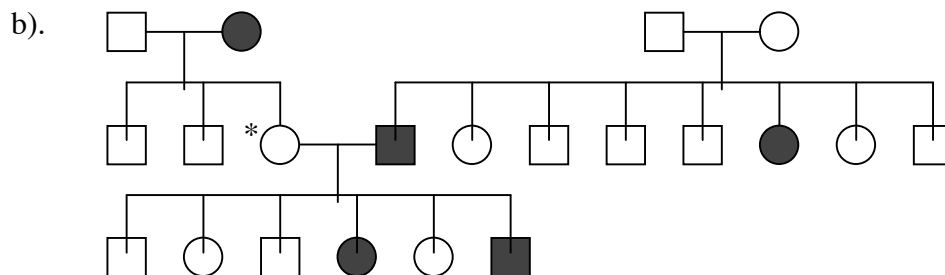
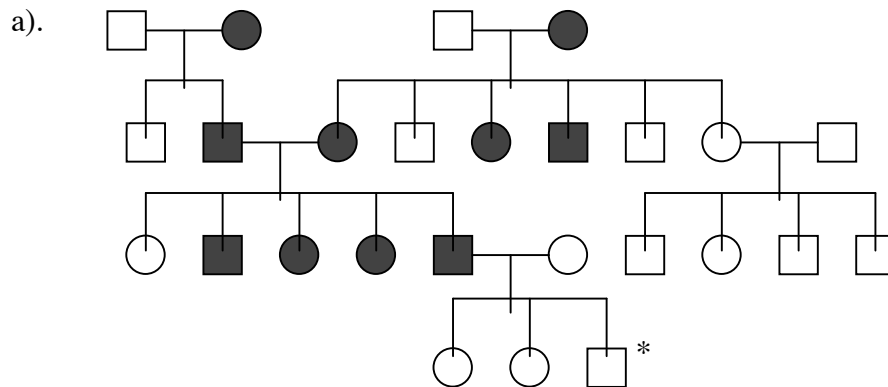


Genetics 201 Extra Mammalian Problems

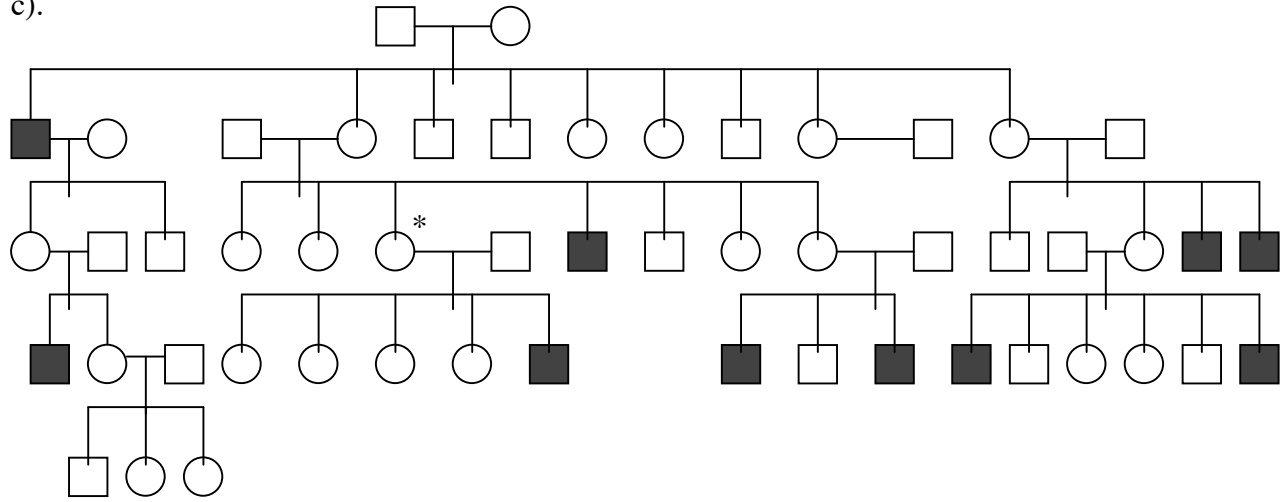
1). You are a human geneticist interested in understanding the genetic basis of inherited diseases. After a great deal of networking with your clinician friends, you were able to collect pedigrees for three different rare disorders. It is now your task to deduce the inheritance pattern for each of these disorders. In each of the following pedigrees, males are represented by squares, females are represented by circles, and affected individuals are represented by solid symbols. You may assume that each of these rare traits is completely penetrant.

