Study Questions for Topic 7: Linkage Analysis in Mice and Men

Reading Assignment: Chapter 5, section 5.4; Handout 2, pages 213 through 222

Principles and Key Concepts:

1. Principles of linkage covered earlier apply to mammalian genetics.
2. Special statistics are required to assess recombination frequencies estimated from human pedigrees.
3. LOD scores are used to assess the statistical significance of linkage estimates made using information from human pedigrees.
4. Groups of linked alleles on a single chromosome are referred to as a haplotype. For example, ***A b C*** and ***a B c*** are two different haplotypes at the same genetic locus.
5. Within a population, some allele combinations in a haplotype are more frequent than others. For example, in certain populations, ***b*** may always be associated with ***A*** and ***C*** and never with ***a*** and ***C*** or ***A*** and ***c***. This is referred to as linkage disequilibrium (LD).
6. In human populations, clusters of alleles at many chromosomal locations are in linkage disequilibrium. These clusters are called LD groups.
7. LD groups are haplotypes that are useful in mapping disease genes to precise chromosomal locations.

Study guide for the Matching section of test 7:

The assigned reading, my Key Term List, all the study questions.

Study guide for the short answer/problem solving section of test 7:

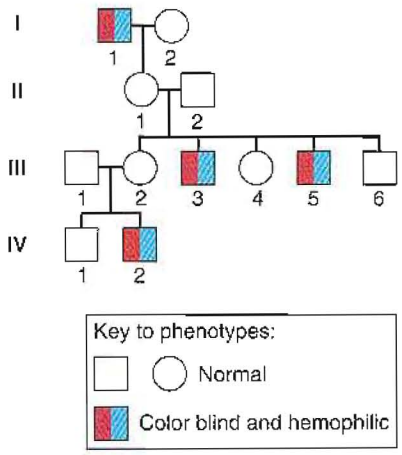
Genetic Mapping using information from pedigrees:

- DNA markers: 5.11
- Estimating map distance: 5.8; 5.10; NOTE: Caculate an LOD score for the genetic distance you estimate for 5.8 and 5.10
- Calculating probabilities based on recombination frequencies 5.9

Additional Questions from other sources:

- Question 7 on hemophilia and color blindness (next page of this study guide, "p169")
- Question 7.41 and 7.42 (third page of this study guide, p175)
- Questions 1 through 4 (last two pages of this study guide)

7. The following pedigree shows four generations of a family described in 1928 by M. Madlener. The great-grandfather, I-1, has both color blindness and hemophilia. Letting *c* represent the allele for color blindness and *h* represent the allele for hemophilia, what are the genotypes of the man's five grandchildren? Do any of the individuals in the pedigree provide evidence of recombination between the genes for color blindness and hemophilia?



Answer: The genes for color blindness and hemophilia are X-linked. Because I-1 has both color blindness and hemophilia, his genotype must be *c h*. His daughter, II-1, is phenotypically normal and must therefore carry the nonmutant alleles, *C* and *H*, of these two X-linked genes. Moreover, because II-1 inherited both *c* and *h* from her father, the two nonmutant alleles that she carries must be present on the X chromosome she inherited from her mother. II-1's genotype is therefore *C H/c h*—that is, she is a coupling heterozygote for the two loci. III-2, the first granddaughter of I-1, is also a coupling heterozygote. We infer that she has this genotype because her son has both color blindness and hemophilia (*c h*), and her father is phenotypically normal (*C H*). Evidently, III-2 inherited the *c h* chromosome from her mother. Among the grandsons of I-1, two (III-3 and III-5) of them have both hemophilia and color blindness; thus, these grandsons are genotypically *c h*. The other grandson (III-6) is neither color blind nor hemophilic; his genotype is therefore *C H*. The genotype of the remaining granddaughter (III-4) is uncertain. This woman inherited a *C H* chromosome from her father. However, the chromosome she inherited from her mother could be *C H*, *c h*, *C h*, or *c H*. The pedigree does not allow us to determine which of these chromosomes she received. The most we can say about III-4's genotype is that she carries a chromosome with the *C* and *H* alleles.

None of the four grandchildren to whom we can assign genotypes provides evidence of recombination between the genes for color blindness and hemophilia. Neither do the two great-grandchildren shown in generation IV. One of these great-grandchildren is genotypically *C H*; the other is genotypically *c h*. Thus, in the pedigree as a whole there is no evidence for recombination between the *C* and *H* genes.

