Problem Sets

Fall 1993

7.03 Problem Set 1

due in class Friday, September 24

All four problems will be graded. Some parts of these problems are quite difficult, if you get stuck try doing the other problems and come back to the hard parts later.

1. Consider the following experiments designed to identify genes in yeast that are required for the synthesis of the amino acid arginine. Yeast mutants that are defective in arginine synthesis and are therefore called Arg^- can be identified because they can not grow on medium that is not supplemented with arginine. Ten Arg^- strains are isolated by screening mutagenized yeast colonies for those colonies that grow on minimal medium with arginine but will not grow on minimal medium without arginine. Five of the mutants are isolated in a haploid yeast strain of mating type α (strains 1 - 5) and five of the mutants are isolated in a haploid strain of mating type **a** (strains 6 - 10). Remember that an **a** strain will only mate with an α strain and that **a** will not mate with **a** and α will not mate with α. Pairwise matings are performed between different strains as indicated in the table below. When the resulting diploid can not grow on minimal medium without arginine a (–) is indicated at the intersection of the two parental strains. When the resulting diploid can grow without arginine a (+) is indicated.

		strains of mating type α					
		1	2	3	4	5	wild type
strains of mating type **a**	6	+	+	–	+	–	+
	7	+	+	+	–	–	+
	8	+	+	–	+	–	+
	9	+	+	+	–	–	+
	10	+	+	–	+	–	+
	wild type	+	+	+	+	–	+

(a) Explain the unusual behavior of strain 5. Are any of the complementation tests with this mutant meaningful?

(b) How many different genes or, more precisely, complementation groups are represented by these ten mutations. Indicate which mutations are in the same complementation group.

(c) In order to change its mating type, strain 1 is crossed to wild type, the diploid is sporulated, and a haploid spore colony is isolated that is Arg$^-$ and mating type **a**.

It is now possible to perform complementation tests between mutation 1 and the mutations in the strains of mating type α. Based on the results of these tests shown below, how many Arg$^-$ complementation groups do you now think that there are?

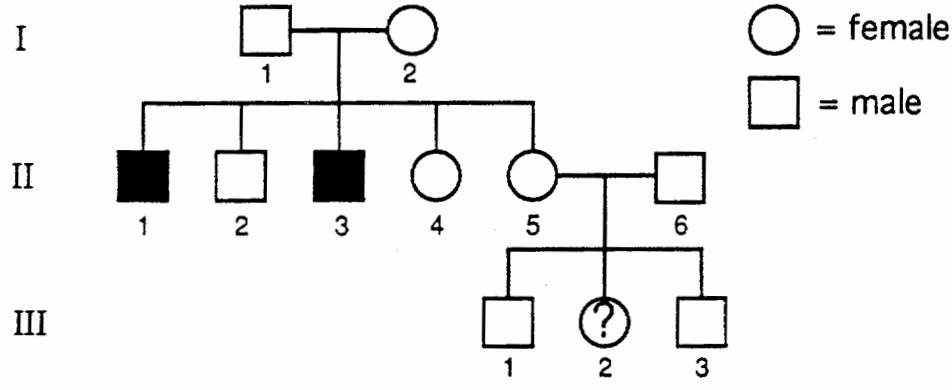
		mating type α				
		1	2	3	4	5
mating type **a**	1	–	–	+	+	–

2. **(a)** In a large mouse breeding experiment, an unusual mouse with six digits on the front paws is discovered in one of the litters. When this mouse is crossed to a wild type mouse about half of the F1 are normal and half have six digits on the front paws. Describe in detail the crosses that you would perform in order to produce a true breeding strain of mice with the six digit trait.

(b) In a separate line of experiments in another lab, a true breeding strain has been developed that exhibits six digits on the hind paws. Thinking that the two mutations might be related you decide to perform a cross between mice of the two strains. All of the F1 progeny of this cross have six digits on both the front and back paws. There are two reasonable explanations for this result. First the two mutant strains could have dominant alleles in two different genes. Alternatively the two different strains could have codominant alleles of the same gene. (If this second possibility does not make sense to you, try to relate these ideas to multiple alleles for the ABO blood antigens in humans described on p. 89-90 of *Suzuki.*) When the F1 mice are crossed, about half of the F2 mice have six digits on both front and back paws, some have six digits in the front, some have six digits on the back and a few are normal. Based on these results which explanation best fits the data? Which phenotypic class of F2 mice is most important in your decision and why?

(c) When you propagate the true breeding strains from parts (a) and (b) you notice that although for the most part the traits appear to breed true, about 5% of the progeny appear to be wild type. When these apparently wild type mice are crossed to true wild type mice most of the resulting progeny are mutant. These results can be explained as a result of incomplete penetrance. Propose a cross that would allow the two possibilities outlined in part (b) to be distinguished even with 5% incomplete penetrance and give the outcomes that you would expect for the two cases. There are a number of reasonable solutions to this problem.

3. **(a)** You are a geneticist studying a rare human disorder that appears to be inherited. Presented with the following family where two sons (II-1 and II-3) have the disorder you propose that the trait is either X-linked recessive or autosomal recessive. Since you have been trained at MIT you decide to put these ideas to a quantitative test. Using the chi-squared test, determine the probability that the inheritance pattern exhibited by children II-1 – II-5 is significantly different from that expected for autosomal recessive inheritance. Can you draw a conclusion about the mode of inheritance based on this family?



(b) Your plan is to perform a more significant test of the mode of inheritance by collecting data from more families that exhibit the disorder. Before conducting this study, it would be helpful to know how much additional data is needed to get a significant answer. Assume that the disorder is due to an X-linked recessive allele. On average, how many children from families exhibiting the disorder would you need to look at in order to show a pattern of inheritance that differs significantly (ie $p<.05$) from the pattern expected for an autosomal recessive trait? (Assume equal frequencies of males and females among the children in families exhibiting the disorder.)

4. Sickle cell anemia is one of the most prevalent genetic diseases debilitating about 1.8 per 1000 U. S. blacks. The pathology of this disease stems from the circulating erythrocytes which have a sickled shape that obstructs capillaries and causes excessive erythrocyte destruction. The genetic basis of sickle cell disease was unraveled when it was discovered that within the U. S. population there was a milder and much more prevalent form of the disease known as sicklemia. Sicklemic individuals are usually have no symptoms of anemia and their blood cells in circulation are not sickled. However, blood removed from sicklemic individuals will sickle under conditions of unusually low oxygen pressure. On testing of random blood samples it turns out that about 8% of U. S. blacks are sicklemic. Incidentally, the sicklemic condition was first appreciated during World War II when many aviators were found to be debilitated when flying to high altitude in unpressurized aircraft.

Two hypotheses were originally proposed to explain the inheritance of sicklemia and sickle cell disease. The first hypothesis postulates that the sickle cell allele is recessive and only individuals homozygous for the disease allele have sickle cell disease whereas heterozygotes have sicklemia. The second hypothesis postulates that the allele for sickle cell disease is dominant but is incompletely penetrant. In this case, most individuals heterozygous for the disease allele are sicklemic and only about 1/50 heterozygous individuals with the allele exhibit the more severe phenotype of sickle cell disease.

(a) Under the first hypothesis that the disease allele is recessive and given the frequencies of sicklemic and sickle cell disease individuals, what is the frequency of the sickle cell disease allele within the U. S. black population? Do the phenotypic ratios agree with expectation of the Hardy-Weinberg principle for the sickle cell trait?

(b) Under this hypothesis that the disease alleel is dominant and incompletely penetrant, what is the frequency of the sickle cell disease allele within the U. S. black population? Do the phenotypic ratios agree with expectation of the Hardy-Weinberg principle for the sickle cell trait?

(c) A large study was conducted where the parents of individuals with sickle cell disease were tested for sicklemia. Under the second hypothesis that the sickle cell allele is dominant what fraction of sickle cell individuals would have only one sicklemic parent? What fraction of sickle cell individuals would have both parents sicklemic?

This study was actually carried out in the late 1940s and it was found that essentially all parents of sickle cell individuals are sicklemic. This finding established that the first hypothesis is correct — the sickle cell allele is recessive and heterozygotes are sicklemic.

