Course: Fundamentals of Genetics

Class: - Ist Year, IInd Semester

Lecture No. IV

Title of topic: - Monohybrid crosses, Di-hybrid crosses, Test cross and Back cross

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Monohybrid crosses:—Crosses between parents that differed in a single characteristic. The character (S) being studied in a monohybrid cross are governed by two or multiple variations for a single locus.

The Mendel's first law i.e. Law of segregation or purity of gametes can be explained by considering the monohybrid ratio i.e. by studying inheritance of only one character. For example: In pea, round seed shape is dominant over wrinkled seed shape.

Generation — Parental

Parents — Female — X — Male

Phenotype — Round — X — Wrinkled

Genotype — RR — X — rr

Gametes — R — r

Generation — F1 Rr (Heterozygous) Round

On selfing:

Parents — Female — X — Male

Phenotype — Round — X — round

Genotype — Rr — X — Rr

Gametes — R, r — R, r

Generation — F2

♀ \ ♂	R	r
R	RR Round	Rr Round
r	Rr Round	rr Wrinkled

Phenotype ratio: 3 round : 1 wrinkled Genotypic ratio: 1 RR : 2Rr : 1rr

Two different alleles of the same gene i.e. 'R' and 'r' were brought together in the hybrid (F_1). Even though the hybrid was round seeded in the next generation (F_2) it produced both round and wrinkled seeded progeny. Thus both the alleles for round shape (R) and wrinkled shape (r) remained together in the hybrid without contaminating each other. In F_2 generation (selfing of (F_1) hybrid), the different phenotypes could be recovered because the two alleles in F_1 remained pure and did not contaminate each other thus producing two types of gametes from F_1 i.e. (R) and (r). The separation of homologous chromosomes during anaphase I of meiosis may be regarded as the reason for segregation of the two alleles of a gene. This is because the alleles of a gene are located in an identical position in the two homologous chromosomes.

The conclusions that Mendel developed about inheritance from his monohybrid crosses have been further developed and formalized into the principle of segregation and the concept of dominance.

The principle of segregation states that each individual organism possesses two alleles that can encode a characteristic. These alleles segregate when gametes are formed, and one allele goes into each gamete. The concept of dominance states that, when the two alleles of a genotype are different, only the trait encoded by one of them—the "dominant" allele—is observed.

Di-hybrid crosses: A di-hybrid cross describes a mating experiment between two organisms that are identically hybrid for two characters. A hybrid organism is one that is heterozygous, which means that is carries two different alleles at a particular genetic position, or locus. Therefore, a di-hybrid organism is one that is heterozygous at two different genetic loci.

Mendel's second law i.e. Law of independent assortment can be explained by studying the inheritance of two characters at a time, simultaneously.

Independent segregation for two genes can be explained by assuming that the two genes are located in two different chromosomes. The two alleles of a gene will be located in the two homologues of the concerned chromosome. Independent separation of these two pairs of chromosomes at anaphase I of meiosis will lead to the independent segregation of the genes located in them. Thus any allele of one gene is equally likely to combine with any allele of the other gene and pass into the same gamete. Independent segregation of two genes produces four different types of gametes in equal proportion. A random union among these gametes gives rise to sixteen possible zygotes. These zygotes yield a 9:3:3:1 phenotypic ratio, which is known as the typical dihybrid ratio.

When two pairs of independent alleles enter into F1 combination, both of them have their independent dominant effect. These alleles segregate when gametes are formed But the assortment occurs independently at random and quite freely.

Example: when plants of garden pea with yellow round seeds (Y Y RR) were crossed with plants having green wrinkled seeds (yyrr), yellow round seed plants (YyRr) were obtained in F1. Thus yellow colour of seed exhibited dominance over green and round seed shape over wrinkled seed shape independently. The F1 produces four types of gametes YR, Yr, yR and yr. Selfing of F1 gives rise to yellow round, yellow wrinkled, green round and green wrinkled individuals in 9:3:3:1 ratio. This is possible only when the alleles of two genes controlling the two characters assort independently to one another.

