

GENETICS Module II

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OVERVIEW

In the mid-1800's, an Austrian monk named Gregor Mendel conducted crosses with pea plants in order to understand the basic patterns of inheritance. Mendel's approach provided the basis for modern genetics. Although Mendel used garden peas for his experimental crosses, genetics is not just for peas! In this module, we will use worms, corn, and humans to analyze a variety of genetic problems.

- In the first set of experiments, you will set up and analyze genetic crosses of the nematode worm *Caenorhabditis elegans*. In these experiments, your goal will be to deduce unknown parental genotypes by analyzing the phenotypes of their “children” and “grandchildren”. *C. elegans* is widely employed as a genetic model organism because of its small size, large broods, and rapid (3 day) generation time. Interestingly, *C. elegans* populations consist of males and self-fertile hermaphrodites, so your genetic analysis will include crosses between males and hermaphrodites as well as hermaphrodite self-crosses.
- Although Mendel’s critical insight was that genetic inheritance follows predictable mathematical patterns, the actual numbers gathered in genetic studies often deviate from these mathematical expectations. As part of this module, you will collect phenotypic data and employ a commonly used statistical test (Chi-square) to test whether your results deviate from the expected values in a statistically significant or statistically non-significant manner.
- To see how basic genetic principles have real life consequences, you will work through medical case studies to predict the inheritance patterns of human disease genes.

A REVIEW OF KEY CONCEPTS

1. **Genes are located on chromosomes and genes come in pairs:** In most organisms, individuals have two alleles of each gene. Homozygous individuals have two identical alleles of a given gene. Heterozygous individuals have two distinct alleles. During the meiotic divisions associated with the maturation of eggs and sperm, the two alleles separate so that each egg or sperm possesses only a single allele. This principle is true for diploid organisms; genetics works differently in haploid organisms such as bacteria which possess only a single copy of each gene.
2. **Dominant and Recessive Alleles:** When two distinct alleles responsible for a single character (gene product) are present in a single individual, one allele is typically **dominant** to the other **recessive** allele. The phenotype of the recessive allele is observed only in its homozygous state; it is masked in the presence of the dominant allele. Usually the dominant allele produces a functional product while

the recessive allele produces a non-functional or partially-functional product. However there are also deleterious dominant alleles such as the mutation associated with Huntington's disease which produce a stable but malfunctioning form of the protein. Within a population of organisms, there can be multiple alleles of a particular gene, each of which differs in DNA sequence such that the function of the resulting RNA and/or protein is affected.

3. Mendel's Principles:

- a. During the meiotic divisions associated with the formation of gametes (eggs or sperm), the two alleles of a single gene separate (segregate) randomly so that each gamete receives one or the other with equal likelihood.
- b. When two different genes/traits (e.g. body shape and physical mobility) are located on different chromosomes, the alleles of each gene assort independently of each other.

4. Linkage, sex chromosomes, and the complexities of genetic recombination

- a. When two different genes are located on the same chromosome, they can sometimes sort independently of each other. If the two genes on the same chromosome are closely linked, they almost always segregate together. However if they are far apart on the same chromosome, they can separate from one another as a result of genetic recombination.
- b. As a general rule, if a gene is on a sex chromosome, not every gamete will receive an allele of that gene.

Additional Definitions

Gene: a unit of heredity that occupies a specific position on a chromosome and codes for one macromolecule. Many genes are transcribed into RNA molecules which are processed mature mRNA, exported from the nucleus, and then translated into polypeptide chains. Other genes encode RNAs that are themselves end-products; for instance, the small nuclear RNAs, ribosomal RNAs, transfer RNAs that function directly in mRNA processing and translation.

Genotype: All of the alleles of every gene present in a given individual. In practice, we often talk about the genotype for only one or a few genes.

Genome: All of the heredity information in an organism, including not only genes but also other non-gene stretches of DNA.

