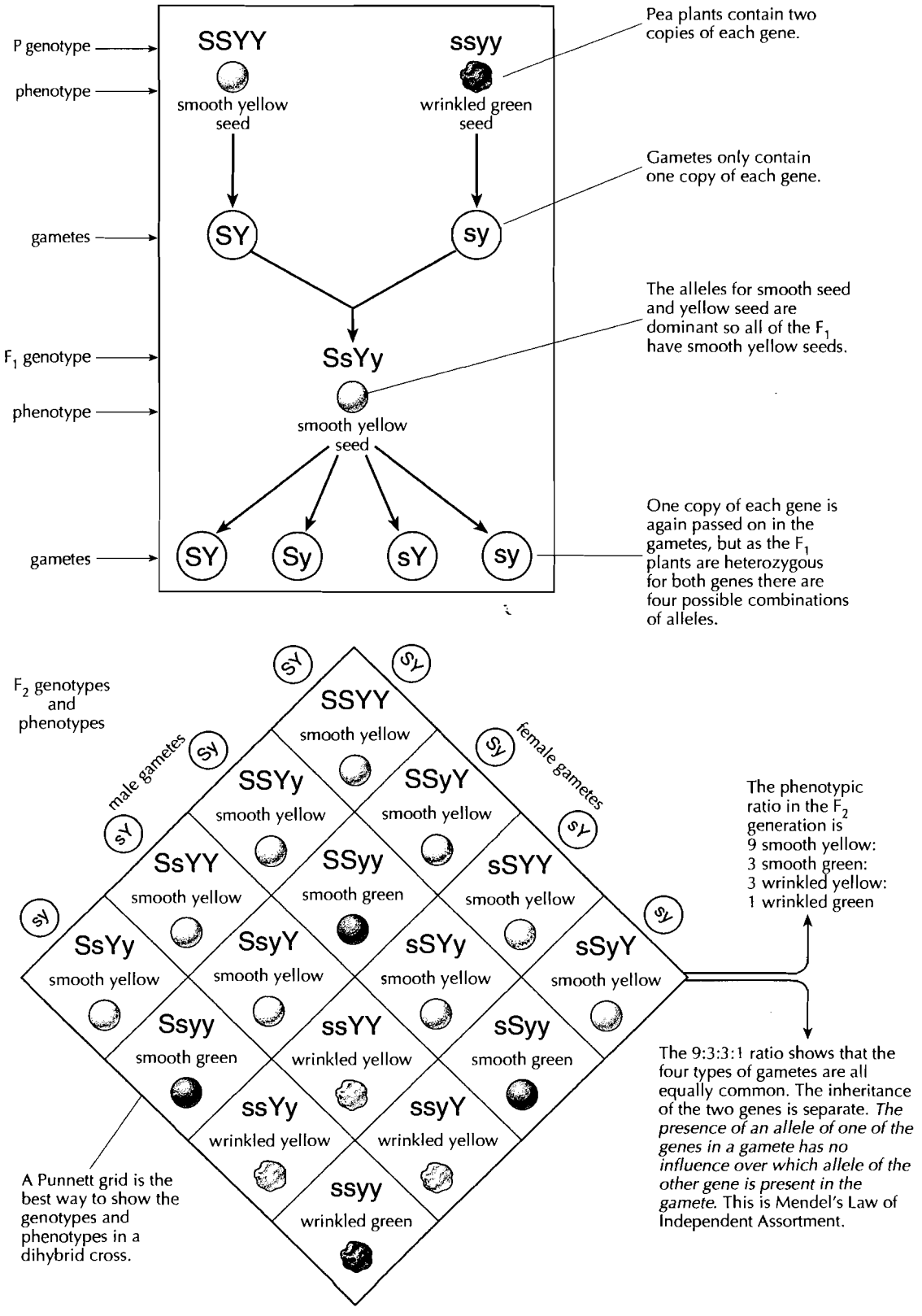
Mendel's Law of Independent Assortment

Gregor Mendel discovered the Law of Segregation by doing monohybrid crosses with pea plants. He discovered another law of inheritance by doing crosses in which the parents differed in two characteristics, that are controlled by two different genes. These are called **dihybrid crosses**. Mendel did his dihybrid crosses with pea plants. An example of one of his crosses is shown below. The parents in this cross differ in seed shape, controlled by one gene and in seed colour, controlled by a different gene.