For the sake of an exercise in population genetics, imagine a disease like sickle cell anemia that we will call "star cell anemia". This disease is like sickle cell anemia in every respect except that rather than being autosomal the gene for star cell anemia is on the X chromosome. Further assume that the allele for star cell disease is present in the population at the frequency q=0.04.

(d) For autosomal sickle cell allele the ratio of sickle cell individuals to sicklemic individuals is 1/50. If the star cell disease allele is recessive as in part (a), what would the ratio of star cell individuals to "starlemic" individuals be? Assume equal numbers of males and females in the population.

(e) Under conditions of part (d)— for individuals with the star cell disease what would the ratio of males to females be?

(f) Now assume that the star cell allele is dominant with incomplete penetrance as in part (b). For individuals with the star cell disease what would the ratio of males to females be? Assume 1/50 of either males or females with the disease gene will have star cell disease.

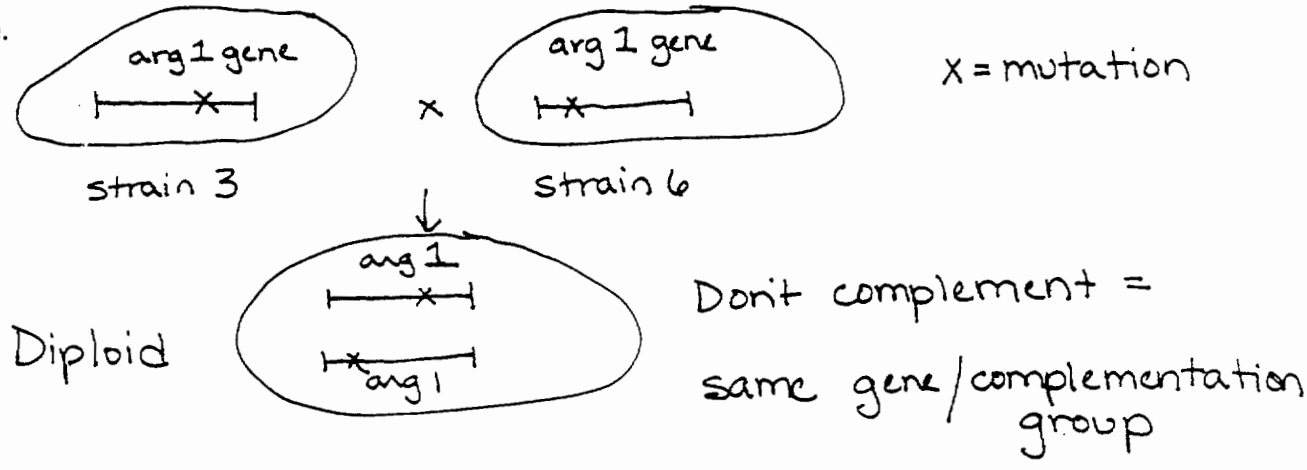
Answers to Problem Set #1, 7.03

Problem 1

a) In order to do a complementation test, one must first determine whether the mutations are dominant or recessive. This is done by crossing each haploid mutant strain to a wild-type haploid strain to produce a diploid, heterozygous strain. If the resulting diploid can grow without arginine, then the mutation is recessive. If the diploid cannot grow without arginine, then the mutation is dominant. From the table, we can see that all the mutations are recessive, with the exception of strain 5, which must carry a dominant mutation. Therefore, none of the complementation tests with this strain are meaningful. One cannot conduct a complementation test with a dominant mutation, since a diploid resulting from a cross between the dominant strain and any other recessive mutant strain will not be able to grow without arginine.

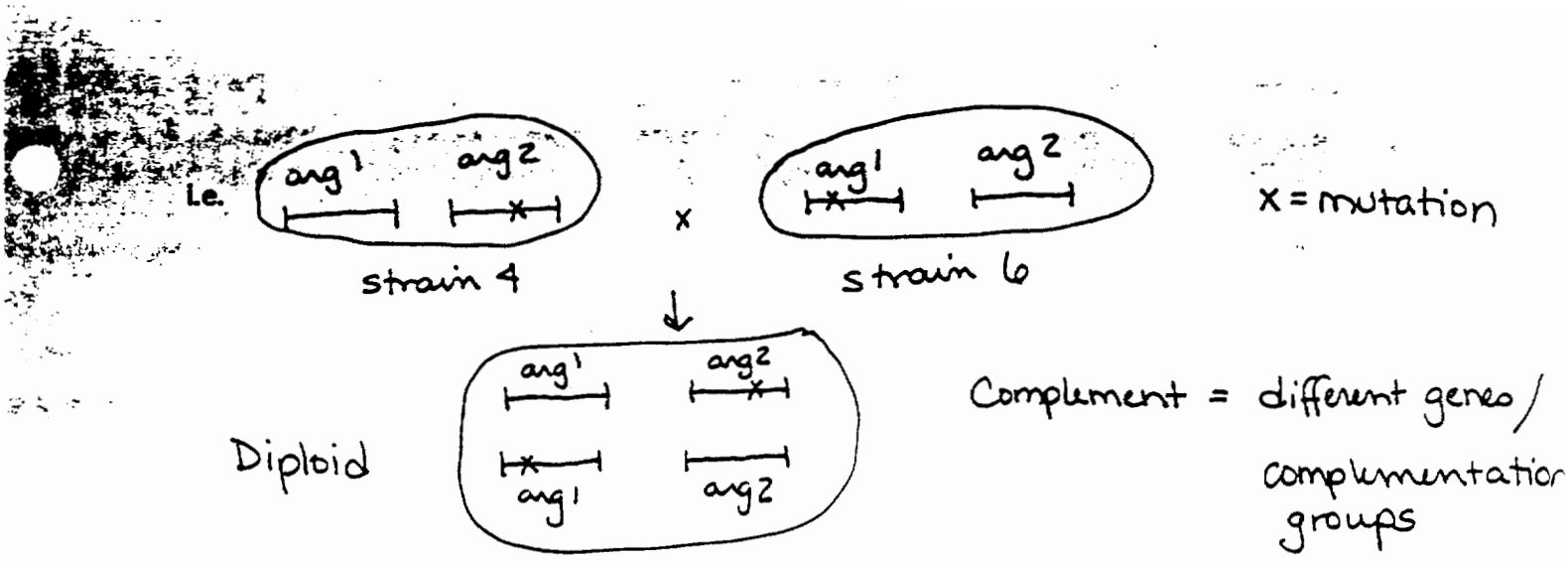
b) If two mutations are in the same gene, or complementation group, then the diploid generated by crossing the two mutant haploid strains will not be able to grow without arginine. This is because the diploid has two mutant copies of the same gene.

i.e.



This situation is represented as a "-" in the chart.

If two mutations are in different genes, or complementation groups, then the diploid generated will be able to grow without arginine. Thus, the two mutants "complement " each other in the diploid by providing a wild-type copy of the gene in which the mutation in the other strain lies.



This situation is represented by a "+" in the chart.

It follows that strains 3,6,8,10 comprise one complementaion group, whereas strains 4,7,9 comprise another, separate, complementation group. Strain 5 cannot be placed in a group, since it carries a dominant mutation (see part a.) It cannot be determined if strains 1 and 2 are in the same, or different complementation groups (see part c.) However, neither are in the same group as either of the two other complementation groups.

c) From the last result, we can now determine that the mutations in strains 1 and 2 are in the same complementation group, which is distinct from the other two groups. Therefore, there are 3 complementation groups, with strain 5 not determined.

3,6,8,10	4,7,9	1,2	5
1 group	1 group	1 group	undetermined

Helpful hint: When a mutant is first identified usually the first thing to do is to cross it to the wild type strain. If the progeny are all wild type then the mutation is recessive and the genotype of the first isolate must have been m/m. If some of the progeny are mutant then the mutation is dominant. It is reasonable to assume that the first animal with a dominant mutation which is identified is a heterozygote +/M.

