

Methods to Determine the Phenotypic Ratio of a Hybrid Cross

Carter Cunningham
Department of Mathematics
Northwest University
Kirkland, WA 98033

Faculty Advisor: Dr. Millicent Thomas

Abstract

The character variation between two organisms or phenotypes are the starting point of gene analysis. The discovery of Mendelian phenotype contributes a wide knowledge and understanding of the gene functions, gene regulations and the development of the biological mechanism. There are various techniques that are effectively utilized to analyze the genetic constitution of an individual or population. The Punnett Square can be used to predict the genotypes and phenotypes of offspring in a genetic cross. Punnett Squares are an effective and straightforward way to examine simple genetic crosses. Unfortunately, it becomes cumbersome to count each square in finding the phenotypes and genotypes when there are more than two genes. Punnett Square methods are time consuming when the inheritance of multiple characters is examined. Here I describe few methods that are used to calculate the precise mathematical ratios for phenotypic ratios.

Keywords: Variability, Dominant Traits, Phenotypic Ratio

1. Introduction

Determining genotypes and phenotypes is common when studying genetics because they both serve as descriptors for individual organisms. While genotypes tell us the genetic make-up of an organism, the phenotypes are the observable traits that the genotypes create.

To determine the phenotype and genotype of an organism, Gregor Mendel created the Punnett Square [1]. The Punnett Square is a simple way to cross the genes of two parent organisms to discover the probability that the resulting organism will have certain traits. It is important to note that one of two or more alternative forms of a gene is called an allele. Assuming complete dominance, here is an example of a Punnett Square where 'A' is the dominant allele and 'a' is the recessive allele:

This cross of genes is called a monohybrid cross. This is when only one pair of genes are being crossed in a Punnett Square. When Mendel created the square, he wanted to see if the prediction made by the Punnett Square of pea plants' offspring was consistent with the results. He started with breeding parental plants to test their genetic inheritance. This parental generation created the first filial generation F_1 generation of all heterozygous plants. Next, he let the plants self-fertilize so that the result was just as the one shown in figure 1.

	A	a
A	AA	Aa
a	aA	aa

Figure 1

The probability of the resulting F_2 generation were

$$p(AA) = \frac{1}{4}, \quad p(Aa \text{ or } aA) = \frac{1}{2}, \quad p(aa) = \frac{1}{4}.$$

Hence it was proved that the genotypic ratio of the F_2 generation is 1:2:1 and the phenotypic ratio is 3:1.

Mendel thought of what would happen if there were more than one gene being checked. He created a dihybrid cross creating a Punnett Square for 2 unlinked genes. The Punnett square for these two unlinked genes has 16 squares. In this genotypic analysis, we see a phenotypic ratio 9:3:3:1. This allows us to easily determine the probability of the genotypes and phenotypes of the offspring.

The Punnett Square can be used to predict the genotypes and phenotypes of offspring in a genetic cross. Punnett Squares are an effective and straightforward way to examine simple genetic crosses. Unfortunately, it becomes cumbersome to count each square in finding the phenotypes and genotypes when there are more than two genes. Punnett Square methods are time consuming when the inheritance of multiple characters is examined. For example, if there is a Punnett Square with a tetrahybrid cross there would be $4^4=256$ possibilities. In general, for every 'n' number of genes there are 4^n possibilities.

Here I describe two methods that are used to calculate the precise mathematical ratios for phenotypic ratios of any number of genes in a hybrid cross.

2. Method I. Using Binomial Coefficients in the Binomial Distribution Expansion

A more effective way is to use the two basic laws of probability, the Addition Rule and the Multiplication Rule of Probabilities [4].

In a family of 3 children the sample space consists of the 8 possibilities {BBB, BBG, BGB, BGG, GBB, GBG, GGB, GGG}. The probabilities are as follows:

$$p(\text{all girls}) = \frac{1}{8}; \quad p(\text{2 girls and 1 boy}) = \frac{3}{8}; \quad p(\text{1 girl and 2 boys}) = \frac{3}{8}; \quad p(\text{all boys}) = \frac{1}{8}.$$