KEY TO SYMBOLS

S = allele for smooth seed.
s = allele for wrinkled seed.
Y = allele for yellow seed.
y = allele for green seed.



Dihybrid crosses

PREDICTING RATIOS IN DIHYBRID RATIOS

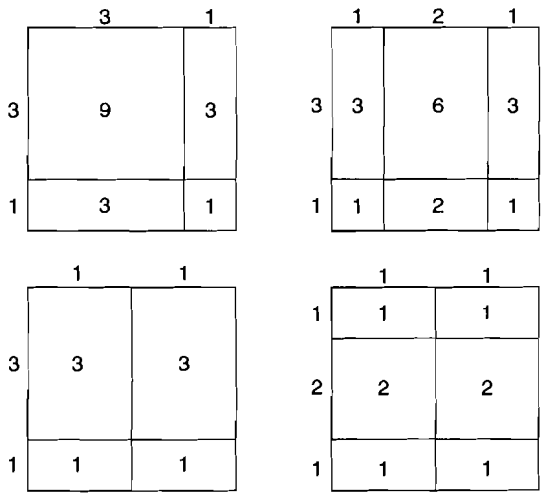
The 9:3:3:1 ratio is often found when parents that are heterozygous for two genes are crossed together. The ratio is the product of two 3:1 ratios – each of the two genes would give a 3:1 ratio in a monohybrid cross between two heterozygous parents. In a dihybrid cross they follow Mendel's Law of Independent Assortment because they are unlinked.

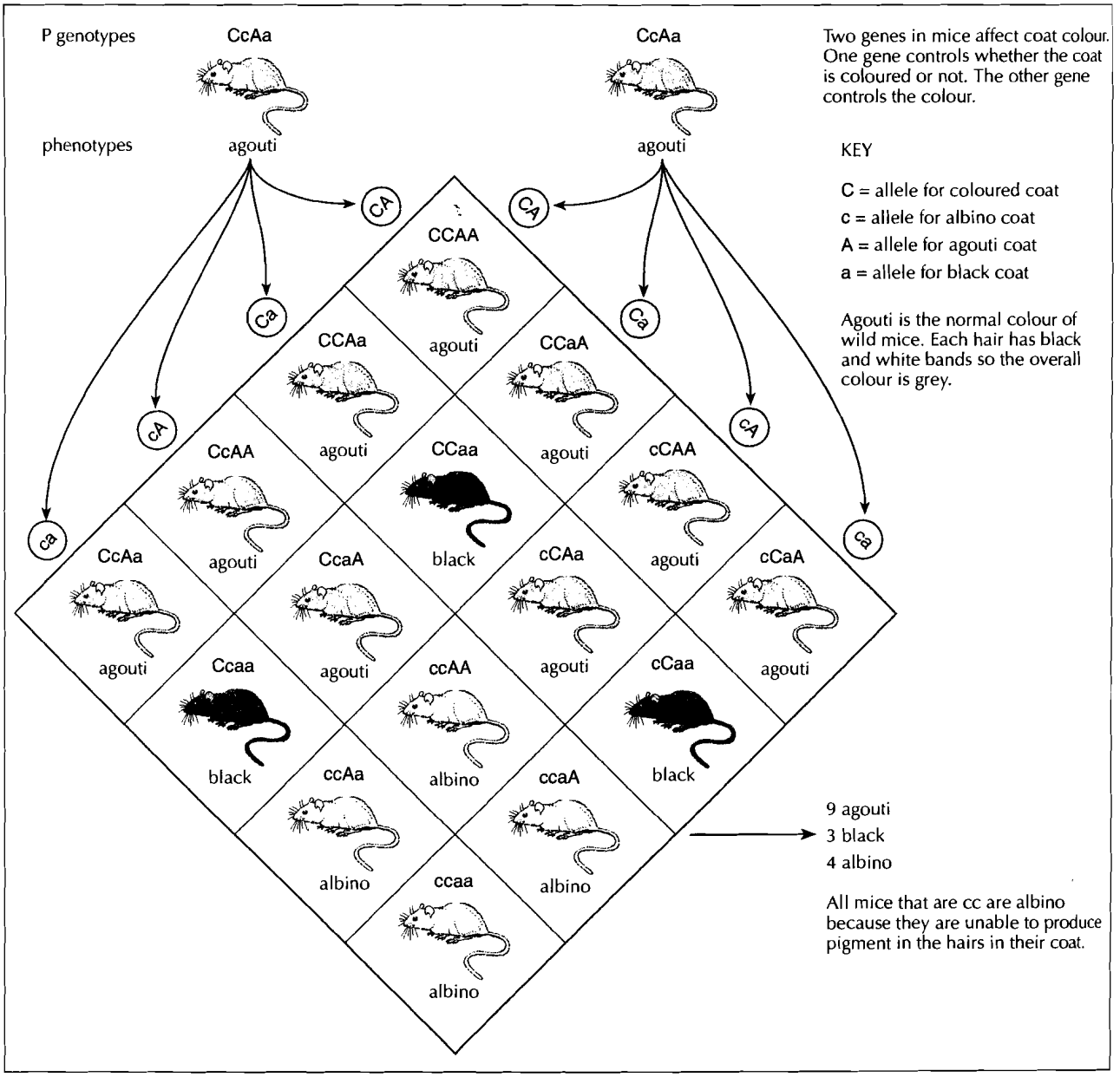
Dihybrid crosses can give other ratios if:

- either of the genes has codominant alleles,
- either of the parents is homozygous for one/both of the genes,
- either of the genes is sex linked. Sex-linked genes are located on sex chromosomes instead of on autosomes (non-sex chromosomes).

The figure (right) shows ratios that these types of genes could give. Another cause of unusual ratios is interaction between genes. The figure (below) shows an example of a dihybrid cross where there is interaction between genes.

Possible ratios in dihybrid crosses





Polygenic inheritance

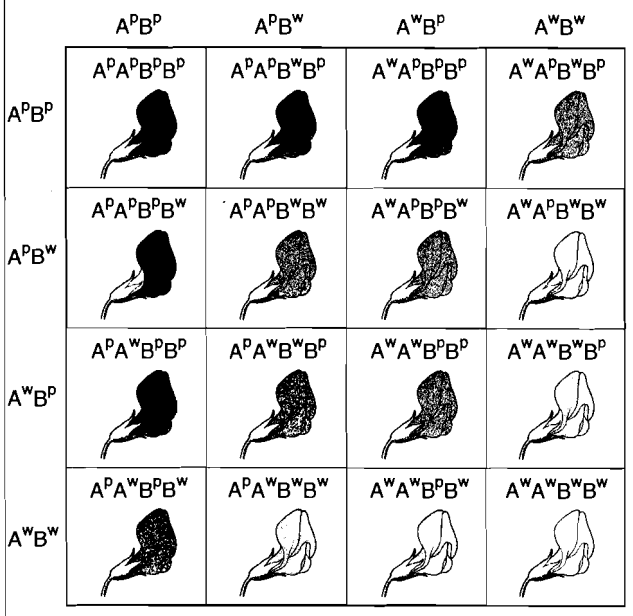
THE DISCOVERY OF POLYGENIC INHERITANCE

Some characteristics are influenced by more than one gene. This is called polygenic inheritance. Gregor Mendel discovered an example of **polygenic inheritance**, when he crossed a purple-flowered species of bean with a white-flowered species. The F_1 offspring were all purple, so he expected a 3:1 ratio of purple to white flowers in the F_2 offspring. Instead, he found a much smaller proportion of white flowers and a wide variety of shades of purple flower. Mendel suggested that two or three genes might be involved. If these were codominant genes, each with two alleles, one for purple flowers (A^P and B^P) and one for white (A^W and B^W), there could be five shades of flower colour (right).

POLYGENIC INHERITANCE AND CONTINUOUS VARIATION

Most examples of polygenic inheritance involve more than two genes with codominant alleles. As the number of genes involved increases, the number of possible phenotypes increases. Eventually, it becomes impossible to divide individuals into discrete groups – the variation is continuous.