Phenotype: Any of the observable characteristics of an individual. Commonly includes physical, physiological, and behavior characteristics.

References for more information

Freeman text – Chapter 13

Websites for genetic practice problems

<http://biology.clc.uc.edu/courses/bio105/geneprob.htm>

<http://www.k-state.edu/biology/pob/genetics/intro.htm>

Worms on the Web

Caenorhabditis elegans WWW Server (worm anatomy, links to worm labs, movies, search of the worm literature) <http://elegans.swmed.edu>

Wormbase (main hub for all *C. elegans* genomic and genetic data and information for large-scale expression and RNAi screens) <http://wormbase.org>

OVERALL LAB SCHEDULE

Week One

- Using a dissecting microscope
- *C. elegans*: Worm basics (observing, manipulating, and sexing worms)
- *C. elegans*: Set up your Monohybrid Cross.

Week Two

- *C. elegans*: Score the F1 phenotypes of your Monohybrid
- *C. elegans*: Create Punnett squares to predict the possible outcomes of your Monohybrid Cross

Week Three

- *C. elegans*: Dihybrid Cross - Analyze F2 phenotype to deduce linkage
- Chi square analysis
- *C. elegans*: Worm Base exercise to find out more about your mutant alleles

No Friday discussion section

Week Four

No Lab – Fall Break

Week Five

- Lab Report turned in at the beginning of class
- Human Genetics – case studies
- Class presentation of research results

C. elegans Genetics Lab

WEEK #1: More about *C. elegans*

Caenorhabditis elegans is a small, free-living soil nematode that is commonly found in many parts of the world. For use in laboratory settings, the worms are typically grown on agar media plates spread with *E. coli*. The worms are only about 1 mm long as adults, so it is easy to grow millions of worms in a small amount of lab space. Because of their size, worms are handled by viewing them under a dissecting microscope, and individual worms are transferred from one plate to another using a worm “pick” (a small hand-made platinum microspatula).

Worms have two sexes; XX self-fertile hermaphrodites which produce sperm and then eggs, and XO males. Each hermaphrodite can produce about 300 progeny. Worms develop from embryos through four distinct larval stages before becoming fertile adults. The entire process normally takes 3-5 days depending on the growth temperature. For these experiments, worm growth can be slowed down in order to accommodate a one week schedule by temporarily maintaining the worms at 4°C.

Many top biomedical research laboratories use *C. elegans* to analyze the function of specific genes. *C. elegans* was the first multi-cellular organism to have its genome completely sequenced and several labs are cooperating to create genetic mutants for each and every *C. elegans* gene.

Exercise One – Stereo Microscopes Rule

You will work in pairs today and share a scope. It is crucial to the success of the genetics module that you become very comfortable using the stereo microscopes in lab.

1. Adjust your stool and optics of the dissecting microscope so that you can view the letter “e” slide on the stage. Don’t forget to turn on the light box. Your TA will help point out the many parts of the dissecting microscope and show you how to adjust the focus and the magnification.
2. Draw what you see in the space provided. Don’t forget to use both eyes when looking thru the eyepieces. Ask your TA for help if you are having a hard time seeing a single field.

3. Try to touch the “e” with the tip of your pencil/toothpick. Is it easier at low magnification? Keep trying until you get really comfortable touching the slide with the pencil / toothpick as you look thru the eyepieces.

Exercise Two – Intro to the “Ways of the Worm” and Worm Picking

Before you can set up your genetic crosses, you must first develop the necessary hand-eye coordination to manipulate these 1-mm long worms without either killing the worms or scratching the agar plates.