A First note that since half the F1 progeny exhibit the mutant phenotype the mutation is dominant. The cross is +/M x +/+ and it gives two +/+ (normal mice) and two +/M (mice with six digits). We need to get a strain that is true breeding, or homozygous mutant (M/M). Mate a six-toed male (+/M) and a six-toed female (+/M) from the F1 to generate the F2. 1/4 of the F2 progeny from this cross will be +/+ with normal paws, 1/2 of the F2 will be +/M with six toes, and 1/4 of the F2 will be M/M with six toes. To distinguish between these last two classes it is necessary to perform a test cross. Take several six-toed F2 mice and cross them to wild type mice (+/+). If any normal mice are recovered from this cross then the genotype of the F2 mouse if +/M. Conversely if a large number of progeny are generated which all have six toes then it is highly probable that the F2 mouse in this test cross has the genotype M/M. Based on the results of these test crosses select male and female mice which you have deduced are M/M and cross them to each other to generate a true breeding six-toed mouse strain.

B If the two mutations are co-dominant alleles of the same gene then the genotype of the F1 mice should be M1/M2, while their parents were M1/M1 and M2/M2. When two F1 M1/M2 mice are crossed to each other three classes of F2 progeny should be observed: 1/4 M2/M2 mice with normal front paws and six-toed hind paws, 1/2 M1/M2 mice with six digits on all paws, and 1/4 M1/M1 mice with six-toed front paws and normal hind paws (see top table below). No completely normal mice should be recovered!

If the mutations are in separate genes then the genotype of the F1 mice is +/M1, +/M2, while their parents were M1/M1, +/+ and +/+, M2/M2. In this case when two F1 mice are crossed four phenotypic classes of progeny should be observed (see table below). Note that 1/16 of the progeny will have normal paws (+/+, +/+), 3/16 will have six toes on the front paws (M1/any, +/+), 3/16 will have six toes on the hind paws (+/+, M2/any), and 9/16 will have six toes on all paws (M1/any, M2/any). Normal mice are recovered from this cross. Thus, the normal mice are the most important class of F2 mice as their existence rules out the hypothesis of co-dominant alleles of the same gene.

See table below for the genotypes that could result from this cross.

If the mutations are co-dominant alleles of the same gene:

gametes	M1	M2
M1	**M1/M1** (6 toes on front paws)	**M1/M2** (6 toes on all paws)
M2	**M1/M2** (6 toes on all paws)	**M2/M2** (6 toes on rear paws)

If mutations are in two different genes then the possible progeny are as follows:

gametes	+, +	+,M2	M1, +	M1, M2
+,+	+/+, +/+	+/+, +/M2	+/M1, +/+	+/M1, +/M2
+,M2	+/+, M2/+	+/+, M2/M2	+/M1, M2/+	+/M1, M2/M2
M1,+	M1/+, +/+	M1/+, +/M2	M1/M1, +/+	M1/M1, +/M2
M1,M2	M1/+, M2/+	M1/+, M2/M2	M1/M1, M2/+	M1/M1, M2/M2

genotype			phenotype	ratio
+/+, +/+			normal paws	1
+/+, +/M2	+/+, M2/+	+/+, M2/M2	6 toes on hind paws	3
+/M1, +/+	M1/+, +/+	M1/M1, +/+	6 toes on front paws	3
all others			6 toes on all paws	9

C Incomplete penetrance means that not all of the animals that carry a mutation will exhibit the phenotype associated with that mutation. For example, a M1/M1 mouse might have a normal number of toes even though most other mice of this genotype will have six toes on their front paws. This confounds the test described in part b to determine whether M1 and M2 are co-dominant alleles of the same gene or in separate genes because wild type progeny will be recovered from the F1 cross in either case. One way to distinguish these two models is to cross the F1 to the wild type. If the alleles are co-dominant then only 5% of the mice should be wild type. If the mutations are in different genes then 1/4 or 25% (plus (.05)x(3/4) for the incomplete penetrance) of the progeny will be phenotypically normal. A second way is to test the genotype of the phenotypically wild type F2 mice by crossing these mice back to wild type mice. If the mutations are co-dominant then all but 5% of the progeny from these test crosses will have six toes on one set of paws or the other. If the mutations are in different genes then some of the mice put through the test cross will give all wild type progeny. Note that you will need to put a number of phenotypically normal mice through a test cross to distinguish between these two models. Think about why this is the case!!!

3a. The hypothesis we will test is that the inheritence pattern of children II-1 to II-5 is that expected if the disorder is inherited in an autosomal recessive manner.

We will test this idea using the chi squared test. All that this test can tell us is how likely it is the observed data fit the expected pattern by chance. If the observed data are very unlikely to fit the expected pattern by chance, then we can reject our hypothesis, the expected pattern, as being improbable. Thus we can never prove anything with this test, but we can show that one possibility is highly unlikely.

What is the expected pattern? If the disorder is autosomal recessive and some of the II generation have the disorder, then both the mother (I-2) and father (I-1) must be carriers of the disorder allele.

In this case we would expect a 3:1 ratio of healthy children to affected children, both for male and female offspring. Since we can not classify our observed healthy children into homozygous, DD, or heterozygous, Dd, we will lump them all together.

Calculating chi squared:

	Expected ratio	Expected ratio for five children	Observed ratio	$(O-E)^2$	$(O-E)^2/E$
healthy	.750	3.75	3	.5625	.15
disordered	.250	1.25	2	.5625	.45
	1.000	5.00	5		$X^2=0.600$

Looking up the p value:

Because we have divided our expected frequencies into two classes the degrees of freedom= 2-1= 1.

For one degree of freedom a chi squared value of 0.600 gives a p value of between 0.5 and 0.1. There is between a 50% and 10% chance that the observed data fit the expected pattern by chance. Therefore we can not reject the hypothesis as being improbable. We can not draw a conclusion about the mode of inheritence based only on this family.

3b. To solve this problem let us assume that the observed data are consistant with X-linked inheritence and that the expected data is for autosomal recessive inheritence. I am going to assume that because the disorder is rare the case of disordered parents having children will not happen frequently and we can ignore those cases in our calculations.

	Expected ratio	Observed ratio
male, healthy	.375	.250
male, disordered	.125	.250
female, healthy	.375	.500
female, disordered	.125	.000
	1.000	1.000

The number of observed healthy males is equal to N, the total number of observed individuals times the frequency of the group, N(.250). By this method we can calculate all the O and E values from all the groups.

Calculating chi squared:
In order for the results to be significant at the p=.05 level with our three degrees of freedom, then chi squared must be equal to or greater than 7.815.

$$\chi^2 = \sum \frac{(O-E)^2}{E} = \frac{[N(.250)-N(.375)]^2}{N(.375)} + \frac{[N(.250)-N(.125)]^2}{N(.125)} + \frac{[N(.500)-N(.375)]^2}{N(.375)} + \frac{[N(.00)-N(.125)]^2}{N(.125)}$$

$$= \frac{N^2(.125)^2}{N(.375)} + \frac{N^2(.125)^2}{N(.125)} + \frac{N^2(.125)^2}{N(.375)} + \frac{N^2(.125)^2}{N(.125)}$$

$$= \underline{(.0156)N\left(\tfrac{8}{3}+8+\tfrac{8}{3}+8\right)} = (.0156)N(21.33) = .3328(N) = \chi^2$$

$\chi^2 > 7.815 < .3328(N)$, $N > 23.4$ ← Look at at least 24 kids

So we need to examine at least 24 children before we can get enough data to reject the autosomal recessive hypothesis.

The problem can also be solved with an alternative form of chi squared.*

$$\chi^2 = \sum \frac{O^2}{E} - N = \frac{[N(.250)]^2}{N(.375)} + \frac{[N(.250)]^2}{N(.125)} + \frac{[N(.500)]^2}{N(.375)} + \frac{[N(.00)^2]}{N(.125)} - N$$

$$= N(.166 + .50 + .666 + 0) - N = 1.33N - N = N(1.33-1)$$

$$= N(.33)$$

$\chi^2 = N(.333) > 7.815$

$N > 23.4$ or at least 24 kids.

* the alternative form can be fairly easily derived from the original formula.

3c. The first insight you need to make is that you are going to apply bayes theorum to the mother of the granddaughter (II-5) and not to the granddaughter herself. The question is really- what is the probablility that II-5 is a carrier given that she had two normal sons.

The probability that the granddaughter, III-2, is a carrier is then 1/2 of the chance that her mother is a carrier. This is true because the granddaughter has a 1/2 chance of receiving the disordered X from her carrier mom.

