

Chapter 7

GENES AND HYPOTHESIS

Inheritance has intrigued people for centuries. Countless proposals, many of which seem humorous today, have been advanced to explain the phenomena of inheritance. It was long accepted that characteristics were passed from parents to their offspring. Theories such as pangenesis, which suggested that bits of heritable information from cells of each body part were contributed to germ cells, and blending were developed to accommodate the observations that offspring usually appeared similar to their parents or showed characteristics intermediate between those of their parents. While intuitively likely and acceptable in their times, these theories were largely incorrect. It remained for Gregor Mendel in the 1860s and another generation of biologists at the beginning of the Twentieth Century to elucidate the correct mechanisms of inheritance. In honor of its original proponent, this aspect of the study of inheritance is named Mendelian Genetics.

I. MENDELIAN GENETICS

While genetic experimentation of sorts had been conducted as early as the time of Aristotle, Mendel revolutionized the science by developing and adhering to a new set of rules. Indeed Mendel succeeded where all others had failed because of his method of investigation:

1. Mendel followed the pattern of inheritance of single characteristics through several generations while others attempted to deal with hybridizing and analysis of organisms as a whole.
2. Mendel limited his interest to characteristics that occurred in discrete alternative forms.
3. Mendel kept exact records of all his crosses and all his results - even those results which did not fit his expectations.
4. Mendel employed mathematical analysis to quantify and simplify his data so that he could recognize the underlying pattern of inheritance, test his hypotheses, and predict the results of future matings.

By the elegant use of the scientific method Mendel unraveled the heretofore mysterious manner of transmission of heritable characteristics. The achievement is all the more remarkable when one realizes that Mendel was not aware of the function or indeed the existence of egg and sperm in life cycles of sexually reproducing organisms, not to speak of the existence and nature of chromosomes that contain the genetic material DNA. To summarize the findings of Gregor Mendel's genetic experiments, we may state the following rules of inheritance:

1. Each characteristic or trait is the result of the expression of inheritable factors, now called genes. Most organisms possess two copies of the gene for any particular trait, i.e. there are two so-called alleles present for each particular gene. If the alleles of a gene are identical, the organism is homozygous, if they are different, the organism is heterozygous.
2. During the reproductive activities of the organism the members of each pair of alleles segregate from each other so that only one allele is passed to any given offspring.

3. In a heterozygous (hybrid) individual, one member of a pair of alleles may dominate over the other, however the recessive one, though not expressed, may be passed unchanged to the next generation.
4. The pair of alleles for a particular characteristic segregates independently of alleles for other characteristics, resulting in the independent assortment of the genes that a parent donates to any given offspring.

Note the distinction between the genetic constitution, the genotype, of an individual and its observable characteristics, its phenotype. Homozygous individuals can pass only a single form of a gene to their offspring, because the alleles of that gene are identical. Therefore, when bred amongst themselves, homozygous individuals will be true breeding, i.e. their offspring will always show the parental characteristics. The systematic analysis of inheritance patterns, however, involves crosses between true breeding strains that differ in one or more traits. Crosses which involve analysis of one characteristic (monohybrid crosses) and those involving two different characteristics (dihybrid crosses) illustrate the Mendelian principles of inheritance.

A. *Drosophila* crosses

In this lab we will investigate the patterns of inheritance of two dihybrid crosses using the fruit fly *Drosophila melanogaster*. In the first experiment, female flies with normal, bright red eyes and tiny wings (vestigial wings) were crossed with male flies with dark brown eyes (sepia eyes) and normal wings; in the second experiment, female flies with normal eyes and a dark body color (ebony body) were crossed with male flies with dark brown eyes (sepia eyes) and normal, light body color. The genes that control the respective traits are named after the phenotype of the mutants: vestigial (vg), sepia (se), and ebony (e). The corresponding “wild-type” alleles which underlie the normal phenotype abundant in nature are designated vg^+ , se^+ , and e^+ , respectively. In each case the wild-type allele is dominant over the mutant allele, so that heterozygous flies appear normal.

Let's follow the segregation of alleles for the first cross applying the Mendelian rules of inheritance stated above:

Parental generation (P) $vg/vg, se^+/se^+ \times vg^+/vg^+, se/se$

Gametes $vg, se^+ \qquad \qquad \qquad vg^+, se$

First filial generation (F₁) $vg^+/vg, se^+/se \times vg^+/vg, se^+/se$

Gametes and second filial generation (F₂) shown in Punnett Square

male female	vg^+, se^+	vg^+, se	vg, se^+	vg, se
vg^+, se^+	$vg^+/vg^+, se^+/se^+$	$vg^+/vg^+, se^+/se$	$vg^+/vg, se^+/se^+$	$vg^+/vg, se^+/se$
vg^+, se	$vg^+/vg^+, se^+/se$	<u>$vg^+/vg^+, se/se$</u>	$vg^+/vg, se^+/se$	<u>$vg^+/vg, se/se$</u>
vg, se^+	$vg^+/vg, se^+/se^+$	$vg^+/vg, se^+/se$	$vg/vg, se^+/se^+$	$vg/vg, se^+/se$
vg, se	$vg^+/vg, se^+/se$	<u>$vg^+/vg, se/se$</u>	$vg/vg, se^+/se$	<u>$vg/vg, se/se$</u>

