Parent A'	gamete type (parental or nonparental)
long red	43		
short red	8		
long yellow	9		
short yellow	40		

4d. Perform a χ^2 test to assess whether the R and S loci are linked or unliked. State your null hypothesis and conclusion. If appropriate, provide an estimate for the map distance separating the two loci.

5. Using the data shown in the table at right, construct a linkage map (gene order and map distance) for the following genes:

Genes: a/+
              b/+
              c/+

Parents: a/+   b/+   c/+
              a/a   b/b   c/c

5a. Identify the order of the genes.
5b. Calculate the % of recombinant products.
5c. Draw the linkage map, showing gene order and map distance.

Progeny Phenotypes			Observed Counts	NCO, SCO, or DCO?
a+	b+	c+	525	SCO
c+	a	b	45	DCO
a	b+	c	4180	NCO
b	c	a	532	SCO
b	a+	c	226	SCO
b+	a+	c	53	DCO
c+	b	a+	4205	NCO
a	b+	c+	234	SCO
			Total = 10,000	

6. That farmer in the Skagit Valley just won't shut up about his tulips. He tells you of other varieties of tulips he's found. One is short-stemmed, and examination of monohybrids suggests it is recessive to normal-stemmed. Another trait is red; to him, this is a mystery because when red tulips are crossed to white, all the progeny are pink, but when the pinks are selfed, they produce red, white and pink

progeny. He wishes to map these traits with respect to each other and to his fringed petaled-plants (remember, recessive to normal-shaped), so he can plant efficiently for marketing hybrid varieties. He has a short, white, fringed true-breeding stock, and a red true-breeding stock of normal height and petal shape.

6a. What is the first cross you tell him to do?

The farmer performs the cross that you recommend, testcrosses the trihybrid to short, white, fringed plants, and obtains the following results:

> Short, white, fringed 623
> Short, pink, fringed 2
> Short, white 62
> Short, pink 29
> White, fringed 25
> White 3
> Pink, fringed 63
> Pink 630

6b. Draw a genetic map of these loci, including map distances, gene order, and any interference.

6c. The farmer wants to establish a true-breeding stock of his short, pink, fringed plants. Can he do this? If so, explain how he it could be done. If not, explain why it could not be done.

7. You cross an *a trp1 HIS5* yeast strain to an *α TRP1 his5* strain and then proceed to conduct tetrad analysis with the diploid strain. These experiments yield the following results:

TETRAD		Mating type	Growth on media lacking:		# of tetrads
			tryptophan	histidine	
I	A	a		+	172
	B	a		+	
	C	α	+		
	D	α	+		
II	A	a		+	82
	B	a	+	+	
	C	α			
	D	α	+		
III	A	a		+	14
	B	a	+		
	C	α		+	
	D	α	+		
IV	A	a		+	92
	B	a			
	C	α	+	+	
	D	α	+		

("+" indicates the spore grew on the plate lacking that particular nutrient.)

7a. What do these findings allow you to conclude about the TRP1 and HIS5 genes?

8. A cross was performed between two different haploid yeast strains of opposite mating type that each require methionine and lysine for growth (*met⁺ lys⁺*). One hundred asci were dissected and

colonies were grown for the four spores in each ascus. Cells from these colonies were tested for their ability to grow on Petri plates containing either minimal medium (min), min + lysine (lys), min + methionine (met), or min + lys + met. The asci could be divided into two groups based on this analysis:

Group 1: In 89 asci, cells from two of the four spore colonies could grow on all four kinds of media, while the other two spore colonies could only grow on min + lys + met.

Group 2: In 11 asci, cells from one of the four spore colonies could grow on all four kinds of petri plates. Cells from a second one of the four spore colonies could grow only on min + lys plates and on min + lys + met plates. Cells from a third of the four spore colonies could only grow on min + met plates and on min + lys + met plates. Cells from the remaining colony could only grow on min + lys + met.

8a. What are the genotypes of the spores within each of the two types of asci?

8b. Are the *lys* and *met* genes linked? If so, what is the map distance between them?

8c. If you could extend this analysis to many more asci, you would eventually find some asci with a different pattern. For these asci, describe the phenotypes of the four spores. List this as the ability of dissected spores to form colonies on the four kinds of petri plates.