Bayes theorum allow one to calculate the probability of a cause based on an observed effect, probability of a given b, p(a/b)

In this case a= probability that II-5 is a carrier given
b= she has two healthy sons.

p(b/a)= probability that II-5 has two healthy sons given that she is a carrier. This is (1/2)(1/2)= 1/4

p(a)= probability that II-5 is a carrier.

Assuming X-linked recessive inheritence, II-5 has a 1/2 chance of being a carrier. She definitely got a wild-type X chromosome from her father and had a 50-50 chance of getting the disorder X chromosome from her mother.

p(b/not a)= probability that II-5 has two healthy sons given that she is not a carrier.
If she is not a carrier than there is a 100% chance her sons will not have the disorder.

p(not a)= the probability that II-5 is not a carrier. This is 1-p(a)=1/2.

PLUG IN THE NUMBERS:

$$p(a/b) = \frac{p(b/a)\,p(a)}{p(b/a)\,p(a) + p(b/\text{not a})\,p(\text{not a})} = \frac{(1/4)(1/2)}{(1/4)(1/2) + (1)(1/2)} = 1/5$$

So if II-5 has a 1/5 chance of being a carrier, given that she had two healthy sons, her daughter, III-2, has a (1/5)(1/2)= 1/10 chance of being a carrier.

PROBLEM 4

a) Under the first hypothesis that the disease allele is recessive, the affected individuals are of the genotype ss (homozygote mutants). We know that .18% of the population are affected, this means that $q^2 = 0.0018$ (Remember that ss= q^2, Ss=2pq and SS=p^2). We can now calculate q which is equal to $(0.0018)^{1/2}$, q= 0.042 and p=1-q, p=0.958.

2pq which is the number of heterozygotes carriers of the disease and in this case the sicklemic should be equal to 2x0.042x0.958= 0.08. If the population were in Hardy-Weinberg equilibrium then 8% should be heterozygotes for the sickle cell allele. Since the heterozygotes have a distinctive phenotype (sicklemic) which is present in 8% of the population we can conclude that the population is in equilibrium.

b) Under the second hypothesis that the disease allele is dominant and incompletely penetrant we can calculate the frequency of the disease allele by writing the following equation:

q = f (A) = f (AA) + 1/2 f (Aa)

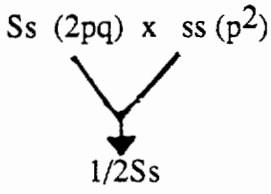
Since q is a very small number the expression f(AA) = q^2 ,which are the homozygotes mutants, is negligible compared to 1/2 f (Aa) and we can write:

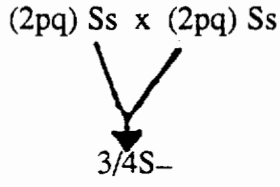
q = 1/2 f (Aa) = the sum of sicklemic and sickle cell individuals = 1/2 0.0818 = 0.041

In this problem you can't say anything about Hardy-Weinberg equilibrium since you can't distinguish in the sickle cell population which ones are heterozygotes and which ones are homozygotes for the sickle allele.

This problem can be solved in a number of ways. As long as you justify your assumptions and your reasoning is correct you are going to get full credit.

c) Under the hypothesis that the disease is dominant and incompletely penetrant you want to know what fraction of the individuals with sickle cell disease have one parent sicklemic (Ss) or both parents sicklemic (we are going to assume that the parents of the individuals with sickle cell disease have the genotype Ss, the homozygotes for the disease are a very small fraction).





The probability of a mating between a heterozygote and a homozygote wild type is equal to $2pq \times p^2 \times 2$ You multiply times two because there are two possibilities to get this mating — the mother is heterozygote and the father wildtype or that the mother is wild type and the father heterozygote for the sick allele. You then have to multiply the possibility of the mating times the possibility that the mating gives you sickle cell progeny which is 1/2.

The probability of having one parent sicklemic is $2pqxp^2x2x1/2= 2p^3q$

The probability of mating between two heterozygotes is 2pq x 2pq. The probability that this mating will give rise to sickle progeny is 3/4.

The probability of having both parents sicklemic is $2pq \times 2pq \times 3/4 = 3p^2q^2$

The ratio is $2p3q / 3\ p^2q^2 = 2p/3q = 2 \times 0.958 / 3 \times 0.042 = 15.2$

Approximately 1/16 of the sickle cell individuals are going to have both parents sicklemic.

d) Given that star cell anemia is X-linked, we need to determine how to represent the affected males in the population. If .04, or 1/25 of the <u>total</u> X chromosomes in the population carry the star cell allele, and 2/3 of the X's are in females whereas 1/3 are in males, we can set up an equation as follows:

let n=number of individuals in the population.

$1/2n + 2(1/2n)$ = number of X chromosomes in population = $3/2n$
$\delta s \quad \varphi s$

Therefore, $1/25(3/2n)$ = number of X's carrying star cell allele, and $1/3(1/25)(3/2n)$ = number of X's carrying the star cell allele in males = $1/50n$. This number divided by the total number of males in the population (n/2), gives the number of males with the star-cell disease. $\frac{n/50}{n/2} = 1/25 = q$!!!!!

So, we can represent males with the disease merely as q.

Now, the ratio of star cell to "starlemic" individuals is represented by: $q + q^2 / 2pq =$ .04 + .0016 / .0768 = 0.54, which is roughly a 1:2 ratio.

e) Ratio of star cell males to star cell females:

$q/q^2 = .04/.0016 = 25:1.$

f) Ratio of star cell males to females given incomplete penetrance:

$\frac{q/50}{q2/50 + 2pq/50} = \frac{.0008}{.000032 + .001536} = 0.51$, which is roughly a 1:2 ratio.

7.03 Problem Set 2

due in class Wednesday, October 13

1 **(a)** The human genetic map is comprised of many hundreds of loci. Allelic differences at these loci allow recombination in families to be detected and from the recombination frequencies genetic map distances are derived. The total genetic length of the human genome in has been determined to be about 2,500 m.u. If we consider there to be 500 loci on the map distributed at random what is the average distance between neighboring markers? What is the chance in one meiosis of a genetic crossover between an average pair of neighboring markers?

(b) When homologous chromosomes cross over during meiosis, structures form at the sites of crossing over that are called chiasmata. Meiosis in humans can be observed in the light microscope by examining the division of specialized diploid cells known as spermatocytes in the production of sperm. During meiosis in spermatocytes chiasmata can be seen and counted. Given the total human map length of 2,500 m.u. how many chiasmata would you expect to see in the average spermatocyte meiosis? Assume that one crossover between homologous chromosomes occurs at each chiasma. Check your answer carefully — it is very easy to make two fold errors in this calculation.

(c) Map distances calculated from recombination in human females do not exactly match map distances in males. The total human map length from female recombination rates is 3,900 m.u. and the total length from male recombination rates is 2,000 m.u. In one sentence explain what this must mean about the relative recombination rates in human males and females.

2 By crossing two true breeding *Drosophila* strains you produce F1 flies that are heterozygous at three autosomal loci — genotype: A/a, B/b, C/c. You test cross female F1 flies to homozygous recessive males— genotype: a/a, b/b, c/c. The phenotypes and numbers of the progeny are given below with + indicating the wild type trait and lower case letters indicating the mutant traits.

+ + +	669
a b +	139
a + +	3
+ + c	121
+ b c	2
a + c	2,280
a b c	653
+ b +	2,215

(a) What are the genotypes of the true breeding parents of the F1 flies?

(b) What is the order of the a, b and c markers?

(c) In a similar cross, female flies that are heterozygous at loci B/b, C/c, D/d are test crossed to b/b, c/c, d/d males and the phenotypes of the progeny are scored.

b c d	8
b + +	441
b + d	90
+ c d	376
+ + +	14
+ + d	153
+ c +	64
b c +	141

Draw a map of of this region of the chromosome showing the order of the four loci and the three distances between neighboring loci in map units.

3 You are interested in finding the chromosomal map position of MET14 and MET20, two yeast genes required for methionine synthesis. Mutations in these genes cause yeast to be phenotypically Met^-. That is, these mutations prevent yeast from growing on minimal medium that does not contain methionine. You cross a $met14^-$ strain of mating type α with a $met20^-$ strain of mating type **a**. The resulting diploid is sporulated and tetrads are dissected. Three different tetrad types are found with respect to ability to grow on minimal medium without methionine.