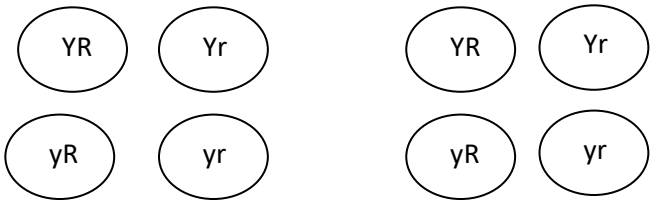
Parents	Female		X	Male
Phenotype	Yellow round	X		Green wrinkled
Genotype	YYRR	X		yyrr

Gametes: YR ⇩ yr

Generation: F1 YyRr (Yellow round) (Heterozygous)

On selfing/ intermating

Parents	Female		X	Male
Phenotype	Yellow round	X		Yellow round
Genotype	YyRr		X	YyRr



Generation F2

	YR	**Yr**	**yR**	**yr**
YR	YYRR Yellow round	YYRr Yellow round	YyRR Yellow round	YyRr Yellow round
Yr	YYRr Yellow round	YYrr Yellow wrinkled	YyRr Yellow round	Yyrr Yellow wrinkled
yR	YyRR Yellow round	YyRr Yellow round	yyRR green round	yyRr green round
yr	YyRr Yellow round	Yyrr Yellow wrinkled	yyRr green round	yyrr green wrinkled

Phenotypic ratio:
9 Yellow round : 3 Yellow wrinkled : 3 green round : 1 green wrinkled
Genotypic ratio:
1 YYRR : 2 YYRr : 1 YYrr : 2 YyRR : 4 YyRr : 2 Yyrr : 1 yyRR : 2 yyRr : 1 yyrr
This analysis is predicated on two assumptions:
(1) That each gene segregates its alleles, and (2) that these segregations are independent of each other.
Mendel conducted similar experiments with other combinations of traits and in each case observed that the genes segregated independently. The results of these experiments led him to a third key principle:
The Principle of Independent Assortment: The alleles of different genes segregate, or as we sometimes say, assort, independently of each other.

Test cross: A testcross is a cross between an individual with an unknown genotype and one with a homozygous recessive genotype. A testcross tests, or reveals, the genotype of the first individual. A test cross can help determine whether a dominant phenotype is homozygous or heterozygous for specific trait.

Suppose you were given a tall pea plant with no information about its parents. Because allness is a dominant trait in peas, your plant could be either homozygous (*TT*) or heterozygous (*Tt*), but you would not know which. You could determine its genotype by performing a testcross. If the plant were homozygous (*TT*), a testcross would produce all tall progeny (*TT* _ *tt* n all *Tt*); if the plant were heterozygous (*Tt*), half of the progeny would be tall and half would be short (*Tt* _ *tt* n1/2 *Tt* and 1/2 *tt*). When a testcross is performed, any recessive allele in the unknown genotype is expressed in the progeny, because it will be paired with a recessive allele from the homozygous recessive parent.

Backcross: - In a back cross, two lines are cross to yield a hybrid. Next, selected individual from the progeny are crossed with one of the parents (or with an organism genetically similar to the parent). In plant breeding, a backcross is very valuable, because breeder can hybridize a high yielding variety with another variety to introduce a desired trait (such as disease resistance), then backcross to make sure the progeny have the same desirable characteristics as the high yielding variety.

Procedure for backcross method The Plan of backcross method would depend upon whether the gene being transferred is recessive or dominant. The plan for transfer of a dominant gene is simpler than that for a recessive gene.

First Year	Non-Recurrent		Recurrent
	Parent B	x	Parent A
	Resistant to rust		Susceptible to rust

	F1	Rr	x	rr	BC1
	Resistant				
rr		Rr	x	rr	BC2
rr		Rr	x	rr	BC3
rr		Rr	x	rr	BC4
r		Rr	x	rr	BC5

Back cross upto 6th or 7th generation. After 7th BC rust resistant lines are self pollinated. Harvest is done on single plant basis.

