Mendelian Principle of Inheritance

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Genetics is the study of genes and their variation and heredity among organisms. Long before DNA was recognised as the genetic material, Gregor Mendel through his studies predicted the presence of such a factor responsible for heredity. Heredity had been observed in nature for centuries, but Mendel studied this phenomenon in a scientific manner, performed experiments and put forth his hypothesis that has withstood the test of time. Although some variations to principles of Mendelian inheritance have been observed, the basic framework of genetic inheritance initially proposed by him in essence remains true.

2.1 Mendelian's Monohybrid Cross

Gregor Mendel is widely regarded as the founder of genetics. He was a priest in an abbey in Brno where he conducted his experiments on pea plants. Mendel carried out his experiments on breeding of pea plants for about 7 years from 1856 to 1863 and presented his findings at the Brno Natural Science meetings in 1865. His paper which underlined the basic principles of inheritance was published in 1866. However, Mendel's work remained unnoticed until 1900 when other scientists like botanists Hugo de Vries, Erich von Tschermak and Carl Correns obtained similar results while working independently with plant breeding. They interpreted their results in the context of Mendel's theories and published their work supporting and drawing attention to Mendel's original work.

Mendel was successful in obtaining and interpreting his results due to a number of factors. One of the most important factors was his scientific approach and analytical reasoning. Others before him had crossed plants and described their results. Mendel, however, was able to formulate a hypothesis based on initial observations and design

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suitable experiments to test them. He also recorded the number of plants with different features across various crosses and tried to interpret these observations to fit a single framework of inheritance. One of the other reasons for his success was the choice of garden pea as his experimental model.

2.1.1 The Garden Pea

Pisum sativum, commonly known as the garden pea, was the ideal choice for genetic breeding experiments. Since Mendel worked in a monastery at Brno, he could easily access the monastery garden and greenhouse. Pea plants are easy to cultivate and can grow relatively rapidly with a life cycle of 1 year. He therefore invested years in following several generations of the plants. Pea plants are also able to produce numerous seeds which allowed Mendel to calculate mathematical ratios in the traits of offspring (seeds). Different varieties of peas are available and Mendel was able to choose those that differed in various traits and were purebred. He also chose to study features that were present in two easily distinguishable forms/traits like round seeds versus wrinkled seeds. He avoided those features which had a range of variable traits. He chose to study seven features which are shown in Fig. 2.1. Apart from these, some references also mention an eighth feature: seed coat colour which can be either green or white. Mendel noticed that a coloured seed coat always gives rise to plants bearing purple flowers, while the white seed coat gave rise to plants having white flowers. Seed coat colour is therefore sometimes mentioned instead of flower colour as the traits studied by Mendel.

With advances in molecular biology, studies have been performed to identify the genes and particular mutations responsible for the traits studied by Mendel. Genes can be classified into groups based on the structural or functional similarities of proteins they produce. They may also be grouped together if they all participate in a

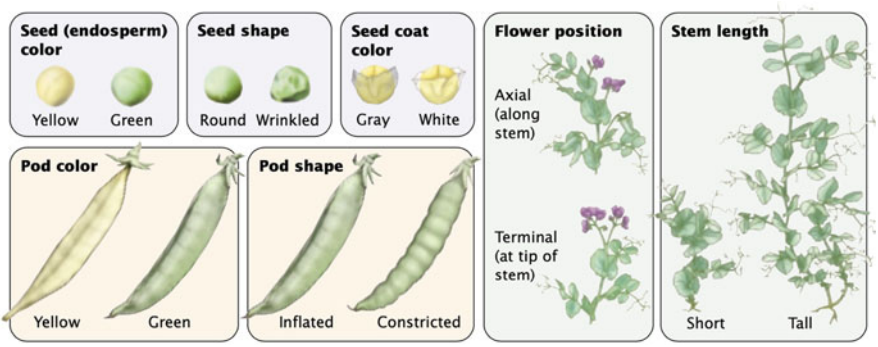

Fig. 2.1 The traits or plant characteristics studied by Mendel in his experiments on pea plants: Mendel studied seven different traits in pea plant for conducting his experiments. Traits being studied were present in either one of the two forms in the different varieties of pea plant (Pierce 2010)

Table 2.1 Group symbols and their functions for the traits studied by Mendel: Molecular studies to understand the traits studied by Mendel have resulted in identification of the genes responsible for the trait and their function. Of these R, LE, A and I have been cloned and well-studied. Less is known about the other genes

Trait	Group symbol	Gene function
Seed shape	R	Starch branching enzyme 1
Stem length	LE	GA3 oxidase 1
Flower colour	A	bHLH transcription factor
Pod colour	GP	Chloroplast structure in pod wall
Pod form	V	Sclerenchyma formation in pods
Position of flowers	FA	Meristem function
Seed colour	I	Stay-green gene

particular process. Gene group symbol and their functions for the traits studied by Mendel are given in Table 2.1.

The seed shape can be either round or wrinkled. The seeds differed in their starch, sugar and lipid content. Wrinkled seeds possess a higher amount of fructose, glucose and sucrose resulting in higher water retention due to osmotic pressure. A mutation in the *R* gene which codes for a starch branching enzyme 1 affected starch biosynthesis. This also further affected the protein and lipid biosynthesis in the seed ultimately changing its shape. Stem length was controlled by the *LE* gene. This gene codes for one of the GA3 oxidase genes which convert the gibberellin to an active form GA1. Gibberellin is a plant hormone which regulates the development of the plants including its length. Seed colour is influenced by the *I* gene which is important in chlorophyll degradation. Mutation in this gene leads to the appearance of green seeds. The flower colour is influenced by a gene which exhibits pleiotropic effects. This means that mutations in the gene can affect multiple traits. The gene codes for a basic helix-loop-helix (bHLH) transcription factor. It regulates multigene family-chalcone synthesis (CHS) genes which are responsible for flavonoid production. Flavonoids are secondary metabolites produced in plants and are responsible for pigmentation in plants, thus governing the flower colour. Other genes associated with the traits studied by Mendel have not been cloned and studied in molecular detail (Reid and Ross 2011).

To perform his experiments, Mendel crossed different varieties of pea plants. In order to understand how he achieved this, we need to know a little bit about the plant reproductive system. The male reproductive organ in a plant is called a stamen. It is composed of the filament and anther. The filament holds up the anther where pollen is produced. This pollen is carried by either wind, water or wildlife to the female reproductive organ. The female reproductive organ is called a pistil which consists of the stigma, style and ovary. The style holds the sticky stigma at the distal end, while the ovary is present at its proximal end. Stigma captures the pollen and allows it to germinate. Sperm carried in the pollen reaches the ovary through tubes formed during germination. Fertilisation occurs and an embryo is formed which is stored in the seed capsule. The seed remains dormant until favourable environmental conditions allow it to develop into a plant.

Pea plants often undergo self-pollination. This means that pollen from the flower will fall on the stigma of the same flower due to its close proximity. This happens even before the flower has opened. This type of pollination reduces genetic variability as the pollen and egg come from the same plant allowing them to maintain their characteristics. Plants which always pass on a specific trait to their offspring are called purebred varieties. Mendel grew pea plants for around 2 years in this manner to obtain purebred varieties for each trait. He also wanted to cross plants with different traits to see what traits were seen in the offspring. To achieve this, he opened the flowers and removed their anthers to prevent self-pollination. He then manually dusted pollen from the desired plant on the stigma of a flower from a different variety. This is called cross-pollination and resultant offspring are called hybrids. He obtained seeds from these cross-pollinated plants and observed their traits. He also grew these seeds through the next season to observe the traits of the hybrid plants (Fig. 2.2).

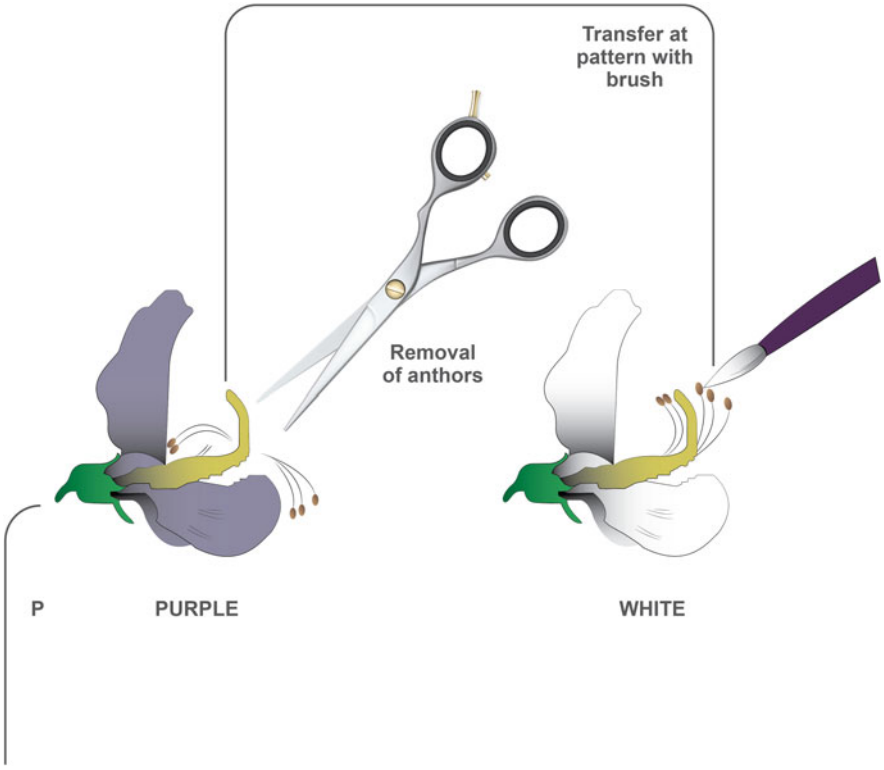

Fig. 2.2 Figure illustrating the male and female reproductive organs in a plant: To cross different varieties of plants, Mendel removed anthers from the flower to prevent self-pollination. He then dusted pollen from desired plant onto the stigma of this flower (Griffiths et al 2011)

2.1.2 Concept of Dominant and Recessive Traits

Mendel crossed different varieties of plants to study the traits inherited by the resultant offspring. He started by conducting monohybrid crosses, i.e. crosses between plants which differed by a single trait. Let us take the example of seed colour which can be either green or yellow. When Mendel crossed plants having green seed colour with those having yellow seed colour (referred to as the parental generation—P), he found that all the offspring called the first filial generation (F1) had yellow seed colour. He also carried out reciprocal crosses where instead of taking pollen from yellow seed plant and dusting it on the stigma of a green seeded plant, he took pollen from a green seeded plant and dusted it on the stigma of a yellow seed plant. In both cases, he found that plants in F1 generation had yellow seeds. Similarly, in crosses for the rest of the traits, he found that F1 generation always showed a single parental trait. It wasn't a mix of the parental traits, nor did the outcome change with various repetitions. This trait which was observed in the F1 generation was called the dominant trait and the trait which was lost was called the recessive trait. Mendel took this experiment one step further and allowed the plants from F1 generation to undergo self-pollination to create the F2 (second filial) generation. Most of the plants in the F2 generation had yellow seed colour, but surprisingly, there were a few plants in which seed colour was green. He counted the number of these plants and found that the number of plants having yellow seed colour was roughly thrice the number of plants having green seed colour.