1. Transferring worms between plates is best done at 10X and 25X. What magnification setting will you use on your stereo microscopes for a total magnification of 10X?... of 25X? (Ask TA if you need to be reminded how to calculate the total magnification of a microscope)
2. Ask your TA for an agar plate. Set your magnification to the lowest setting. Remove the top of the plate. Describe what you see on the surface of the plate?
3. Increase the magnification to the highest setting. Take some time to get comfortable moving the plate around on the stage at different magnification settings. Most expert worm pickers leave their hand on the focus knob to make adjustments as they move the plate on the stage.
4. When you are done with this part of the exercise place the cover back on the worm plate. Why do we keep the worm plate covered when we are not using it?
5. Now you are ready to ask your TA for a worm pick. This is a very valuable tool that you will use to move worms from place to place. A standard “worm pick” is a short length of platinum wire fashioned into a microspatula and melted into a glass pasteur pipet. If you or your partner were to drop and break the pick, please alert your TA right away. Why is the worm pick made of platinum wire?
6. Place your agar plate on the stage of your stereo microscope and remove the lid. Brace your “worm picking “ hand on the side of the scope (Use your dominate hand). While looking through the microscope at the lowest setting, try to touch the agar plate without scratching the surface. Flame your Pick. Try it again at a higher magnification. Flame your pick. Why are we trying to avoid damaging the surface of the agar plate?

**REMEMBER TO
FLAME YOUR PICK AFTER EACH USE TO
STERILIZE THE PLATINUM WIRE**

7. When you get confident that you can touch the surface of the plate without scratching it, try to scoop up some of the bacteria. It is often easiest to scoop the bacteria from the edge of the bacterial lawn. When you are done, flame your pick.
8. Ask your TA for your first worm plate. The plate contains wild type worms. In terms of genetics, what does wild type mean? Remove the top of the plate and view the worms at low and high magnification. The worm plate should be full of worms. What details can you see at low magnification? ... at high magnification?
9. While still looking through the scope, lift the plate of wild type (normal) worms about 1/2 cm off the microscope and then let it back down. The associated vibration gets the worms “scrambling”. How do they move? (Describe in a few sentences and draw a picture of “worm tracks”.)
10. The plates that you have been given contain adult hermaphrodites, embryos, and probably some larvae. Draw side-by-side picture of an adult hermaphrodite and an embryo while trying to accurately represent their relative sizes. Can you see the very young embryos that are still present within the mid-section of the adult hermaphrodite?
11. Next... At 20X, use the pick to GENTLY stroke a worm on the head (the clear end). How does it respond? What happens when you stroke its tail?
12. Stroke the pick on the edge of the bacterial lawn in order to coat it with bacteria. Now see if you can pick up a single worm by touching it with a bacteria-coated worm pick. Try putting it back down on another section of the plate by touching the “worm-loaded” pick to the plate and holding it there until the worm crawls off. Repeat this step, multiple times until you can do this repeatedly WITHOUT either killing the worm or scratching the agar plate. Worm picking requires a gentle touch in both picking up the worms and placing them down. But if you leave them in the air too long, they will die. Repeat at least 5-6 times.
13. Cover the plate and put it to the side for now.

Exercise Three – Learning to Distinguish Worms by Sex and Phenotypes

1. Ask your TA for a plate with males. Remove the top. How do these animals differ from adult hermaphrodites? How do their tails compare? Do males contain embryos within their mid-body region? Take a look at the supplemental pictures of adult *C. elegans* placed on your lab benches.

2. As you continue to look at your plate and begin to see the physical differences between the sexes, can you begin to see differences in their behavior? How do the males move when they are near a hermaphrodite? ... when they are alone?

3. Now that you have spent some time with the wild type worms, it is time to check out your morphological mutants. Ask your TA for your set of three plates. Record the phenotypes of your “unknown” mutants.
 - a. Red Strain: _____
 - b. Black Strain: _____

4. Examine the set plates. Were you able to identify the phenotype? Check the side of the plate to check your answer.

5. How do these hermaphrodites differ from each other? How do they differ from the wildtype worms that you have been working with up until this point?
 - a. Red Strain:

 - b. Black Strain:

