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Genetics 201

Final exam

December 18, 2007

PUT YOUR NAME ON EVERY PAGE.

THERE ARE THREE MULTI-PART QUESTIONS ON THIS EXAM. THE POINT VALUE FOR EACH PART IS INDICATED AT THE END OF EACH QUESTION. THE TOTAL VALUE FOR THE THREE QUESTIONS IS LISTED BELOW.

QUESTION 1 – 45 POINTS

QUESTION 2 – 24 POINTS

QUESTION 3 – 31 POINTS

WE RECOMMEND THAT YOU LOOK THROUGH THE EXAM AND ANSWER THE EASIER QUESTIONS FIRST.

PLEASE TRY TO GIVE SIMPLE AND STRAIGHTFORWARD ANSWERS.

WRITE ALL YOUR ANSWERS IN THE SPACE PROVIDED. WE HAVE OFTEN LEFT MORE SPACE THAN IS NECESSARY FOR YOUR ANSWER. IF YOU NEED EXTRA SPACE FOR ANY ANSWERS, USE THE BACKS OF PAGES.

1. You are studying a mouse model of myopia. You have two strains of inbred mice of different backgrounds, one severely myopic (Myo) and one with normal vision (wild-type, WT). Myopia segregates as a fully penetrant autosomal dominant phenotype in these mouse strains. You want to use linkage analysis to map the gene responsible for myopia in this model system.

1a. You cross Myo mice to wild-type mice to generate F1 mice, which are all myopic. You then backcross the F1 mice to the wild-type strain to create a backcross (BC) population.

How many informative chromosomes are present in each BC mouse, and which parent(s) are they from? (3 points)

1b. You then genotype the BC progeny with a panel of SSR markers distributed across the genome. For an SSR on chromosome 5, you obtain the data shown below. This SSR has two alleles: one with 2 CA repeats (allele 2), found in the Myo strain, and one with 4 CA repeats (allele 4), found in the WT strain.

BC Class	Phenotype	Marker genotype	# of BC mice
1	Myopic	2/4	88
2	Myopic	4/4	13
3	Wild-type	2/4	17
4	Wild-type	4/4	82
Total BC progeny			200

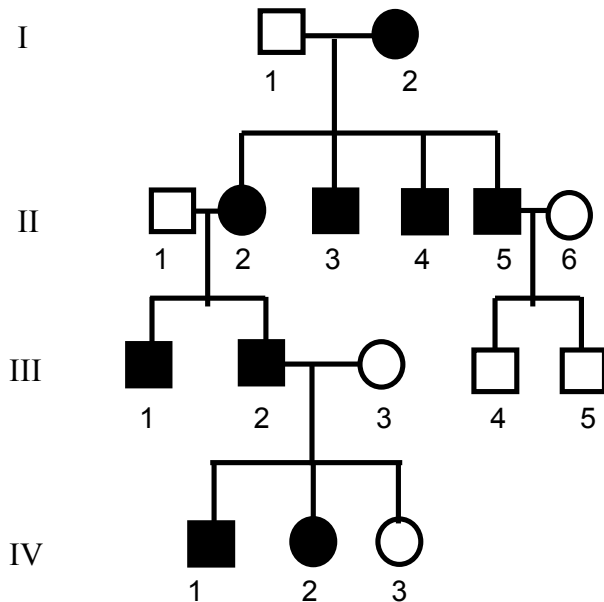
1bi. List the classes of BC mice that contain recombinant chromosomes. (2 points)

1bii. What is the LOD score for linkage between the myopia gene and the chromosome 5 SSR for a theta of 0.1? Please show your calculations. (4 points)

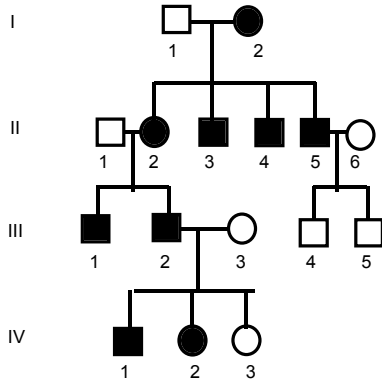
1biii. Is this significant evidence for linkage between the chromosome 5 SSR and the myopia gene? Why or why not? (2 points)

1c. You are eager to see if your findings in the mouse are relevant to human myopia. To reduce any potential locus heterogeneity in your analysis, you begin by looking for pedigrees of human families in which myopia is inherited. You plan to use DNA from these families for linkage analysis.