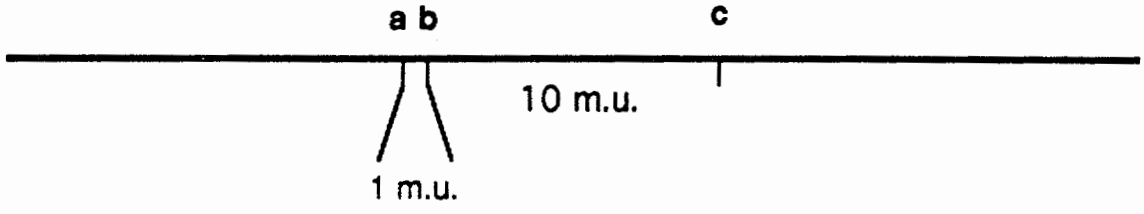
Type I	Type II	Type III
4 Met^-	3 Met^-, 1 Met^+	2 Met^-, 2 Met^+

(a) Classify each of the tetrad types as being either PD, NPD or T type tetrads.

(b) In a complementation test, if you mated a $met14^-$ mutant to one of the two Met^- spore clones from a type III tetrad would the resulting diploid be Met^+ or Met^-?

(c) Of 100 tetrads dissected 75 are of type I, 22 are of type II, and 3 are of type III. What is the distance in map units between MET14 and MET20?

4 In a phage cross, bacteria are simultaneously infected with wild type phage and a phage that is mutant at three different loci: **a**, **b**, and **c**. Each bacterial cell is infected with more than one phage of each genotype thereby allowing recombination between the phage to occur. Millions of progeny phage can be produced from such a cross allowing recombination frequencies to be determined very accurately. The genetic map for the phage is shown below. The distance between **a** and **b** is 1 m.u. and the distance between **b** and **c** is 10 m.u. What are the eight possible genotypes that would result from this cross and what fraction of the total progeny would each represent? You can designate the mutant alleles with lower case letters and wild type alleles with +.



PROBLEM SET 2 ANSWERS

Question 1

a) The average distance between two neighboring markers is the length of the human genome divided by the total number of loci or 2500 mu / 500 loci, which is equal to 5 mu. The chance in one meiosis of a genetic crossover between an average pair of neighboring makers is 10% or 0.1.
The average distance between neighboring markers is 5 mu which means that there are 5 recombinants gametes for neighboring markers in a total of 100 gametes. One meiosis gives rise to four gametes and if a crossover occurs between adjacent markers only 1/2 of them are recombinants. The chance of crossover in one meiosis is

1/20 x 4 x 1/2 = 0.1 or 10% chance (1/20 = 5/100)

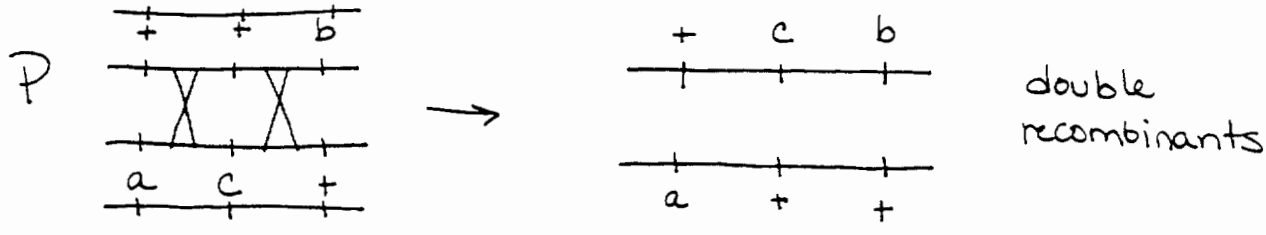
b) In part a) you have calculated the probability of a crossover between any pair of neighboring markers. Given that the human genome has a total of 500 loci separated by and average distance of 5 mu and there is a 10 % chance of crossover between any of them per meiosis, you would expect to see 50 chiasmas per spermatocyte meiosis, which is 0.1 x 500.

c) The recombination rate of human females is almost twice that of human males.

Problem 2:

a) In order to determine the genotype of the parents of the F1 flies, one must find the classes which have the largest number of progeny in the F2. These are the a+c, and +b+ classes. Therefore, one parent's genotype was **a/a, +/+, c/c,** and the other was **+/+, b/b, +/+.**

b) In order to determine the order of the markers, one must look at the classes with the least number of progeny in the F2. These classes are the result of double crossovers, and will be rare. The classes that fit this criteria are a++, and +bc. Now, one must determine which marker was "switched " during the double crossover. It will be the one marker that looks different from the parental setup. In comparing the parental genotypes with those of the double recombinant classes, it is apparent that marker **c** is in the middle. This can be more clearly illustrated below:



Therefore, the gene order is **a-c-b**.

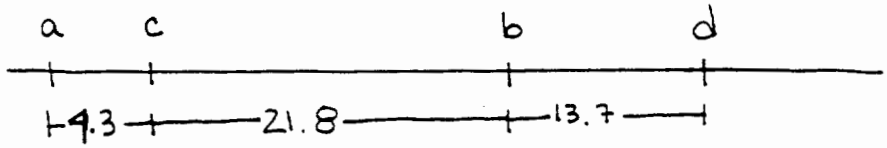
c) Now we have one more marker to consider. We use the same techniques as outlined above to first determine the order of the markers. The parental genotypes were +/+, b/b, +/+, and c/c, +/+, d/d. The double crossover classes show us that b is in between c and d. Therefore the marker order is: **a-c-b-d**. Now we can determine the map units separating the four markers. I will use the data from the first part of the problem to calculate the distances between a-c, and c-b, and clearly the data from the second part to determine the distance between b-d. (If you use the data from the second part to determine the distance between c-b, you will still get full credit.)

a-c: $\frac{139+3+121+2}{6082} * 100 =$ **4.3 mu**

c-b: $\frac{669+3+2+653}{6082} * 100 =$ **21.8 mu**

b-d: $\frac{8+90+14+64}{1287} * 100 =$ **13.7 mu**

The linear map is therefore:



* not exactly to scale

Solution to Problem 3

Before classifying the tetrads we don't know if the two genes are linked or not. First classify the tetrads. Let + designate the wild type allele, and metX- the mutant allele. We know the two parental types were met14-/+ and +/met20. Draw out the genotype of each spore in each tetrad:

Type I Parental ditype (PD)		Type II Tetratype (T)		Type III Non-parental ditype (NPD)	
genotype>>>phenotype		genotype>>>phenotype		genotype>>>phenotype	
+,met20-	Met-	met14-,met20-	Met-	met14-,met20-	Met-
+,met20-	Met-	met14-,+	Met-	met14-,met20-	Met-
met14-,+	Met-	+,met20-	Met-	+,+	Met+
met14-,+	Met-	+,+	Met+	+,+	Met+

a Note that type II has four different genotypes but only two different phenotypes. This is the tetratype class (T). Type one has only the parental gene combinations and thus is the parental ditype class (PD). Type three has only two genotypes and both are different from the parents so this is the non-parental ditype (NPD) class.

b Note that the genotype of a Met- spore from Type III tetrads (NPD) is met14-/met20-. When this is crossed to a met14- haploid the resulting diploid is met14-/met14-, +/met20-. If we assume that met14- and met20- are in different genes then it is clear that the diploid will be **Met-** because it does not have any good copy of met14. Note that at this point we do not know if these mutations are dominant or recessive but this information is not necessary for this question.

c Note that PD does not equal NPD so the genes are linked.
mu = T + 6NPD/2total = 22 + 3(6)/200 = 40/200 = **20 mu**

Solution to problem 4:

The eight types of genotypes that could result from this cross are

1) a b c (PT) parental types
2) + + +

3) a + + (SR1) single recombinants type 1
4) + b c

5) a b + (SR2) single recombinants type 2
6) + + c

7) a + c (DR) double recombinants
8) + b +

First notice that the progeny are divided into four classes. No recombination leads to types 1 and 2. A crossover between loci a and b leads to classes 3 and 4. Every crossover leads to one of type 3 and one of type 4, so the frequencies of types 3 and 4 must be equal. A crossover between loci b and c leads to types 5 and 6. When two crossovers occur, one between a and b and one between b and c you generate types 7 and 8. The frequency of type 5 must be equal to the frequency of type 6, and the frequency of type 7 must be equal to the frequency of type 8. In this problem I will assume the cells were infected with equal numbers of parental type phage so that the frequencies of the non-recombinant types, 1 and 2, will also be equal.