Results of a cross between red and white flowered beans

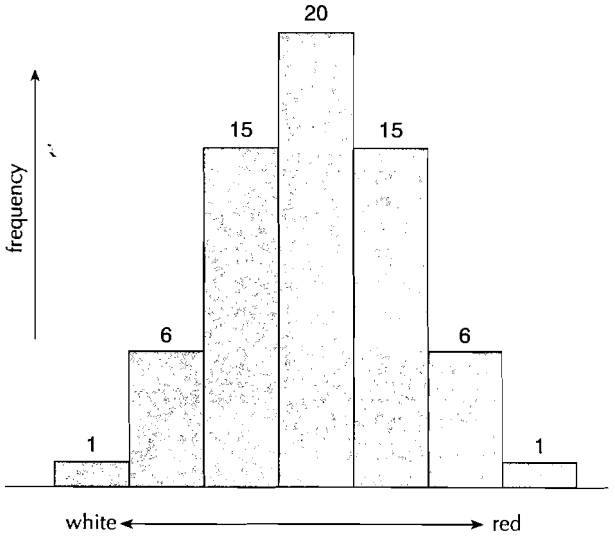


EXAMPLES OF POLYGENIC INHERITANCE

Grain colour in wheat

Wheat grains vary in colour from white to dark red, depending on the amount of a red pigment they contain. Three genes control the colour. Each gene has two alleles, one that causes pigment production and one that does not. Wheat grains can therefore have between 0 and 6 alleles for pigment production. The figure (right) shows the expected distribution of grain colour from a cross between two plants that are heterozygous for each of the three genes.

Distribution of grain colour in wheat



Skin colour in humans

The colour of human skin depends on the amount of the black pigment melanin in it. There is a continuous distribution of skin colour from very pale (little melanin) to black (much melanin). At least four and possibly more genes are involved, each with alleles that promote melanin production and alleles that do not. There is therefore a wide range of possible genotypes with anything from no alleles promoting melanin production to many.
The figure (below) shows humans with a range of skin colour.

Skin colour variation in humans

Statistical testing

USING STATISTICAL TESTS

Biologists use statistical tests to find out whether the differences between sets of results are significant and whether observed results differ significantly from theoretical expectations. In the chi-squared test and other commonly used tests, the significance level is shown as a percentage. A 5% significance level means that there is a 5% probability (1 in 20) that the differences are due to the samples being, by chance, unrepresentative. Thus there is a 95% probability that the differences are due to a real difference between the populations or treatments being investigated. With less than 95% confidence, there is too great a probability that the differences are due to chance for the conclusion to be trusted. The 5% level of significance is therefore usually the minimum that is accepted in biological investigations.

EXPECTED AND OBSERVED RESULTS IN GENETIC CROSSES

In monohybrid and dihybrid crosses, the **expected results** are calculated using the appropriate genetic ratio. For example, Mendel expected a ratio of 3 round seeds to one wrinkled seed in one of his crosses. 7324 seeds were produced in total in the cross, so the expected results were 5493 round seeds and 1831 wrinkled seeds. The actual numbers of each type of offspring in a cross are the **observed results**. The observed results of Mendel's cross were 5474 round seeds and 1850 wrinkled seeds.

DIFFERENCES BETWEEN EXPECTED AND OBSERVED RESULTS

In the example above, Mendel's observed results are close but not identical to the expected results. In monohybrid and dihybrid crosses the observed results are rarely identical to the expected results. There are two possible reasons for this.

1. *Chance effects of random fertilization*
   The probability of each type of male gamete successfully fertilizing the female gamete depends on the proportions of each type of male gamete. However, each fertilization is random and so the actual numbers of successful male gametes of each type will not usually match these proportions exactly.

2. *The results do not fit the expected ratio*
   If the pattern of inheritance is not as expected, the ratio used to calculate the expected results will not be correct. The observed results will be different from the expected results by a greater amount than differences due to chance.

The chi-squared test can be used to distinguish between these two reasons.