The cross between the two true breeding parent strains yields an F₁ generation that is heterozygous for both genes and phenotypically wild-type. Applying Mendel's rules of segregation and independent assortment allows a prediction about the distribution of genotypes in the F₂ generation: the Punnett square shows 9 different genotypes that occur in a ratio of 1:2:1:2:4:2:1:2:1. With the wild-type alleles of both genes being dominant, these genotypes result in 4 different phenotypes: normal eyes and normal wings (normal typing in the Punnett square), sepia eyes and normal wings (underlined), normal eyes and vestigial wings (bold), and sepia eyes and vestigial wings (double underlined), in a ratio of 9:3:3:1.

PROCEDURE

1. Obtain the flasks with your F₂ flies. Each flask should contain a few hundred flies by now.
2. Anesthetize the F₂ animals of the first cross with "FlyNap" and put them under the dissecting microscope.
3. Identify the different phenotypes and count the number of flies in each phenotypic class. Record your data in the table below.
4. Repeat this procedure for the F₂ flies of your second cross.
5. Analyze your counts with the χ^2 -test, i.e. find out, if your hypothesis derived from the Mendelian rules of inheritance is supported by your data (see "Testing the Hypothesis", p. 70).
6. Write up a group lab report on your breeding experiments, following the form of a scientific paper as outlined in the appendix to chapter 4.

Phenotype	Checklist of Flies	Number of Flies (n)
normal wings, normal eyes		
vestigial wings, normal eyes		
normal wings, sepia eyes		
vestigial wings, sepia eyes		
	Total	

Table 2. Phenotypic Distribution of F₂ individuals from <i>Drosophila</i> Cross ebony x sepia		
Phenotype	Checklist of Flies	Number of Flies (n)
normal body, normal eyes		
ebony body, normal eyes		
normal body, sepia eyes		
ebony body, sepia eyes		
	Total	

PROBLEMS

The following problems are designed to aid you in analyzing genetic crosses. A strong working knowledge in formal genetic analysis still provides the foundation for many applications of genetic research, for instance genetic counseling. Work the problems in the space provided, and bring any questions you may have to your instructor's attention.

1. Assume that brown eyes are caused by a dominant allele, and blue eyes by a recessive allele of a gene that controls eye color in humans. If a dark-haired, brown-eyed man and a blue-eyed blonde woman have a blue-eyed son, what is the genotype of that son? What is the genotype of the mother? The father?

2. In peas seed shape is controlled by one gene, with round being dominant over wrinkled.
 - a. Diagram the cross of true breeding parents for round and wrinkled pea seeds. Define your symbols! Show the gametes of each parent, and the F₁ offspring.

- b. Now cross two F_1 plants and show the F_1 gametes and F_2 progeny. How would the F_2 ratio differ if dominance were not exhibited in this characteristic?
3. In the summer squash plant, white colored fruit is due to a dominant allele, yellow fruit is caused by the corresponding recessive allele. Which is more likely to occur, two white fruit squash producing yellow fruit offspring, or two yellow fruit squash producing white fruit offspring? Explain your answer by means of diagramming the two potential crosses.
4. In chickens, R = rose comb, r = recessive single comb. How would you determine if a rose combed rooster were homozygous or heterozygous? Diagram the crosses you would make. What are these crosses called?
5. The shape and color of radishes are controlled by two independent pairs of alleles showing no dominance. Therefore each genotype is distinguishable phenotypically. Possible genotypes:
 RR = red RR' = purple $R'R'$ = white
 LL = long LL' = oval $L'L'$ = round
- a. Diagram the cross of two parents true breeding for color. Carry to the F_2 .

b. Diagram the cross of two parents true breeding for shape. Carry to the F_2 .

c. Diagram the cross of two parents true breeding for both color and shape.
(Dihybrid Cross)

6. In humans two well known recessive traits are lack of freckles and red hair.
R = non red hair F = freckles
r = red hair f = lack of freckles

How could a freckled dark-haired man and a non-freckled red-haired woman produce a red-haired, non-freckled child?

7. The eye color of a hypothetical mammal is controlled by two genes. Allele A of the first gene acts to suppress eye color (white eyes), while the alternative allele, a, allows eye pigment to form. The alleles of the second gene are responsible for brown eye pigment (allele B), or blue pigment (allele b). Dominance is complete for both pairs of alleles, with the capital letters designating the dominant alleles. Cross $AAbb \times aaBB$. What eye color classes are found in the F_2 ?

II. HYPOTHESES

A. Statistical Tests

Scientific experiments regardless of how well conducted seldom have results which exactly fit the investigator's expectation. Many times the results deviate from the expected outcome simply by chance, i.e. the results fit the hypothesis but show some random deviation.

Statistical tests have been devised to aid experimenters in their decision making process. These tests indicate the amount of deviation from the expected results which could be accounted for by chance alone. While statistics cannot make decisions for the investigator, it can simplify and clarify the data and indicate how likely the decision is to be correct.