Based on these results, Mendel made certain assumptions and put forth a hypothesis. Although the F1 generation always showed a single parental trait, the second parental trait reappeared in the F2 generation. This led him to assume that the F1 generation might have received genetic factors for both parental traits. Unless the F1 generation inherited genetic factors from both parents, it is impossible to explain the appearance of both parental traits in F2 generation. He hypothesised that offspring must inherit genetic factors from both parents and there must be two genetic factors in the plant for a single trait. The two genetic factors described here are what we now know as alleles. Alleles are, simply put, different forms of the same gene and are designated by a single letter. In this case, the allele for yellow seed colour is designated as Y and that for green seed colour is designated as y. Since the parental generation was purebred, the parental generation with yellow seed colour would have the alleles YY and the one with green seed colour would have the alleles yy. This composition of the alleles (YY or yy) is referred to as the genotype, and the trait physically expressed by the plant is called the phenotype (green or yellow seed colour).

He next assumed that the alleles separate when forming gametes and each allele gets segregated into one gamete. So the parental yellow seed coloured plants formed the gametes having allele Y and those from green coloured seed formed the gamete with allele y. In the F1 generation, these two gametes united and they had the genotype Yy. All F1 generation only had yellow coloured seeds. This trait was called the dominant trait. Although the allele for green coloured seed was present, it was masked and not expressed in the presence of Y. This was called the recessive

trait. He concluded that, of the two parental traits, one trait is the dominant and the other is recessive. Only the dominant trait gets expressed even in the presence of the recessive trait.

2.1.3 Segregation of Alleles

He further hypothesised that the F1 generation having genotype Yy forms gametes having Y and y with equal probability. Therefore, half the gametes will have allele Y and the other half will have allele y and these will get paired randomly in the F2 generation. The resulting progeny might have the genotypes YY, Yy, yY and yy. Since yellow coloured seed is the dominant character, YY, Yy and yY will have yellow coloured seeds, and only yy will have green coloured seeds. This explains the 3:1 ratio of phenotypes observed by Mendel in his experiments. This ratio can only be obtained if we assume that the alleles get segregated with equal probability while forming gametes. When the genotype of a plant consists of the same alleles, they are called homozygous (YY, yy), and when the alleles differ they are called heterozygous (Yy) (Fig. 2.3).

2.1.4 Mendel's Analytic Approach

The findings from Mendel's monohybrid cross are formally stated in two laws known as law of segregation and law of dominance. Law of segregation states that during the formation of gametes, two alleles in an individual will separate such that each gamete will have one allele. Law of dominance states that hybrids of different alleles will express only one of the parental traits called the dominant trait. Mendel was able to draw meaningful insights from his work due to the analytic approach towards his experiments. The ratios Mendel obtained from his experiments were not perfect. Plants may die or wither before their characteristics can be noted. Some plants may fail to germinate. Therefore, the ratio of monohybrid cross that Mendel obtained was almost but not exactly 3:1. However, Mendel obtained numbers for multiple experiments and noted that the ratios were approximately 3:1 in all cases. He further went on to self-pollinate plants obtained from F2 generation to confirm his findings.

Let us take the example here of round vs wrinkled seeds. When plants having round seeds are crossed with those having wrinkled seeds, the resulting F1 generation has all round seeds. Here, round seeds is the dominant trait and represented by the allele R, while wrinkled seeds are represented by the allele r. We can therefore say that the parental generation had a genotype of RR and rr and the F1 generation has the genotype Rr. When these F1 plants are self-pollinated, 1/4 seeds are wrinkled (rr) and 3/4 are round (RR and Rr) in the resulting F2. On further self-pollinating the F2 generation, he observed that all the wrinkled seeds gave rise to wrinkled seeds on selfing. This can be explained by the fact that as the genotype of wrinkled seeds is rr, they will always form gametes carrying the allele r. Therefore, on self-pollination,

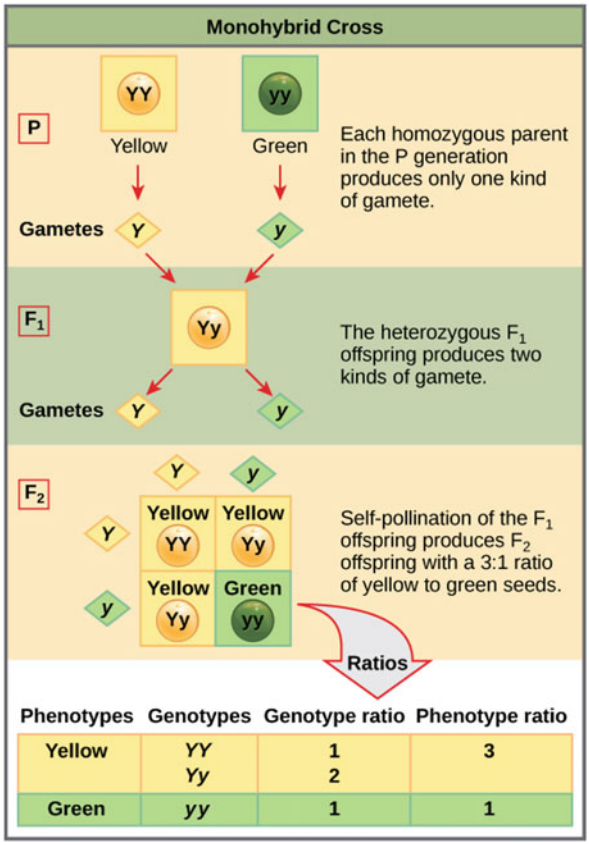

Fig. 2.3 Figure illustrating monohybrid cross between plants having yellow and green seed colour: The parental generation is homozygous and produces only one type of gametes. These get united in the F1 generation resulting in all plants having yellow coloured seeds. The F1 hybrids on selfing give rise to yellow and green seeds in a ratio of 3:1. The genotypic ratio is 1: 2:1 (The Punnett Square Approach for a Monohybrid Cross 2020)

they will always give rise to wrinkled seeds. Among the round seeds, 1/3 of the plants gave rise to only round seeds on self-pollination. The remaining 2/3 gave rise to a mix of round and wrinkled seeds in the ratio of 3:1. It follows that the seeds always giving rise to round seeds had the genotype RR. The remaining 2/3 seeds had the genotype Rr which, similar to the F1 generation, gives rise to the seeds in a ratio of 3:1. The results of these F3 generations give further evidence to support Mendel's hypothesis. These analytic approaches followed by Mendel were one of the strongest reasons why Mendel was able to come up with a reasonable hypothesis and support his claims through further experimentation (Fig. 2.4).

2.1.5 Test Cross: One Character

Homozygotes for the dominant allele as well as heterozygotes will display the dominant trait. To verify if the plant was homozygous or heterozygous for the dominant allele, Mendel crossed it with a plant showing the recessive trait and observed their progeny. This cross between plants showing dominant phenotype

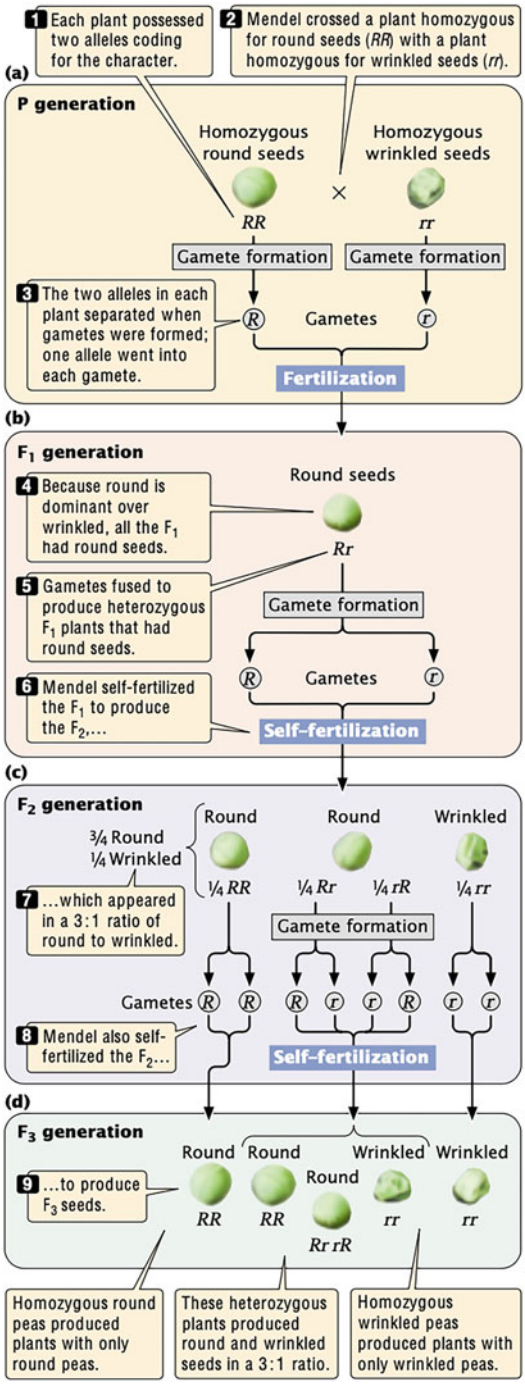

Fig. 2.4 Monohybrid cross till F3 generation shows Mendel's analytic approach: Mendel crossed plants with round seeds and wrinkled seeds and obtained F1 generation having all round seeds. On selfing the F1 generation, he obtained the F2 generation in a ratio of 3 round:1 wrinkled as expected. He further allowed the F2 to undergo self-pollination to obtain the F3 generation. He found that wrinkled seeds always gave rise to wrinkled seeds. Of the round seeds, 1/3 always gave rise to round seeds and the remaining 2/3 gave rise to seeds in the ratio of 3 round:1 wrinkled like the F2 generation. This provides further evidence to support Mendel's theory of segregation and dominance (Pierce 2010)