USING THE CHI-SQUARED TEST

An example (above right) shows how the statistic chi-squared is calculated. It is not necessary to memorize the equation for chi-squared, but it is important to understand how the calculated statistic is used. The larger the calculated value of chi-squared, the greater is the difference between the observed and expected results. The calculated value is compared with a table of critical values of chi-squared (right). The correct line of the table must be used, corresponding to the number of degrees of freedom. In the example (above right) there is one degree of freedom and chi-squared is 0.262. This is much lower than the critical value for a significance level of 5%. This shows that there is no significant difference between observed results and the results expected with a ratio of 3:1.

EXAMPLE OF CHI-SQUARED CALCULATION

Total number of offspring = 7324
Expected F_2 ratio = 3 round : 1 wrinkled.

	Round	Wrinkled
Expected results	5493	1831
Observed results	5474	1850

$$\text{Chi-squared} = \sum \frac{(O-E)^2}{E}$$

Where O = observed result
E = expected result

$$\text{Chi squared} = \frac{(5474-5493)^2}{5493} + \frac{(1850-1831)^2}{1831}$$

$$= \frac{361}{5493} + \frac{361}{1831}$$

$$= 0.065 + 0.197$$

$$= 0.262$$

$$\text{Degrees of freedom} = \text{number of classes} - 1$$
$$= 2 - 1$$
$$= 1$$

CRITICAL VALUES FOR THE CHI-SQUARED TEST

Degrees of freedom (df)	Level of significance (P)						Level of significance (P)					
	0.05	0.025	0.01	0.005	0.001		0.05	0.025	0.01	0.005	0.001
1	3.84	5.02	6.63	7.88	10.83	20	31.41	34.17	37.57	40.00	45.32
2	5.99	7.38	9.21	10.60	13.81	21	32.67	35.48	38.93	41.40	46.80
3	7.31	9.35	11.34	12.84	16.27	22	33.92	36.78	40.29	42.80	48.27
4	9.49	11.14	13.26	14.86	18.47	23	35.17	38.08	41.64	44.18	49.73
5	11.07	12.83	15.09	16.75	20.52	24	36.42	39.36	42.98	45.56	51.13
6	12.59	14.45	16.81	18.55	22.46	25	37.65	40.65	44.31	46.93	52.62
7	14.07	16.01	18.48	20.28	24.32	26	38.89	41.92	45.64	48.29	54.05
8	15.51	17.53	20.09	21.98	26.13	27	40.11	43.19	46.96	49.64	55.48
9	16.92	19.02	21.67	23.59	27.88	28	41.34	44.46	48.28	50.99	56.89
10	18.31	20.48	23.21	25.19	29.59	29	42.56	45.72	49.59	52.34	58.30
11	19.68	21.92	24.73	26.76	31.26	30	43.77	46.98	50.89	53.67	59.70
12	21.03	23.34	26.22	28.30	32.91	40	43.77	46.98	50.89	53.57	59.70
13	22.36	24.74	27.69	29.82	34.53	50	67.50	71.42	76.16	79.49	86.66
14	23.68	26.12	29.14	31.32	36.12	60	79.08	83.30	88.38	91.95	99.61
15	25.00	27.49	30.58	32.80	37.70	70	90.53	95.02	100.43	104.22	112.32
16	26.30	28.85	32.00	34.27	39.25	80	101.88	106.63	100.43	104.22	112.32
17	27.59	30.19	33.41	35.72	40.79	90	113.15	118.14	124.12	128.30	137.21
18	28.87	31.53	34.81	37.16	42.31	100	124.34	129.56	135.81	140.17	149.44
19	30.14	32.85	36.19	28.58	43.82						

In dihybrid crosses some offspring inherit a combination of characteristics that one of the parents had and other offspring inherit new combinations of characteristics. Offspring with a new combination of characteristics are called **recombinants**, because the new combinations are formed by **recombination**. *Recombination is the reassortment of genes or characteristics into different combinations from those of the parents.*

RECOMBINATION OF UNLINKED GENES

Unlinked genes are located on different types of chromosome, so when homologous chromosomes pair up in meiosis they are on different pairs. The pairs of homologous chromosomes are called **bivalents**. The bivalents are orientated randomly on the equator and so the pole to which the each allele of a gene moves is not affected by the pole to which alleles of other unlinked genes are moving. This is how independent assortment of unlinked genes occurs (below). It also allows the recombination of unlinked genes – combinations of alleles inherited from a parent are broken up and new combinations can then be formed by random fertilization.