For each of the following pedigrees, please provide:

- i) the most likely mode of inheritance, along with an explanation of your reasoning.
- ii) the probable genotype of the individual marked with an asterisk (*), along with a brief explanation of any symbols that you use.



c).



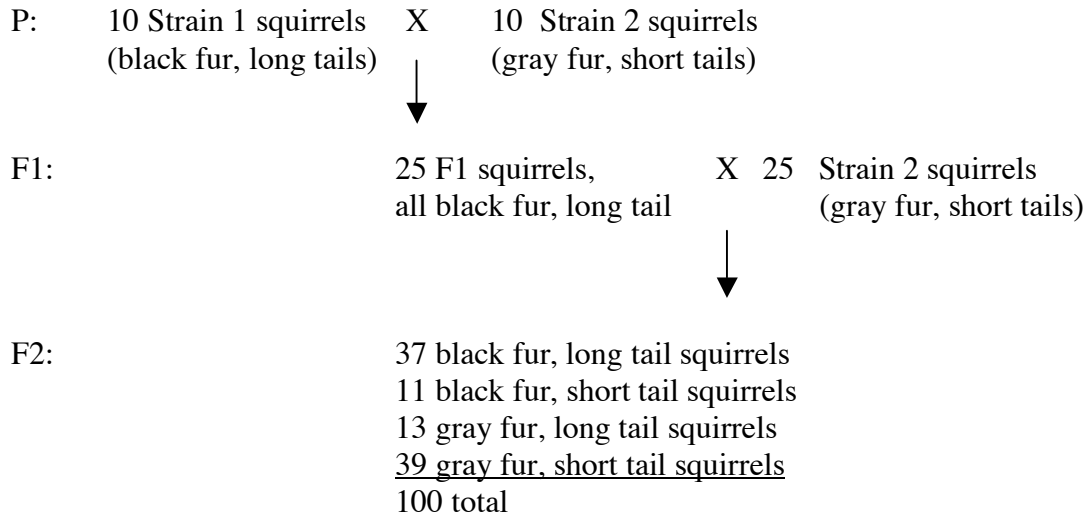
2). You are studying the squirrel population on an isolated college campus in western Massachusetts. You have created two strains of inbred squirrels:

Strain 1: squirrels have black fur and long tails

Strain 2: squirrels have gray fur and short tails

Both the fur color and tail length traits are controlled by single genes. The traits show an autosomal dominant inheritance pattern and are fully penetrant.

You want to create a preliminary genetic map of the squirrel. You begin by mapping the fur color locus relative to the tail length locus to determine if the two genes are linked.



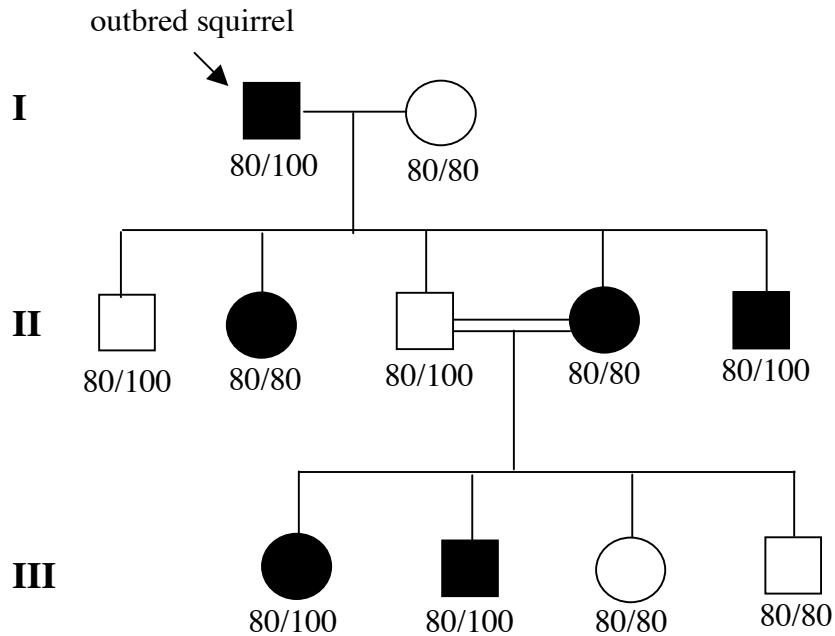
a). Is phase known in the above set of crosses? What is the number of informative chromosomes from the above set of crosses?

b). Calculate the LOD score for linkage between the fur color gene and the tail length gene at a distance of 20 cM.

c). Is this LOD score significant evidence for linkage between the two genes? Justify your answer in a sentence.

d). An outbred male squirrel with black fur manages to slip into your squirrel colony and mate with an inbred gray fur female. You type the two parents and their progeny (which have also reproduced) with a new SSLP marker that you have been testing. Note that the SSLP marker amplifies alleles of two sizes, 80 base pairs and 100 base pairs.