9. A tomato farmer wants to grow and sell fuzzy, yellow, beaked (pear-shaped) tomatoes. Each of these traits is determined by a single recessive gene (*fz, y* and *bk*, respectively). Although he has a true-breeding stock containing all these mutations, it is very unhealthy and not practical for growing lots of tomatoes. He therefore wants to construct a new stock. He knows that *beaked* and *fuzzy* are normally 38 map units apart in normal tomato plants, and he knows that *yellow* is normally unlinked to these genes. He plans to cross *bk fz / bk fz* plants to *y / y* plants, cross the trihybrid to his sick testcross stock, and isolate the resulting *bk fz y* recombinant plants.

9a. When he does this, what percentage of the progeny will be *fuzzy*, *yellow* and *beaked*? Do <u>not</u> ignore recombination between *bk* and *fz*.

9b. The farmer also knows of a tomato variant generated in a research lab at the University of Washington in which a portion of a chromosome bearing the *yellow* locus has translocated onto the chromosome bearing the *fuzzy* and *beaked* genes. In this variant the *yellow* locus is now linked to both *fuzzy* and *beaked*. Furthermore, a map of these variants has been determined: *beaked* -38cM- *fuzzy* -18cM -*yellow*. His alternate plan is to obtain the + + *yellow* variants from the UW lab and cross them to the corresponding *bk fz* + variants. He will then testcross this trihybrid, and isolate the resulting *bk fz y* recombinant plants. Using this approach, what percentage of the progeny will be *fuzzy*, *yellow* and *beaked*? Assume all the chromosomes used for this have the same translocation, and again, do not ignore recombination between *bk* and *fz*.

9c. What do you think is therefore the most economical way for the farmer to obtain the plants he wants?

10. Male beetles expressing the recessive mutations sp (spotted), ll (long leg), fs (foot spike), and b (black) were crossed to phenotypically wildtype females and 3288 progeny were observed with the following phenotypes (if a phenotype is not listed assume that for those alleles the wildtype is expressed):

653	black, spotted, long leg, foot spike
670	spotted, long leg, foot spike
675	wildtype
655	black
71	black, spotted
73	spotted
73	black, long leg, foot spike
74	long leg, foot spike

87   black, spotted, long leg
84   spotted, long leg
86   black, foot spike
83   foot spike
1   black, spotted, foot spike
1   spotted, foot spike
1   black, long leg
1   long leg

10a.   Diagram the genotype of the female parents.
10b.   Map these loci. Give relative chromosomal locations with map distances.
10c.   Is there interference? How much?

11. The following is a genetic map of a region of a chromosome:

   gene a -10cM- *gene b* - 5cM- *gene c* -20cM- *gene d*

Start with a heterozygote with all the mutant alleles of these genes in cis, perform a testcross with this tetrahybrid as one of the parents, and examine 10,000 progeny:
11a. How many progeny do you expect to have the phenotype *a + + +*?
11b. How many will have the phenotype *a b + +*?
11c. How many will have the phenotype *a + c +*?

PROBLEM SET 4 – LINKAGE AND RECOMBINATION
(covering lectures 8 & 9 and QS 5 from week 5)

Answers posted by Jeff Kidd. Attend office hours for explanations/questions.

1. What is wrong with your conclusion in each scenario?
1a. You are examining two loci, A and B, in a haploid yeast strain. The yeast strain has genotypes A and b at the two loci. You conclude that the strain is heterozygous.

The strain is haploid and only has a single allele at each locus.

1b. A genetic map shows that loci A and B are on the same chromosome and are 60cM away from each other. You conclude that A and B are linked.

The maximum proportion of recombinant gametes is 50%. The two loci are far enough apart that they will behave as if they are unlinked even though they are on the same chromosome.

1c. Loci A and B each code for a protein involved in adenine biosythesis. If a haploid yeast strain has alleles A and B, then it will grow on –ade media, but if it has genotypes a/B or A/b, then it will fail to grow on –ade media. You conclude from this that Gene A is epistatic to gene B.

Neither gene masks the phenotype caused by the other.

1d. You give up working on yeast and decide to examine a tooth development phenotype in humans. You find a family with a high rate of a hereditary tooth development problem, and all of the affected individuals have a mutation in the AMELY gene on the Y chromosome. Because everyone who has one copy of the mutation is affected, you conclude that the mutation is dominant.

Each of the affected individuals has only a single Y chromosome. Dominant/recessive is not meaningful in this scenario since there is only a single allele of the locus present in these affected individuals.

2. While at the Skagit Valley Tulip festival, you were chatting with a kindly farmer about some of his tulip varieties. He tells you of two true-breeding varieties he grows, one gives red flowers, and the other gives yellow flowers. He tells you that when he crosses a true-breeding red with a true breeding yellow, he only gets red flowers. However, if he then self-fertilizes these monohybrids, he observes 286 red flowers, and 108 yellow flowers.