WHAT FRACTION OF PROGENY WILL HAVE EACH GENOTYPE?

We are told that a and b are 1 map unit apart..

m.u.=1 = 100(# phage recombinant for a and b)/(total # of phage)
1= 100[# of SR1 class + # of DR class]/total # of phage

I prefer to write this in terms of frequency and not absolute numbers:
1 = 100[f(SR1) + f(DR)]
equation 1 .01 = f(SR1) + f(DR), this is also the chance of having a crossover between a and b.

We are told that the map distance between b and c is 10.
10 = 100(# of phage recombinant for b and c)/(total # phage)
10 = 100[(# of SR2 class) + (# of DR class)]/ total # of phage
10 = 100 [f(SR2) + f(DR)]
equation 2 .10 = f(SR2) + f(DR), this is also the chance of having a crossover between b and c.

The frequency of having a double crossover is equal to the frequency of having a crossover between a and b AND the frequency of having a crossover between b and c.

f(DR) = (Freq of crossover between a and b)(freq of crossover between b and c)
f(DR) = [f(SR1) + f (DR)][f(SR2) + f(DR)]
f(DR) = (.01)(.10)= .001

We can plug this value, F(DR)= .001, into equations 1 and 2 to get the values for f(SR1) and f(SR2).

.01 = f(SR1)+f(DR)
.01 = f(SR1) + .001
.009 = f(SR1)

.10 = f(SR2) + f(DR)
.10 = f(SR2) + .001
.099 = f(SR2)

Since the frequencies of all four classes must total to one, we can calculate the frequency of the non-recombinant class, f(PT):
f(PT) + f(SR1) + f(SR2) + f(DR) = 1
f(PT) + .009 + .099 + .001 = 1
f(PT) = .891

The question asks for the frequency of each of the eight genotypes and not for the frequency of the four classes, so we must divide each value by 2.

1)	a b c	.4455	.891	(PT) parental types
2)	+ + +	.4455		
3)	a + +	.0045	.009	(SR1) single recombinants type 1
4)	+ b c	.0045		
5)	a b +	.0495	.099	(SR2) single recombinants type 2
6)	+ + c	.0495		
7)	a + c	.0005	.001	(DR) double recombinants
8)	+ b +	.0005		

7.03 Problem Set 3

due in class Wednesday, November 3

Note: Question #1 is purposely omitted.

2 A mutant strain of *E. coli* (strain II) which requires each of five nutrients (A, B, C, D, and E) was isolated from a wild type strain (strain I) in a series of five mutational steps. Assume that the genotype of strain II is **a^- b^- c^- d^- e^-** and the genotype of strain I is **a^+ b^+ c^+ d^+ e^+**. In order to determine the linkage relationships among these five markers, strain II was infected with phage P1 (a generalized transducing phage) that had previously been grown on strain I. The following data describe the frequencies with which transductants were found that no longer required the five nutrients, taken singly and in pairs:

Growth in the absence of	Frequency (transductants per input phage)	Growth, cont'd	Frequency, cont'd
A	10^{-4}	A and E	10^{-8}
B	10^{-4}	B and C	10^{-5}
C	10^{-4}	B and D	10^{-8}
D	10^{-4}	B and E	6×10^{-6}
E	10^{-4}	C and D	10^{-8}
A and B	10^{-5}	C and E	10^{-8}
A and C	9×10^{-5}	D and E	9×10^{-6}
A and D	10^{-8}		

Hint for interpreting this data: $10^{-8} = (10^{-4})(10^{-4})$

(a) Draw a map consistent with the data given above (but don't attempt to be quantitative in assigning relative distances). Indicate any ambiguities in the order of markers.

(b) Assume the ready availability of strains with any combination of markers in their genotype that can be used as donors and recipients in transduction. Design a specific experiment that would resolve any ambiguities in the map order and indicate how you would interpret the results.

3 Consider the hypothetical **Suc** operon which is required for *E. coli* to grow on sucrose. The **Suc** operon controls the synthesis of the enzyme sucrase whose enzymatic activity can be assayed under different growth conditions and in strains of different genotypes. The synthesis of sucrase is regulated by the availability of sucrose in the medium as shown by the units of sucrase activity below:

	+sucrose	-sucrose
wild type	1000	1

The structural gene for sucrase is **sucA**. Sucrase assays of **sucA**$^-$ mutants give less than 1 unit of activity in either the presence or absence of sucrose. Two different **F'** episomes are available that carry the entire **Suc** operon. One of the episomes was constructed from a wild type strain and the other was constructed from a **sucA**$^-$ strain. Sucrase activity assays with different genotypes are given below:

	+sucrose	-sucrose
sucA$^+$/F'sucA$^+$	2000	2
sucA$^-$/F'sucA$^+$	1000	1
sucA$^-$/F'sucA$^-$	<1	<1

A number of mutations in the **Suc** operon that alter sucrase regulation have been isolated and will be designated by a different number for each mutation. Note that it is possible for two different mutations to be in the same gene.

(a) Three mutations that give constitutive synthesis of sucrase, **suc8**, **suc11**, and **suc14** are analyzed below:

	+sucrose	-sucrose
suc8	1000	1000
suc11	1000	1000
suc14	1000	1000
suc8/F'sucA$^+$	2000	2
suc11/F'sucA$^+$	2000	2000
suc14/F'sucA$^+$	2000	2000 1000
suc8/F'sucA$^-$	1000	1
suc11/F'sucA$^-$	1000	1000
suc14/F'sucA$^-$	1000	1000
suc8, sucA$^-$/F'sucA$^+$	1000	1
suc11, sucA$^-$/F'sucA$^+$	1000	1000
suc14, sucA$^-$/F'sucA$^+$	1000	1

Propose a simple model to explain the nature of the defects in **suc8**, **suc11**, and **suc14**.

(b) Three mutations that give reduced induction of sucrase, **suc21**, **suc23** and **suc25** are analyzed below:

	+sucrose	-sucrose
suc21	50	1
suc23	50	1
suc25	<1	<1
suc21/F'sucA+	1000	1
suc23/F'sucA+	2000	1
suc25/F'sucA+	1000	1
suc21/F'sucA-	50	<1
suc23/F'sucA-	2000 1000	1
suc25/F'sucA-	<1	<1

Propose a simple model to explain the nature of the defects in **suc21**, **suc23**, and **suc25**.

(c) Describe what do you think the phenotype of a **suc8**, **suc23** double mutant would be. Express your answer as sucrase levels in the double mutant with or without sucrose.

Problem 4 (corrected version)

You are interested in isolating some of the genes in the **Suc** operon. Starting with a library of random segments of the *E. coli* chromosome inserted into a plasmid vector you transform a **suc23** mutant strain selecting for ampicillin resistance which is the selectable marker on the vector. The **suc23** mutant strain, unable to induce high levels of sucrase, does not grow well on sucrose. Of 1000 ampicillin resistant clones, 3 allow the **suc23** mutant strain to grow well on sucrose.

(a) One of the three clones (clone 3) causes the sucrase activity induced by sucrose to be at least five times greater than the induced sucrase levels in a wild type strain. Given that the vector is present in cells at a copy number of about ten how would you explain the extraordinarily high levels of sucrase induction.

(b) To determine which functions are carried on the three plasmid clones, each plasmid is transformed into each of three different mutant strains and sucrase activity is measured.

	+sucrose	-sucrose
suc23/clone1	1000	1
suc23/clone2	1000	1
suc23/clone3	5000	1
suc8/clone1	1000	1000
suc8/clone2	1000	1
suc8/clone3	5000	5000
$sucA^-$/clone1	<1	<1
$sucA^-$/clone2	<1	<1
$sucA^-$/clone3	5000	1

Which of the functional genes identified in problem 3 are included on each of the three cloned chromosomal fragments.