**CONGRATULATIONS! ... YOU CAN BEGIN
TO YOUR GENETIC CROSS**

Exercise Four –MonoHYBRID CROSS experiment

Each lab pair will be setting a genetic cross between homozygous wildtype males and hermaphrodites that are homozygous for either Dpy or Unc mutation (your unknown). By analyzing the phenotypes of the offspring of this cross, your goal will be to determine

- a) Whether the wildtype (+) allele is recessive or dominant relative to the Dpy (dpy-dumpy)
- b) Whether the wildtype (+) allele is recessive or dominant relative to the Unc (unc-uncoordinated movement)

– Parental cross

- Each student in a team should set up a cross.
 - 1) a cross with 2 Dpy homozygous hermaphrodites and 4 homozygous wildtype males,
 - 2) a cross with 2 Unc homozygous hermaphrodites and 4 homozygous wildtype males.Be sure to place the wildtype males directly on the bacterial lawn. Notice that when we describe the phenotype alone, we capitalize the first letter of the name.
- How will you distinguish self-cross and out-cross progeny?
- Label the bottom of each cross plate with your name and date. Also indicate the genotypes of the homozygous parents. Ask your TA for the gene name and allele designation for the mutant strains. The wildtype alleles of the males can be designated as (+)/(+) Use a permanent marker provided by your TA.
- Record the genotypes on your worksheet.
- Why did you write on the bottom of the plate?
- Take a look at your plates under the dissecting scope and evaluate how well you did. The best plates have:
 - 2 hermaphrodites (morphological mutants only) and 4 wildtype males. The males should be actively crawling on the plate. If they are not moving, you will need to try to transfer more males to the cross plate.
 - no gouges and few scratches on the surface of the agar.
- How did you and your partner do?

- The parental cross will be incubated upstairs for you until next week. We will remove the adults for you so that the worms that you see next week will be the progeny of the mutant hermaphrodites.
- Predict the possible outcomes of the two crosses.
 - First, what will happen if the mutant allele inherited from the hermaphrodite is dominant to the wildtype allele inherited by the male?
 - Second, what will happen if the mutant allele is recessive to the wildtype allele?
 - Third, what will happen if the mutant allele is semi-dominant to the wild type allele?
- Rubberband plates and hand to your TA.

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WEEK #2: Scoring Monohybrid crosses and Getting Accustomed to using Punnett Squares with *C. elegans* Nomenclature

PART I: SCORING MONOHYBRID CROSS

– SCORING F1 progeny of a Monohybrid Cross

- Examine the phenotypes of the F1 progeny. Which allele is dominant and which is recessive?
- Which prediction is supported by your data?

Part II: PUNNETT SQUARES

– Making genetic predictions

A Punnett square is a visual device that helps you understand what is happening in a cross and also what progeny genotypes to expect. A cross involves a male and a female individual, each contributing one allele for each gene. The columns of the Punnett square represent the female contribution to progeny (through egg cells), and the rows represent the male contribution to progeny (through sperm cells). There are two columns and two rows because each parent has two alleles that may be passed to the next generation. The tops of the columns are labelled with the female's alleles, and the sides of the rows are labelled with the male's alleles. The cells of the table represent the genotypes of progeny that can result from the cross, each containing both an allele from the mother and an allele from the father. Since any particular progeny individual could inherit either maternal allele and either paternal allele, there are four possible combinations in total.

For a cross between a heterozygous female (Cc) and a heterozygous male (Cc), the Punnett square would look like this:

Punnett Square for Cc X Cc

| | | |
|---|----|----|
| ♀ | C | c |
| ♂ | C | c |
| C | CC | cC |
| c | Cc | cc |

For single traits involving dominant and recessive alleles. [assume Normal (d), Dpy (r)] One quarter of the progeny will inherit the C allele from both parents, and will thus be morphologically normal. One quarter will inherit the C allele from the mother and the c allele from the mother, and will also be morphologically normal (being heterozygotes). One quarter will inherit the c allele from the mother and the C allele from the father, making normal heterozygotes again. The last quarter of the the progeny will inherit the c allele from both parents and will be dumpy. Thus, the genotypic ratio is 1 CC:2 Cc:1 cc (the two classes of heterozygotes are combined), and the phenotypic ratio is 3 normal:1 dumpy. In some years, we have included an exercise on corn genetics during this second week. In scoring the corn kernals, students are able to score large numbers of progeny and are exposed to several differrent mathematicall patterns of inheritance.