2a. What do you think are the genotypes of the P, F1 and F2 plants? Define and use your own symbols.

R=red (dominant)
r= yellow (recessive)

P=RR (red) and rr (yellow)
F1=Rr (red)
F2=RR(red), RR (red), rr (yellow)

2b. Does the observed phenotypic ratio fit with a 1:1 segregation, or with a 3:1 segregation? Test each possibility using a χ^2 test, and state the genetic hypothesis you will propose to the farmer.

Test for 1:1 segregation
H0=the ratios of the classes are 197:197
p<0.01 Reject the null hypothesis

Test for 3:1 segregation
H0=the ratios of the classes are 296:98 (a 3:1 ratio)
p=0.269
Fail to reject the null hypothesis

The data are consistent with two alleles for a single gene and that the F1s are heterozygous.

2c. Impressed with your knowledge, the farmer then tells you of a different variety he is growing. The petals of these flowers have serrated, fringed edges. He tells you of another cross he performed, using a red, serrated parent and a yellow, normal parent. All of the offspring had red, normal-shaped petals. He then self-crossed these dihybrids, and observed the following progeny: 56 red, plain; 20 red, serrated; 24 yellow, plain; and 12 yellow, serrated. Propose a genetic hypothesis and test it with a χ^2 test, defining your genetic symbols.

N=normal petals (dominant)
n=serated petals (recessive)

Hypothesis: parents are RRnn X rrNN. The F1s are therefore RrNn. The two loci are independent and the F2 classes should follow a 9:3:3:1 ratio.
H0=the phenotype classes of F2s are in a 9:3:3:1 ratio. p=0.2. Fail to reject the null hypothesis. The data are consistent with two independently assorting loci.

3. If the a and b loci are 20 map units apart in humans, and an AB/ab woman marries an ab/ab man, what is the probability that their first child will be Ab/ab?

The probability of an Ab/ab child is 10%

4. Leaf color and stem length in a certain species of diploid flowers are controlled separately by the R and S locus. The R̲ allele (dominant) results in red leaves while r results in yellow leaves. S̲ (dominant) results in short stems and s results in long stems.

A cross is performed between a true breeding long-stemmed red plant and a true breeding short-stemmed yellow plant.

4a. What is the genotype and phenotype of the resulting F1 plants?

Parents are RRss and rrSS
F1s are RrSs (short stems and red)

The resulting F1 plants are crossed with long-stemmed plants with yellow leaves:

F1 plants   **X**   Long-stemmed plants with yellow leaves
(parent 'A')   (parent 'B')

100 offspring are collected from this cross, and the following phenotype counts are observed.

phenotype	count
long red	43
short red	8
long yellow	9
short yellow	40

4b. What are the corresponding genotypes for each of the four classes of the offspring from this cross?

See below

4c. Using the information above, determine the genotype of the gametes contributed by "parent A" to each of the resulting offspring. Label the gamete types as parental or nonparental.

phenotype	count	genotype of gametes contributed by 'Parent A'	gamete type (parental or nonparental)
long red	43		
short red	8		
long yellow	9		
short yellow	40		

phenotype	count	genotype	genotypes of gamets from 'Parent A'	gamete type
long red	43	Rrss	Rs	parental
short red	8	RrSs	RS	nonparental
long yellow	9	rrss	rs	nonparental
short yellow	40	rrSs	rS	parental

4d. Perform a χ^2 test to assess whether the R and S loci are linked or unliked. State your null hypothesis and conclusion. If appropriate, provide an estimate for the map distance separating the two loci.

H0. Loci are unlinked and the gamete types (and therefore the observed offspring phenotype classes) from parent A are in a 1:1:1:1 ratio.

Conclusion: reject null hypothesis (X-squared = 43.76, df = 3, p< 0.01).   Conclude that the loci are likely linked. Estimated map distance as   17 cM.

5. Using the data shown in the table at right, construct a linkage map (gene order and map distance) for the following genes:
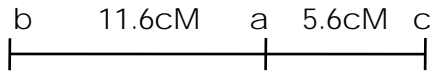
Genes: a/+
                b/+
                c/+

Parents: a/+   b/+   c/+
                a/a   b/b   c/c

5a. Identify the order of the genes.
5b. Calculate the % of recombinant products.
5c. Draw the linkage map, showing gene order and map distance.