The following pedigree shows the two squirrels and their progeny. For each squirrel, coat color phenotype is indicated by open circles (gray fur) or shaded circles (black fur). The SSLP alleles detected in DNA from each squirrel are indicated immediately below it in the pedigree.

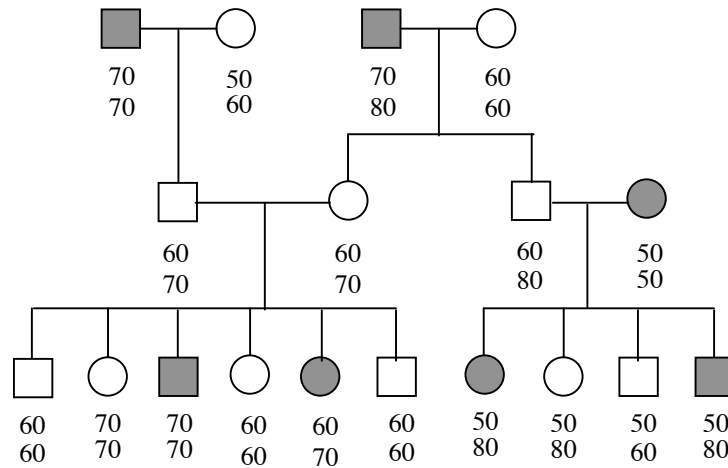


You wish to test whether the SSLP marker shows linkage to the fur color gene, in the hopes of adding it to your genetic map of the squirrel. The following questions all refer to the fur color locus and the SSLP locus.

i). What is the total number of informative chromosomes in generation II?
 What is the total number of informative chromosomes in generation III?

ii). Given all the chromosomes in the pedigree, calculate the LOD score for linkage between the fur color locus and the SSLP marker at a distance of 10 cM.

3). The pedigree below shows a family with a highly penetrant, autosomal, recessive disease that causes people to instantly fall asleep when they attempt to do schoolwork. The individuals affected by the disease are indicated by shaded circles or squares. You hypothesize that the disease locus is linked to an SSLP marker that your lab has identified. You therefore genotype the family members at this particular SSLP locus. The results are shown below each individual.

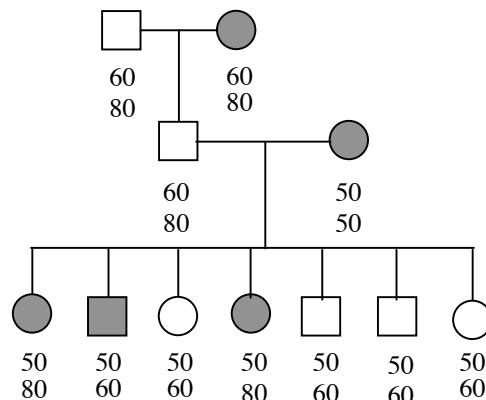


a). If you were to use the meioses captured in the third generation to calculate linkage, is the phase known? Why or why not?

b). i. How many informative chromosomes are there in the third generation? (*Remember that each individual may have 0, 1, or 2 informative chromosomes. For a chromosome to be informative, one must be able to tell if a recombination event has or has not occurred in the parents to produce that chromosome*).

ii. Use these informative chromosomes to calculate the LOD scores for linkage between the disease gene and the SSLP marker at recombination fractions of $\theta = 0.2, 0.25$ and 0.3 . Of these three θ values, which one is the best estimate of the distance between the disease gene and the SSLP marker? Are any of the LOD scores significant? Why or why not?