(c) Try to draw a map summarizing all that you have learned about the **Suc** operon. Show each of the genes that you have hypothesized and their relative positions, show which gene is inactivated by each of the mutations, and show which gene/s are included in each of the clones.

Answers to 703 Problem Set # 3

PROBLEM 2

a) The higher the frequency of transductants the closer the genes are in the chromosome. A higher cotransduction frequency means that the chance of two markers being in the same piece of DNA packaged by the virus proteins is high and that once inside the recipient cell the chance of a cross over event beween the two markers with concomitant loss of one of them is low because the distance between them is small.

According to the data the map could be drawn either as:

1) _____

C A B E D

OR

2) _____

A C B E D

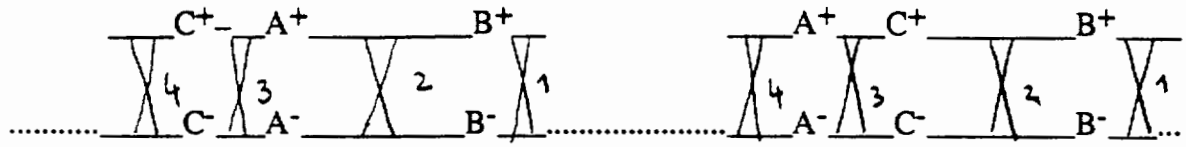
You can't determine whether C is on one side or the other of A. Note: A frequency of 10^{-8} is the frequency of two independent events, the probability of one marker being transduced times the probability of the other marker being transduced, or the chance that two independent molecules of DNA get incorporated independently.

b) In order to resolve the ambiguities of this map you could set up the following experiment (there is more than one way of doing it)

You could grow P1 in a strain with the following genotype, $B^+ C^+ A^+$, and the lysate used to infect a strain that is $B^- C^- A^-$. Selection of B^+ incorporation is done by growing the bacteria in a medium without B and supplemented with C and A. After selection you then screened for incorporation of the other markers. There are four possible classes:

A^-C^+
A^+C^-
A^+C^+
A^-C^-

There are two possible orders:



order 1 **order 2**

When you select for B you are selecting for a crossover to the right of B and the different classes are generated by different locations of the second crossing over or by the event of four crossovers.

	order 1 crossovers	**order 2 crossovers**
A^+C^+	1+4	1+4
	(2)	(2)
A^-C^-	1+2	1+2
	(2)	(2)
A^+C^-	1+3	1+2+3+4
	(2)	(4)
A^-C^+	1+2+3+4	1+3
	(4)	(2)

The two orders predict a different least frequent class or the class generated by four crossovers. Determining the frequency of A^+C^- and A^-C^+ and comparing their frequency should allow you to determine the order of the genes. If A^+C^- is the least frequent then order 2 is the correct order and if A^-C is the least frequent then order 1 is correct.

Problem 3:

a) The suc8/F'sucA+ cross shows that the suc8 mutation is recessive, and from the two other F' crosses we can determine that the mutation acts in trans. Since it is constitutive, recessive, and trans-acting, the most likely explanation is that the mutation lies in a **repressor** for the operon.

The suc11 mutation is dominant, and also acts in trans. Since we know that a repressor exists for control of this operon, the suc11 mutation is analagous to the I^{-d} mutation in the lac repressor. Therefore, the suc11 mutation is a **dominant negative mutation in the repressor.**

The suc14 mutation is also constitutive and dominant, but only acts when in cis to a functional sucA+ gene. The simplest explanation for this mutation is that it lies in an **operator** site that normally binds the repressor. This mutation is analagous to the O^c mutation in the lac operon.

b) The suc23 mutaton is uninducible, recessive and trans-acting. The simplest explanation is that it lies in an **activator** for the operon. One can postulate that if there is no activator present, as in a suc23- mutant, then only low levels of sucrase can be made.

The suc21 mutation is uninducible and recessive, but only acts when in cis to a functional sucA+ gene (as shown in the suc21/F' sucA- merodiploid). The most likely explanation is that this mutation lies in an **operator site that the activator binds.** This site can be different than the site that binds the repressor, or it can be the same site. However, if it is the same site, one must stipulate that the suc21 mutation only affects binding of the activator, and not binding of the repressor. Moreover, the suc8 mutation affects repressor binding, but not activator binding.

The suc25 mutation has the same characteristics as the suc21 mutation, except that no sucrase activity is detected in the suc25 mutant. There are two possible explanations for this result. One is that the suc25 mutation is in the **promoter** for the operon. One would expect no enzyme activity if RNA polymerase could not transcribe the region. Alternatively, the mutation could lie within the **sucA** gene itself.

c) The suc8 suc23 double mutant would be defective in both repressor and activator function. Therefore, the phenotype would be constitutive low levels of enzyme production, or **50 units with or without sucrose.**

Problem 4 solution:

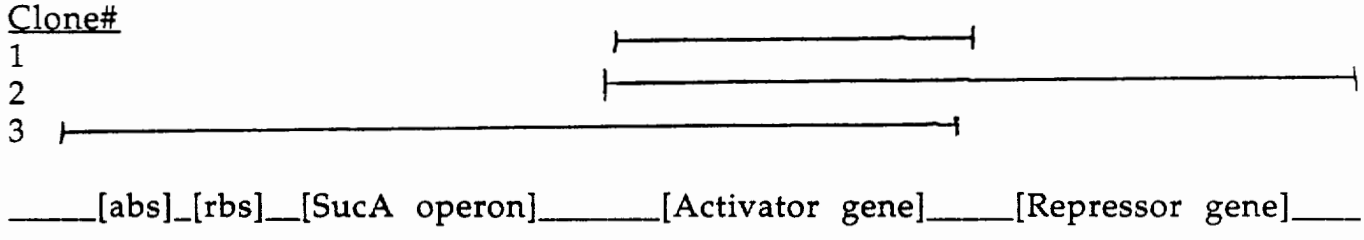
Note that the plasmid library was made from random DNA fragments from the E coli genome. This has two implications. First, all genes, binding sites and other sequences carried in the plasmid inserts are wild type alleles. Second, each plasmid has only a single continuous DNA insert; in other words the order of genes in a plasmid insert represents their order in the bacterial chromosome. Recall that the suc23 mutation is recessive. The three clones that grow well on sucrose must carry the suc23+ gene.

a) Clone three shows high sucrose expression levels because it carries the sucA+ gene (see below) and its regulatory sequences in addition to suc23+. Since the plasmid, and thus suc23+ and sucA+, is present in many copies per cell, sucrase expression is very high under inducing conditions in strains carrying clone 3. This data shows that sucA, and not the activator (suc23+), is limiting in sucrase expression.

b) Clones that alleviate the mutant phenotype complemented the recessive mutation in the recipient strain, and thus carry a functional copy of the mutated host gene (suc23, suc8 or sucA). All three clones carry suc23+. Only clone 2 carries suc8+, and only clone 3 carries $sucA^+$ and its regulatory sequences. We know that clone three must carry the regulatory sequences because normal activation and repression of the plasmid $sucA^+$ genes is observed. If the repressor binding site were missing there would be several thousand sucrase activity units in the absence of sucrose. Similarly, if the activator binding site were missing there would be only fifty sucrase units from each plasmid in the presence of sucrose, for a total of several hundred sucrase units .

c) Remember that the inserts contain continuous DNA inserts derived from the wild type E coli chromosome. There are two possible gene orders based on these three clones:
sucA...suc23...suc8 or suc8...suc23...sucA.

A schematic map based on the first order is presented below.



Gene/regulatory element	Mutations
SucA gene	suc25
Activator gene	suc23
Activator binding site (abs)	suc21
Repressor	suc8 (recessive), suc11 (dominant)
Repressor binding site (rbs)	suc14

Other regulation models may be consistent with the data, but this is the simplest.

7.03 PROBLEM SET 4

November 17, 1993

Due: Wed. Nov. 24, 1993

Please remember to note your name, recitation day, time and instructor on your answers.