If the random number x - represents the number of boys in the family then the above probabilities can be represented by the probability distribution table:

x	0	1	2	3
p(x)	1/8	3/8	3/8	1/8

The numerators of these probabilities are 1,3,3,1. We see that obtaining these numerators in these probabilities are reliable from the Binomial Expansion:

$$(p + q)^n = p^n + \binom{n}{1}p^{n-1}q + \binom{n}{2}p^{n-2}q^2 + \dots + \binom{n}{r}p^{n-r}q^r + \dots + q^n \quad - (1)$$

$$(p + q)^3 = p^3 + \binom{3}{1}p^{3-1}q + \binom{3}{2}p^{3-2}q^2 + q^3 = p^3 + 3p^2q + 3pq^2 + q^3 \quad - (2)$$

$$(p + q)^2 = p^2 + \binom{2}{1}p^{2-1}q + q^2 = p^2 + 2pq + q^2 \quad - (3)$$

When $n=2$, the coefficients are 1:2:1,
 $n=3$, the coefficients are 1:3:3:1 and for the general case
 $n=n$, the coefficients are $1: \binom{n}{1}: \binom{n}{2}: \binom{n}{3}: \dots : \binom{n}{n-r}: \dots : 1$

2.1 Note 1:

- For gender calculations if p = probability of boys and q = the probability of girls then $p = q = \frac{1}{2}$.
- In ‘dominant’ and ‘recessive’ phenotype calculations $p \neq q$.
- For Mendelian Inheritance of the type {AA, Aa, aA, aa} all are dominant except for ‘aa’ which is recessive. Here $p = \frac{3}{4}$; $q = \frac{1}{4}$.

Let n be the number of genes. Then the binomial expansion equation (1) becomes,

$$\left(\frac{3}{4} + \frac{1}{4}\right)^n = \binom{n}{0}\left(\frac{3}{4}\right)^n + \binom{n}{1}\left(\frac{3}{4}\right)^{n-1}\left(\frac{1}{4}\right) + \binom{n}{2}\left(\frac{3}{4}\right)^{n-2}\left(\frac{1}{4}\right)^2 + \dots + \binom{n}{r}\left(\frac{3}{4}\right)^{n-r}\left(\frac{1}{4}\right)^r + \dots + \left(\frac{1}{4}\right)^n \quad - (4)$$

2.2. Observed Results:

Monohybrid cross: $n=1$. The above equation (4) becomes

$$\left(\frac{3}{4} + \frac{1}{4}\right)^1 = 1 \cdot \frac{3}{4} + 1 \cdot \frac{1}{4}.$$

Observing the right-hand side of the result, the denominator will show the number of possibilities while the numerator shows the phenotypic ratio which is 3: 1.

Dihybrid cross: $n=2$. The above equation (4) becomes

$$\left(\frac{3}{4} + \frac{1}{4}\right)^2 = 1 \cdot \frac{9}{16} + 2 \cdot \frac{3}{16} + 1 \cdot \frac{1}{16}.$$

It is better showed in the dihybrid cross. The coefficients show the number of times the numerator will be repeated in the ratio. The phenotypic ratio is 9: 3: 3: 1.

Trihybrid cross: $n=3$. The above equation (4) becomes

$$\left(\frac{3}{4} + \frac{1}{4}\right)^3 = 1 \cdot \frac{27}{64} + 3 \cdot \frac{9}{64} + 3 \cdot \frac{3}{64} + 1 \cdot \frac{1}{64}$$

The coefficients show the number of times the numerator will be repeated in the ratio. The phenotypic ratio is 27: 9: 9: 9: 3: 3: 3: 1.

Notice when $n=2$, the equation is identical to the Hardy-Weinberg Principle ($p^2 + 2pq + q^2$). Absent of evolution within a population, the Hardy-Weinberg Principle observes the allele and genotypic behavior within a population under random mating [3]. This principle relates to a single gene of diploid organisms. However, it is shown here that

n may be any integer, and therefore may be expanded to include any number of unlinked genes. The observed results are a consequence of the Hardy-Weinberg Principle, given that the binomial expansion of 2 alleles within a single gene presents the equation.