8th Season:-Individual plant progenies grown a) homozygous plants having resistance and resembling parent A are selected harvested and bulked

9th season:-Yield trials.

Course: Fundamentals of Genetics

Class: - Ist Year, IInd Semester

Lecture No. V

Title of topic: - Dominance relationships- Incomplete, Co-Dominance, Over dominance

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Introduction:-

One of Mendel's important contributions to the study of heredity is the concept of dominance—the dea that an individual organism possesses two different alleles for a characteristic but the trait encoded by only one of the alleles is observed in the phenotype. With dominance, the heterozygote possesses the same phenotype as one of the homozygotes.

Complete and incomplete dominance:-

Complete dominance: - Dominance can be understood in regard to how the phenotype of the heterozygote relates to the phenotypes of the homozygotes. In the example presented in the top panel of flower colour potentially ranges from red to white. One homozygous genotype, *A*1*A*1, produces red pigment, resulting in red flowers; another, *A*2*A*2, produces no pigment, resulting in white flowers. Where the heterozygote falls in the range of phenotypes determines the type of dominance. If the heterozygote (*A*1*A*2) produces the same amount of pigment as the *A*1*A*1 homozygote, resulting in red, then the *A*1 allele displays **complete dominance** over the *A*2 allele; that is, red is dominant over white. If, on the other hand, the heterozygote produces no pigment resulting in flowers with the same colour as the *A*2*A*2 homozygote (white), then the *A*2 allele is completely dominant, and white is dominant over red.

Incomplete dominance: - When the heterozygote falls in between the phenotypes of the two homozygotes, dominance is incomplete. With **incomplete dominance**, the heterozygote need not be exactly intermediate between the two homozygotes; it might be a slightly lighter shade of red or a slightly pink shade of white. As long as the heterozygote's phenotype can be differentiated and falls within the range of the two homozygotes, dominance is incomplete.

Incomplete dominance is exhibited when the heterozygote has a phenotype intermediate between the phenotypes of the two homozygotes. When a trait exhibits incomplete dominance, a cross between two heterozygotes produces a 1 : 2 : 1 phenotypic ratio in the progeny.

Example:-1. Incomplete dominance is also exhibited in the fruit color of eggplant. When a homozygous plant that produces purple fruit (*PP*) is crossed with a homozygous plant that produces white fruit (*pp*), all the heterozygous F1 (*Pp*) produce violet fruit. When the F1 are crossed with each other, 1/4 of the F2 are purple (*PP*), 1/2 are violet (*Pp*), and 1/4 are white (*pp*). Note that this 1 : 2 : 1 ratio is different from the 3 : 1 ratio that we would observe if eggplant fruit color exhibited complete dominance.

2. Another example of incomplete dominance is feather color in chickens. A cross between a homozygous black chicken and a homozygous white chicken produces F1 chickens that are gray. If these gray F1 are intercrossed, they produce F2 birds in a ratio of 1 black : 2 gray : 1 white. We should now add the 1 : 2 : 1 ratio to those phenotypic ratios for simple crosses presented in Chapter 3 (see Table 3.3). A 1 : 2 : 1 phenotypic ratio arises in the progeny of a cross between two parents heterozygous for a character that exhibits incomplete dominance (*Aa* × *Aa*). The genotypic ratio among these progeny also is 1 : 2 : 1. When a trait displays incomplete dominance, the genotypic ratios and phenotypic ratios of the offspring are the *same*, because each genotype has its own phenotype. The important thing to remember about dominance is that it affects the phenotype that genes produce but not the way in which genes are *inherited.*

Co-dominance Another type of interaction between alleles is **co-dominance**, in which the phenotype of the heterozygote is not intermediate between the phenotypes of the

homozygotes; rather, the heterozygote simultaneously expresses the phenotypes of both homozygotes.