Progeny Phenotypes			Observed Counts	NCO, SCO, or DCO?
a+	b+	c+	525	SCO
c+	a	b	45	DCO
a	b+	c	4180	NCO
b	c	a	532	SCO
b	a+	c	226	SCO
b+	a+	c	53	DCO
c+	b	a+	4205	NCO
a	b+	c+	234	SCO
			Total = 10,000	

Answer:

b   11.6cM   a   5.6cM   c
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Reverse map is also correct.

6. That farmer in the Skagit Valley just won't shut up about his tulips. He tells you of other varieties of tulips he's found. One is short-stemmed, and examination of monohybrids suggests it is recessive to normal-stemmed. Another trait is red; to him, this is a mystery because when red tulips are crossed to white, all the progeny are pink, but when the pinks are selfed, they produce red, white and pink progeny. He wishes to map these traits with respect to each other and to his fringed petaled-plants (remember, recessive to normal-shaped), so he can plant efficiently for marketing hybrid varieties. He has a short, white, fringed true-breeding stock, and a red true-breeding stock of normal height and petal shape.
6a. What is the first cross you tell him to do?

RR=red, Rr=pink, rr=white

Tell him to cross $s\,rf\,/\,s\,rf$ to $+\,R\,+\,/\,+\,R\,+$ (s is short, f is fringed) to generate the $s\,rf\,/\,+\,R\,+$ heterozygote.

The farmer performs the cross that you recommend, testcrosses the trihybrid to short, white, fringed plants, and obtains the following results:

Short, white, fringed 623
Short, pink, fringed 2
Short, white 62
Short, pink 29
White, fringed 25
White 3
Pink, fringed 63
Pink 630

6b. Draw a genetic map of these loci, including map distances, gene order, and any interference.

s--4.1cM--r--9.0cM--f

Interference: 0.057

6c. The farmer wants to establish a true-breeding stock of his short, pink, fringed plants. Can he do this? If so, explain how he it could be done. If not, explain why it could not be done.

He will not be able to establish true-breeding stocks, because the pink color is the result of incomplete dominance of the *R* allele to *r*, and he will always get some *RR* (red) and *rr* (white) homozygotes back out of the pink parents.

7. You cross an *a trp1 HIS5* yeast strain to an *α TRP1 his5* strain and then proceed to conduct tetrad analysis with the diploid strain. These experiments yield the following results:

TETRAD		Mating type	Growth on media lacking:		# of tetrads
			tryptophan	histidine	
I	A	a		+	172
	B	a		+	
	C	α	+		
	D	α	+		
II	A	a		+	82
	B	a	+	+	
	C	α			
	D	α	+		
III	A	a		+	14
	B	a	+		
	C	α		+	
	D	α	+		
IV	A	a		+	92
	B	a			
	C	α	+	+	
	D	α	+		

("+" indicates the spore grew on the plate lacking that particular nutrient.)

7a. What do these findings allow you to conclude about the TRP1 and HIS5 genes?

The three loci are linked to each other. The order and map distances are :

TRIP1 --- 13.3 cM --- MAT --- 14.7 cM --- HIS5

8. A cross was performed between two different haploid yeast strains of opposite mating type that each require methionine and lysine for growth (*met⁺ lys⁺*). One hundred asci were dissected and colonies were grown for the four spores in each ascus. Cells from these colonies were tested for their ability to grow on Petri plates containing either minimal medium (min), min + lysine (lys), min + methionine (met), or min + lys + met. The asci could be divided into two groups based on this analysis:
    Group 1: In 89 asci, cells from two of the four spore colonies could grow on all four kinds of media, while the other two spore colonies could only grow on min + lys + met.
    Group 2: In 11 asci, cells from one of the four spore colonies could grow on all four kinds of petri plates. Cells from a second one of the four spore colonies could grow only on min + lys plates and on min + lys + met plates. Cells from a third of the four spore colonies could only grow on min + met plates and on min + lys + met plates. Cells from the remaining colony could only grow on min + lys + met.
8a. What are the genotypes of the spores within each of the two types of asci?

Note: The wording is unclear but this question is supposed to imply that the parental strains are (met+ lys+) and (met- lys-).
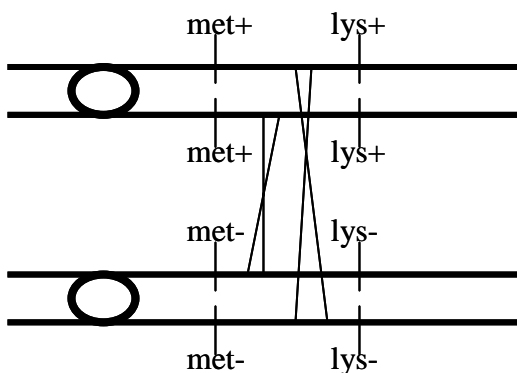
Group 1: *met⁺,lys⁺* and *met⁻,lys⁻*
Group 2: *met⁺,lys⁺* and *met⁺,lys⁻*, and *met⁻,lys⁺* and *met⁻,lys⁻*

8b. Are the *lys* and *met* genes linked? If so, what is the map distance between them?