2.3 Note 2: To easily calculate the Binomial Coefficients $\binom{n}{r}$.

- May use the formula $\binom{n}{r} = \frac{n!}{r!(n-r)!}$.
- May use TI Calculators by choosing Math- Prob- 3 option.
- May use the Pascal's Triangle [see Figure 2 and 3]:

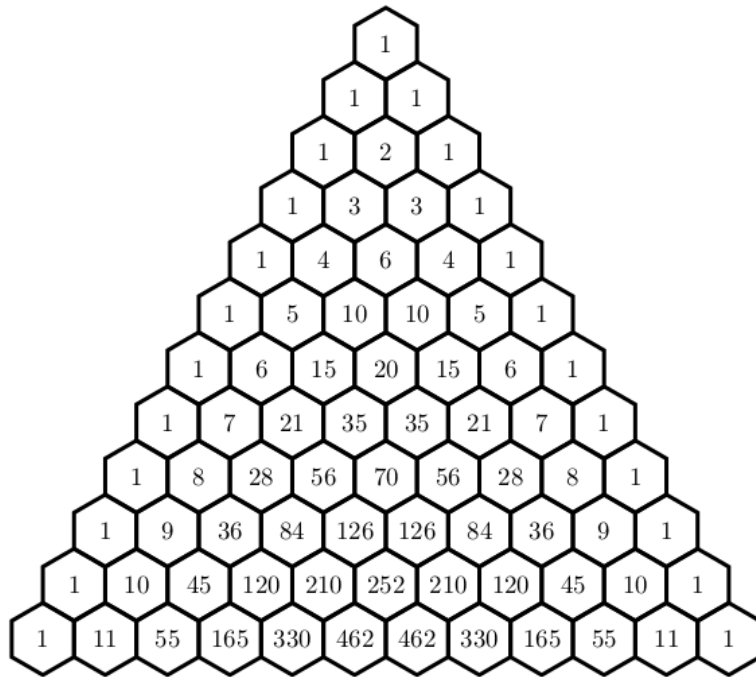


Figure 2. Pascal's triangle
<https://i.stack.imgur.com/DyuOO.png>

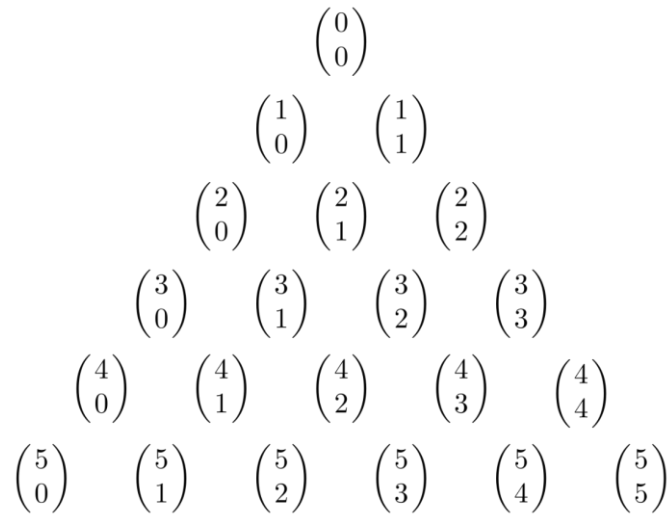


Figure 3. Binomial coefficients arranged to form Pascal's Triangle
<https://i.stack.imgur.com/nqaiV.png>

Pascal's Triangle and Binomial Theorem are inextricably linked. When n is positive and a whole number the coefficients of the Binomial expression $(a + b)^n$ is the same as the elements of the n th row of the Pascal's Triangle. [2]

3. Method II. Using the product of the probabilities and the Pascal's Triangle.

Phenotype calculation may be easily done using just the product of the probabilities $(p^m)(q^n)$ where $m+n =$ the number of genes being crossed in the binomial expansion equation (4).

$$\left(\frac{3}{4} + \frac{1}{4}\right)^n = \binom{n}{0} \left(\frac{3}{4}\right)^n + \binom{n}{1} \left(\frac{3}{4}\right)^{n-1} \left(\frac{1}{4}\right) + \binom{n}{2} \left(\frac{3}{4}\right)^{n-2} \left(\frac{1}{4}\right)^2 + \dots + \binom{n}{r} \left(\frac{3}{4}\right)^{n-r} \left(\frac{1}{4}\right)^r + \dots + \left(\frac{1}{4}\right)^n .$$

3.1 Observed Results:

3.1.1 monohybrid cross: $m + n = 1$. the above probability product becomes,

$$\left(\frac{3}{4}\right)^1 \left(\frac{1}{4}\right)^0 = \frac{3}{4} \text{ (once)}$$

$$\left(\frac{3}{4}\right)^0 \left(\frac{1}{4}\right)^1 = \frac{1}{4} \text{ (once)}$$

The number of times a number is repeated in the ratio is determined by the Pascal's Triangle given above. The Pascal's triangle shows 1-1 and hence the phenotypic ratio is 3:1 which are the numerators in the right-hand side.