of unknown genotype with a plant of homozygous recessive genotype is called a test cross. Following Mendelian laws, if the plant being examined is homozygous for the dominant allele, all its progeny with the homozygous recessive parent will display the dominant phenotype. In the example below, if the purple flower is homozygous (having the genotype PP), it will produce gametes having P, and so all the F1 plants will show purple flowers. If, on the other hand, the purple flower is heterozygous (having the genotype Pp), it will produce two types of gametes having P or p. These, on crossing with p from the recessive parent, will give progeny with either purple or white flowers in a ratio of 1:1.

One of the recessive alleles will be provided by the parent showing the recessive phenotype as it will only produce gametes with the recessive allele. If a plant appears in the progeny displaying the recessive phenotype, it is clear that the other parent was carrying the recessive allele implying that the parent was heterozygous. If all the progeny show the dominant trait, the other parent has only provided gametes having the dominant allele implying that the parent was homozygous for the dominant allele. Test cross is a powerful method to examine unknown genotypes of an organism showing the dominant trait. This information is useful for breeders who would wish to choose homozygous plants for further breeding but cannot estimate the genotype based on observation of phenotype alone (Fig. 2.5).

2.2 Mendelian Dihybrid Cross

We have seen the results of crosses between plants differing in one trait. Mendel's next step was to study the pattern of inherited traits in crosses of plants differing in two traits. Let us take two traits in the pea plant: round vs wrinkled seeds and green vs yellow seeds. When Mendel crossed green round seeds with yellow wrinkled seeds, he observed that all the F1 progeny were yellow and round. In the monohybrid cross for each of the above traits, the F1 progeny expressed the dominant trait which was yellow colour and round shape. In the dihybrid cross too, the F1 hybrids expressed the two dominant traits. On selfing the F1 hybrids, he observed four phenotypes in the F2: yellow and round, green and round, yellow and wrinkled and green and wrinkled. On counting the number of plants in each category, he surmised that the plants were approximately in a ratio of 9:3:3:1 for the above combination of traits.

To make sense of the ratio that he obtained, Mendel made some logical deductions. He counted the number of yellow vs green and round vs wrinkled seeds and observed that they were in a ratio of 3:1 similar to the monohybrid cross. Mendel deduced that of all the F2 plants, 3/4 had yellow seeds and the remaining 1/4 had green seeds. Of the 3/4 yellow seeded plants, 3/4 had round seeds and 1/4 had wrinkled seeds. Similarly, 3/4 of the green seeds had round seeds and 1/4 had wrinkled seeds. This calculation gives the 9:3:3:1 ratio seen above. It appeared therefore that the dihybrid cross was a combination of 3:1 ratio for two traits. This will be easier to understand in the branched diagram in Fig. 2.6.

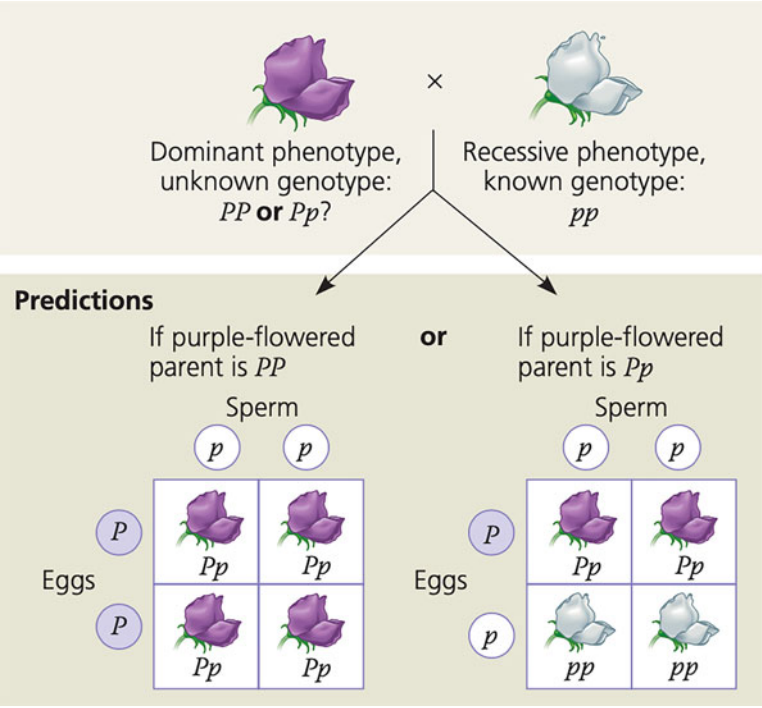

Fig. 2.5 Test cross involves crossing of a plant of unknown genotype with a plant showing the recessive trait: If the parent plant is homozygous, all its progeny will show the dominant trait. If the parent plant is heterozygous, half of its progeny will show the dominant trait and the other half will show the recessive trait. In the above illustration, test cross of a homozygous purple flower plant will result in all its progeny showing purple flowers. Test cross of a heterozygous purple flower plant will give flowers in the ratio of 1 purple:1 white (Reece et al 2011)

2.2.1 Independent Assortment

Mendel performed the dihybrid cross for a number of combinations of traits and always got a phenotypic ratio of 9:3:3:1. Let us now understand this ratio in biological terms. When a plant having yellow wrinkled seeds with the genotype YYrr is crossed with a plant having green round seeds with the genotype yyRR, hybrids with yellow round seeds having the genotype YyRr are produced. These hybrids can produce four gametes having four different combinations: YR, Yr, yR and yr. Each gamete carries one allele for each trait. On self-pollination, these gametes can merge in a variety of different combinations giving a phenotypic ratio of 9:3:3:1 as seen in the figure below (Fig. 2.7).

The fact that the dihybrid cross ratio is a combination of 3:1 ratio for each trait tells us that the gametes for each trait can assort independently. It means that allele Y has equal probability of pairing with either allele R or r to form a gamete. If one of the alleles in the cross above assorted preferentially with another allele, we would

¾ yellow seeds	¾ round seeds	¾ X 3/4 = 9/16 yellow round seeds
	¼ wrinkled seeds	¾ X ¼ = 3/16 yellow wrinkled seeds
¼ green seeds	¾ round seeds	¼ X ¾ = 3/16 green round seeds
	¼ wrinkled seeds	¼ X 1/4 = 1/16 green wrinkled seeds

Fig. 2.6 Phenotypic ratios obtained in a dihybrid cross: Each of the traits gives a 3:1 ratio. In the example above, we obtain seeds in a ratio of 3 yellow:1 green seeds. Each of these phenotypes also shows a ratio of 3 round:1 wrinkled seeds. This gives the overall 9:3:3:1 phenotypic ratio seen in a dihybrid cross

not obtain a phenotypic ratio of 9:3:3:1. This is called the law of independent assortment which states that different gene pairs can assort independently during gamete formation. However, genes which are close to each other on the same chromosome do not assort independently because they are held together on the same chromosome. In this case, alleles for different genes which are on the same chromosome always assort together during meiosis. The modified law of independent assortment can therefore be stated as 'Gene pairs present on different chromosomes assort independently of each other during formation of gametes'.

The tendency of genes which are close to each other to be inherited together is called linkage. Genes which get inherited together are classified into a single linkage group. Therefore, if any of the genes studied by Mendel belonged to same linkage group, their phenotypic ratios would have differed from the ones defined by Mendel. Mendel did not observe linkage between the genes that he studied and hence put forth the law of independent assortment. Mendel's work has been criticised on the basis that his data fits too well with his hypothesis and does not show as much variation due to chance as expected. Mendel's critics also cite the lack of evidence of linkage as one of the reasons to doubt Mendel's work. Recent work has, in fact, shown that the seven traits that Mendel studied belong to five different linkage groups of which only stem length and pod form show strong linkage. Mendel might have been lucky in his choice of traits for dihybrid cross. He might not have performed dihybrid crosses for this particular combination of stem length and pod form, or he would have been surprised by the resulting phenotypic ratios. Seed shape and pod colour show weak linkage, and all other traits are not linked allowing Mendel to obtain the same ratio for most of his dihybrid crosses. The debate about the validity of Mendel's work is discussed in detail in Box 2.1.

