Genetics Lab Biology 322 Fall 2013

***CAENORHABDITIS ELEGANS* AND MENDEL'S SECOND LAW REVISITED:**

- Independent assortment versus linkage of gene pairs during gamete formation
- Allele and genotype symbolism: application of symbol conventions in the interpretation of genetic data
- the χ 2 (chi square) statistical test
- Review of basic probability
- The funky world of gene names
- The relationship between genotype and phenotype

Reading assignment: In your genetics text (or an online text), review basic Mendel, basic probability, basic linkage, probability and χ^2 (chi square) analysis.

Introduction to Mendel revisited : When a new mutant strain is identified, the initial genetic analysis of such a strain typically includes experiments designed to determine the chromosomal location of the mutation (that is, the gene defined by the mutation). Such an analysis involves looking at the genetic behavior of the mutation relative to standard genetic markers on each chromosome. If the new mutation and the particular genetic marker show independent assortment, then the genes are unlinked and the mutation is probably on a different chromosome from the marker. If the mutation and the marker do not show independent assortment, then the two are linked and the mutation is located on the same chromosome as the genetic marker. Additional experiments would map the mutation more precisely relative to other genes on the same chromosome.

The nematode *C. elegans* is a diploid animal with n=6 (5 autosomes and an X chromosome). Although sex is determined genetically, there is no equivalent to the Y chromosome in this species. In this exercise we will use the mutant ***dpy-11(e224)*** **V** as a genetic marker for chromosome V.

In this exercise you will determine whether a mutation called e189 (which defines the unc-32 gene) is located on Chromosome V or is located on a different chromosome.

C.elegans* Nomenclature:** In *C. elegans* nomenclature, gene names often reflect the phenotype of mutations in the gene. For example, a gene defined by a mutation that results in a paralyzed or *unc*oordinated phenotype would be designated as an *unc* gene. The phenotype would be referred to as an Unc phenotype. Likewise, if mutations in a gene result in a short, fat or "*d*um*py*" phenotype, the gene is cally *dpy* and the phenotype is a Dpy phenotype. The formal genetic nomenclature for the Dpy strain that we will be working with is ***dpy-11(e224) V.

- *The* ***number 11*** identifies the specific dpy gene. There are many genes in *C.elegans* that produce Dpy phenotypes when mutated.
- *The* ***number e224*** identifies the specific mutant allele of the *dpy-11* gene
- *The* ***roman numeral V*** indicates that this gene is located on Chromosome V

Genotypes of the three strains used in this experiment

Be sure to copy this genotype info into your lab notebook

By convention only genes with mutant alleles are listed in the formal genotype. For example, the *him-5* strain has a mutation in the *him-5* gene but carries wildtype alleles of the *unc-32* and *dpy-11* genes. So only the *him-5* gene is listed in the genotype for this strain and wildtype alleles are implied for the other two genes. [NOTE though that when you track genotypes for each generation in this experiment, you will need to indicate both the mutant and wildtype alleles -- more later on this].

Alleles Symbols + = wild-type allele
e224 = recessive mutant allele of the *dpy-11* gene
e189 = recessive mutant allele of the *unc-32* gene
e1490 = recessive mutant allele of the *him-5* gene

Genotypes of Strains used in this experiment using standard conventions

NOTE: ♀ = hermaphrodite
Wildtype C. elegans -- carries the wildtype allele of the *him-5*, *unc-32* and *dpy-11* genes ♀
him-5(e1490) V ♀ and ♂
unc-32 (e189) • dpy-11(e224) V ♀

- implies no information about the linkage of the two genes

him strain An unmated wild-type hermaphrodite produces mostly hermaphrodite and a small (<1%) fraction of male self-progeny. To facilitate setting up this first cross, the males will come from mutant strains that produces a ***h***igh ***i***ncidence of self-progeny ***m***ales. This *him* strain is wild-type for all other genes, including the *unc-32* and *dpy-11* genes.