GENE LINKAGE

Some pairs of genes do not follow the Law of Independent Assortment. The expected 9:3:3:1 ratio is not found when parents that are heterozygous for the two genes are crossed. The figure (right) shows the first example to be discovered. The results show that there were more offspring than expected with the parental character combinations – purple long and red round. There were fewer than expected with the new combinations – purple round and red long. Combinations of genes tend to be inherited together. This is called **gene linkage**. Gene linkage is caused by pairs of genes being located on the same type of chromosome.

RECOMBINATION OF LINKED GENES

Pairs of linked genes are located on the same type of chromosome. Recombination of linked genes involves a special process called **crossing-over**, which happens during the early stages of meiosis.

Gene linkage in *Lathyrus odoratus*

P genotypes → PPLL ppll

phenotypes → purple flowers long pollen; red flowers round pollen

PL pl

F_1 genotype → PpLl

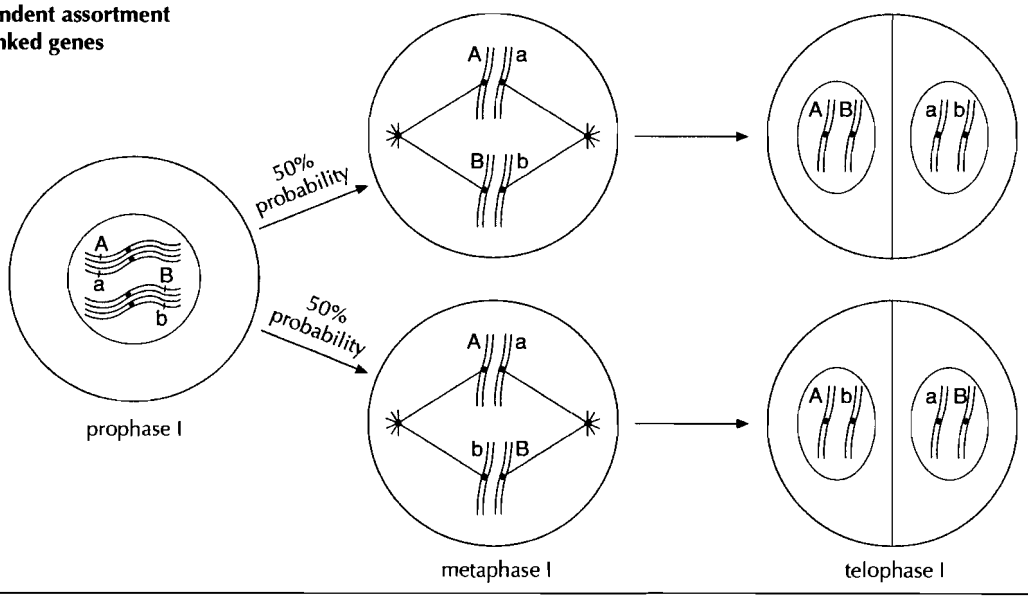
F_1 phenotype → purple flowers long pollen

Self-pollination of F_1 plants to produce F_2 generation.

Expected F_2 ratio	9 purple long	3 purple round	3 red long	1 red round
Expected results (6952 plants in total)	3910.5	1303.5	1303.5	434.5
Observed results	4831	390	393	1338

Chi-squared = 372 at 3 degrees of freedom
Significance level is less than 0.001
So there is 99.9% confidence of a significant difference between the observed and expected results.

Independent assortment of unlinked genes



Crossing-over

EVENTS IN PROPHASE I OF MEIOSIS

Homologous chromosomes pair up in prophase I of meiosis. Each homologous chromosome consists of two sister chromatids. Chromatids of different chromosomes are called non-sister chromatids. While the chromosomes are paired, sections of chromatid are exchanged in a process called **crossing-over**.
The figure (right) shows how crossing-over occurs.

