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Genetics 201

Final exam

December 20, 2005

PUT YOUR NAME ON EVERY PAGE.

THERE ARE FOUR MULTI-PART QUESTIONS ON THIS EXAM. EACH QUESTION IS WORTH A DIFFERENT TOTAL NUMBER OF POINTS. THE POINT VALUE FOR EACH PART IS INDICATED.

QUESTION	TOTAL POINTS
1	29
2	23
3	28
4	20

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.

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1. You are interested in identifying *C. elegans* genes that affect body shape. You decide to undertake a classical genetic screen using EMS as a mutagen. Try to make your screen as efficient as possible. Explain your logic succinctly.

1a. Design a simple screen that will allow you to isolate both dominant and recessive mutations that cause a body shape phenotype. Be sure to indicate gender used, whether you are using multiple or single worms, and how you will identify multiple independent mutant strains that will correspond to multiple genes. Indicate at what generation you can identify dominant and recessive mutations. (6 points)

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1b. One of the mutant strains you isolate from your screen has a round, fat body; you name the corresponding gene, *plumpy-1 (plp-1*). The *plp-1* mutant phenotype is recessive and completely penetrant. You want to identify the chromosome on which the *plp-1* gene is located.

You cross homozygous *plp-1* males to hermaphrodites homozygous for the *unc-32* marker (chromosome III). You select nonPlp nonUnc hermaphrodites, self singly, and score the resulting F2 progeny for their Plp and Unc phenotypes. Note that the *unc-32* marker is recessive.

i. List the expected phenotypic classes of progeny and their frequency if the *plp-1* mutation maps to an autosome other than chromosome III. (2 points)

ii. If *plp-1* is a recessive mutation on the X chromosome, how will this change the expected phenotypic ratios in the F2 progeny? (2 points)

iii. List the expected major phenotypic classes of progeny and their frequency if the *plp-1* mutation DOES map to chromosome III, to a location extremely close to the *unc-32* gene. (2 points)

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1c. You have been working hard to map the *plp-1* gene via recombinational mapping. Using this approach, you have managed to map the gene to a small region on chromosome III. You now wish to test if *ORF-1*, a predicted gene in the region, is the *plp-1* gene.

For each method listed below, choose if it could potentially provide convincing evidence as to whether or not the *ORF-1* gene is the same as the *plp-1* gene. In a sentence, describe the type of result that would provide such evidence, or why such a method would not be useful for this purpose . **(2 points each)**

i. Approach: Sequence *ORF-1* in *plp-1* animals
Could this provide convincing evidence? (circle one) YES NO
Why /why not?

ii. Approach: Perform mosaic analysis on *plp-1* gene
Could this provide convincing evidence? (circle one) YES NO
Why /why not?

iii. Approach: Inject double-stranded *ORF-1* RNA into wild-type nematodes
Could this provide convincing evidence? (circle one) YES NO
Why /why not?

iv. Approach: Construct *plp-1* animals expressing a wild-type copy of *ORF-1*
Could this provide convincing evidence? (circle one) YES NO
Why /why not?

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1d. Normally all animals from the *plp-1* strain are Plp. However, someone accidentally left your *plp-1* plate under a UV light last week. Several days later, before you toss the irradiated *plp-1* animals, you notice a hermaphrodite that is nonPlp (wild-type) on the plate. You single this animal and find that the progeny produced (mostly hermaphrodites and some rare males) are 1/3 Plp and 2/3 nonPlp.

Propose a genetic explanation for these results. Identify the genotype and phenotype of each phenotypic class. Please define your nomenclature. (4 points)

1e. Complementation tests indicate that you have also isolated a second allele in the *plumpy* gene, *plp-1(xx2)*. Like your original *plp-1* allele, which is named *xx1*, the *xx2* allele has a recessive phenotype. To analyze this allele further, you begin genetic analysis. (Note: you may assume that appropriate phenotypic markers are used to distinguish cross progeny from self progeny.) First, you cross a homozygous *plp-1(xx2)* hermaphrodite to a phenotypically wild-type male from part d; the F1 cross progeny are 1/2 Plumpy and 1/2 nonPlumpy. You take the nonPlumpy hermaphrodites from the cross progeny and cross them to *plp-1(xx2)* males. You observe that in the F2 cross progeny, 3/4 are Plumpy and 1/4 are nonPlumpy.

Provide a genetic explanation for the observed cross results. Your answer should include the genotypes of the P, F1, and F2 cross progeny and explain the ratios in which they are present. (5 points)

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2. You find a spontaneous mutant in your mouse colony that has a very short tail (but not too short for a tail DNA prep). You save the mouse, breed it, and observe that the mutation appears to be fully penetrant and to have an autosomal dominant inheritance pattern; it is also homozygous viable.