One family showing segregation of myopia is drawn below. Shaded = individuals affected with myopia; unshaded = normal vision.



Consider the modes of inheritance listed on the following page. For each mode, state whether or not it is consistent with the pedigree data. If your answer is no, briefly (one sentence) state the reason for your conclusion, citing the individuals in the pedigree that led you to this conclusion. (pedigree repeated on following page)



1ci. X-linked recessive inheritance

Consistent with pedigree data? (circle one) Yes No
 If no, briefly state your reasons, citing particular individuals. (3 points)

1cii. X-linked dominant inheritance

Consistent with pedigree data? (circle one) Yes No
 If no, briefly state your reasons, citing particular individuals. (3 points)

1ciii. Autosomal dominant inheritance

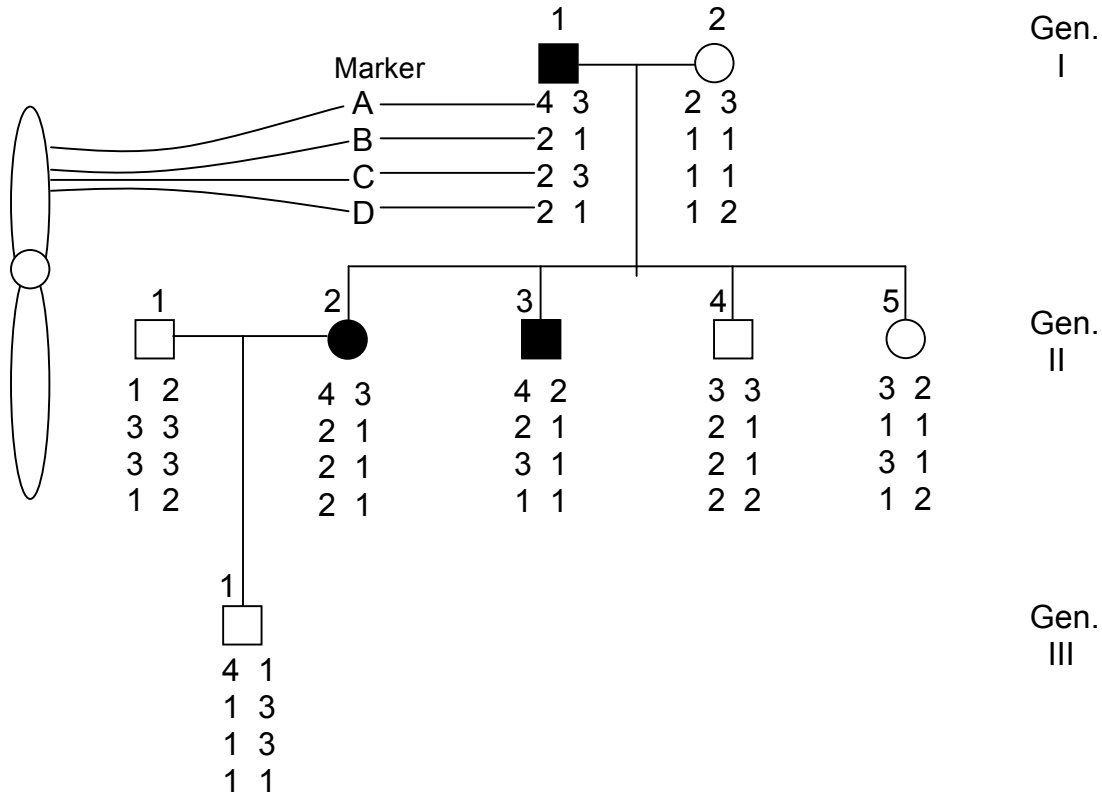
Consistent with pedigree data? (circle one) Yes No
 If no, briefly state your reasons, citing particular individuals. (3 points)