Punnett squares can also be used to analyze the assortment of two traits. To see how these squares work, read section 13.2 of Freeman and make a copy of figure 13.6 to include in your lab notebook.

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WEEK #3: Scoring F2s from the *Dpy Unc* Dihybrid Cross, Chi square and WormBase

Part I: Scoring F2 Progeny from a Dihybrid Cross

1. Find your F2 plate and score the worms. Record your counts in the table below.

| Class of Mutant | Count |
|-----------------|-------|
| Wildtype | |
| dpy nonunc | |
| nondpy unc | |
| dpy unc | |

Chi-Square Calculation for dumpy and unc: F₂

| Class of mutant | Observed # | Expected # | (o - e) | (o - e) ² | (o - e) ² /e |
|-----------------|------------|------------|---------|----------------------|-------------------------|
| Wildtype | | | | | |
| dpy nonunc | | | | | |
| nondpy unc | | | | | |
| dpy unc | | | | | |
| | | | | TOTAL | |

- What's the p value and what's your interpretation of this result?
- If you did not see an expected distribution of phenotypes for your F₂s, how can you explain this result?

Part II: WormBase Searches

WormBase is an international consortium of biologists and computer scientists dedicated to providing the research community with accurate, current and accessible information concerning the genetics, genomics and biology of *C.elegans* and related nematodes. Founded in 2000, Wormbase is the repository of mapping, sequencing and phenotypic information for *C. elegans* (and some other nematodes). The information on the database is updated every other week by the nearly 30 full time curators. The search engine, Textpresso, was first developed for WormBase and has been adapted for databases in other scientific communities therefore, searching for information about genes on WormBase is very similar to searching on other model system databases.

The first step when you want to look up a gene is to log onto the computer and go to www.wormbase.org. The first page should look something like this

Enter your gene of interest in the space (ie. unc-4)

The second step is to type in your gene of interest in the space indicated by the arrow. The next page that comes up on the screen is the Gene Summary Page. The gene summary page provides information on gene identification, gene location, function, available alleles, protein similarities, and research papers that cite the gene. The pages also contain hypertext links to pages containing detailed information on the information, where available. Complete the following exercise using WormBase's Gene Summary Page for your gene of interest.

The Gene Summary Page for your Gene of Interest

1. Scroll to **Identification**. What is your gene name (CGC name)? The CGC name is the official designation approved by the *C. elegans* **Genetics Center**.
2. What is its sequence name?
3. Click on the sequence name and scroll down to see the DNA sequence information for this gene. Researchers interested in the molecular details of the gene use this page to download the DNA sequence data to their computer.
4. Return back to the Gene Summary Page by closing the DNA sequence data window. Under **Identification**, find the section that lists the **Concise Description** of the gene. What does your gene encode? (Most genes encode a protein. What type of protein is listed?)
5. Now that you have identified the type of protein that the gene encodes, how is the molecular product related to the mutant phenotype? (the dumpy phenotype is defective in body shape and the uncoordinated phenotype is defective in movement)
6. Scroll down to the **Gene model (s)**. How many amino acids make up the protein? Click on the number listed and it will take you to a window that lists the amino acid sequence. Researchers can copy and download this protein sequence information for further analysis.
7. Return back to the Gene Summary Page by closing the window. Scroll down to **Location**. Look for **Genetic Position**. What is the genetic position of your gene? Is your gene in the center ... on the right ... or on the left side of the chromosome?