2a. You decide to perform SSLP mapping to localize the mutation, which you have named *Short tail* (*St*). Because the mutation arose on a C57BL/6 genetic background (B6 for short), you begin by crossing your homozygous mutant short tail strain to wild type 129 mice. You then backcross the F1 animals to the 129 strain to create a backcross population (BC1). You score 100 BC1 progeny for a panel of sixty SSLP markers that span the mouse chromosomes. Note that each marker is polymorphic between the B6 and 129 strains.

i. Is phase known in these crosses? (2 points)

ii. What is the total number of informative chromosomes present in the BC1 generation? (2 points)

2b. The results from 4 SSLP markers typed in the BC1 animals are shown below. "129/129" indicates that the animal is homozygous for the 129 allele at that marker; "B6/129" indicates that the animal is heterozygous for the B6 allele and the 129 allele at that marker.

	Number of BC animals			
Marker	B6 / 129 short tail	129 / 129 short tail	B6 / 129 normal tail	129 / 129 normal tail
D1Mit10	26	25	26	23
D2Mit20	39	11	8	42
D3Mit30	22	27	29	22
D4Mit40	34	14	16	36

i. Which marker is most closely linked to the short tail gene? (2 points)

ii. Provide a LOD score calculation for the most closely linked marker for a distance of θ = 0.1 to support your answer, and indicate whether this LOD score provides significant evidence for linkage. (6 points)
(Some space is left at the top of the next page if you need it.)

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2c. You have successfully localized the *Short tail* mutation to a region of a chromosome. You now wish to type the backcross progeny for several SSLP markers that lie within this small region. Because you are getting ready to leave for the holidays, you want to do as few genotyping reactions as possible.

What is the minimum number of animals you will need to genotype in order to be able (theoretically) to obtain a significant LOD for linkage between the *Short tail* mutation and one of your markers at a distance of θ = 0.01? Please show your calculations. (5 points)

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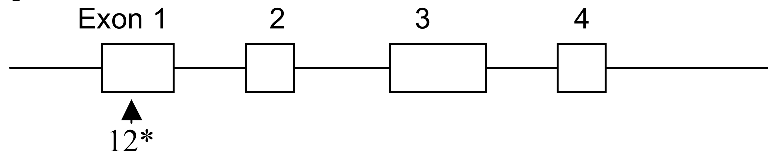
2d. After much work, you clone and sequence the *Short tail* gene. You find that the gene encodes a Ras-like GTPase. Your original Short tail strain contained a loss-of-function mutation in the *Short tail* gene.

You are interested in creating a mouse model that expresses a particular gain-of-function mutation in the Short-tail gene. To begin, you use PCR-directed mutagenesis to make a point mutation in the gene; this mutation causes an amino acid substitution in codon 12. Based on homology to Ras, you expect that this substitution will cause the protein to be constitutively active. You observe that the codon 12 mutant protein shows increased GTPase activity when tested in vitro.

Short tail gene:



To make your mouse model, you want the codon 12* Short tail allele to be expressed under control of its own endogenous promoter, with as few extra DNA sequences as possible present in the mouse.

How can you utilize the Cre-LoxP system to create your mouse model? List the steps for your experiment, including any necessary selections. Please draw your targeting vector and any recombination events. You may assume wild-type ES cells and any necessary sequences and plasmids are available. (6 points)

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3. You decide to perform a screen to identify mutations on the *Drosophila* X chromosome that affect eye color.

You mutagenize wild type males and cross them to attached $\widehat{XX}$/Y females.
(Recall that in Drosophila, attached $\widehat{XX}$ chromosomes always co-segregate. Also recall that gender is determined by the X to autosome ratio, not by the presence of a Y chromosome). The cross is shown below.

P: Virgin females $\widehat{XX}$/Y x X/Y males, fed EMS

F1 viable progeny X*/Y males $\widehat{XX}$/Y females

(There are also inviable progeny: $\widehat{XX}$X and YY.)

The male flies from this cross carry a mutagenized X chromosome.

3a. From your screen, you obtain a male fly with white eyes. You cross this male to wild-type females to generate progeny and to characterize the mode of inheritance of the mutation. You find it behaves like an X-linked dominant trait, so you name this mutation *Wh* (for *White eye*).

Describe the observed classes of progeny from your test cross and their relative frequency (gender and eye color phenotype). (Note: You may assume the white eye phenotype is due to a single, fully penetrant mutation. Please define your nomenclature clearly.) **(6 points)**

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3b. You now need to create a homozygous stock of your new *Wh* mutation. Hint: You will need to use a balancer chromosome to accomplish this efficiently.