Example:-An example of co-dominance is seen in the MN blood types. The MN locus encodes one of the types of antigens on red blood cells. Unlike antigens foreign to the ABO and Rh blood groups (which also encode red-blood-cell antigens), foreign MN antigens do not elicit a strong immunological reaction; therefore, the MN blood types are not routinely considered in blood transfusions. At the MN locus, there are two alleles: the *L*M allele, which encodes the M antigen; and the *L*N allele, which encodes the N antigen. Homozygotes with genotype *L*M*L*M express the M antigen on their red blood cells and have the M blood type. Homozygotes with genotype *L*N*L*N express the N antigen and have the N blood type. Heterozygotes with genotype *L*M*L*N exhibit co-dominance and express both the M and the N antigens; they have blood-type MN.

Differences between dominance, incomplete dominance,and codominance

Type of Dominance	Definition
Dominance	Phenotype of the heterozygote is the same as the phenotype of one of the homozygotes
Incomplete dominance	Phenotype of the heterozygote is intermediate (falls within the range) between the phenotypes of the two homozygotes.
Codominance	Phenotype of the heterozygote includes the phenotypes of both homozygotes

Over dominance: - In many instances heterozygotes have a higher degree of fitness than homozygotes for one or the other allele. This situation, known as heterosis or overdominance, leads to the stable coexistence of both alleles in the population and hence contributes to the widespread genetic variation found in populations of most organisms.

Example:- An example in humans is that of the sickle cell anemia. This condition is determined by a single polymorphism. Possessors of the deleterious allele have lower life expectancy, with homozygotes rarely reaching 50 years of age. However, this allele also yields some resistance to malaria. A thus in region where malaria exerts or has exerted a strong selective pressure, sickle cell anemia has been selected for its conferred partial resistance to the disease. While homozygotes will have either no protection from malaria or a dramatic propensity to sickle cell anemia, heterozygotes have fewer physiological effects and a partial resistance to malaria.

Course: Fundamentals of Genetics

Class: - Ist Year, IInd Semester

Lecture No. VI

Title of topic: - Probability and Chi-square

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Probability:-

Probability expresses the likelihood of the occurrence of a particular event. It is the number of times that a particular event occurs, divided by the number of all possible outcomes.

Example-

I. A deck of 52 cards contains only one king of hearts. The probability of drawing one card from the deck at random and obtaining the king of hearts is 1/52, because there is only one card that is the king of hearts (one event) and there are 52 cards that can be drawn from the deck (52 possible outcomes). The probability of drawing a card and obtaining an ace is 4/52, because there are four cards that are aces (four events) and 52 cards (possible outcomes). Probability can be expressed either as a fraction (4/52 in this case) or as a decimal number (0.077 in this case).

In other cases, we determine the probability of an event by making a large number of observations.

II. When a weather forecaster says that there is a 40% chance of rain on a particular day, this probability was obtained by observing a large number of days with similar atmospheric conditions and finding that it rains on 40% of those days. In this case, the probability has been determined empirically (by observation).

Prediction of probability :- Two rules of probability are useful for predicting the ratios of offspring produced in genetic crosses.

1. The first is the **multiplication rule**, which states that the probability of two or more independent events occurring together is calculated by multiplying their independent probabilities. To illustrate the use of the multiplication rule, let's again consider the roll of a die. The probability of rolling one die and obtaining a four is 1/6. To calculate the probability of rolling a die twice and obtaining 2 fours, we can apply the multiplication rule. The probability of obtaining a four on the first roll is 1/6 and the probability of obtaining a four on the second roll is 1/6; so the probability of rolling a four on both is 1/6 _ 1/6 _ 1/36

The key indicator for applying the multiplication rule is the word *and*; in the example just considered, we wanted to know the probability of obtaining a four on the first roll *and* a four on the second roll. For the multiplication rule to be valid the events whose joint probability is being calculated must be independent— the outcome of one event must not influence the outcome of the other. For example, the number that comes up on one roll of the die has no influence on the number that comes up on the other roll; so these events are independent.