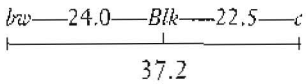
Testing Your Knowledge

INTEGRATE DIFFERENT CONCEPTS AND TECHNIQUES

1. R. K. Sakai, K. Akhtar, and C. J. Dubash (1985, *J. Hered.* 76:140–141) reported data from a set of testcrosses with the mosquito *Anopheles culicifacies*, a vector for malaria in southern Asia. The data involved three mutations: *bw* (*brown* eyes), *c* (*colorless* eyes), and *Blk* (*black* body). In each cross, repulsion heterozygotes were mated to mosquitoes homozygous for the recessive alleles of the genes, and the progeny were scored as having either a parental or a recombinant genotype. Are any of the three genes studied in these crosses linked? If so, construct a map of the linkage relationships.

Cross	Repulsion Heterozygote	Progeny: Parental	Progeny: Recombinant	Percent Recombination
1	*bw* +/+ *c*	850	503	37.2
2	*bw* +/+ *Blk*	750	237	24.0
3	*c* +/+ *Blk*	629	183	22.5

Answer: In each cross, the frequency of recombination is less than 50 percent, so all three loci are linked. To place them on a linkage map, we estimate the distances between each pair of genes from the observed recombination frequencies:



Notice that the recombination frequency between *bw* and *c* (37.2 percent, from Cross 1) is substantially less than the actual distance between these genes (46.5). This shows that for widely separated genes, the recombination frequency underestimates the true map distance.

2. Singed bristles (*sn*), crossveinless wings (*cv*), and vermilion eye color (*v*) are due to recessive mutant alleles of three X-linked genes in *Drosophila melanogaster*. When a female heterozygous for each of the three genes was testcrossed with a singed, crossveinless, vermilion male, the following progeny were obtained:

Class	Phenotype	Number
1	singed, crossveinless, vermilion	3
2	crossveinless, vermilion	392
3	vermilion	34
4	crossveinless	61
5	singed, crossveinless	32
6	singed, vermilion	65
7	singed	410
8	wild-type	3
		Total: 1000

7.39 The order of three genes and the centromere on one chromosome of *Neurospora* is centromere —*x*—*y*—*z*. A cross between + + + and *x y z* produced an ascus with the following ordered array of ascospores (only one member of each spore pair is shown): (+ + *z*) (+ *y z*) (*x* + +) (*x y* +).

(a) Is this ascus most likely the result of a meiotic event in which 0, 1, 2, or 3 crossovers occurred?
(b) In what interval(s) did the crossover(s) most likely occur?
(c) If double or triple crossovers were involved, were they two-strand, three-strand, or four-strand multiple crossovers?

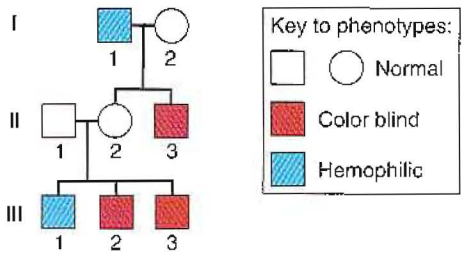
7.40 The following tetrads were produced by a cross of a *Neurospora* strain that had white spores (*w*) and a nutritional requirement for the amino acid arginine (*arg*) with a strain that had dark spores and no arginine requirement:

Spore Pairs	Tetrads 1	2	3	4	5	6
1–2	*w arg*	*w arg*	*w arg*	*w arg*	*w* +	*w* +
3–4	*w arg*	*w* +	+ *arg*	+ +	*w* +	+ +
5–6	+ +	+ *arg*	+ +	+ *arg*	+ *arg*	*w arg*
7–8	+ +	+ +	*w* +	*w* +	+ *arg*	+ *arg*
No.	58	14	15	2	1	10

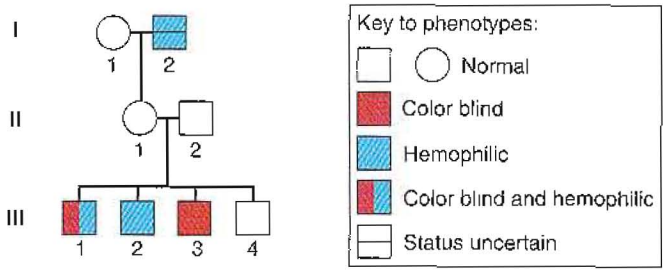
Total = 100

(a) What is the map distance between the *arg* locus and the centromere? (b) What is the map distance between the *w* and *arg* loci? (c) Are the *arg* and *w* loci found on the same or different arms of the chromosome?