You have available the following three strains:

Strain genotype	**Description**
+/+	Wild-type
FM7 / Y	Male flies carrying the X chromosome balancer, FM7. This balancer carries the Bar mutation, which confers a dominant bar-shaped eye phenotype; this phenotype does not affect eye color.
X / FM7	Female flies carrying the X chromosome balancer, FM7, and the Bar marker described above. Note that homozygous FM7 females are sterile.

Starting with a single white eyed mutant male, show the crosses you would use to obtain a homozygous stock of the *Wh* mutant. (8 points)

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3c. To finish the initial genetic characterization of your mutant, you want to map the *Wh* mutation relative to two other known recessive mutations on the X chromosome, *forked bristles* (*f*) and *cut wings* (*ct*).

You cross *Wh* male flies to homozygous *f ct* females. You select F1 females and cross them to *Wh f ct* males. You then analyze the phenotypes present in 2000 F2 progeny.

Phenotype	**Number of F2 flies**
MALES	
White eye	448
Cut wing, forked bristle	442
White eye, cut wing	3
Forked bristle	2
White eye, cut wing, forked bristle	17
Wild-type	18
White eye, forked bristle	36
Cut wing	34
FEMALES	
White eye	466
White eye, cut wing, forked bristle	459
White eye, cut wing	37
White eye, forked bristle	38

Given the data above, calculate the genetic distance between the *White eye, cut wing* and *forked bristle* genes. Draw a map showing the three mutations, their order on the X chromosome, and the distances between them. (5 points)

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3d. You have now completed mapping and cloning of the *White eye* mutation, and have begun functional analysis. Your preliminary experiments indicate that the *white eye* gene encodes a tyrosine kinase; the *White eye* mutation results in a dominant negative version of this kinase. Noting that *White eye* flies lack the R7 photoreceptor cell in the eye, you hypothesize that expression of the mutant kinase in the R7 cell prevents that cell's development.

You now must design a mosaic analysis experiment to test whether expression of the mutant *White eye* protein in R7 cells prevents their development. You have available FRT sequences, a GFP marker, the *White eye* mutation, and the wild-type *white eye* allele (+).

i. Draw the chromosomes you will use for your experiment, including the centromeres and all necessary markers. Please draw them as they will appear before and after FLP-ase induced recombination. (5 points)

ii. State the predicted results from your experiment if your hypothesis is true (i.e., if expression of the mutant White eye protein in R7 cells prevents their development). Include the predicted GFP phenotypes of *white eye* +/+/ , -/-, and +/- cells. (4 points)

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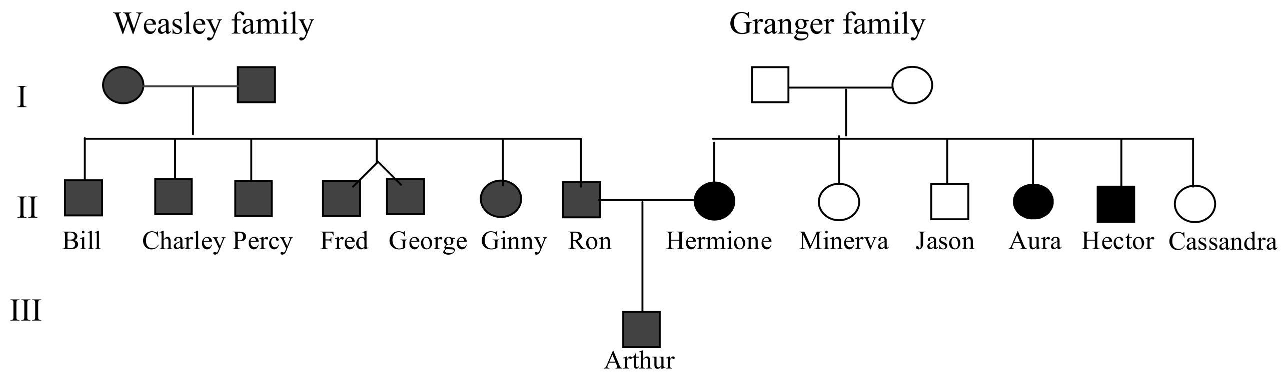
4. You and your entire human genetics laboratory have been mysteriously transported to the England of the Harry Potter books. In this country, wizardry (the ability to perform magic) is an inherited trait. You are eager to understand the mode of inheritance of this trait, and to try to determine its genetic location.

To your astonishment, you find that Ron Weasley and Hermione Granger (two individuals affected with the wizard trait) have married and given birth to a son, who is also affected. You are also surprised to learn that Hermione is one of a large family. With the cooperation of the Weasley and Granger families, you generate the following pedigree: (Note: shaded symbols indicate individuals who are affected by the wizardry trait; unshaded symbols are unaffecteds).