However, if we wanted to know the probability of being hit on the head with a hammer and going to the hospital on the same day, we could not simply multiply the probability of being hit on the head with a hammer by the probability of going to the hospital. The multiplication rule cannot be applied here, because the two events are not independent—being hit on the head with a hammer certainly influences the probability of going to the hospital.

2. **The addition rule** The second rule of probability frequently used in genetics is the **addition rule**, which states that the probability of any one of two or more mutually exclusive events is calculated by adding the probabilities of these events. Let's look at this rule in concrete terms.

To obtain the probability of throwing a die once and rolling *either* a three *or* a four, we would use the addition rule, adding the probability of obtaining a three (1/6) to the probability of obtaining a four (again, 1/6), or 1/6 _ 1/6 _ 2/6 _ 1/3.

The key indicators for applying the addition rule are the words *either* and *or.* For the addition rule to be valid, the events whose probability is being calculated must be mutually exclusive, meaning that one event excludes the possibility of the occurrence of the other event. For example, you cannot throw a single die just once and obtain both a three and a four, because only one side of the die can be on top. These events are mutually exclusive.

The application of probability to genetic crosses:-

- The multiplication and addition rules of probability can be used in place of the Punnett square to predict the ratios of progeny expected from a genetic cross. Let's first consider a cross between two pea plants heterozygous for the locus that determines height, *Tt_Tt.* Half of the gametes produced by each plant have a *T* allele, and the other half have a *t* allele; so the probability for each type of gamete is 1/2.
- The gametes from the two parents can combine in four different ways to produce offspring. Using the multiplication rule, we can determine the probability of each possible type. To calculate the probability of obtaining *TT* progeny, for example, we multiply the probability of receiving a *T* allele from the first parent (1/2) times the probability of receiving a *T* allele from the second parent (1/2). The multiplication rule should be used here because we need the probability of receiving a *T* allele from the first parent *and* a *T* allele from the second parent—two independent events. The four types of progeny from this cross and their associated probabilities are:

TT (*T* gamete and *T* gamete) 1/2 _ 1/2 _ 1/4 tall
Tt (*T* gamete and *t* gamete) 1/2 _ 1/2 _ 1/4 tall
tT (*t* gamete and *T* gamete) 1/2 _ 1/2 _ 1/4 tall
tt (*t* gamete and *t* gamete) 1/2 _ 1/2 _ 1/4 short

- Notice that there are two ways for heterozygous progeny to be produced: a heterozygote can either receive a *T* allele from the first parent and a *t* allele from the second or receive a *t* allele from the first parent and a *T* allele from the second. After determining the probabilities of obtaining each type of progeny, we can use the addition rule to determine the overall phenotypic ratios. Because of dominance, a tall plant can have genotype *TT*, *Tt*, or *tT*; so, using the addition rule, we find the probability of tall progeny to be 1/4 _ 1/4 _ 1/4 _ 3/4. Because only one genotype encodes short (*tt*), the probability of short progeny is simply 1/4.

THE CHI-SQUARE TEST

With DeVries's data, and with other genetic data as well, we need an objective procedure to compare the results of the experiment with the predictions of the underlying hypothesis. This procedure has to take into account how chance might affect the outcome of the experiment. Even if the hypothesis is correct, we do not anticipate that the results of the experiment will exactly match the predictions of the hypothesis. If they deviate a bit, as Mendel's data did, we would ascribe the deviations to chance variation in the outcome of the experiment. However, if they deviate grossly, we would suspect that something was amiss. The experiment might have been executed poorly—for example, the crosses might have been improperly carried out, or the data might have been incorrectly recorded—or, perhaps, the hypothesis is simply wrong. The possible discrepancies between observations and expectations obviously lie on a continuum from small to large, and we must decide how large they need to be for us to entertain doubts about the execution of the experiment or the acceptability of the hypothesis.