7.41 The following pedigree, described in 1937 by C. L. Birch, shows the inheritance of X-linked color blindness and hemophilia in a family. What is the genotype of II-2? Do any of her children provide evidence for recombination between the genes for color blindness and hemophilia?



7.42 The following pedigree, described in 1938 by B. Rath, shows the inheritance of X-linked color blindness and hemophilia in a family. What are the possible genotypes of II-1? For each possible genotype, evaluate the children of II-1 for evidence of recombination between the color blindness and hemophilia genes.



7.43 A normal woman with a color-blind father married a normal man, and their first child, a boy, had hemophilia. Both color blindness and hemophilia are due to X-linked recessive mutations, and the relevant genes are separated by 10 cM. This couple plans to have a second child. What is the probability that it will have hemophilia? color blindness? both hemophilia and color blindness? neither hemophilia nor color blindness?

7.44 Two strains of maize, M1 and M2, are homozygous for four recessive mutations, *a*, *b*, *c*, and *d*, on one of the large chromosomes in the genome. Strain W1 is homozygous for the dominant alleles of these mutations. Hybrids produced by crossing M1 and W1 yield many different classes of recombinants, whereas hybrids produced by crossing M2 and W1 do not yield any recombinants at all. What is the difference between M1 and M2?

7.45 A *Drosophila* geneticist has identified a strain of flies with a large inversion in the left arm of chromosome 3. This inversion includes two mutations, *e* (*ebony* body) and *cd* (*cardinal* eyes), and is flanked by two other mutations, *sr* (*stripe* thorax) on the right and *ro* (*rough* eyes) on the left. The geneticist wishes to replace the *e* and *cd* mutations inside the inversion with their wild-type alleles; he plans to accomplish this by recombining the multiply mutant, inverted chromosome with a wild-type, inversion-free chromosome. What event is the geneticist counting on to achieve his objective? Explain.

Genomics on the Web

at http://www.ncbi.nlm.nih.gov/

Chromosome maps were first developed by T. H. Morgan and his students, who used *Drosophila* as an experimental organism.

1. Find the genetic map positions of the genes *w* (white eyes), *m* (miniature wings), and *f* (forked bristles) on the X chromosome (also denoted as chromosome 1) of *Drosophila melanogaster*.

2. Find the positions of these three genes on the cytogenetic map of the X chromosome of *D. melanogaster*.

Hint: At the web site, click on Genomic Biology and then under Genome resources, click on Insects. From there, open the page on *Drosophila melanogaster*, and then under Related Resources in the

In the example of AMD, a complex disease, a genome-wide association study led to the identification of strongly associated, common SNPs that in turn were in LD with a common coding SNP in the complement factor H gene that appears to be the functional variant involved in the disease. This discovery, in turn, led to the identification of other SNPs in the complement cascade that can also predispose to or protect against the disease. Taken together, these results give important clues to the pathogenesis of AMD and suggest that the complement pathway might be a fruitful target for novel therapies. We expect that many more genetic variants responsible for complex diseases will be successfully identified by genome-wide association with HapMap markers, thereby providing us with powerful insights and potential therapeutic targets for many of the common diseases that cause so much morbidity and mortality in the population.

GENERAL REFERENCES

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USEFUL WEBSITES

GDB Human Genome Database. *http://www.gdb.org/hugo/*
International HapMap Project. *http://www.hapmap.org/*

PROBLEMS

1. The Huntington disease (HD) locus was found to be tightly linked to a DNA polymorphism on chromosome 4. In the same study, however, linkage was ruled out between HD and the locus for the MNSs blood group polymorphism, which also maps to chromosome 4. What is the explanation?

2. Linkage between a polymorphism in the α-globin locus on the short arm of chromosome 16 and an autosomal dominant disease was analyzed in a series of British and Dutch families, with the following data:

θ	0.00	0.01	0.10	0.20	0.30	0.40
LOD scores *(Z)*	$-\infty$	23.4	24.6	19.5	12.85	5.5

$Z_{max} = 25.85$ at $\theta_{max} = 0.05$

How would you interpret these data? What does the value of $Z = -\infty$ at $\theta = 0$ mean?