1civ. Mitochondrial inheritance

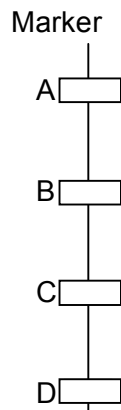
Consistent with pedigree data? (circle one) Yes No
 If no, briefly state your reasons, citing particular individuals. (3 points)

1d. You identify a number of families with autosomal dominant inheritance of myopia. You use genome-wide linkage analysis in these families to map the responsible gene to several cM. You now turn to haplotype analysis to narrow the region further.

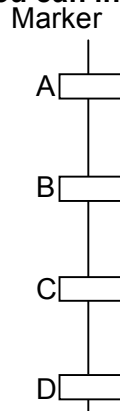
In the family below, shaded individuals are affected with myopia; unshaded individuals have normal vision. You genotype each family member for four linked markers in the region defined by linkage; each marker has multiple alleles. For each individual in the pedigree below, each number represents the allele at that particular marker. For example, at marker A, individual I-1 has allele 4 and allele 3. Each column of numbers represents the linked marker alleles present on a single chromosome (the individual's haplotype).



1di. Based on the haplotype data, shade the most probable region where the myopia gene is located on the map below. (4 points)



1dii. How would your interpretation change if individual III-1 was found to be affected for the myopia trait? You can indicate your answer on the map. (3 points)



1e. To your great happiness, you identify a gene containing six exons that causes a dominant myopia phenotype in both mice and selected human families; you name this gene *myo*. Upon sequencing the affected *myo* allele in humans, you determine that it contains a mutation in exon 5 that alters the amino acid sequence of the protein. You would like to construct a mouse model that contains the same mutation to see if it also has the myopia phenotype. You can assume that the mouse myopia gene is highly homologous to the human gene, and that the sequence for both genes is available.

1ei. Design a targeting construct that can be used to introduce an exon 5 point mutation into the mouse. You can assume you have access to any necessary promoters, sequences, cell lines, mouse strains, and reagents. Your goal should be to create a “clean” mutation (ie, not to leave behind any selectable markers in the genome). (3 points)

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1eii. Briefly list the steps you will perform with your construct from part ei. to generate a mouse heterozygous for the exon 5 point mutation in the *myo* gene. (4 points)

1eiii. Draw any relevant recombination events that will occur in ES cells and/or in mice. Assume you have access to any necessary reagents. (3 points)

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1f. You are now renowned in the field as a myopia expert. You agree to review two papers that have performed association studies on myopia using SNPs. The three top-scoring SNPs from each paper are shown below.

Paper 1: Analyzed 100,000 SNPs distributed across the genome in 2000 myopic and 2000 unaffected individuals, all of similar ethnic background.

SNP	Affecteds	Controls	P value
rs308991, G allele	3418	3346	0.002
C allele	582	654	
rs10988, T allele	2253	2109	0.00001
A allele	1747	1891	
rs200801, C allele	1654	1533	0.0003
T allele	2346	2467	

Paper 2: Analyzed 20 SNPs in a chromosomal region that has been implicated in myopia by linkage studies. Study group consisted of 500 myopic individuals and 500 controls, matched for ethnic background.

SNP	Affecteds	Controls	P value
rs107797, T allele	555	521	0.0319
C allele	445	479	
rs23361, G allele	791	743	0.0005
A allele	209	257	
rs301889, G allele	763	811	0.0001
T allele	237	189	

Which paper's data provide more convincing evidence of an association between a SNP and myopia? Explain briefly, using the data to justify your answer. (5 points)

2. You are a *C. elegans* researcher interested in studying the genetic control of aging. You have performed an F2 screen and identified many mutants that have either a shorter or longer life span than wild-type worms (Age phenotype).