4a. List three modes of inheritance for the wizardry trait that are NOT consistent with the above pedigree data, and justify your logic in a sentence. You may assume the trait is fully penetrant. (2 points each)

1.

2.

3.

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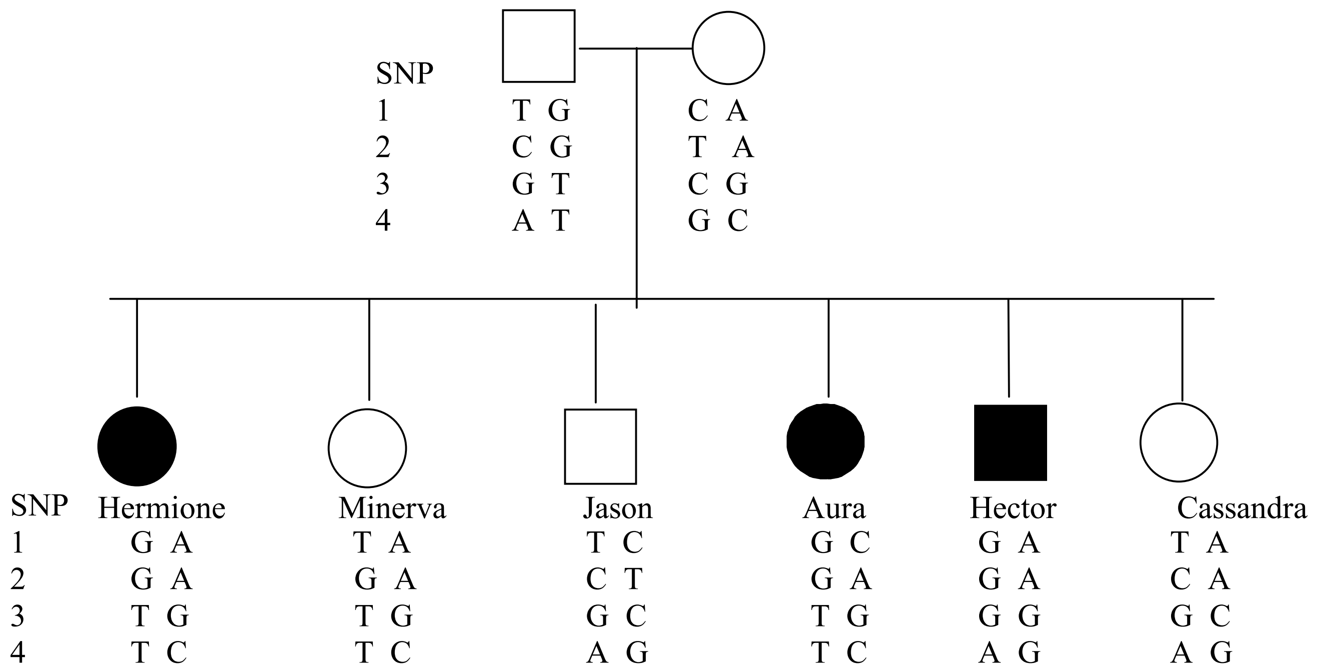
Next, you collect DNA samples from several other magically inclined families and use them for genetic mapping of the wizardry trait. You successfully localize the trait to a region on chromosome 13.

You now decide to perform fine mapping of the trait using haplotype analysis of the Granger family DNA. You take samples of each family member's DNA and type it for four SNP markers in the region, each of which has four alleles (SNPs 1-4, which are linked in numerical order). The results for each individual are shown on the following page.



4b.i. Which chromosome in Mr. Granger carries the wizardry allele? Indicate by writing the haplotype. (2 points)

4b.ii. Which chromosome in Mrs. Granger carries the wizardry allele? Again, indicate by writing the haplotype. (2 points)

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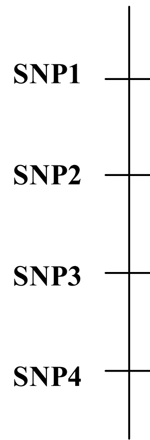
4c. Based on the haplotype data, you determine the location of the wizardry trait relative with SNPs 1-4.

i. Name the two individuals in the pedigree whose haplotypes contain the breakpoints closest to the wizardry trait. (4 points)

ii. Indicate the region in which the wizardry trait maps on the chromosome below. (3 points)



4d. Hermione's sister Cassandra intends to marry a man with wizardry abilities. She wishes to know whether she is a carrier for the wizardry allele, and thus, whether any of their children may express the wizardry trait.

Is Cassandra a carrier for the wizardry trait allele? Briefly justify your answer using haplotype data. (3 points)