Experimental Protocol

THURS SEPT 26 *Each student should set up this cross*

Cross True-breeding Parental Lines

Cross *him-5* ♂ X *unc-32 (e189) dpy-11 (e224)* ♀

- Be careful not to transfer eggs and larvae to the mating plate from the *him-5* plate when you pick up the adult males. But, why is it not a problem if you accidentally transfer eggs and larvae when you pickup UncDpy hermaphrodites?
- Carefully label the side of the bottom of the petri dish –not the lid.
- Record the details of the cross in your lab notebook including the formal genotypes of the strains
- *Put cross plate at ~17°C (check chalkboard for incubator temp)*

*Each **pair** of students should also propagate the unc-32 dpy-11 and the wildtype strains to use as reference stock during the rest of the experiment. Place your new stocks at 17°C. The worm plates you received for today's lab should be placed at 10°C*

TUES OCT 1 ***Self Cross of F1 dihybrid***

Carefully examine WT and DpyUnc reference strains and record short description/drawing in notebook

F1 self cross: Pick F1 ♀♀ and allow to self-fertilize:

- **Pick wild-type** (non-Unc; non-Dpy) **cross-progeny L4*** ♀**:** 1 F1/plate X 3 plates [* see page 6 of *Intro to Worm* for a photomicrograph of an L4]
- Do not transfer eggs and other larvae along with the L4 larvae. Why?
- Each student should set up 3 F1 plates
- Put plates at ~20-23 °C *(check chalkboard for incubator temp)*

*Each **pair** of students should also propagate the unc-32 dpy-11 and the wildtype strain. Place your stocks at ~17* °C

THURS OCT 3 ***Self Cross of F1 dihybrid continues***

F1 self cross: Remove (now adult) F1 parental animal *from each plate. This step is VERY IMPORTANT as it reduces the number of F2 progeny on the plate as well as limiting the extent of age variation. Place the plates in the 16°C incubator*

MON OCT 7 ***Meet in lab to collect phenotypically WT F2 for selfing***

- Pick wild-type (non-Unc; non-Dpy) adult F2 progeny ♀
- (Do not transfer eggs and larvae along with the adults – why not?)
- 1 WT F2 /plate X 8-12 plates (each student sets up 8-12 plates)

Place these new plates at ~24°C.

Return your experimental plates (F1 self) to 16°C.
Place your reference (unc-32 dpy-11 and wildtype) plates at 10°C to hold for Tuesday's and Thursday's labs.

TUES OCT 8 ***F2 Data collection: distribution of animals in each phenotypic category***

Score phenotype of F2 progeny of F1 self cross

Following the instructions given on pg 5, score the phenotype of at least 80* adult F2 progeny [generated by selfing the F1 ♀] . Record the number in each phenotypic class. [*We'll discuss in class why 80 is the minimum.]

Place your reference (unc-32, dpy-11, unc-32 dpy-11 and wildtype) plates at 10°C to hold for Thursday's lab.

THURS OCT 10 ***Genotypes of F2 Wildtypes***

Score F3 progeny of each F2 WT animal and deduce its genotype

Following detailed instructions on pg 6 of this handout, score the progeny of each F2 ♀ for the presence or absence of each phenotypic class. Based on these observations, deduce the genotype of each F2 hermaphrodite. Then collate your results and indicate the number of F2 hermaphrodites in each genotypic class. Also record the combined table and class data.

DETAILED INSTRUCTIONS FOR SCORING F2 PROGENY

Scoring F2 progeny of F1 self

- FIRST: examine reference plates for wildtype, doubly mutant and each single mutant strain (the latter will be provided for you) and in your lab notebook jot down short descriptions (or draw pictures) about the 4 different phenotypic categories. NOTE: it may be easiler to distinguish phenotypic differences under lower rather than higher magnification
- Be confident about your scoring BEFORE you start your actual count
- Take some time to organize your records/data collection in your notebook: raw data versus summary table vs analysis. Leave room for your chi square analysis
- To keep track of where you are on the plate, place it in a lid that has been marked with vertical lines
- Score best of the three plates. Pick plates with limited number of progeny, without contamination and with no breaks in the agar. What if F1 mated and there are males on plate? Is this a serious problem?
- *Score your best plate TWICE. If there are less than 80 progeny on this plate then you should score a second plate*
- Trade plates with your lab partner and score his/her plate and compare notes—you should record your partner's count in your notebook, but you don't have to use his/her data in your chi square analysis
- Do chi square on data at the end of lab (if time) or before the next lab: use the average of your two counts or your second count if you feel more confident about it than the first count. Enter your data in the overhead chart at front of lab (see below)
- ***Photocopy chi square analysis to hand in at the beginning of the next class***

student	Wild type	UNC	DPY	UNC DPY	n	χ 2*	p
expected ratio	**9**	**3**	**3**	**1**			
Raw data							
Observed** ratios							

*Your χ^2 calculations should be carried out to the four decimal places (including your expected values). The χ^2 value itself should be expressed to 3 decimal places -- reflecting the χ^2 values in the table.