2a. You want to identify the chromosome on which each mutation is located. You begin with the *age-100* mutation, which has an autosomal recessive phenotype. You cross homozygous *age-100* males to hermaphrodites from a strain homozygous for the recessive *unc-5* mutation on chromosome IV and the recessive *dpy-11* mutation on chromosome V. You self the hermaphrodite F1 nonUnc nonDpy progeny to generate F2 animals, then score their phenotypes.

F2 phenotype	Number
Wild-type	150
Age	75
Dpy	75
Unc	50
Age Unc	25
<u>Unc Dpy</u>	<u>25</u>
Total	400

2ai. Is *age-100* located on chromosome IV, chromosome V, or a different autosome? (3 points)

2a.ii. Name one phenotypic class that either by its presence or absence, led you to your conclusion in part i above. Explain your logic in a sentence. (3 points)

2b. After careful work, you have mapped and characterized *age-1* and several other *age* mutations. Molecular data indicates that the products of these genes may act in an epistatic pathway. To investigate this, you construct several double mutants that each contain two different *age* mutations. The single and double mutant phenotypes are summarized below. Note that all of the *age* mutations are recessive, loss-of-function mutations.

Genotype	Phenotype
<i>age-100</i>	Extended life span
<i>age-101</i>	Short life span
<i>age-102</i>	Short life span
<i>age-103</i>	Extended life span
<i>age-100 age-101</i>	Extended life span
<i>age-100 age-102</i>	Extended life span
<i>age-101 age-103</i>	Extended life span
<i>age-102 age-103</i>	Short life span

2b. Draw a linear pathway for longevity that is consistent with the above data. Use the appropriate symbols to show activation or repression between each gene in the pathway. (6 points)

2c. You perform a screen to isolate recessive suppressors of the *age-100* mutation. From your screen, you isolate an autosomal mutation, *rt526*, that can suppress the *age-100* phenotype. You move this mutation to a wild-type background and observe that in the absence of any other mutations, homozygous *rt526* animals have the recessive phenotype of short life span. The phenotypes are summarized below.

Genotype	Phenotype
<i>age-100</i>	extended life
<i>age-100 ; rt526</i>	normal life
<i>rt526</i>	short life

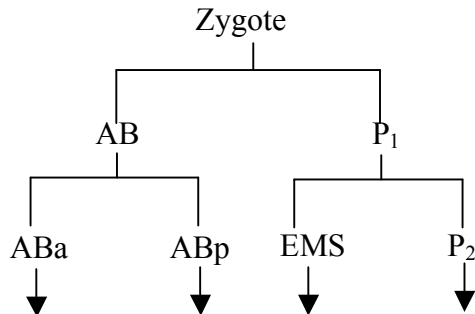
You wish to determine if the *rt526* mutation is in the same gene as the *age-101* mutation, which also causes short life span (see part b). You have available the following strains:

rt526 males and hermaphrodites
age-101 dpy-33 males and hermaphrodites

(The *dpy-33* mutation is a recessive phenotypic marker closely linked to *age-101* that causes a Dumpy phenotype.)

Using your choice of the available strains, list the steps you would take to determine if *rt526* and *age-101* are in the same gene. Please specify the gender and number of animals you will use at each step. Also, include the possible results and how you would interpret them. (4 points)

2d. You would like to carry out some preliminary mosaic analysis to determine whether expression of *age-101* is required primarily in the musculature, the nervous system, or both. Recall that the zygote divides into 2 cells, AB and P₁; AB descendants include most of the nervous system, while P₁ descendants include most of the muscle cells (see pedigree below)



For your experiment, you have a *C. elegans* strain that carries *age-101*, *unc-200*, and *dpy-300* (all closely linked recessive mutations). The wild-type *unc-200* gene product is required in the musculature derived from the EMS progenitor cell; the wild-type *dpy-300* gene product is required in the neurons derived from the AB cell (both ABa and ABp lineages). This strain also carries an extrachromosomal array, *Ex* (+ + +), with the wild-type *age-101*, *unc-200*, and *dpy-300* alleles on it.