One procedure for assessing these discrepancies uses a statistic called **chi-square** (χ^2). A *statistic* is a number calculated from data—for example, the mean of a set of examination scores. The χ^2 statistic allows a researcher to compare data, such as the numbers we get from a breeding experiment, with their predicted values. If the data are not in line with the predicted values, the χ^2 statistic will exceed a critical number and we will decide either to revaluate the experiment—that is, look for a mistake in technique—or reject the underlying hypothesis. If the χ^2 statistic is below this number, we tentatively conclude that the results of the experiment are consistent with the predictions of the hypothesis. The χ^2 statistic therefore reduces hypothesis testing to a simple, objective procedure.

As an example, let's consider the data from the experiments of Mendel and DeVries. Mendel's F_2 data seemed to be consistent with the underlying hypothesis, whereas DeVries's F_2 data showed some troubling discrepancies.

Table-1

F_2 Phenotype	**Observed Number**	**Expected Number**	**$\frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$**
Mendel's dihybrid cross			
Yellow, round	315	313	0.01
Green, round	108	104	0.15
Yellow, wrinkled	101	104	0.09
Green, wrinkled	32	35	0.26
Total	**556**	**556**	**0.51 = χ^2**
DeVries's dihybrid cross			
Red, hairy	70	88.9	4.02
White, hairy	23	29.6	1.47
Red, smooth	46	29.6	9.09
White, smooth	19	9.9	8.36
Total	**158**	**158**	**22.94 = χ^2**

Now imagine carrying out the experiment—carefully and correctly—many times, and each time, calculating a χ^2 statistic. Fortunately, the χ^2 frequency distribution is known from statistical theory, so we don't actually need to carry out many replications of the experiment to get it. The critical value is the point that cuts off the upper 5 percent of the distribution. By chance alone, the χ^2 statistic will exceed this value 5 percent of the time. Thus, if we perform an experiment once, compute a χ^2 statistic, and find that the statistic is greater than the critical value, we have either observed a rather unlikely set of results—something that happens less than 5 percent of the time—or there is a problem with the way the experiment was executed or with the appropriateness of the hypothesis. Assuming that the experiment was done properly, we are inclined to reject the hypothesis. Of course we must realize that with this procedure we will reject a true hypothesis 5 percent of the time. Thus, as long as we know the critical value, the χ^2 testing procedure leads us to a decision about the fate of the hypothesis. However, this critical value—and the shape of the associated frequency distribution—depends on the number of phenotypic classes in the experiment. Statisticians have tabulated critical values according to the **degrees of freedom** associated with the χ^2 statistic (**Table 2**). This index to the set of χ^2 distributions is determined by subtracting one from the number of phenotypic classes.

Table 2: Table of Chi-Square (χ^2) 5% Critical Values[2]

Degrees of Freedom	5% Critical Value
1	3.841
2	5.991
3	7.815
4	9.488
5	11.070
6	12.592
7	14.067
8	15.507
9	16.919
10	18.307
15	24.996
20	31.410
25	37.652
30	43.773

Selected entries from R. A. Fisher and Yates, 1943, *Statistical Tables for Biological, Agricultural, and Medical Research*. Oliver and Boyd, London

In each of our examples, there are 4 -1= 3 degrees of freedom. The critical value for the χ^2 distribution with 3 degrees of freedom is 7.815. For Mendel's data, the calculated χ^2 statistic is 0.51, much less than the critical value and therefore no threat to the hypothesis being tested. However, for DeVries's data the calculated χ^2 statistic is 22.94, very much greater than the critical value. Thus, the observed data do not fit with the genetic hypothesis. Ironically, when DeVries presented these data in 1905, he judged them to be consistent with the genetic hypothesis. Unfortunately, he did not perform a χ^2 test. DeVries also argued that his data provided further evidence for the correctness and widespread applicability of Mendel's ideas—not the only time that a scientist has come to the right conclusion for the wrong reason. To solidify your understanding of the χ^2 procedure, answer the question posed in Solve It: Using the Chi-Square Test.

KEY POINTS

- The chi-square statistic is $\chi^2=\sum$ (observed number - expected number)^2/ expected number, with the sum computed over all categories comprising the data.
- Each chi-square statistic is associated with an index, the degrees of freedom, which is equal to the number of data categories minus one.