With the large inventory of statistical tests to choose from, an investigator will usually be able to find at least one which is appropriate to a specific experiment. Mendelian genetic problems may be analyzed by several straightforward techniques. The issue is generally "goodness of fit", i.e. determining how closely one's results resemble the expected results. We have chosen to use the Chi-square test, χ^2 , to test goodness of fit in the problems and experiments studied in this laboratory. The Chi-square takes into account the number of classes and the sample size and reduces the data set to a single number - the χ^2 value. Probability tables have been calculated to indicate the likelihood of data, represented by each value of χ^2 , fitting the expectation. So by using χ^2 , the investigator simplifies the data to a single value, and determines the probability of the experimental results fitting the theoretical results based on the hypothesis being tested.

It must be pointed out that simply showing a set of data, how well it may be presented and how conclusive it may look, does neither support nor invalidate a hypothesis. Only the application of a statistical test, such as χ^2 , can tell, if a hypothesis can be considered accurate or if it has to be rejected on the basis of the experimental findings.

B. Testing the Hypothesis

A standard method of hypothesis testing is used in most biological experiments. This method may be carried out in the following four steps:

1. Develop a hypothesis. State an expected cause of a particular scientific phenomenon and predict, based on your hypothesis, the outcome of an experiment. Importantly, the hypothesis must be testable by experimentation.
2. Gather data. Conduct an experiment to gain information that will either corroborate or refute your hypothesis. A great deal of importance is placed on the design of experiments so that they actually will test the hypothesis under consideration.
3. Compare obtained data to expectation. By use of a statistical test, measure the degree of concordance between the actual results of the experiment and the expected results based on your hypothesis. For the χ^2 -test you always suppose that your set of data is not different from the expected result (null hypothesis). The χ^2 -test is performed as follows:

- a. Calculate the χ^2 -value using the formula

$$\chi^2 = \sum ((n_{\text{observed}} - n_{\text{expected}})^2 / n_{\text{expected}})$$

with n being the number of individuals in each phenotypic class.

b. Find the degrees of freedom, df, for your experiment. In this lab, we will compute the degrees of freedom as

$$df = z - 1 \quad \text{where } z \text{ is the number of phenotypic classes}$$

c. Consult the χ^2 probability table (Table 3). You will also find these tables in most genetics textbooks. From the table, determine the probability that the deviations you observed were merely the result of chance.

1. Scientists generally agree that deviations which would occur by chance alone in less than 1 out of 20 experiments (probability of occurrence < 0.05 or 5 %) suggest that the obtained data set is significantly different from the expected result. This is the case, if the calculated χ^2 value for your data is greater than the χ^2 value in the table for the 5 % probability level and the appropriate degrees of freedom.

2. If the observed deviations occur with a probability greater than 5 % by chance alone (in more than 1 out of 20 experiments), the data set is considered identical to the expected result. In this case, the calculated χ^2 value is smaller than the value in the table for the 5 % probability level and the appropriate degrees of freedom.

Table 3. Critical χ^2 Values

Degrees of Freedom	Probabilities				significance	
	0.95	0.80	0.50	0.20	0.05	0.01
1	0.004	0.064	0.455	1.642	3.841	6.635
2	0.103	0.446	1.386	3.219	5.991	9.210
3	0.352	1.005	2.366	4.642	7.815	11.345
4	0.711	1.649	3.357	5.989	9.488	13.277
5	1.145	2.343	4.351	7.289	11.070	15.086
6	1.635	3.070	5.348	8.558	12.592	16.812
7	2.167	3.822	6.346	9.803	14.067	18.475
8	2.733	4.594	7.344	11.030	15.507	20.090
9	3.325	5.380	8.343	12.242	16.919	21.666
10	3.940	6.179	9.342	13.442	18.307	23.209

4. Decide to accept or to reject your hypothesis. You will reject your null hypothesis, if your set of data is significantly different from your expectations (χ^2 of data is greater than χ^2 at the 5 % probability level), you will accept your null hypothesis, if your data are not significantly different from your expectations (χ^2 of data is smaller than χ^2 at the 5 % probability level). Be aware that there is always a small chance that you make the wrong decision. For example, you may reject a null hypothesis although it is correct. Statistical testing, however, enables you to estimate, how likely it is that you make this wrong decision.

PROBLEMS

The following problems will give you experience with hypothesis testing using the χ^2 statistics.

1. The results listed below show the F_2 generation of a monohybrid cross for color of petals in flowers. Your hypothesis is that one gene with lack of dominance causes petal color. The numbers given are the observed values. Proceed by following the hypothesis testing method detailed above.

Phenotype of F_2	n_{observed}
red petals	148
pink petals	285
white petals	137
Total	570

2. The following data are the actual numbers obtained by Gregor Mendel in one of his dihybrid garden pea crosses. The numbers given are the observed values of the F_2 generation. Test the hypothesis that seed shape and color are due to two independent genes which operate with complete dominance.

Phenotype of F_2	n_{observed}
yellow - round	315
yellow - wrinkled	101
green - round	108
green - wrinkled	32
Total	556