** Calculate ratio by dividing each number by the number of uncdpy animals.

Instructions for Scoring progeny of F2 wild-types

Each student:

- Set up a table in your notebook to keep track of each F2's progeny.
- Number each F2 plate and then score carefully for the segregation of mutant phenotypes. Record mutant phenotypes observed for each plate in your notebook, but not on the plate itself.
- You do not need to count the number of animals in each class (Why not?). But, for each plate, *estimate* number of animals examined: <20; 20-50; >50 Why? Is 10 enough? Is 25 enough? Is 200 overkill? If you score 10 animals what is the probility that you will miscore a heterozygous animal as a homozygous dominant?
- ***Trade plates with lab partner and rescore plates (blind) MUST DO THIS***
- Resolve any disagreements. A third party can be consulted. DO NOT DISCARD PLATES BEFORE THE END OF THE LAB PERIOD
- Deduce the genotype of each animal. ***In contrast to F2 scoring this assessment should have a very low error rate.***
- Record genotype distribution for your F2 animals on overhead

Each group of 4 students:

- combine data, calculate ratios
- Record "table data" on overhead

Perform a chi squared analysis on the combined class data

Genotype of F2 wild-types (inferred from progeny)

U = wild-type allele u = mutant allele D= wild-type allele d = mutant

	Uu Dd	*Uu DD*	*UUDd*	*UUDD*	n	χ^{2*}	p
expected ratio?							
raw data							
Observed ratios**							

*Your χ^2 calculations should be carried out to the four decimal places (including your expected values). The χ^2 value itself should be expressed to 3 decimal places -- reflecting the χ^2 values in the table.

** Calculate ratio by dividing each number by the number of UUDD animals.

In your lab notebook include:

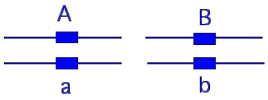
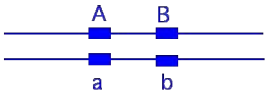
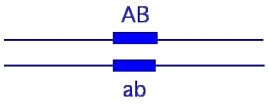
1. **An introductory statement** *(of a sentence or two only)* stating the purpose of this exercise. Don't just copy the introduction in this handout. State it in your own words.
2. **A list of the strains that you will use in the formal *C. elegans* nomenclature AND simple allele designations for each allele of each gene examined.** The notes and records made for this lab exercise will use the simple allele designations and *not* the formal nomenclature.
3. The **genotype(s)** of the animals at each step and the **expected frequency of each genotypic and phenotypic class assuming the genes are on different chromosomes**. Be sure to define your allele symbols.
4. **Results:** Data Sets should be labeled with a descriptive title and clearly presented.
5. **Data Analysis:** State the expected results--phenotypic or genotypic ratios--(assuming the genes are on different chromosomes) of these experiments. Show your calculations and state the results you obtained. If your data deviate from the expected results, the chi-square test can tell you if your experimental data are statistically significant.
6. **Discussion/Conclusions:**
    a. Briefly summarize your data and the results of the χ^2 analysis.
    b. What do your data show?
    c. Be sure to **state in words what the p value means**.
    d. Do your data deviate significantly from the class data or the expected results? If so, how can you explain this deviation?

Other questions to consider:

- Can a statistical test **prove** that your hypothesis is true or false? Explain.
- How does sample size affect the analysis?

How do we know if genes are linked or not?

Gametes formed by a organism who developed from the joining of gametes (AB) and (ab)

Location of genes A and B with respect to each other	PERCENTAGE OF EACH GAMETE TYPE				Percentage of recombination	Description of double heterozygote
	Parental		Recombinant			
	(AB)	(ab)	(Ab)	(Ba)		
I. On different (nonhomologous) chromosomes	25	25	25	25	50	
II. On the same chromosome, but very far apart	25	25	25	25	50	
III. On the same chromosome, neither very far apart nor very close [a]	$\frac{100-x}{2}$	$\frac{100-x}{2}$	$\frac{x}{2}$	$\frac{x}{2}$	x=a number between 50 and 0	
IV. On the same chromosome, very close together	50	50	0	0	0	

a. Only in Cases III and IV do we refer to A and B as linked genes.

Map distance between genes is measured in map units.
1 map unit = 1% recombination, x =# of map units

Note: in a meiotic recombination test, 50% recombination is observed for genes on different chromosomes as well as genes that are very far apart on the same chromosome. Recombination between linked genes never exceeds 50% even if the genes are on opposite ends of the chromosome