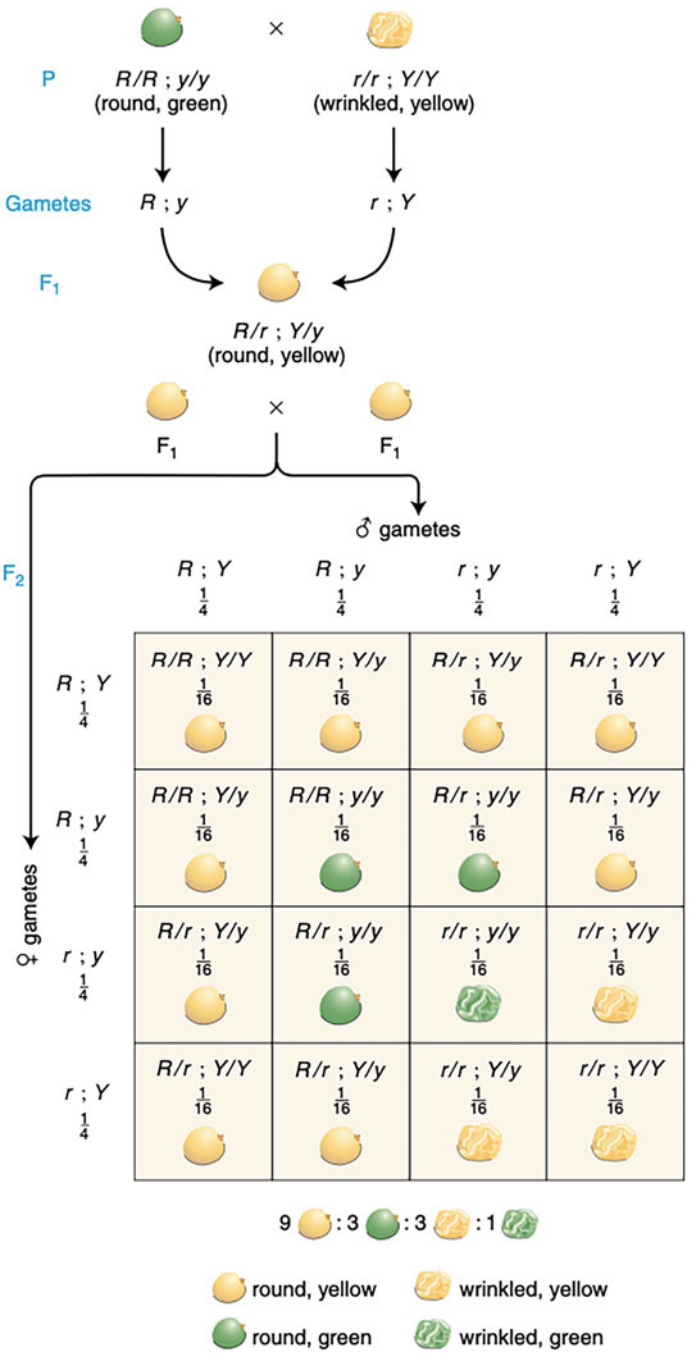

Fig. 2.7 Genotypes of progeny obtained from a dihybrid cross for wrinkled yellow seeds with round green seeds: The F1 progeny has yellow round seeds and can produce four types of gametes. These are shown on the top row and first left column of the square on the right. The various combinations of these gametes give a progeny with a phenotypic ratio of 9:3:3:1 (Griffiths et al. 2011)

2.2.2 Test Cross: Two Characters

Similar to the monohybrid cross, Mendel performed test cross for the dihybrid cross to verify his conclusions. As stated above, test cross involves crossing a plant of unknown genotype with a plant homozygous recessive for the traits under consideration. If we were to perform a test cross for the F1 produced from the dihybrid cross above, the tester (homozygous recessive individual) would be a plant having green and wrinkled seeds. In this case, we would expect the F1 to form gametes with all the combinations, RY, Ry, rY and ry, according to the law of independent assortment. This when fertilised with ry gametes from the tester would give the following different combinations: RrYy (round, yellow), Rryy (round, green), rrYy (wrinkled, yellow) and rryy (wrinkled, green). A phenotypic ratio of 1:1:1:1 would be expected. This is the result that Mendel obtained from his test cross for two characters providing evidence for his law of independent assortment.

The F1 hybrid obtained above is heterozygous for both traits. Let us see how the results would differ if the plant being tested was homozygous for either of the traits. If we take a plant having yellow, round seeds, its genotype could be either YyRr (discussed above), YYRr, YyRR or YYRR. If it is YYRR, it will produce only one type of gamete YR which when crossed with yr will produce all plants of YyRr genotype and single phenotype of yellow round seeds. If it is YYRr, two types of gametes will be produced: YR and Yr. This will give two phenotypes on test cross: yellow, round seeds (YyRr) and yellow, wrinkled seeds (Yyrr) in a ratio of 1:1. Similarly, if the genotype is YyRR, it will produce two phenotypes on performing test cross: yellow, round seeds (YyRr) and green, round seeds (yyRr) in a ratio of 1:1. In this manner, we can detect the genotype of the individual based on the number and ratio of phenotypes produced (Fig. 2.8).

2.3 Mendelian Trihybrid Cross

Mendel's laws can be extended to obtain genotypic and phenotypic ratio of a trihybrid cross, i.e. a cross between plants differing in three traits. As the number of traits increase, the complexity of the ratio increases and several different methods can be used to predict the results. Let us take the example of three genes A, B and C having recessive alleles a, b and c. If a homozygous dominant plant with genotype AABBCC is crossed with a homozygous recessive plant aabbcc, the F1 hybrid will have a genotype of AaBbCc. When the F1 plant is further self-pollinated, it will produce eight different gametes as shown below (Fig. 2.9).

2.4 Application of Mendelian Principles

Mendel's principles of segregation, dominance and independent assortment can be applied to analyse inheritance of multiple traits. Various methods like Punnett square, forked line method or probability method can be utilised to obtain

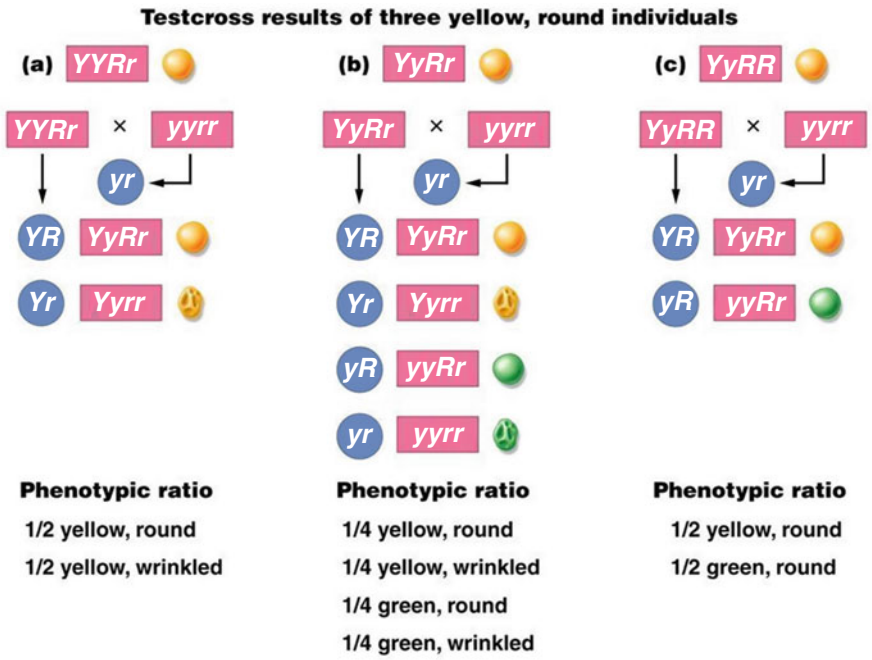

Fig. 2.8 Test cross for a dihybrid cross can reveal the parental genotype: Yellow round seeds with three different genotypes (YYRr, YyRr and YyRR) give rise to different phenotypes and phenotypic ratios in the progeny when subjected to a test cross. This allows us to estimate the genotype of the unknown individual. (Adapted from Klug et al. 2012)

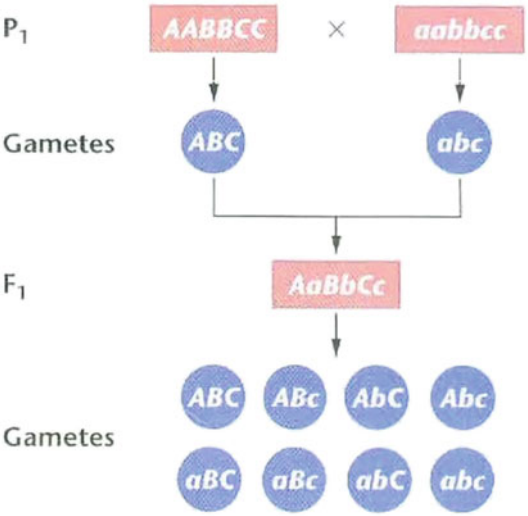

Fig. 2.9 Gametes produced during a trihybrid cross: Eight different gamete combinations of alleles and therefore eight different types of gametes may be produced by an individual heterozygous for three gene pairs (Klug et al. 2012)

phenotypic and genotypic ratios of cross between multiple traits. We will obtain the genotypic ratios for the trihybrid cross mentioned above using both Punnett square and forked line method.

2.4.1 The Punnett Square Method

In the Punnett square method, a grid is created with the gametes from one parent on the upper side and those from the other parent on the left side. Each cell or block within the grid contains a combination of alleles from both parents giving the genotype of the offspring resulting from the combination of the respective gametes. It is named after Reginald C Punnett who devised this method. It is a tabular representation of all possible combinations between the maternal and paternal alleles.

In the image below, gametes produced from F1 hybrid of a trihybrid cross are represented on the top row and left column of the grid. Since this is a self-pollination, the gametes on both sides are identical. In case the parents differ in genotype, gametes from one parent will be in the top row and will differ from gametes produced by the other parent which will be on the left column. The first box in the grid shows the genotype AABBCC which is produced if the gametes ABC and ABC (corresponding parental gametes in first row and first column) were to combine. In this manner, all possible genotypes of the progeny can be represented in the Punnett square in a simplified manner. The genotypes showing the same phenotypes are represented by the same colour of the cell in the grid. From this, we can infer that the phenotypic ratio of a trihybrid cross will be 27:9:9:9:3:3:3:1 (Fig. 2.10).