You hear that another lab has been studying the same peculiar sleeping trait and has also been looking at the possible linkage of the disease gene to the SSLP marker your lab was using. However, this lab used a different family to perform the linkage analysis. The pedigree they used is shown below. Once again the affected individuals are indicated as shaded circles or squares and the SSLP alleles are listed below each individual.



c). Is the phase known for this second pedigree? Why or why not?

d). How many informative chromosomes are there in the third generation of this second pedigree? Use these informative chromosomes to calculate LOD score for linkage of the disease gene and the SSLP marker at recombination fraction of $\theta = 0.2, 0.25$ and 0.3 . Of these three θ values, which one is the best estimate of the distance between the disease gene and the SSLP marker? Are any of the LOD scores significant? Why or why not?

Your competitor lab publishes the results of its linkage analysis (verifying what you calculated in part d) and you wish to combine his data with yours to see if you can get more evidence for linkage between the disease allele and the SSLP.

e). What are the combined LOD scores of the two pedigrees from parts a and c at recombination fractions of $0.2, 0.25$ and 0.3 ? Are any of these significant? Now what is the best estimate of distance between the two loci? What would the minimum number of **additional** informative chromosomes you would need from a phase-known pedigree to result in a significant LOD score (>3) for a recombination fraction of 0.3 ?

4). You are working in a lab that is interested in the genes underlying anemia (red blood cell deficiency) using mice as model organisms for human disease. Your lab has acquired a strain of mice that harbors a spontaneous mutation that, when expressed in the homozygous condition, leads to severe anemia. You name this mutation *lob* (for “loss of blood”) and decide to embark on the task of identifying and analyzing the gene responsible for the mutation in *lob* mice. Mice homozygous for the *lob* mutation can be identified by obtaining a small blood sample and analyzing hemoglobin and hematocrit counts. *Lob* mice have significantly decreased hemoglobin levels and hematocrits, both of which are hallmarks of anemia, but are viable and fertile.

a). Using your choice of the strains listed below, briefly explain how you could genetically map the *lob* mutation. You also have available a full panel of SSLP markers spanning the entire mouse genome.

Strain	Description
C57BL/6- <i>lob/lob</i> mice	<i>Lob</i> mouse strain homozygous for the <i>lob</i> mutation. Present in a C57BL/6 inbred strain background.
C57BL/6 mice	Common inbred mouse strain wild type at the <i>lob</i> locus.
CAST/Ei mice	Wild derived inbred mouse strain commonly used in mapping experiments because of the large number of genetic differences between them and common inbred strains.

b). Your mapping experiments reveal that the *lob* phenotype is caused by a mutation in a single gene, *Sec1*, which encodes a protein involved in trafficking of membrane proteins. Excited by the fact that Sec1 may be involved in iron transport in red blood cells, you decide to generate a mouse containing a knock-out allele of the *Sec1* locus to study the effect of loss of Sec1 on red blood cell development.

Describe the experimental strategy you would use to generate a knock-out allele of *Sec1*. The diagram below shows the mouse *Sec1* locus. Boxes with numbers indicate exons, with the ATG start codon shown in exon 2. Include in your answer a drawing of your targeting vector and the sites where you expect homologous recombination to occur. Also, give a brief description of the steps you would take to go from targeting vector to heterozygous and homozygous lines of your knock-out mouse.



(Note: Exons 4, 5, and 6 are highly conserved regions of the gene and are thought to encode functional domains of the protein. You may assume that you have access to the complete sequence of the *Sec1* gene).

c). Your gene targeting strategy is successful, and after a few months of work, you obtain animals that are heterozygous for the knock-out allele. However, you are disappointed to find that after crossing two heterozygous animals, you recover no live-born pups that are homozygous for the knock-out allele ($sec1^{ko}/sec1^{ko}$). You genotype several litters at various stages of embryogenesis and are able to recover embryos with the genotype $sec1^{ko}/sec1^{ko}$ only up until day 14 of gestation (the mouse gestational period is about three weeks).

Based on this information, what conclusion can you draw about the knock-out model you have generated?

d). You decide to make another attempt at knocking out *Sec1*, but this time you decide to generate a conditional allele using the Cre-loxP system. Briefly describe the experimental strategy you would use to create a conditional knock-out allele at the *Sec1* locus. Please draw the targeting vector and indicate the locations where you expect homologous recombination to occur. Also, briefly list the steps you would perform with your targeting vector to create a mouse expressing the loxP allele.

e). You are concerned that widespread loss of the Sec1 protein will cause systemic defects and/or embryonic lethality in your mice. Using the loxP mouse you generated above, how might you be able to generate a mouse that lacks the Sec1 protein only in red blood cells?