You take *age-101 unc-200 dpy-300; Ex(+ + +)* hermaphrodites and self them singly. Some of these animals rarely and randomly lose the extrachromosomal array, resulting in mosaic animals. You analyze mosaic animals for their Unc, Dpy and Age phenotypes; the phenotypic classes of animals you find are listed below.

Mosaic animal phenotypes:

Dpy
Unc Age
Age

2di. For the Dpy mosaic animals, circle the earliest cell listed below in which the array could have been lost. (2 points)

(circle one) AB ABa ABp P₁ EMS P₂

2dii. For the Unc Age mosaic animals, circle the earliest cell listed below in which the array could have been lost. (2 points)

(circle one) AB ABa ABp P₁ EMS P₂

2diii. For the Age mosaic animals, circle the earliest cell listed below in which the array could have been lost. (2 points)

(circle one) AB ABa ABp P₁ EMS P₂

2div. Based on the mosaic results, in what tissue(s) does *age-100* appear to be required for normal life span? (2 points)

(circle one) Nervous system Musculature Both nervous system and musculature

3. You are studying the genetic control of flight in adult *Drosophila melanogaster*. Specifically, you would like to identify genes on chromosome 3 that when mutated, produce recessive phenotypes of poor or no flight.

3a. You have available wild-type flies, *Tb/ TM3 Sb* flies, and a source of EMS. *Tb* (*Tubby*) is a chromosome 3 marker that confers a dominant tubby body phenotype. *TM3* is a chromosome 3 balancer that confers a dominant Stubble bristle phenotype (*Sb*) and a recessive lethal phenotype. You also have the ability to screen for flight ability using flight tests.

Design a screen to identify recessive mutations on chromosome 3 that affect flight ability. Please include gender and number of flies used at each step, and indicate the phenotypes you will use to identify the desired class of progeny. Clearly draw out any crosses you plan, showing the relevant genotypes. (6 points)

3b. You recover many mutants with recessive phenotypes in your screen which you call flightless mutants (*fli-1*, *fli-2*, *fli-3*, etc). You also save a dominant mutant, *Fli-4*. All of these mutations have been mapped. You now want to characterize these mutations. You obtain strains that carry either large deletions (*Del*) or duplications (*Dup*) that include these loci and you have available the new mutations as either homozygotes or as heterozygotes in combination with the *Del* and *Dup* mutations.

3bi. You want to compare the phenotypes of two strains to determine if *fli-1* is a null allele.

List the genotypes of the two strains you will use to determine if *fli-1* is a null allele and briefly describe the predicted results if it is a null. (3 points)

Strain1 genotype:_____

Strain 2 genotype:_____

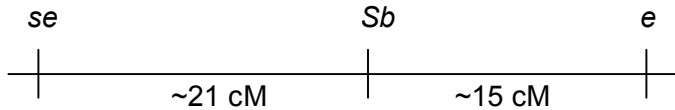
3bii. You also want to compare the phenotypes of two strains to determine if your dominant allele, *Fli-4*, is a hypermorphic allele.

List the genotypes of the two strains you will use to determine if *Fli-4* is a hypermorphic allele, and state the predicted results if it is a hypermorphic mutation. (3 points)

Strain 3 genotype:_____

Strain 4 genotype:_____

3c. You now plan to use recombinational mapping to determine the position of *fli-1* on chromosome 3 relative to known markers on the chromosome. You cross *fli-1* males to *se Sb e / se + e* virgin females. These females are homozygous for the recessive *sepia eyes* (*se*) and *ebony body* (*e*) mutations, and heterozygous for the dominant Stubble (*Sb*) mutation. *se*, *Sb*, and *e* are linked on chromosome 3 in the order listed (see map below).



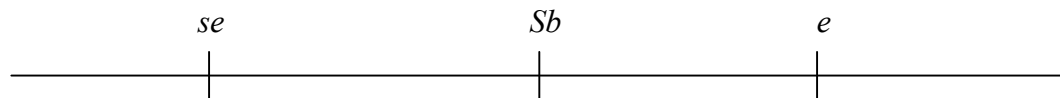
You take *Sb* F1 virgin female flies and cross them to *fli-1 se e* males (no order is implied by listing *fli-1* first). You then score the phenotypes of the F2 flies, shown in the table below.