8. Click on the **Genomic Position**. Scroll down and look for the **Overview** section (highlighted in blue) of the new page. What is the name of the gene to the right of your gene? ... to the left? Geneticists use this page to help them position their gene of interest relative to all of the others on the chromosome.

9. Return back to the Gene Summary Page and scroll down to **Function**. Look for the section, **Mutant Phenotype**. Does the description of the mutant phenotype listed on WormBase match your description (see Exercise 3)? List any other details that this description helps you see.

10. Scroll down to **Alleles**. What is the reference allele? How many alleles are listed for your gene? How do alleles for a gene differ from one another? Give an example from Dr. Shakes' discussion lecture last Friday.

11. Scroll back up to **Mutant Phenotype**. Are there any allele specific differences in the mutant phenotypes? If there are differences, how might you explain them?

12. Scroll down to **Similarities**. How many other organisms are known to have a similar gene? List two and the % similarity listed by WormBase.

13. Write out your hypothesis for your genetic experiment with the dpy unc dihybrid cross.

14. Read through the handout "Scientific Lab Report" before you leave class. If you have any questions, please ask your TA. Visit LabWrite for Students at www.ncsu.edu/labwrite for additional information (and tutoring assistance) on writing a scientific lab report.

15. The Written Assignment for the GENTICS MODULE is a lab report.

The lab report that you will write for the Genetics Module will contain only information pertaining to your hypothesis for your dpy unc dihybrid cross. It will contain a title, introduction, materials/methods, results, discussion and references. The report will be 2-3 pages in length (not including references). The report will be typed and 1.5-spaced. Standard fonts and font sizes will be used (Times, Helvetica or Courier, size 12). **Your report will be due by your next lab.** This is the week after Fall Break.

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WEEK #5: Human Genetics – Case Studies

This lab is designed to be a “seminar” experience. What is a seminar experience? It is a group meeting where information is shared and ideas are exchanged. The success of your experience depends upon you. Each person is expected to contribute to the discussion. How? You should come to lab prepared with comments on your assigned case study, answers to the questions posed in the reading, conduct your own research about the genetic disease and come to class with some new ideas or questions to discuss. Each student member of the group will be responsible for leading some part of the discussion. How do you lead an In-class Discussion?

The goals should always be to 1) help the class come up with some consensus about the major arguments, assumptions or conclusions of the reading and 2) uncover some new and interesting points to discuss. Your group may choose the way or method that you all feel is best to lead a discussion of your assigned human genetic case study.

1) Presentation: Focus your discussion by presenting the reading to the class. It is helpful to use overheads that summarize your points for your audience. This is a common way that members of a research lab discuss their latest results with one another in a weekly lab meeting.

2) Group Discussion: The discussion is fostered around a series of well defined questions. The types of questions could be a blend of those that are included in your reading and those that you come up with in your own. If you choose this format, you will need to be flexible enough to carefully listen to the audience responses and incorporate their contributions in your responses.

3) Small-Group Discussion: More people actually participate or at least have a better chance of participating when they are part of a small group discussion. The larger group can break into smaller groups tasked to discuss a question or issue brought up in the reading. After 10-15 minutes, the groups can reconvene and present their ideas to the entire class.

4) Skits or Debate: Some groups may have a predisposition to act out the reading as a “scene” from a play. Each member of the group plays a part and then members of the group can pose questions to the audience. Or, members of the group can take different positions and debate the questions posed in the reading.

5) Combination: Your group can be creative and come up with your own format to lead the discussion of your case study.

Your TA will assign one of three Human Genetics Case Studies

1. Sometimes it is all in the genes- Adapted from a case study by Anne Galbraith and David Howard (Univ. of Wisconsin/ La Crosse)
2. The Death of Baby Pierre by Clyde Freeman Herreid (SUNY/Buffalo)
3. Breast Cancer Susceptibility and the BRCA I Gene- Adapted from a case study by La Shawn Alexander (Univ. of Michigan- Dearborn)

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