AaBbCc / AaBbCc	ABC	ABc	AbC	aBC	Abc	aBc	abC	abc
ABC	AABBCC	AABBCc	AABbCC	AaBBCC	AABbCc	AaBBCc	AaBbCC	AaBbCc
ABc	AABBCc	AABBcc	AABbCc	AaBBCc	AaBbcc	AaBBcc	AaBbCc	AaBbcc
AbC	AABbCC	AABbCc	AAbbCC	AaBbCC	AAbbCc	AaBbCc	AabbCC	AabbCc
aBC	AaBBCC	AaBBCc	AaBbCC	aaBBCC	AaBbCc	aaBBCc	aaBbCC	aaBbCc
Abc	AABbCc	AABbcc	AAbbCc	AaBbCc	AAbbcc	AaBbcc	AabbCc	Aabbcc
aBc	AaBBCc	AaBBcc	AaBbCc	aaBBCc	AaBbcc	aaBBcc	aaBbCc	aaBbcc
abC	AaBbCC	AaBbCc	AabbCC	aaBbCC	AabbCc	aaBbCc	aabbCC	aabbCc
abc	AaBbCc	AaBbcc	AabbCc	aaBbCc	Aabbcc	aaBbcc	aabbCc	aabbcc

Fig. 2.10 Punnett square showing genotypes produced from a trihybrid cross: The genotypic and phenotypic ratio for a cross between individuals of genotype AaBbCc and AaBbCc is shown in the Punnett square above. The gametes produced from each parent are shown in the top row and left column. Each grid represents a combination of the gametes in the respective row and column. The individuals showing the same phenotype are in the same colour. We can see that a phenotypic ratio of 27:9:9:9:3:3:3:1 is obtained

2.4.2 The Forked Line Method

Although useful, Punnett square can be too cumbersome to use when more than three traits are being analysed. In such instances, forked line method, also called the branch diagram, can be very useful. In this method, the genotypic or phenotypic outcome for one gene pair is first predicted. Then, outcome of the next gene pair is computed in conjunction with earlier gene pair. This method is followed for all the remaining gene pairs. In the figure below, phenotypic ratio of trihybrid cross is predicted using the forked line method. Here, three traits are considered: round vs wrinkled seeds, green vs yellow seeds and grey-brown vs white seed coat. We know the dominant phenotype for the other traits except seed coat where grey-brown is dominant over white. According to Mendel's law of segregation and random fertilisation, a monohybrid cross between round and wrinkled seeds will result in 3/4 seeds being round and the remaining 1/4 being wrinkled. This is the outcome for the first trait. Now, of the round seeds, 3/4 seeds would be yellow and 1/4 green, which is the outcome for the second trait. Similarly, 3/4 of the wrinkled seeds will be yellow and 1/4 green. Next, of the round and yellow seeds, 3/4 will have grey-brown coat and 1/4 will have a white coat. This gives us a total of 27 round, yellow and grey-white coat seeds (3/4 round $\times$ 3/4 yellow $\times$ 3/4 grey-brown coat = 27/64). Similarly, proportion of round, yellow and white seed coat will be 3/4 $\times$ 3/4 $\times$ 1/4 = 9/16. We can calculate the proportion for all phenotypes in this manner and obtain a phenotypic ratio of 27:9:9:9:3:3:3:1.

Calculations for genotypic ratio can be done in a similar manner. Although the phenotypic ratio for a monohybrid cross is 3:1, we need to bear in mind that the genotypic ratio is 1:2:1 (1 AA, 2 Aa and 1 aa). The genotypic calculation for a trihybrid cross using forked line method is illustrated below. There are a few rules of thumb that can be used to cross-check your calculations. First, count the number of heterozygous gene pairs in the cross. In a cross AaBbCc X AaBbCc, heterozygous gene pairs 3. 2^n will be the number of different gametes that can be produced from each parent. 3^n will be the number of different genotypes produced after fertilisation, and 2^n will be the number of different phenotypes produced in this cross. In the above example, therefore, $2^3 = 8$ different gametes are formed from each parent, $3^3 = 27$ different genotypes are produced in this cross and it gives rise to $2^3 = 8$ different phenotypes. These are the numbers that we get from our calculations with the forked line method as well as Punnett square method (Fig. 2.11).

2.4.3 The Probability Method

Probability is a mathematical tool that predicts the likelihood of occurrence of an event.

We can easily use probability to predict the outcome of a genetic cross. Probability can be calculated as the number of times an event occurs divided by the total number of events. For example, the probability of picking a queen of hearts from a deck of cards would be 1/52. This is because there is only one queen of hearts (one

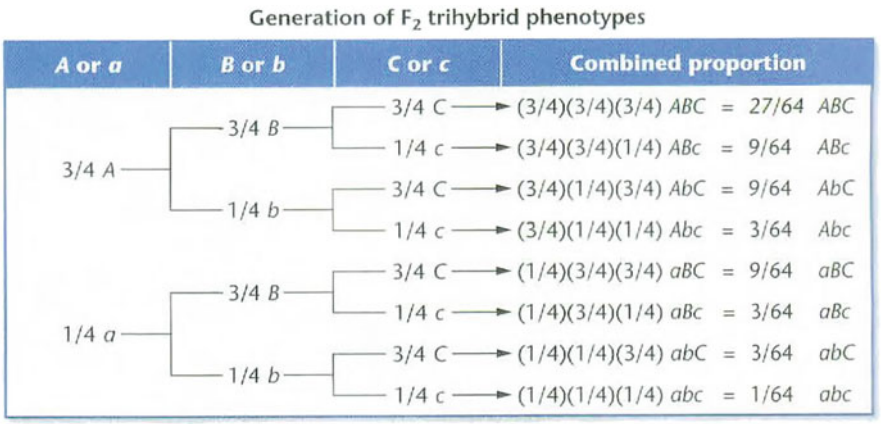

Fig. 2.11 Forked line method for obtaining genotypic and phenotypic ratios in a trihybrid cross: For a monohybrid cross, genotypic ratios of 1:2:1 are obtained. Then for each of the genotypes, the ratio for the next set of genes is calculated and further on and the proportions multiplied at the end. Similarly, phenotypic ratios can be calculated by multiplying 3:1 ratios for each trait (Klug et al. 2012)

event) in a deck of 52 playing cards (all possible events). If, however, we were to calculate the probability that a card picked would be any queen, this probability would be 4/52 as there are four queen cards in a deck. There are two rules to be followed for the calculation of slightly more complicated probabilities.

1. **Multiplication rule**: The probabilities of co-occurrence of two or more events can be calculated by the product of their individual probabilities. This rule can only be used if the events are occurring independent of each other. If the occurrence of one event affects the probability of occurrence of the next event, then their combined occurrence cannot be a simple product of their independent probabilities. For example, the probability that two consecutive rolls of a dice are a three and a six in that order is $1/6 \times 1/6 = 1/36$. The individual probabilities of a three and a six in a roll of dice are 1/6 and 1/6, respectively. Their product will give the probabilities of their co-occurrence. We have already used the multiplication rule while calculating the dihybrid and trihybrid cross ratio in the branch diagram or forked line method. Multiplication rule is applied when the word *and* is used. In the above example, we wanted to find the probability of 'a three *and* a six in a roll of dice'.
2. **Addition rule**: We can calculate the probability of either of two or more mutually exclusive events occurring together by the addition rule. For example, if we just wanted to obtain the probability of occurrence of a three or a six in a roll of the dice, we would find the sum of individual probabilities. Thus, the probability is $1/6 + 1/6 = 2/6 = 1/3$.

The multiplication and addition rules can be used in predicting the outcome of genetic crosses instead of Punnett square or forked line method. Let us consider the cross between two plants having round seeds with genotypes Rr and Rr. The probability of wrinkled seeds can be calculated using multiplication rule. The probability of obtaining r allele from one parent is 1/2 and from the other parent is also 1/2. For a wrinkled seed, the genotype needs to be rr and its corresponding probability is $1/2 \times 1/2 = 1/4$. If we were to calculate probability of round seeds, both multiplication and addition rules need to be used. Round seeds can occur because of three genotypes: RR, Rr and rR. Their individual probabilities are as follows:

$$1/2\ \mathrm{R} \times 1/2\ \mathrm{R} = 1/4\ \mathrm{RR}$$

$$1/2\ \mathrm{R} \times 1/2\ \mathrm{r} = 1/4\ \mathrm{Rr}$$

$$1/2\ \mathrm{r} \times 1/2\ \mathrm{R} = 1/4\ \mathrm{rR}$$

Their combined probabilities would therefore be 1/4 + 1/4 + 1/4 = 3/4 round seeds. It is easier to use probability method for calculation of complex crosses with multiple traits as compared to Punnett square or forked line method.

2.4.3.1 Binomial Theorem

Binomial theory can be utilised when we want to calculate the occurrence of a specific set of outcomes among a large number of potential events. Let us consider the case of galactosemia. In this disorder, mutation in one of the galactose metabolising genes prevents the individual from converting galactose to glucose. The affected individual may show symptoms like lethargy, failure to gain weight and liver damage. This disorder can only occur if both copies of the gene are mutated. Thus, the parents of a child may each be carrying one mutated allele and not express the disorder. Only if the child inherits both mutated copies will he/she be affected. Let us assume that the gene affected here is a and its wild-type/normal allele is A. If we now want to find the probability that both kids of heterozygous parents are affected. The probability of one child being affected (aa) is 1/4 (Aa X Aa = 1/4 aa, 3/4 A-) and that of two children being affected will be $1/4 \times 1/4 = 1/16$.