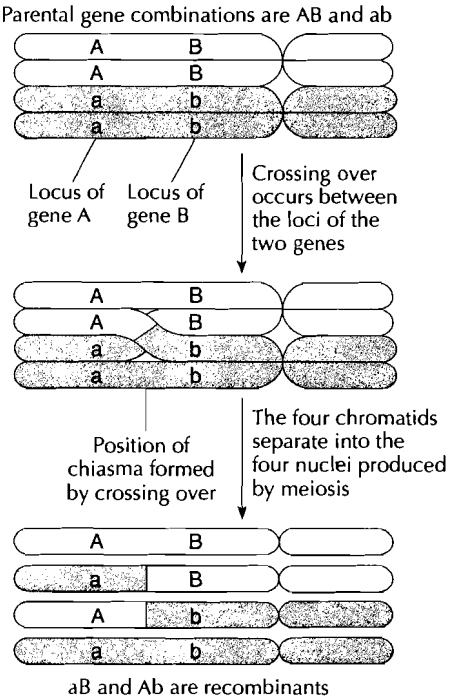
BENEFITS OF CROSSING-OVER

Crossing-over has two important consequences.

1. It creates chiasmata which hold homologous chromosomes together in pairs called bivalents, during the later stages of prophase I and metaphase I until microtubules have attached.
2. It allows recombination of linked genes. All of the genes that have their loci on the same chromosome type form a **linkage group**.

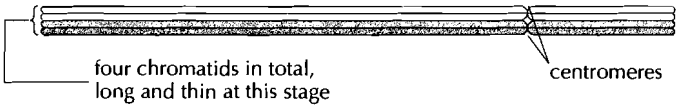
Recombination of genes in a linkage group cannot occur without crossing-over. The point where crossing-over occurs along chromosomes is random – it can occur at a vast number of different points. Meiosis can therefore produce an almost infinite amount of genetic variety.
The figure (below) shows how crossing-over can cause recombination of linked genes.
The figure (right) shows an example of a cross involving gene linkage, using bars to represent the chromosomes on which the genes are linked. A test cross was done on the F_1 plants.

Recombination of linked genes

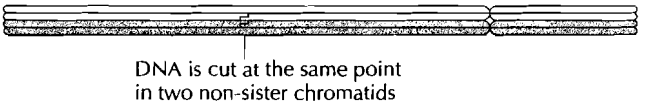


The process of crossing over

At one stage in prophase I all of the chromatids of two homologous chromosomes become tightly paired up together. This is called *synapsis*.

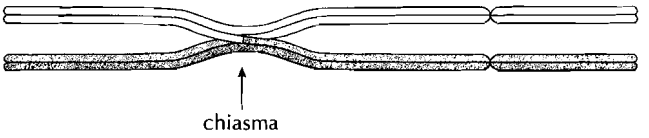


The DNA molecule of one of the chromatids is cut. A second cut is made at exactly the same point in the DNA of a non-sister chromatid.

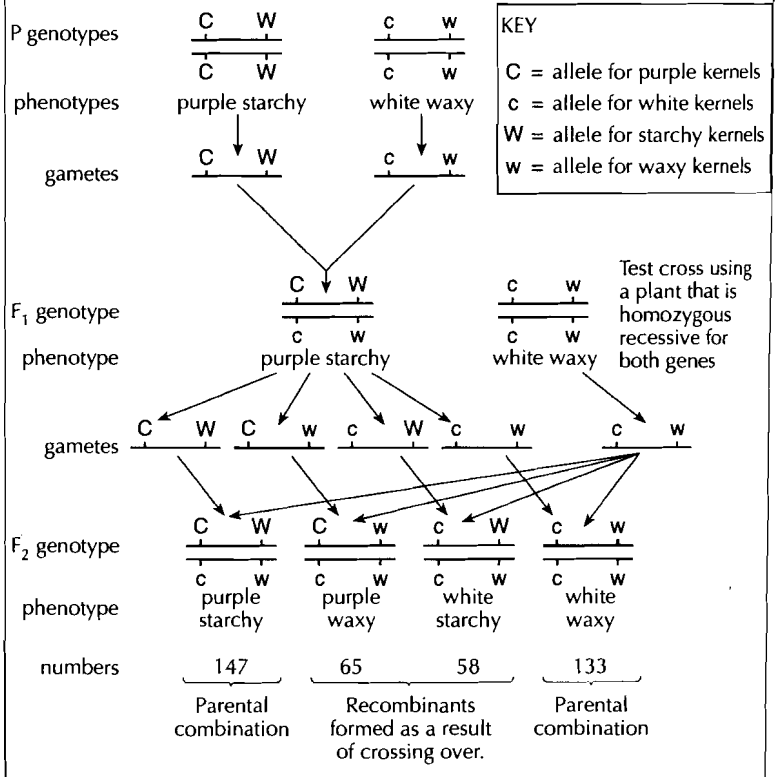


The DNA of each chromatid is joined up to the DNA of the non-sister chromatid. This has the effect of swapping sections of DNA between the chromatids.