Yes. Map distance= 5.5 cM

8c. If you could extend this analysis to many more asci, you would eventually find some asci with a different pattern. For these asci, describe the phenotypes of the four spores. List this as the ability of dissected spores to form colonies on the four kinds of petri plates.

met⁻,lys⁺ and *met⁺,lys⁻* (rare recombinants in which both homologous chromosome pairs underwent a recombination event between the *met* and *lys* genes). This is diagrammed below (note: order relative to centromere is not known):

9. A tomato farmer wants to grow and sell fuzzy, yellow, beaked (pear-shaped) tomatoes. Each of these traits is determined by a single recessive gene (*fz, y* and *bk*, respectively). Although he has a true-breeding stock containing all these mutations, it is very unhealthy and not practical for growing lots of tomatoes. He therefore wants to construct a new stock. He knows that *beaked* and *fuzzy* are normally 38 map units apart in normal tomato plants, and he knows that *yellow* is normally unlinked to these genes. He plans to cross *bk fz / bk fz* plants to *y / y* plants, cross the trihybrid to his sick testcross stock, and isolate the resulting *bk fz y* recombinant plants.

9a. When he does this, what percentage of the progeny will be *fuzzy*, *yellow* and *beaked*? Do <u>not</u> ignore recombination between *bk* and *fz*.

15.5%

9b. The farmer also knows of a tomato variant generated in a research lab at the University of Washington in which a portion of a chromosome bearing the *yellow* locus has translocated onto the chromosome bearing the *fuzzy* and *beaked* genes. In this variant the *yellow* locus is now linked to both *fuzzy* and *beaked*. Furthermore, a map of these variants has been determined: *beaked* -38cM- *fuzzy* -18cM -*yellow*. His alternate plan is to obtain the + + *yellow* variants from the UW lab and cross them to the corresponding *bk fz* + variants. He will then testcross this trihybrid, and isolate the resulting *bk fz y* recombinant plants. Using this approach, what percentage of the progeny will be *fuzzy*, *yellow* and *beaked*? Assume all the chromosomes used for this have the same translocation, and again, do not ignore recombination between *bk* and *fz*.

5.5%

9c. What do you think is therefore the most economical way for the farmer to obtain the plants he wants?

He should use his own stocks and not bother with the translocation stock.

10. Male beetles expressing the recessive mutations sp (spotted), ll (long leg), fs (foot spike), and b (black) were crossed to phenotypically wildtype females and 3288 progeny were observed with the following phenotypes (if a phenotype is not listed assume that for those alleles the wildtype is expressed):

653	black, spotted, long leg, foot spike
670	spotted, long leg, foot spike
675	wildtype
655	black
71	black, spotted
73	spotted
73	black, long leg, foot spike
74	long leg, foot spike
87	black, spotted, long leg

84 spotted, long leg
86 black, foot spike
83 foot spike
1 black, spotted, foot spike
1 spotted, foot spike
1 black, long leg
1 long leg

10a. Diagram the genotype of the female parents.

Since we observe all possible phenotype combinations the female parent must be heterozygous for all loci.

10b. Map these loci. Give relative chromosomal locations with map distances.

sp--9cM--ll--10.5cM--fs   The b locus is unlinked from the others (> 50 cM away or on a different chromosome)

10c. Is there interference? How much?

Yes. Interference = 0.89

Predicted DCO=0.009, Observed DCO=0.001.
COC=(0.001/0.009)=0.11

11. The following is a genetic map of a region of a chromosome:

gene a -10cM- gene b - 5cM- gene c -20cM- gene d

Start with a heterozygote with all the mutant alleles of these genes in cis, perform a testcross with this tetrahybrid as one of the parents, and examine 10,000 progeny:
11a. How many progeny do you expect to have the phenotype a + + +?

380 progeny. (Hint: expect 1,000 recombinants between a and b, half of which will be a. However, one must also consider cross-overs between the other loci and subtract recombinants as appropriate.)

11b. How many will have the phenotype a b + +?

180

11c. How many will have the phenotype a + c +?

5