Now, if we suppose the couple has three children and we want to find the probability that two children are unaffected and one child is affected. There are three scenarios in which this is possible:

$$1/4 \times 3/4 \times 3/4 = 9/64\ (\text{child 1 affected, other 2 unaffected})$$

$$3/4 \times 1/4 \times 3/4 = 9/64\ (\text{child 1 and 3 unaffected, child 2 affected})$$

$$3/4 \times 3/4 \times 1/4 = 9/64\ (\text{child 1 and 2 unaffected, child 3 affected})$$

$$\text{Total probability} = 9/64 + 9/64 + 9/64 = 27/64$$

This calculation becomes more complex for situations with more number of children and multiple different combinations. If we want to find the probability of this couple having five children, three of whom are affected and the remaining two are not, we can use the binomial expression. The binomial expression is of the form $(a + b)^n$ where a and b are probabilities of two alternate events and n is the number of times the event occurs. In the above case, we can define a as the probability that the child suffers from galactosemia (1/4), while b is the probability that the child remains unaffected (3/4). n here is the number of children which will be 5. The binomial can be expanded as follows:

$$(a+b)^5 = a^5 + 5a^4b + 10a^3b^2 + 10a^2b^3 + 5ab^4 + b^5$$

It follows the rule:

$$(a+b)^n = a^n + a^{n-1}b + a^{n-2}b^2 + a^{n-3}b^3 + \ldots.b^n$$

The expansion of $(a + b)^n$ consists of $n + 1$ terms. Each of these terms has a numerical coefficient. The coefficient of the first term is always 1. The second term has the coefficient same as the power to which the binomial is raised. So, in this case, it is 5. For the next coefficient, multiply the coefficient of the previous term with the exponent of a in that term. Divide this by the number of the term in the equation. Thus, to calculate the coefficient for a^3b^2 in the above expansion, $(5 \times 4)/2 = 10$ where coefficient of earlier term is 5 and exponent of a in that term is 4 and it is the second term in that equation. Similarly for the coefficient of a^2b^3, it can be calculated as $(10 \times 3)/3 = 10$. We can calculate the coefficients for the rest of the terms and expand the binomial.

Another method to calculate the coefficients in the equation is to use Pascal's triangle. We can determine the coefficients for each term in the binomial expression from the terms in front of the corresponding n. Notice that all terms other than 1 in Pascal's triangle are the sum of terms directly above them (Fig. 2.12).

Once we have the equation, we can obtain the probability of any combination of events by simply inserting the values of a and b. For example, to obtain the probability of three out of five children having galactosemia, the term we use is $10a^3b^2$:

$$10a^3b^2 = 10 \times (1/4)^3 \times (3/4)^2 = 90/1024 = 0.087$$

In this manner, we can easily calculate the probability of any combination of events. There is another method to do the above calculation. It uses the formula:

$$P = \frac{n!}{s!t!} \times a^s b^t$$

P is the overall probability of co-occurrence of two events X and Y. Event X has a probability of a occurring s times, while event Y has probability b occurring t times.

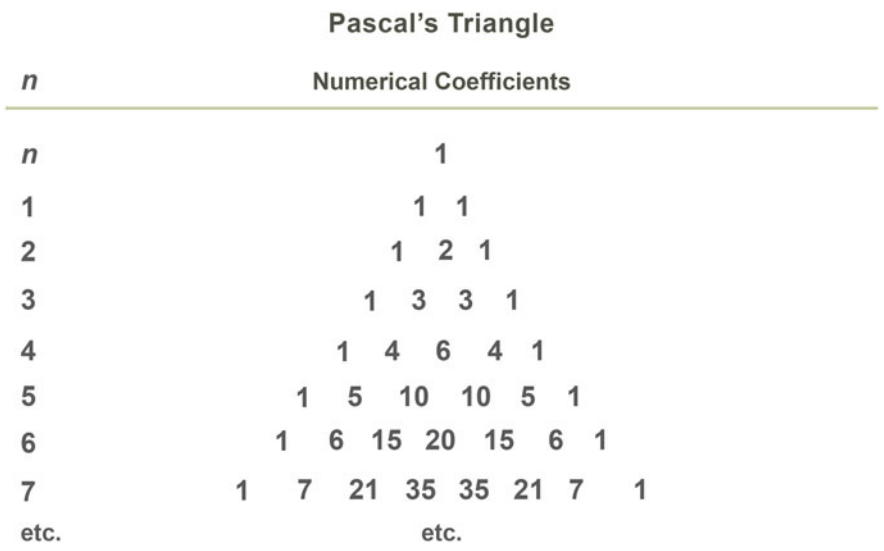

Fig. 2.12 Pascal's triangle: Pascal's triangle can be used to obtain coefficients for terms in the binomial expansion for any n. The terms other than 1 in Pascal's triangle are a sum of the terms directly above them

In the above case, X is the probability that the child is affected. Therefore, a is 1/4 and s is 3. Event Y is the probability that the child is unaffected. Here, b is 3/4 and t is 2. N is the total number of events which is 5 in this case. The symbol ! is for a factorial which is the product of all positive integers from 1 to n. For example, $5! = 5 \times 4 \times 3 \times 2 \times 1$.

The calculation therefore is:

$$P = \frac{5!}{3!2!} \times (¼)^3 (¾)^2$$
$$= \frac{5 \times 4 \times 3 \times 2 \times 1}{(2 \times 1)(3 \times 2 \times 1)} \times (¼)^3 (¾)^2$$
$$= 0.087$$

This value is the same as that obtained from binomial theorem.

2.5 Test of Genetic Hypothesis

Crosses between two individuals of known genotypes yield a certain genotypic and phenotypic ratio. Based on Mendel's laws, we can predict a certain ratio. However, the experimental ratios may not match the expected values. Other than technical difficulties (like death of plants before the phenotype can be observed), chance plays a very important role in this deviation. This is easily illustrated with the example of a coin flip. We know that the probability of getting a heads or tails in a coin flip is 1:1.

If we do the coin toss for a large number of times, say 1000, we can expect that we will get a number close to 1:1. However, if we toss the coin only ten times, we might get seven heads and three tails or two heads and eight tails. This deviation from expected ratio is just a chance event.

Genetic ratios however can also be different, if there is some linkage between the traits being studied or if the gene is following some non-Mendelian pattern of inheritance. An experimenter needs to know if the deviation from expected ratios is just a matter of chance or it is of some biological significance. In such cases, we can make use of a chi-square test.

2.5.1 The Chi-Square Test

Chi-square test, also written as χ^2 test, is used to evaluate how well the observations support the null hypothesis. It is calculated from the sum of squared errors or sample variance. A chi-square test can only tell us if the resulting ratio of genetic crosses is deviating from the expected ratio merely due to chance. It cannot tell us if there is a mistake during crossing or during calculation of expected ratios or there are some complex inheritance patterns involved. In other words, it gives us a probability that the difference in observed and expected ratio can be due to chance alone.

Let us take an example to understand how to use the chi-square test. A monohybrid cross between two tall plants resulted in a progeny of 100 tall plants and 40 short plants. If we were to assume that the genes involved followed a Mendelian inheritance pattern, we would expect a ratio of 3 tall:1 dwarf plants. For a total of 140 plants, $3/4 \times 140 = 105$ plants should be tall and $1/4 \times 140 = 35$ plants should be short. We see that the observed ratio differs slightly from the expected ratio. Is this merely an effect of chance?

We start by establishing a null hypothesis (H_o). The null hypothesis is called so because it assumes that there is no real difference between our expected and observed outcomes and any deviation is a result of chance events. Through the probability derived from the chi-square test, we can then accept or reject the null hypothesis. Our null hypothesis for this example will be that the inheritance follows a ratio of 3:1. The formula for chi-square test is:

$$\chi^2 = \Sigma \frac{(O-E)^2}{E}$$

where E = expected value for that category

O = observed value for that category

Σ = sum of calculated values for all categories

Plugging in the above values in this equation:

$$\chi^2 = \frac{(100-105)^2}{105} + \frac{(40-35)^2}{35}$$
$$= 25/105 + 25/35$$
$$= 0.238 + 0.714 = 0.952$$

After this calculation, we have to determine the degrees of freedom (df) which is $n-1$ where n is the number of different categories the value may fall into. Here, the plant can be tall or short. Thus, $n = 2$ and df $= 1$. Degrees of freedom are considered because more categories introduce more deviation in the results. We now have to interpret the χ^2 value in terms of its corresponding probability value. This calculation is very complex, and we instead make use of a standard table which provides probability values for different χ^2 values for each degree of freedom.

In the table below (Fig. 2.13), we can see that the calculated value of 0.952 for df 1 lies between p value of 0.5 and 0.1. We can interpret this as the probability that the observed deviation from expected value is due to chance is between 10 and 50%. Traditionally, scientists have accepted a p value cut-off of 0.05. That is to say that if the p value is above 0.05, we can accept the null hypothesis. If the p value is less than 0.05, it means that the probability that the deviation is due to chance is less than 5%. In this case, the null hypothesis is rejected. In our example, we can accept the null hypothesis and conclude that the variation seen in the ratios is a product of chance and that the inheritance indeed follows a 3:1 ratio.

Table 3.4 Critical values of the χ^2 distribution

	P								
df	.995	.975	.9	.5	.1	.05	.025	.01	.005
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801

P, probability; df, degrees of freedom.

Fig. 2.13 Probability values for χ^2 distribution: Figure giving probability values for estimated χ^2 values at different degrees of freedom. The probability value keeps decreasing towards the right, while the χ^2 values keep increasing

Let us take another example. In a cross between plants having violet flowers and white flowers, violet flowers were observed in F1. On self-fertilisation, it was seen that 790 of the progeny had violet flowers and 210 had white flowers. Can we ascertain if this follows the Mendelian pattern of inheritance?