3.1.2 dihybrid cross: $m + n = 2$. the above probability product becomes,

$$\left(\frac{3}{4}\right)^2 \left(\frac{1}{4}\right)^0 = \frac{9}{16} \text{ (once)}$$

$$\left(\frac{3}{4}\right)^1\left(\frac{1}{4}\right)^1 = \frac{3}{16} \text{ (twice)}$$

$$\left(\frac{3}{4}\right)^0\left(\frac{1}{4}\right)^1 = \frac{1}{4} \text{ (once)}$$

The Pascal's triangle shows 1-2-1 and hence the phenotypic ratio is 9: 3: 3: 1 which are the numerators in the right-hand side.

3.1.3. trihybrid cross: $m + n = 3$. the above probability product becomes,

$$\left(\frac{3}{4}\right)^3\left(\frac{1}{4}\right)^0 = \frac{27}{64} \text{ (once)}$$

$$\left(\frac{3}{4}\right)^2\left(\frac{1}{4}\right)^1 = \frac{9}{64} \text{ (thrice)}$$

$$\left(\frac{3}{4}\right)^1\left(\frac{1}{4}\right)^2 = \frac{3}{64} \text{ (thrice)}$$

$$\left(\frac{3}{4}\right)^0\left(\frac{1}{4}\right)^3 = \frac{1}{64} \text{ (once)}$$

The Pascal's triangle shows 1-3-3-1 and hence the phenotypic ratio is 27: 9: 9: 3: 3: 3: 1.

3.1.4 tetrahybrid cross: $m + n = 4$. the above probability product becomes,

$$\left(\frac{3}{4}\right)^4\left(\frac{1}{4}\right)^0 = \frac{81}{256} \text{ (once)}$$

$$\left(\frac{3}{4}\right)^3\left(\frac{1}{4}\right)^1 = \frac{27}{256} \text{ (4 times)}$$

$$\left(\frac{3}{4}\right)^2\left(\frac{1}{4}\right)^2 = \frac{9}{256} \text{ (6 times)}$$

$$\left(\frac{3}{4}\right)^1\left(\frac{1}{4}\right)^3 = \frac{3}{256} \text{ (4 times)}$$

$$\left(\frac{3}{4}\right)^0\left(\frac{1}{4}\right)^4 = \frac{81}{256} \text{ (once)}$$

The Pascal's triangle shows 1-4-6-4-1 and hence the phenotypic ratio is 81: 27: 27: 27: 27: 9: 9: 9: 9: 9: 3: 3: 3: 3: 1.

3.1.5 pentahybrid cross: $m + n = 5$. the above probability product becomes,

$$\left(\frac{3}{4}\right)^5\left(\frac{1}{4}\right)^0 = \frac{243}{1024} \text{ (once)}$$

$$\left(\frac{3}{4}\right)^4\left(\frac{1}{4}\right)^1 = \frac{81}{1024} \text{ (5 times)}$$

$$\left(\frac{3}{4}\right)^3\left(\frac{1}{4}\right)^2 = \frac{27}{1024} \text{ (10 times)}$$

$$\left(\frac{3}{4}\right)^2\left(\frac{1}{4}\right)^3 = \frac{9}{1024} \text{ (10 times)}$$

$$\left(\frac{3}{4}\right)^1\left(\frac{1}{4}\right)^4 = \frac{3}{1024} \text{ (5 times)}$$

$$\left(\frac{3}{4}\right)^0\left(\frac{1}{4}\right)^5 = \frac{1}{1024} \text{ (once)}$$

The Pascal's triangle shows 1-5-10-10-5-1 and hence the phenotypic ratio is 243:81:81: 81:81:81: 27:27:27: 27:27:27: 27:27:27: 27:9:9: 9:9:9: 9:9:9: 9:9:3: 3:3:3: 3:1 and so on.

The two methods presented in this paper are faster and easier methods in determining the phenotypic ratio of any hybrid cross. These methods are also less prone to mistakes especially when using a hybrid cross that contains greater than two genes.

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5. References:

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