In the later stages of prophase I the tight pairing of the homologous chromosomes ends, but the sister chromatids remain tightly connected. When each cross-over has occurred there is an X-shaped structure called a chiasma



AN EXAMPLE OF GENE LINKAGE AND TEST-CROSSING IN *ZEA MAYS*



Phases of meiosis

Meiosis involves two divisions. Each division is divided into four phases. The main events of each phase are listed below.

PROPHASE I

- Chromosomes start to coil up and so become shorter and thicker.
- Homologous chromosomes pair up.
- Crossing over occurs.
- Centrioles move to the poles in animal cells.
- Nucleoli break down.
- At the end of prophase I the nuclear membrane breaks down.

METAPHASE I

- Chromosomes continue to shorten and thicken.
- Spindle microtubules attach to the centromeres.
- Bivalents line up on the equator.
- Chiasmata slide towards the ends of the chromosomes, causing the shapes of the bivalents to change.
- At the end of metaphase I the chromosomes start to move.

ANAPHASE I

- The two chromosomes of each bivalent move to opposite poles. This halves the chromosome number. Each chromosome consists of two chromatids. Because of crossing over the two chromatids are not identical.
- At the end of anaphase I the chromosomes reach the poles.

TELOPHASE I

- Nuclear membranes form around the groups of chromosomes at each pole.
- The cell divides to form two haploid cells.
- The chromosomes uncoil partially.
- At the end of telophase I the two cells either enter a brief period of interphase or immediately proceed to the second division of meiosis. The DNA is not replicated.

PROPHASE II

- Chromosomes become shorter and thicken again by coiling.
- Centrioles move to the poles in animal cells.
- At the end of prophase II the nuclear membranes break down.

METAPHASE II

- Spindle microtubules attach to the centromeres.
- Chromosomes line up on the equator
- At the end of metaphase II the centromeres divide.

ANAPHASE II

- The two chromatids of each chromosome move to opposite poles.
- At the end of anaphase II the chromatids reach the poles.

TELOPHASE II

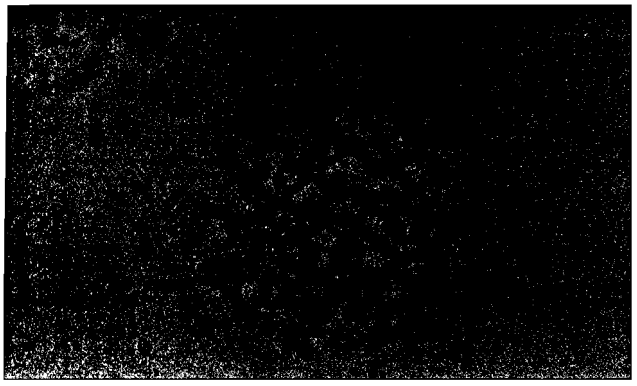
- Nuclear membranes form around the groups of chromatids at each pole. Each chromatid is now considered to be a chromosome.
- The two cells each divide to form to four cells in total.
- The chromosomes uncoil.
- Nucleoli appear.
- In most organisms the cells formed at the end of telophase II develop into gametes.

SUMMARY OF MEIOSIS

1. Meiosis involves two divisions. One cell or nucleus divides to form four cells or nuclei.
2. The chromosome number is halved, from diploid to haploid.
3. An almost infinite amount of genetic variety is produced, as a result of crossing-over in prophase I and the random orientation of bivalents in metaphase I.

The figure (below) shows micrographs of four stages in meiosis in cells from the testis of a locust.

Early prophase I



Late prophase I

Metaphase I

Telophase I