Null hypothesis—The pattern of inheritance follows Mendelian genetics and does not differ from a ratio of 3:1:

$$\chi^2 = \Sigma \frac{(O-E)^2}{E}$$

E: If the flower colour inheritance followed Mendelian genetics, we would see that 3/4 of the total flowers would be violet and 1/4 would be white since violet is the dominant character (because F1 flowers were violet). The expected numbers would therefore be:

Violet $3/4 \times 1000 = 750$, white $1/4 \times 1000 = 250$

$$\chi^2 = \frac{(790-750)^2}{750} + \frac{(210-250)^2}{250}$$
$$= 2.133 + 6.4 = 8.533$$

Degree of freedom here too is 1, as only two characters are being observed. *P* value for χ^2 8.533 at df 1 is less than 0.005

We can therefore reject the null hypothesis. The probability that deviation in ratio is purely due to chance is very less and the gene is probably following some non-Mendelian pattern of inheritance.

2.6 Application of Mendelian Principles in Human Genetics

Mendel's work has shed light on the inheritance of genes and traits. We can use this knowledge to analyse inheritance of various genetic diseases and traits in humans too. We can do this by obtaining information regarding occurrence of the trait being studied in the family of the affected individual.

2.6.1 Pedigree Analysis

Pedigree analysis is similar to a family tree for a specific trait. It is basically a chart which illustrates which family members have the traits being studied. This aids in understanding the method of inheritance of the trait. We can also predict the possible genotype of individuals for that trait which can help in predicting probability of inheritance of the trait in future generations.

A set of standardised symbols are used for illustrating a pedigree. Squares represent males, and circles represent females. A shaded box denotes individuals that expressed the phenotype being studied. A horizontal line between two

individuals denotes mating. Their progeny are represented in the order of birth on a horizontal line connected to the parental mating line. Different generations are represented on descending levels. A double line connecting two individuals denotes consanguineous marriage. A marriage between second cousins or even more closely related individuals is referred to as a consanguineous marriage. Many studies have shown that consanguinity is one of the major contributors of birth defects and abnormalities. If an individual has a recessive gene, his progeny might inherit the gene but not express the phenotype. Thus, individuals belonging to the same family have a greater probability of carrying the recessive gene. A marriage between members of a family increases the probability of a child from this union inheriting two copies of a recessive gene and therefore suffering from a genetic disorder inherited in an autosomal recessive manner. Although consanguineous marriages have reduced over the years, they are still prevalent in the Middle East and parts of Asia and Africa. More symbols and their meanings are given in Fig. 2.14.

Let us examine the pedigree shown in Fig. 2.15. Individual 4 from generation III is the proband. This means that this individual was the first to be investigated for this

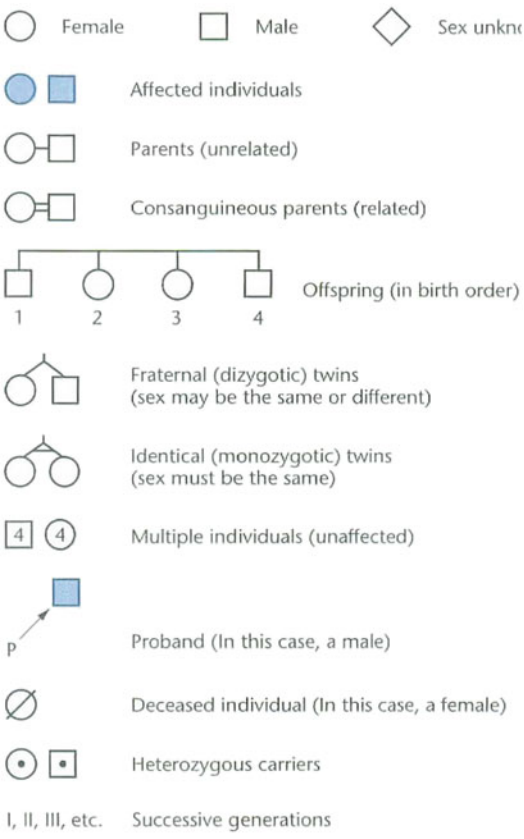

Fig. 2.14 Standard symbols used while drawing a pedigree chart: Proband denotes the first individual studied in the family for this trait. Consanguineous marriage means marriage between individuals from the same family, usually first cousins (Klug et al. 2012)

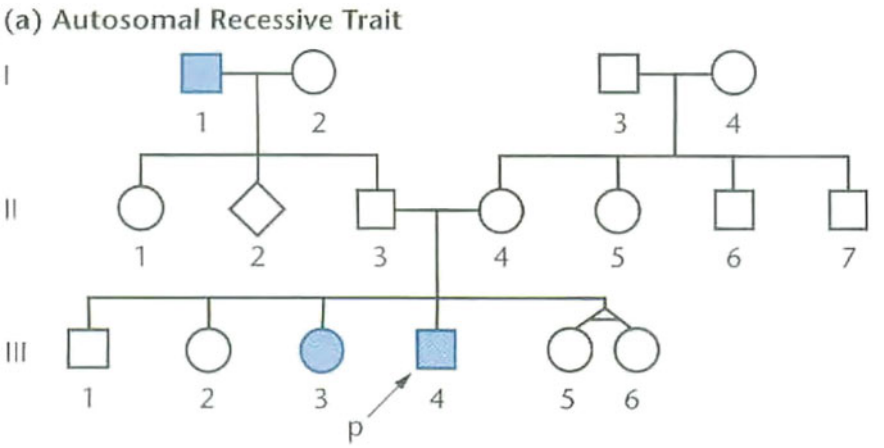

Fig. 2.15 Pedigree showing autosomal recessive mode of inheritance: The pedigree shown above shows that the character has skipped a generation. Fewer members of the family are affected and the trait is evenly distributed between males and females. Based on this, we can conclude that the mode of inheritance of the gene being studied is autosomal recessive (Klug et al. 2012)

phenotype and prompted construction of the pedigree. We can see that one of the siblings of individual 4 is also affected. None of the parental generation (Gen II) has any affected members. Among the grandparental generation (Gen I), individual 1 is affected. We can draw a few conclusions from this information. First is that the trait being studied is recessive. Based on Mendel's law of dominance, if the trait was dominant, at least one parent of the affected individual would have expressed the trait. Since none of the parents show the trait, they are most likely carriers of the recessive allele. This skipping of generation in expression of traits is a characteristic feature of recessive traits. Second, although there aren't many affected individuals, the trait seems to be passed equally between males and females (Gen III individuals). We can therefore assume that the recessive trait is on an autosome. Of the 23 pairs of chromosomes that are present in humans, 22 are autosomes and 1 pair is a sex chromosome (X and Y chromosomes). This means that the 22 pairs are inherited randomly between males and females. However, the sex chromosomes determine the sex of the individual. In humans, XX determines a female and XY determines males. Therefore, the inheritance of traits present on sex chromosomes will not follow Mendelian patterns and instead show different ratios for males and females. For example, genes on Y chromosomes will only be passed on to males and not to the females. The probability of inheritance of a mutated gene between males and females remains the same for an autosomal disorder irrespective of which parent carries the mutated gene. In case of recessive disorders, however, the probability of inheritance of mutated gene between the sons and daughters will differ based on whether the father or the mother is carrying the mutated gene. We will discuss this further in the next chapter. For the context of this discussion, it is enough to

understand that any trait which seems to be passed equally between males and females is most likely present on an autosome.

2.6.2 Mendelian Segregation

We can also deduce from this pedigree that either individual I-3 or I-4 was heterozygous for the allele being studied. For individual III-4 to be affected, both his parents need to be heterozygous for the allele in question. Based on Mendel's law of segregation, for individual III-4 to be homozygous recessive, he has to inherit one recessive allele from each of his parents. Individual II-3 could have obtained the recessive trait from individual I-1 since he was affected. For individual II-4 to be a carrier, either individual I-3 or I-4 would have to be a carrier as they do not show the phenotype. We can determine the pattern of inheritance and composition of the genotype of an individual from a pedigree based on Mendel's laws of dominance and segregation.

Some examples of autosomal recessive disorders are cystic fibrosis, sickle cell anaemia and Tay-Sachs disease. Cystic fibrosis is caused by a defect in both copies of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The bodily fluids become thick and sticky. Due to this, the individuals suffer from respiratory and digestive problems. The abnormal mucous clogs airways and damages the pancreas. Tay-Sachs disease is a progressive neuronal disorder that affects the neurons in the brain and spinal cord. It is a rare disease in which infants start showing symptoms after 3–6 months. Their development slows and they develop muscle weakness. Progression of disease leads to loss of hearing, paralysis and seizures. The disease is caused due to two defective copies of the *HEXA* gene. This gene codes for the hexosaminidase A enzyme that plays a role in the breakdown of a fatty substance called GM2 ganglioside. Build of GM2 ganglioside is toxic for the neurons.

Let us now take the example of an autosomal dominant disorder. A typical pedigree is shown in Fig. 2.16 for inheritance of an autosomal dominant trait. We can immediately observe that this pedigree has at least one affected member in each generation. This is a typical characteristic of inheritance of a dominant allele. We can also see that the disorder has been passed on to both the males and the females. We can therefore infer that the allele is present on the autosomes. An example of autosomal dominant disorder is the Marfan syndrome. Marfan syndrome affects the connective tissue due to which a number of abnormalities in the heart, bones, joints, eyes and blood vessels can be observed. Marfan syndrome patients are tall and slender with long narrow faces. Their arm span exceeds their body height and they have elongated fingers and toes. Marfan syndrome is caused by a mutation in the *FBN1* gene which codes for the fibrillin 1 protein. Fibrillin 1 is instrumental in the formation of microfibrils. Microfibrils are threadlike filaments that provide strength and flexibility to the connective tissue. They also bind to growth factors

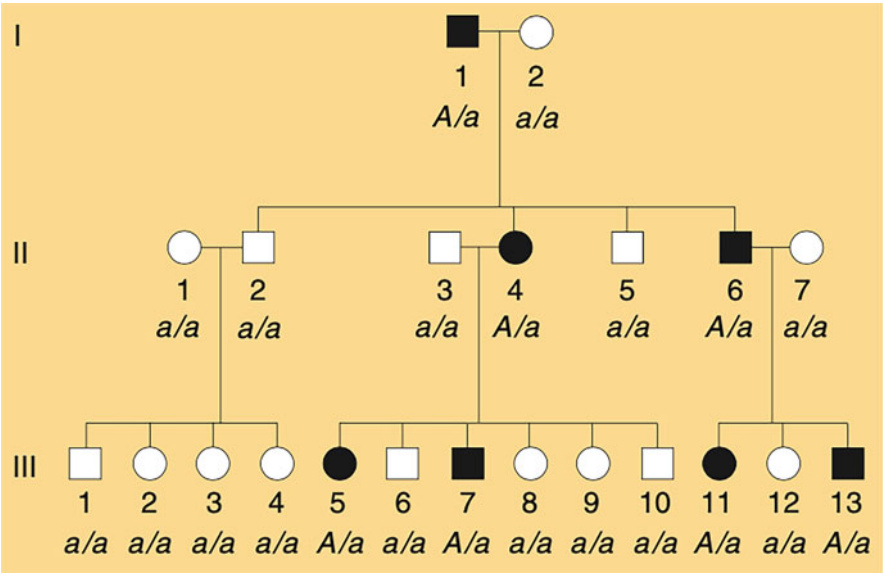

Fig. 2.16 Pedigree showing autosomal dominant mode of inheritance: The pedigree shown above has an affected member in each generation. A number of members of the family are affected and the trait is evenly distributed between males and females. Due to this, we can assume that the mode of inheritance of the trait being studied is autosomal dominant (Griffiths et al. 2011)

and control their release. Absence of functional fibrillin 1 reduces the amount of microfibrils leading to lack of control in the availability of growth factors. An excessive amount of available growth factors leads to overgrowth and abnormal tissue formation. Being an autosomal dominant disorder, the presence of even one mutated allele is sufficient for the manifestation of this disease.

Neurofibromatosis type 1 is also an autosomal dominant disorder. It is associated with a range of symptoms. Individuals suffering from the disease show a pigmentation change with appearance of dark patches of the skin. Benign tumours (non-cancerous) grow along the nerves in the brain and other parts of the body. In some cases, these tumours may turn cancerous. Additionally, these individuals may suffer from hypertension, macrocephaly, skeletal abnormalities and abnormal curvature of the spine. Some affected individuals may develop learning disabilities or attention deficit/hyperactivity disorder (ADHD). Neurofibromatosis type 1 is caused due to mutations in the *NF1* gene which codes for a protein called neurofibromin. This protein is produced in the neurons as well as glial cells like oligodendrocytes and Schwann cells. Neurofibromin acts as a brake for cell division and is known as a tumour suppressing gene. Non-functional neurofibromin leads to lifting of this brake and rampant and uncontrolled cell division leading to formation of tumours as seen in the disease.

Observations of the pedigree charts given above will make it clear that we do not always see expected Mendelian ratios in these inheritances. This is mainly because we do not have a large number of progeny which can be observed to reach the expected ratio. The inheritance of gametes is dependent on chance, and as discussed in chi-square analysis, we can see vastly different ratios than expected for a small sample size. The second factor is that in a population, some alleles are more commonly found than others. Most people are carriers of the rare allele and very few are homozygous for the rare allele. Thus, mating usually happens between individuals who are either heterozygous or homozygous for the most common allele, making the appearance of individuals homozygous for the rarer allele very uncommon.

2.6.3 Genetic Counselling

Pedigree analysis can also be used to predict the probability of the progeny inheriting a certain trait or disease. Couples having certain disorders running in the family or who themselves are affected may wish to know the probability of their children inheriting the disease. Couples with one of their children affected with a genetic disorder may seek to understand the possibility of their next child having the disease. Genetic counselling may help in such situations. Genetic counsellors will obtain information from the couples about affected family members and draw a pedigree. From this, they can deduce the mode of inheritance and further calculate the possibility of their unborn offspring inheriting the disorder. They can provide information and educate as well as address concerns of the family members regarding the disorders and provide support. They can also inform individuals about their genetic predisposition to certain diseases and lifestyle changes if any that can prevent or manage the disorder.

The Human Genome Project completed in 2003 was a 13-year-long study aimed at sequencing the entire human genome. This sequencing was carried out at multiple labs around the world and DNA was taken from a number of donors. The sequence is therefore a mosaic and not from any one individual. This prompted the 100,000 genome project in the UK which aims at sequencing 100,000 individuals comprising people with rare diseases, their families and cancer patients. With the mapping of these genomes, we can hope to understand more and more about our genes and the functions that they play in health and disease. We may be able to pinpoint the causes of a number of genetic diseases which remain unknown till now. Genomic sequences from patients will aid in developing diagnostics and therapeutics for individuals suffering from Mendelian disorders. It may allow us to get closer to personalised medicine where the analysis of an individual's genome may provide clues as to what treatment would be most effective for the individual.

Box 2.1 Scientific Concept: Gregor Mendel's Genetic Experiments: A Statistical Analysis After 150 Years (Jan Kalina)

Gregor Mendel is not only regarded as the founder of genetics but also as one of the pioneers of applying statistical principles in their experiments. Mendel's parents were peasants and bred fruit trees. After completing his secondary education, Mendel joined the St Thomas Augustinian abbey where he was ordained as a priest after some years. The abbey fostered an interest in science and encouraged Mendel to carry out his plant breeding experiments for which he was also given the use of one of the greenhouses in the abbey. In 1866, Mendel published his landmark paper which defined basic laws of genetics. He could not, however, enjoy the recognition of his work in his lifetime. Mendel passed away in 1884, aged 61 years, due to chronic nephritis. His work was rediscovered in 1900 independently by three scientists. In 1936, Robert Fisher reanalysed Mendel's data in light of the new statistical methods available. After a detailed analysis of the data, he concluded that Mendel's data seemed to be too much in agreement with his theoretical expectations. Given the variation caused by chance, his data seemed 'too good to be true'. He therefore concluded that Mendel must have falsified most, if not all, of his data to fit his theoretical assumptions (Kalina 2016).

Since then, a large number of papers have come out analysing both sides of the debate. In 2008, many scientists from different fields came together to write a review on the subject hoping to put an end to the argument. The authors were able to refute all arguments except the too good to be true claim for the data. There were few Mendel supporters like Pilgrim, who found fault with the way in which Fisher had performed his analysis (Pilgrim 1986). However, his arguments were later refuted by Edward (Edwards 1986). Then, there was a group of people who believed Fisher's analysis but found it too stringent and sought ways to analyse the data in an alternative manner. They too however found that Mendel might have adjusted the data to suit his hypothesis. Then there are those who believe that Fisher's analysis holds true given the assumptions. However, they try to provide suggestions for explaining the high p values other than deliberate malpractice. Some of the explanations provided are stopping experiments when the results seem to be good, error carried out by some anonymous assistant and discarding plants due to suspicion of some errors like pollen contamination and data selection for presentation (Novitski 2004).

In spite of the range of explanations provided, the bias towards expected values seen in Mendel's data has not been completely resolved. New statistical models to explain the bias are being proposed to provide a satisfactory explanation and end the controversy regarding falsification in Mendel's data. Based on the distribution of p values observed in Mendel's data and different simulation approaches, a plausible explanation of a two-stage model has been

(continued)

Box 2.1 (continued)
put forth. It states that Mendel repeated some of his experiments, presumably those which showed the largest deviation from expected values. He then reported only the best of two or a combination of results of the two which would result in values closer to those that were expected. This is called as a two-stage model where experiments were performed and results evaluated in second stage again for those experiments which did not meet the distribution. This speaks of an unconscious bias that Mendel introduced in his experimental approach but cannot be called as intentional scientific fraud (Pires and Branco 2010; Kalina 2016). Mendel's laws have stood the test of time and are still accepted today. His data might have been biased or adjusted but his conclusions about the nature of genes and their inheritance are still relevant today. We must therefore focus on Mendel's contribution to the field of genetics and quantitative biology and put an end to the debate as we seem to have some possible explanations.

2.7 Summary

- Gregor Mendel's painstaking decade long experiments and theories derived from them have laid the foundation of genetics, and he is known as the father of genetics. Mendel's three laws of genetics provide a framework for understanding the inheritance of genes. His work was rediscovered independently by three botanists in 1900.
- In monohybrid crosses, plants which differed in only one trait were crossed. The F1 hybrids carry the alleles for both parental traits but only express one of the parental traits which was termed as the dominant trait. The parental trait that was not expressed in the F1 hybrid was termed as the recessive trait. This was called as the law of dominance.
- The F1 hybrid produces gametes possessing the dominant and recessive alleles with equal probability. These can get paired randomly in F2 generation. This was called the law of segregation of alleles.
- Mendel also carried out dihybrid crosses where he crossed plants differing in two traits. He observed that the traits were inherited independently of each other. This was referred to as the law of independent assortment.
- He developed the method of test cross to determine the genotype of plants. Plants showing a dominant trait may either be heterozygous or homozygous for the dominant allele. Test cross involved crossing the plant in question with a plant showing the recessive trait. The ratio and phenotype of progeny from this cross could indicate heterozygosity or homozygosity of the plant being studied.
- Phenotypic and genotypic ratios for a cross with multiple traits can be predicted by using the methods of Punnett square, forked line method or probability

method. Statistical methods like chi-square goodness of fit test can be used to determine if the observed ratio from a cross vary from the expected ratios purely due to chance.

- Application of Mendelian principles can contribute to understanding and predicting genetic disorders in humans. Pedigrees can be constructed for a particular trait to understand mode of inheritance of a gene. Genetic counselling may help in counselling parents suffering from genetic disorders who want to understand the chances of their children inheriting the disease.

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