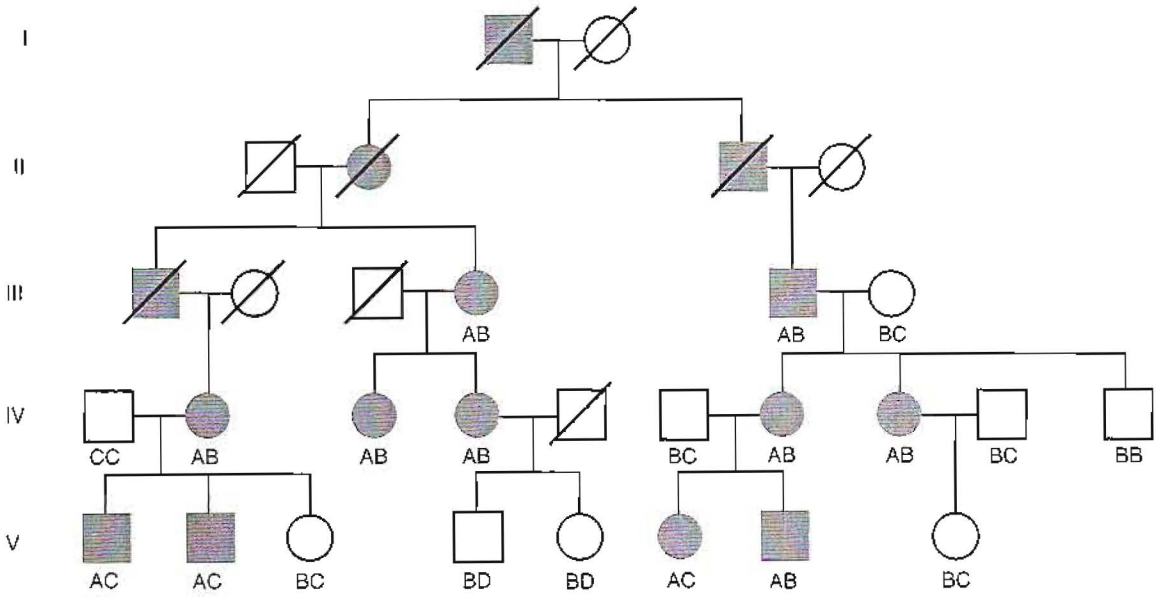
In a subsequent study, a large family from Sicily with what looks like the same disease was also investigated for linkage to α-globin, with the following results:

θ	0.00	0.10	0.20	0.30	0.40
LOD scores *(Z)*	$-\infty$	−8.34	−3.34	−1.05	−0.02

How would you interpret the data in this second study? What implications do these data have for use of linkage information in presymptomatic diagnosis and genetic counseling?

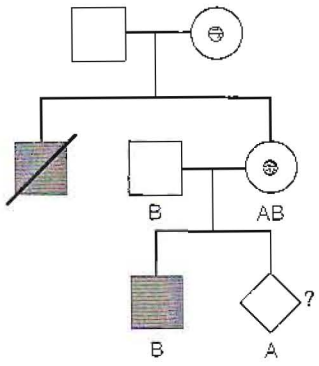
3. This pedigree was obtained in a study designed to determine whether a mutation in a gene for γ-crystallin, one of the major proteins of the eye lens, may be responsible for an autosomal dominant form of cataract. The filled-in symbols in the pedigree indicate family members with cataracts. The letters indicate three alleles at the polymorphic γ-crystallin locus on chromosome 2. If you examine each affected person who has passed on the cataract to his or her children, how many of these represent a meiosis that is informative for linkage between the cataract and γ-crystallin? In which individuals is the phase known between the cataract mutation and the γ-crystallin alleles? Are there any meioses in which a crossover must have occurred to explain the data? What would you conclude about linkage between the cataract and γ-crystallin from this study? What additional studies might be performed to confirm or reject the hypothesis?

PROBLEMS–cont'd



Pedigree for question 3

4. The following pedigree shows an example of molecular diagnosis in Wiskott-Aldrich syndrome, an X-linked immunodeficiency, by use of a linked DNA polymorphism with a map distance of approximately 5 cM between the polymorphic locus and the Wiskott-Aldrich syndrome gene.
   a. What is the likely phase in the carrier mother? How did you determine this? What diagnosis would you make regarding the current prenatal diagnosis if it were a male fetus?
   b. The maternal grandfather now becomes available for DNA testing and shows allele *B* at the linked locus. How does this finding affect your determination of phase in the mother? What diagnosis would you make now in regard to the current prenatal diagnosis?



Pedigree for question 4