F2 phenotypic class	Number
<i>fli</i>	665
<i>se Sb e</i>	671
wild type	9
<i>fli se SB e</i>	8
<i>Sb e</i>	68
<i>fli se</i>	60
<i>fli Sb e</i>	102
<i>se</i>	108
<i>e</i>	0
<i>fli se Sb</i>	1
<i>fli e</i>	126
<i>se Sb</i>	134
<i>fli Sb</i>	14
<i>se e</i>	16
<i>fli se e</i>	10
<i>Sb</i>	8
Total F2 flies	2000

3ci. On the table above, indicate the classes that arise from single, double, and triple crossovers. (3 points)

3cii. On the map below, mark the position of *fli-1* relative to the other three markers. (3 points)

3ciii. Calculate the approximate genetic distances (within 1 cM) between *fli-1* and the two closest markers and mark them on the map below. (4 points)



(The table is reproduced on the next page in case you need more space. Be sure to mark your answer on the map on this page.)

Name _____

Table of data for problem 3c

F2 phenotypic class	Number
fli	665
se Sb e	671
wild type	9
fli se SB e	8
Sb e	68
fli se	60
fli Sb e	102
se	108
e	0
fli se Sb	1
fli e	126
se Sb	134
fli Sb	14
se e	16
fli se e	10
Sb	8
Total F2 flies	2000

3d. You decide to perform mosaic analysis on one of your more interesting mutants, *fli-3*. This mutant has abnormally short neurons innervating the adult muscle. You would like to determine if the neuronal phenotype is cell-autonomous or non-cell-autonomous. You plan to use the MARCM technique to generate GFP-marked clones of homozygous *fli-1* mutant cells. Recall that this system utilizes the Gal genes from yeast, in which Gal4 activates transcription by binding to its UAS. However, activation by Gal4 is inhibited by Gal80.

You have already obtained a strain of flies with the genotype *hs-FLP; neuron-GAL4*. This markers are explained below.

hs-FLP - the FLP recombinase under the control of a heat shock promoter on chromosome 2.

neuron-GAL4 - a copy of *GAL4* expressed specifically in neurons on chromosome 2.

You know that you will need to construct a pair of chromosome 3 homologues with some combination of the following sequences:

Sequence	Description
<i>FRT</i>	Recognition site for FLP-mediated recombination
<i>tub-GAL80</i>	Gal80 repressor under control of tubulin promoter (ubiquitously expressed)
<i>UAS-GFP</i>	Green fluorescent protein and upstream activating sequence
<i>fli-3⁻</i>	Mutant <i>fli-3</i> allele
<i>fli-3⁺</i>	Wild-type <i>fli-3</i> allele

3di. Draw the chromosome 3 homologues you will need to create in order to be able to carry out your mosaic analysis, indicating the placement of each necessary sequence from the table above and the location of the centromere. Keep in mind that your ultimate goal is for *fli3*- neurons to express GFP. (3 points)

3dii. You take embryos of genotype *hsFLP ; neuron-GAL4* that also carry the marked chromosomes you have designed and induce FLP expression via heat shock. Recombination occurs and produces two types of daughter cells.

In the space provided below, draw the chromosomes that will be present in each daughter cell type following recombination and circle the genotype and phenotypes that will be seen.

Daughter cell type 1:

Draw the chromosome 3 homologues as they will appear after recombination. Please include centromeres, label all relevant sequences and genotypes, and circle your answers below. (2 points)

<i>fli-3</i> genotype (circle one):	+/+	+/-	-/-
GFP expression (circle one):	ON	OFF	

Daughter cell type 2:

Draw the chromosome 3 homologues as they will appear after recombination. Please include centromeres, label all relevant sequences and genotypes, and circle your answers below. (2 points)

<i>fli-3</i> genotype (circle one):	+/+	+/-	-/-
GFP expression (circle one):	ON	OFF	

3diii. How will you determine whether the *fli-3* neuronal phenotype is cell-autonomous or non-cell-autonomous? (2 points)