1 (25pts) Two laboratories, one at the University of Indiana, Bloomington and one at the University of Cologne, have identified lethal mutations in a small interval of the Drosophila genome. The results of the two groups are compared by crossing an allele of each complementation group (gene) identified by one lab with a representative allele of each complementation group (gene) identified by the other lab. The results are summarized in the table below:

		BLOOMINGTON					
		FA	FB	FC	FD	FE	FF
COLOGNE	C1	+	+	+	-	+	+
	C2	-	+	+	+	+	+
	C3	+	-	+	+	+	+
	C4	+	+	+	+	+	-
	C5	+	+	+	+	+	+

"+"= viable
"-" = lethal

a) (5pts) Indicate which complementation groups have been identified in both laboratories and which alleles affect the same gene.

Note: Questions #2-5 were purposely omitted.

6. (10pts) The gene for red-green color-vision maps to the X chromosome in humans. Mutations in this gene are recessive to the wild type. An XXY individual is colorblind. His mother and father have normal vision.

a) In which parent did non-disjunction occur?

b) At which stage of meiosis did non-disjunction occur?

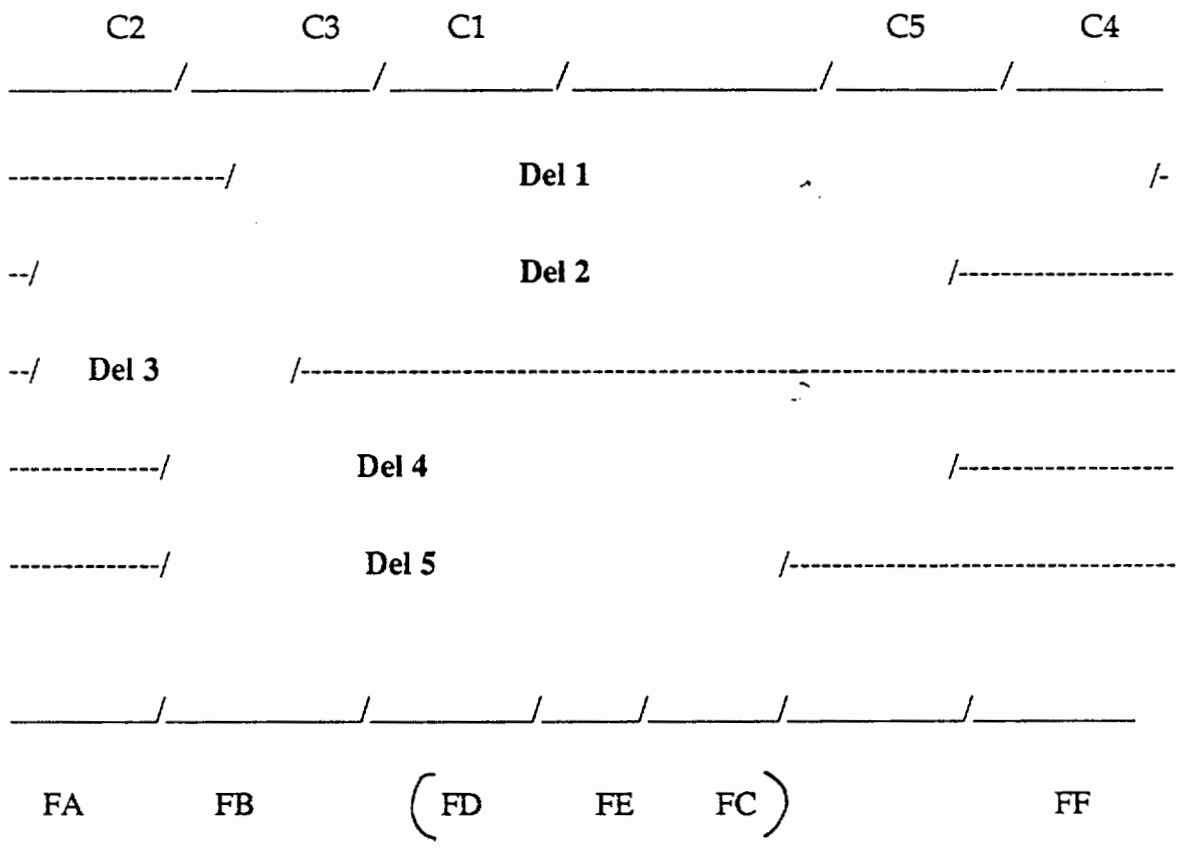
7.03 Problem Set 4

(Solns)

Problem 1

a) The complementation groups FA, FB, FD and FF have been identified by both groups . FA and C2 /FB and C3/ FD and C1/ and FF and C4 , are alleles that affect the same gene (they do not complement each other). The complementation groups FC and FE have only been identified in Bloomington and the complementation group C5 has only been identified in Cologne.

b)



This is just one possible way to write the map. There is ambiguity with respect to the order of FD , FE and FC. These three genes have to be contained between C3/FB and C5 but the order is arbitrary (8 possibilities) [Remember that FE and FC are in different genes since the original crosses were made with a representative allele of each complementation group isolated by each research group]

c) A three factor cross can be done in order to determine the order of the genes. In this case the generation of the transheterozygotes and double mutants is more difficult because this organism is diploid and these mutations can not be bred into homozygozity because they are lethal.

The first step would be to generate a transheterozygote by crossing two single mutants.

$$\frac{m1\ +}{+\ D^*\ +} \quad x \quad \frac{+\ m2}{+\ D^*\ +}$$

* the wild type chromosome carries a marker that gives a dominant (D) non-lethal phenotype

$$\frac{m1\ +}{+\ m2}$$

The progeny that do not carry * dominant marker are the transheterozygotes for the two mutations.

Once you have a female transheterozygote you cross it to a male fly . What you want to isolate is the double mutant which is a product of a recombination event .

$$\frac{m1\ +}{+\ m2} \quad x \quad \frac{+\ +}{+\ +}$$

m1 +/ + + These are the type of progeny that you would get out of
+ m2/ + + such a cross.
m1 m2/ + +

Since you can't distinguish between these possibilities by phenotype you would have to cross each of them to the original single mutants and isolate the progeny that do not complement with either of the single mutants, that is the double mutant.

Once you have each of the double mutants FC FE, FC FD and FD FE you would now be able to do the experiment to determine order.
To do this you would first have to generate a transheterozygote between a double mutant chromosome and a single mutant chromosome(to obtain this think about the transheterozygote generated above) For example:

FD	FC	+	X		Del	
+	+	FE		+	D*	+

The majority of the viable progeny will be of the genotype:
FD FC +/D* and + + FE/D*

Lethal progeny that will not be recovered:
FD FC +/Del and + + FE/Del

Only progeny that is viable in trans to the deletion will be the products of rare "interesting" recombinants of the genotype:
+ + +/Del

Transheterozygous females are crossed to males which carry one of the deletions used in (b) (Del1, Del2, Del4 or Del5) which are deleted for all three genes. To distinguish the chromosome that carries the deletion from its homologue which carries the wild-type alleles for all seven genes studied this chromosome is marked with a dominant marker D* as described above.

Most progeny of this cross carry the chromosome marked with D* in trans to the mutants and you are not interested in those. You search the viable progeny for the products of recombination. Only recombinants that are wild-type for all three genes will be viable in trans to the deletion chromosome.

If FE mapped on either side of FC or FD, wild-type recombinants can be produced by a single cross-over event. If however, FE mapped between FC and FD then the frequency of viable progeny would be very low since it would require two-cross over events to generate the + + + chromosome. You would perform crosses with the three possible combinations (FD FC/FE; FD FE/FC; FC FE/FD). By comparing the frequency of viable progeny in trans to the deficiency you can determine the relative order of the three genes as we did in phage genetics.

Any answer that suggests to resolve the order of the three genes by three factor crosses or by recombination distances will get partial credit.

d) This stock fails to complement the mutant alleles C5, C4/FF, FE and FC. Thus this stock most likely carries a deletion for this region. Since this deletion fails to complement the FC mutation but does complement the lethality caused by the FE and FD/C1 mutations you can refine the order of genes:

FA/C2--FB/C3--(FE--FC)--FD/C1--C5--FF/C4

The relative order of FE and FC is still not determined.

6.

First determine the genotypes of all three individuals:
Let (Xc) represents the X chromosome with the recessive allele for colorblindness.

The son is (Xc)(Xc)Y
The mother is (Xc)(X+)
The father is (X+)Y

Clearly the son must have received the (Xc) chromosomes from his mother. The non-disjunction event that occurred in meiosis two of the mother is diagrammed below: