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A guide for teachers – Years 11 and 12

Gene mapping

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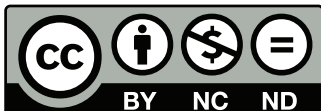*Maths delivers!*
Gene mapping

Dr Michael Evans AMSI

Editor: Dr Jane Pitkethly, La Trobe University

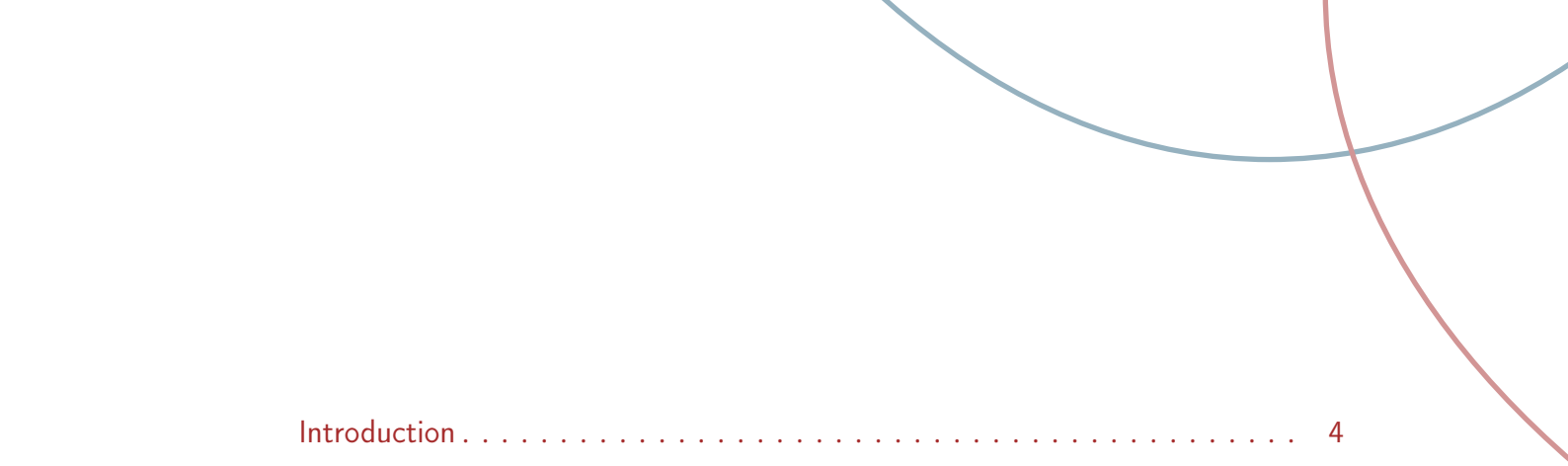Illustrations and web design: Catherine Tan, Michael Shaw

For teachers of Secondary Mathematics

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Gene mapping

Introduction

There has been an explosion of knowledge in the life sciences over the past twenty years. At the center of this explosion is the use of mathematics and statistics. These advances have expanded use of mathematics and statistics beyond the traditional fields of physical science and engineering.

In these notes we hope to give some idea how mathematics and statistics contribute to the study of genetics and in particular gene mapping. Doctors and scientists hope to use our genetic information to diagnose, treat, prevent and cure many illnesses. This knowledge will eventually lead to more effective medicines and treatments. We note that the last ten pages of these notes are derived from the article of Bahlo and Speed cited in the references. It is intended that these notes will be developed further in the future.

Cells, genes and chromosomes

The **cell** is the basic structural, functional and biological unit of all living things. All organisms are composed of one or more cells and all cells come from preexisting cells. Humans contain trillions of cells. Most plant and animal cells are between 1 and 100 micrometres and therefore are visible only under the microscope. The cell was discovered by Robert Hooke in 1665.

We introduce some terms that will be used throughout these notes. There is a diagram illustrating some of them of them on the following page.

A **nucleus** is a membrane-bound part of a cell that contains the cell's chromosomes.

A **chromosome** is a very long DNA molecule. Different organisms carry different amounts of chromosomes. Humans carry 46 chromosomes.

A **gene** is the basic physical unit of inheritance. Genes are passed from parents to offspring and contain the information needed to specify traits. Genes are arranged on chro-

mosomes.

The **locus** of a gene is the location of a gene on a chromosome, like a genetic street address.
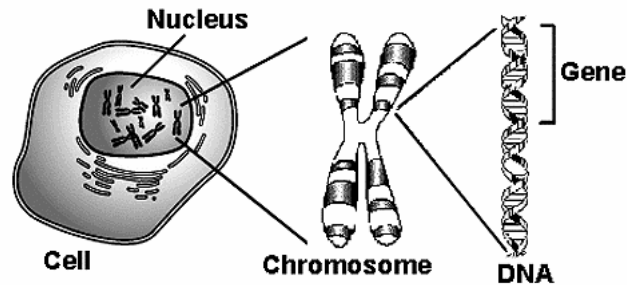
Genes are specific regions along chromosomes, and for much of the 20th century, it was a real challenge to identify and locate genes causing differences. This process is called gene mapping. That is, **Gene mapping** is the process of determining the positions of genes on a chromosome and the distance between them.

With the genetic engineering revolution of the 1970s and 1980s, new genetic tools became available that greatly facilitated this task. Although there are visual aspects to genetics that involve light and electron microscopes and elaborate staining techniques, statistical notions remain essential to the task of gene mapping

Gregor Mendel

An Augustinian friar, Gregor Johann Mendel lived and worked from 1843 in Brno, in the Augustinian Abbey, of which he became the abbot in 1867. At this time Brno was in the Austrian-Hungarian empire. Mendel was known for his activity as a teacher, for his interest in meteorology and in the breeding of bees.

Mendel's experiments on pea plants were started in 1856. In 1865 Mendel presented his paper on pea hybrids, *Experiments in Plant Hybridization*, and in 1866 this was published in the proceedings of the local Society for Natural Sciences. Mendel's article on pea hybrids went almost unnoticed. Over fifty years later, Mendel was to be acclaimed as the father of classical genetics.

How Mendel discovered genes

Mendel's theory shows the power of simple probability models. He collected data for 10 years. His sample sizes were large; he tabulated results from 28,000 pea plants.

The reasons he chose peas could have included the following.

- Peas are easy to grow, and take little space.
- They are inexpensive.
- They have a short generation time compared to large animals so that a large number of offspring can be obtained in a short amount of time.
- They have some distinct characteristics that are easy to recognize.
- These characteristics can be used when trying to determine patterns of inheritance.
- They are easily self-fertilized or cross fertilized.

A portion of Mendel's experiment is the investigation of the colour of the seeds of peas.

Mendel's experiment on pea colour

- Pea seeds can be either yellow or green and a plant can bear seeds of both colours. (Mendel introduced the idea of heredity units, which he called **factors**.The two colours of seeds are examples of Mendel's factors.
- Left alone, peas self-fertilize

- Mendel had bred a pure breeding yellow strain. In this strain every generation had only yellow seeds. He also had a pure breeding green strain.

- He then artificially (that is, by hand) crossed plants of the pure breeding yellow strain with plants of the pure breeding green strain.

  - The first generation of these plants, first-generation (F1) hybrids, were all yellow

  - These first-generation hybrids were then permitted to self cross. Some of the second generation (F2) were green and some yellow. In fact he found 75 % were yellow and 25 % green.

F1 generation

F2 generation
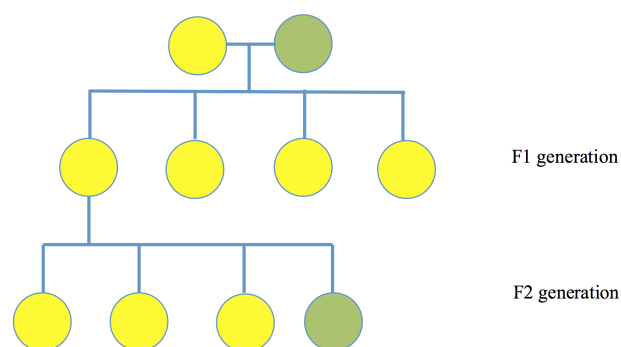
Phenotypes, genotypes and alleles

Mendel's factors are forms of a single gene that determines the character, and we call them alleles.

An **allele** is one of two or more versions of a gene. An individual inherits two alleles for each gene, one from each parent. If the two alleles are the same, the individual is **homozygous** for that gene. If the alleles are different, the individual is **heterozygous.**

We discuss the alleles for pea colour in the following subsection of these notes.

A **genotype** of an individual is the two alleles inherited for a particular gene. The term is also often used to describe an individual's collection of genes.

A **phenotype** is an individual's observable traits, such as height, eye color, and blood type. The genetic contribution to the phenotype is the genotype.
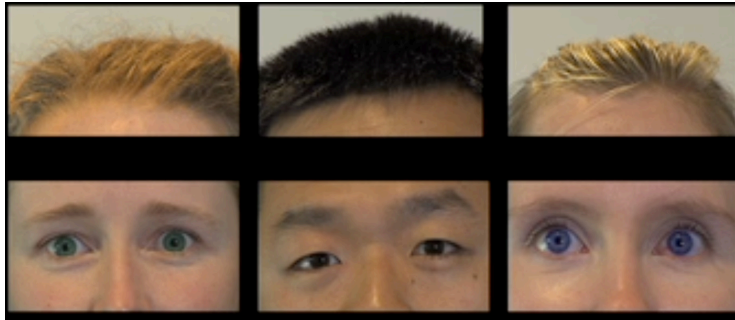
Back to pea seed colour

There are two different alleles of the seed colour gene that we are concerned with. They will be denoted by y (yellow) and g (green).

So there are four different genotypes:

$$y/y, y/g, g/y \text{ and } g/g.$$

The phenotypes are yellow and green

- $y/y, y/g, g/y$ produces yellow
- g/g produces green.

We note that for y/y and g/g the individual is homozygous and for y/g and g/y the individual is heterozygous.

Dominant, recessive and punnet diagrams

Dominant refers to the relationship between the alleles of a gene. Recall that individuals receive two versions of each gene, known as alleles, from each parent. If the alleles of a

gene are different, often, one allele will be expressed; it is the **dominant** gene. The effect of the other allele, called **recessive**, is masked.

It is possible to have **co-dominant** alleles. In this case neither allele is dominant nor recessive with respect to the other allele. We will see an example of this with blood types later in these notes.

Peas yet again

In this case of the peas y is dominant and g is recessive. For the first generation only y/g are produced from a y/y and a g/g. Hence all the peas are yellow since yellow is dominant. But in the second generation we have the following:

		FEMALE	
		y	g
M A L E	y	y/y $(\frac{1}{4})$	y/g $(\frac{1}{4})$
	g	g/y $(\frac{1}{4})$	g/g $(\frac{1}{4})$

The diagram above is called a **Punnett square**.

The probability of obtaining a green pea is $\frac{1}{4}$ and the probability of obtaining a yellow pea is $\frac{3}{4}$ at this second generation stage.

We can ask a simple probability questions at this stage.

Example

Find the probability of the genotype y/y of the seed colour given a phenotype yellow.

Solution

There are three equally likely possible genotypes yielding the yellow phenotype.

Therefore $\Pr(y/y|\text{ yellow}) = \frac{1}{3}$.

Mendel's postulates

1 Plants have factors (genes) that determine the inheritance of each character. Genes are never blended together.

2 One form (allele) of a gene may be dominant over another.

3 **Law of segregation** Each individual has two alleles at each locus, and transmits one to each of its offspring (segregation). This can be restated as: An individual passes on to its offspring either its paternally or matenally inherited allele, each with probabilty $\frac{1}{2}$, with the transmitted allele for different offspring being independent.

4 **Law of independent segregation of alleles at two or more loci.**. If alleles a and b are segregating at locus 1 and alleles c and d are segregating at locus 2 then the combinations ab, ad, cb, cd segregate jointly each with probabilty $\frac{1}{4}$.

Mendel's first law of segregation has been found to be generally true at single locus, but his second law holds only for certain pairs of loci, and is not generally true. The following example is one of where the fourth postulate is apparently true.

Example of Mendel's fourth postulate

Mendel also worked with trait of seed shape. The two shape alleles are round (r) and wrinkled (w). Round is dominant over wrinkled and yellow is dominant over green. We will consider the locus with seed colour and the locus with seed shape. The combinations in this case are:

$$yr \qquad yw \qquad gr \qquad gw$$

These were found to segegate jointly with probabilty $\frac{1}{4}$ each. Colour and shape are independent. The punnett diagram can be formed as shown. The phenotypes will be denoted with capital letters in bold and brackets. For example (**YR**) means the seed is yellow and round.

	yr	yw	gr	gw
yr	$yyrr$(**YR**)	$yyrw$(**YR**)	$ygrr$(**YR**)	$ygrw$(**YR**)
yw	$yywr$(**YR**)	$yyww$(**YW**)	$ygwr$(**YR**)	$ygww$(**YW**)
gr	$gyrr$(**YR**)	$gyrw$(**YR**)	$ggrr$(**GR**)	$ggrw$**GR**)
gw	$gywr$(**YR**)	$gyww$(**YW**)	$ggwr$**GR**)	$ggww$**GW**)

We can see from this:

- $\Pr(\text{Yellow seed}) = \dfrac{3}{4}$

- $\Pr(\text{Yellow round seed}) = \dfrac{9}{16}$

- $\Pr(\text{Yellow wrinkled seed}) = \dfrac{3}{16}$

- $\Pr(\text{Green seed}) = \dfrac{1}{4}$

- $\Pr(\text{Green round seed}) = \dfrac{3}{16}$

- $\Pr(\text{Green wrinkled seed}) = \dfrac{1}{16}$

We can associate the genotypes with the phenotypes:

Genotypes	Phenotype
$ggwr, ggrw, ggrr$	green and round
$ggww$	green and wrinkled
$yyrr, yyrw, ygrr, ygrw, yywr, ygwr, gywr, gyrr, gyrw$	yellow and round
$ygwr, gyww, yyww$	yellow and wrinkled

Because of the independence of segregation at the two loci we can use

$$\Pr(A \cap B) = \Pr(A)\Pr(B)$$

to determine probabilities.

For example we know that $\Pr(green) = \dfrac{3}{4}$ and $\Pr(round) = \dfrac{3}{4}$.

Hence $\Pr(Green \text{ and } Round) = \dfrac{3}{4} \times \dfrac{3}{4} = \dfrac{9}{16}$.

Exercise 1

Find the probabilty of

a the genotype $yyww$ given that the pea is yellow and wrinkled.

b the genotype $yyww$ given that the pea is yellow

c yyxx given that the pea is yellow (Here the xx denotes that any combination of r and w is possible.)

d xxrr given that the pea is round (Here the xx denotes that any combination of y and g is possible.)

Suppose 20 pea seeds are randomly selected from a large population of pea seeds. Find the probability of

a   at least 5 seeds being green

b   at least 5 seeds being green and round

c   less than 10 seeds being yellow and round.

Hardy-Weinberg Law

An important result in population genetics is the Hardy-Weinberg theorem. It is not in the video but it is an interesting result obtained by simple mathematics. G. H. Hardy (1877 - 1947) was an English mathematician, known for his achievements in number theory and mathematical analysis. He writes in his short paper of 1908.

(We note: Brachydactyly is a medical term which means shortness of the fingers and toes.)

To The Editor of Science: I am reluctant to intrude in a discussion concerning matters of which I have no expert knowledge, and I should have expected the very simple point which I wish to make to have been familiar to biologists. However, some remarks of Mr. Udny Yule, to which Mr. R. C. Punnett has called my attention, suggest that it may still be worth making. In the Proceedings of the Royal Society of Medicine (Vol I., p. 165) Mr. Yule is reported to have suggested, as a criticism of the Mendelian position, that if brachydactyly is dominant 'in the course of time one would expect, in the absence of counteracting factors, to get three brachydactylous persons to one normal. It is not difficult to prove, however, that such an expectation would be quite groundless.

He adds in the footnote.

P. S. I understand from Mr. Punnett that he has submitted the substance of what I have said above to Mr. Yule, and that the latter would accept it as a satisfactory answer to the difficulty that he raised. The 'stability' of the particular ratio 1:2:1 is recognized by Professor Karl Pearson (Phil. Trans. Roy. Soc. (A), vol. 203, p. 60).

We assume a large random-mating population. Suppose that at any locus only two alleles may occur A_1 and A_2. The corresponding three genotypes are $(A_1 A_1)$, $(A_1 A_2)$ and $(A_2 A_2)$. Suppose that the fractions of the total population of each of these are P, $2Q$ and R respectively. Furthermore suppose that the numbers are fairly large, and that the

mating may be regarded as random with respect to genotype, that the sexes are evenly distributed among the three genotypes, and that all are equally fertile. The table below gives the fraction of the total matings of the different combinations of the genotypes. For example the fraction of the matings of an $A_1 A_1$ with $A_1 A_1$ is P^2. We have the following:

Male

	$A_1 A_1$	$A_1 A_2$	$A_2 A_2$
$A_1 A_1$	P^2	$2PQ$	PR
$A_1 A_2$	$2QP$	$4Q^2$	$2QR$
$A_2 A_2$	PR	$2QR$	R^2

Female (label to the left of the table)

1 How do we end up with the genotype $A_1 A_1$ from these matings?

- We obtain $A_1 A_1$ from $A_1 A_1 \times A_1 A_1$ every time. That is, the proportion of offspring is 1.
- We obtain $A_1 A_1$ from $A_1 A_1 \times A_1 A_2$ half the time. That is, the proportion of offspring is $\frac{1}{2}$.
- We obtain $A_1 A_1$ from $A_1 A_2 \times A_1 A_2$ one quarter of the time. That is, the proportion of offspring is $\frac{1}{4}$.

The proportion P' of $A_1 A_1$ of the offspring generation is:

$$P' = P^2 + \frac{1}{2}(4PQ) + \frac{1}{4}4Q^2 = (P + Q)^2 \qquad (1)$$

2 How do we end up with the genotype $A_1 A_2$ from these matings?

- We obtain $A_1 A_2$ from $A_1 A_1 \times A_1 A_1$ half the time. That is the proportion of offspring is $\frac{1}{2}$.
- We obtain $A_1 A_2$ from $A_1 A_2 \times A_1 A_2$ half the time. That is the proportion of offspring is $\frac{1}{2}$.
- We obtain $A_1 A_2$ from $A_1 A_1 \times A_2 A_2$ everytime. That is theproportion of offspringis 1.
- We obtain $A_1 A_2$ from $A_1 A_1 \times A_2 A_2$ everytime. That is the proportion of offspring is 1.

The proportion $2Q'$ of $A_1 A_2$ of the following generation is:

$$2Q' = \frac{1}{2}(4PQ) + \frac{1}{2}(4Q^2) + 2PR + \frac{1}{2}(4QR) = 2(P + Q)(Q + R) \qquad (2)$$

3 The proportion R' of $A_1 A_2$ of the offspring generation is:

$$R' = \frac{1}{4}(4Q^2) + \frac{1}{2}4QR + R^2 = (Q + R)^2 \qquad (3)$$

Exercise 3

Assume that the proportion of the genotypes $(A_1 A_1)$, $(A_1 A_2)$ and $(A_2 A_2)$ are 0.64, 0.16, 0.04 respectively. These are the values of P, Q and R.

- Show that in the next generation the proportions of genotypes are the same.
- Show that $Q^2 = PR$

To find the proportions P'', Q'' and R'' in the third generation we substitute P', Q' and R' in equations (1), (2) and (3) respectively.

Hence,

$$P'' = P'^2 + \frac{1}{2}(4P'Q') + \frac{1}{4}4(Q')^2$$

$$= (P' + Q')^2$$

$$= ((P + Q)^2 + (P + Q)(Q + R))^2$$

$$= (P + Q)^2(P + Q + Q + R)$$

$$= (P + Q)^2(P + 2Q + R)$$

$$= (P + Q)^2$$

$$= P'$$

Similarly it can be shown that

$$Q'' = Q' \qquad \text{and} \qquad R'' = R'.$$

Clearly from this every succeeding generations will have the genotypes $(A_1 A_1)$, $(A_1 A_2)$ and $(A_2 A_2)$ with proportions P', Q' and R'

It is clear that $Q'^2 = P'R'$

Theorem

A necessary and sufficient condition for the second generation to have genotypes $(A_1 A_1)$, $(A_1 A_2)$ and $(A_2 A_2)$ with proportions P, Q and R is

$$Q^2 = PR$$

(that is the proportions of genotypes stay the same if this condition holds).

Proof

First assume

$$P = (P + Q)^2 \qquad (4)$$

$$Q = (P + Q)(Q + R) \qquad (5)$$

$$R = (Q + R)^2 \qquad (6)$$

Divide (4) by (5)

$$\frac{P}{Q} = \frac{P + Q}{Q + R}$$

Multiply by (6).

$$\frac{PR}{Q} = (P + R)(Q + R) = Q$$

Hence we have

$$Q^2 = PR$$

Conversely if $Q^2 = PR$

$$P' = P^2 + 2PQ + Q^2 = P^2 + 2PQ + PR = P(P + 2Q + R) = P$$

$$Q' = (P + Q)(Q + R) = PQ + PR + Q^2 + QR = PQ + Q^2 + Q^2 + QR = Q(P + 2Q + R) = Q$$

$$R' = (Q + r)^2 = Q^2 + 2QR + R^2 = PR + 2QR + R^2 = R(P + 2Q + R) = R$$

$\square$

From this discussion we see that If we let $p = P + Q$ the frequency of the A_1 allele and $q = Q + R$ the frequency of the A_2 allele the the proportions of the three genotypes are $P^2, 2PQ, Q^2$.

Theorem (Hardy-Weinberg)

Under the assumptions stated, a population having genotypic frequencies

- *P of $A_1 A_1$*
- *$2Q$ of $A_1 A_2$*
- *R of $A_2 A_2$*

achieves, after one generation of random matings, stable genotypic frequencies $p^2, 2pq, q^2$ where $p = P + Q$ and $q = Q + R$.

If the initial frequencies are already of the form $p^2, 2pq, q^2$ then these frequencies are stable for all generations.

It is worth noting again the model assumes ideal conditions, and for example a particular

genotype doesn't improve the chance of surviving.

The importance of the theorem is that it shows that there is no intrinsic tendency for any variation in the population to disappear. Here we talk about the variation caused by the three genotypes $(A_1 A_1)$, $(A_1 A_2)$ and $(A_2 A_2)$. The imporance of this is that evolution through natural selection can occur only if, within the population, there is variation upon which selective forces can act. This obviously depends on the population satisfying conditions some of which are listed above.

The Hardy-Weinberg theorem does not always apply to humans. One reason that it doesn't is that random mating, a condition of Hardy-Weinberg Equilibrium, does not occur. Random mating implies that mating should be arbitrary with regard to the locus being considered. Humans tend to mate with individuals that are similar to themselves, especially with respect to evident or visible traits such as height and ethnicity.

Example

You have sampled a population in which you know that the percentage of the homozygous genotype $A_1 A_1$ is 49 %. Using that 49 % and assuming Hardy-Weinberg conditions, calculate an estimate of the following:

1   The frequency of the $A_1 A_1$ genotype.

2   The frequency of the A_1 allele.

3   The frequency of the A_2 allele.

4   The frequencies of the genotypes $A_1 A_2$ and $A_2 A_2$

Solution

1   The frequency of $A_1 A_1$ is 0.49.

2   The frequency of A_1 allele is 0.7.

3   The frequency of the A_2 allele is 0.3.

4   The frequency of the genotypes $A_1 A_2 = 2 \times 0.7 \times 0.3 = 0.42$. The frequency of the genotypes $A_2 A_2 = 0.3^2 = 0.09$

Exercise 4

You have sampled a population in which you know that the percentage of the heterzygous genotype $A_1 A_2$ is 18 %. Using that 18 % and assuming Hardy-Weinberg conditions, calculate an estimate of the following:

a   The proportion of the A_1 allele.

b The proportion of the $A_1 A_1$ genotype.

c The proportion of the A_2 allele.

d The proportion of the $A_2 A_2$ genotype.

Human blood types

Karl Landsteiner discovered the ABO system in 1900, distinguishing it as one of the most important blood group systems in transfusion medicine.

With the classical ABO system there are three alleles. We write these as I^A, I^B and I^O.

There are 6 genotypes. These are $I^A I^A$, $I^A I^B$, $I^A I^O$, $I^B I^B$, $I^B I^O$ and $I^O I^O$.

I^A is dominant to I^O and I^B is dominant to I^O. I^A and I^B are codominant.

There are four possible phenotypes: A, B, AB and O.

In the table below the 6 genotypes and the associated phenotypes are given.

Phenotype	Genotype
A	$I^A I^A$ or $I^A I^O$
B	$I^B I^B$ or $I^B I^O$
AB	$I^A I^B$
O	$I^O I^O$

The percentages of each phenotype vary according to the country. For example in Australia the approximate distribution is

- O 49 %
- A 38 %
- B 10 %
- AB 3 %

The ABO system remains by far the most significant for transfusion. In an emergency, anyone can receive type O red blood cells, and type AB individuals can receive red blood cells of any ABO type. Therefore, people with type O blood are known as 'universal donors,' and those with type AB blood are known as 'universal recipients'.

Remember that the alleles I^A and I^B are dominant to I^O. An $I^A I^B$ father and an $I^O I^O$

mother will produce children who are either blood type *A* or blood type *B* .None of the children will be of blood type *O*.

Male

		I^O	I^O
Female	I^A	$I^A I^O$	$I^A I^O$
	I^B	$I^B I^O$	$I^B I^O$

A mating between two AB individuals will result in $\dfrac{1}{2}$ of the children with AB blood type like their parents, $\dfrac{1}{4}$ with blood type A, and $\dfrac{1}{4}$ with blood type B.

Male

		I^A	I^B
Female	I^A	$I^A I^A$	$I^A I^B$
	I^B	$I^B I^A$	$I^B I^B$

The probability of two AB parents having three children with blood type A is $\dfrac{1}{64}$.

Exercise 5

A child has a father with type A blood with genotype $I^A I^O$ and a mother with type B blood with genotype $I^B I^O$. What is the probability of the child having type *O* blood?

Exercise 6

Assume Hardy Weinberg conditions in the following. In a given population, only the I^A and I^B alleles are present in the *ABO* system; there are no individuals with type *O* blood or with I^O alleles in this particular population. If 200 people have type *A* blood, 75 have type *AB* blood, and 25 have type *B* blood, what are the alleleic frequencies of this population?

The Hardy Weinberg theorem can be extended to three alleles and in particular to blood. The genotypes are $I^A I^A, I^A I^B, I^A I^O, I^B I^B, I^B I^O, I^O I^O$. In this case we can use the expansion

$$(p_1 + p_2 + p_3)^2 = p_1^2 + p_2^2 + p_3^2 + 2p_1 p_2 + 2p_1 p_3 + 2p_2 p_3$$

Each of the six terms of the expansiom can be associated as a probability with the geno-

types of the *ABO* system in the following way.

Genotype	Probabilty
$I^A I^A$	p_1^2
$I^B I^B$	p_2^2
$I^O I^O$	p_3^2
$I^A I^B$	$2p_1 p_2$
$I^A I^O$	$2p_1 p_3$
$I^B I^O$	$2p_2 p_3$

We know that $p_1 + p_2 + p_3 = 1$. Also, p_1, p_2 and p_3 are the frequencies of the alleles I^A, I^B and I^O respectively.

Exercise 7

Find the probabilities of the *ABO* blood phentotypes, *A*, *B*, *AB* and *O* in terms of p_1, p_2 and p_3.

Exercise 8

Out of 2000 people, 37.8 % are type *A*, 14.0 % type *B*, 4.5% type *AB*, and 43.7% type*O*. Find the values of p_1, p_2 and p_3.

Exercise 9

A study of the blood types in France produced information in the table below. What fraction of type A individuals are $I^A I^O$ heterozygotes? Determine the frequencies of the alleles I^A, I^B, and I^O.

Blood Type	*O*	*A*	*B*	*AB*
Frequency	0.441	0.435	0.090	0.034

Mouse coats

Mendel also looked at multiple traits at the same time, for example wrinkled versus non-wrinkled peas and green versus yellow peas. These are the results of combinations of alleles at two different genes on different chromosomes. They behave independently. This is not always the case.

Here is a mouse example where we consider two different genes at two different loci for coat colour.

The video *Gene Mapping* refers to an experiment which resulted in 132 intercross offspring (F2's). The data referred to in this document is from that source. It was originally given in the article of Bahlo and Speed.

The first gene controls whether the mouse is brown (agouti) or black. The agouti allele A is dominant. The allele a is non-agouti and is recessive. This genetic locus is known in mouse genetics as the **agouti locus**. The genotypes for this gene are A/A, A/a and a/a.

The second locus controls whether the mouse is white (albino) or not white. This is known as the **albino locus**. If the mouse is homozygous (c/c) for the white allele c, at the albino locus, the mouse is always white. The c allele is recessive. If the mouse has only one or no copies of the c allele then the mouse can show whatever colour is bestowed upon it by the other coat colour locus in the mouse genome. (The genome is the entirety of an organism's hereditary information.) The allele C is non-white and is dominant. The genotypes for this gene are $c/c, c/C,$ and C/C.

The two genes are not independent. The alleles at the two loci interact. This interaction is called **epistasis** in genetics. The resulting phenotypes of the intercross are as follows:

- The phenotype agouti corresponds to genotypes of the form AxCx.
- The phenotype black corresponds to genotypes of the form aaCx.
- The phenotype albino corresponds to genotypes of the form xxcc. That is, it doesn't matter what alleles are at the agouti locus. The mouse will be albino.

The x in the notation xxcc indicates that it doesn't matter what allele is in that position, the resulting phenotype is determined by cc at the albino locus. Similarly aaCx indicates that the mouse will be black. The last two alleles can be CC or Cc.

The figure below illustrates the mating of a black ($aaCC$) male mouse with an female Albino ($AAcc$) female mouse. These are termed 'parents'.The C57/B16 × NOD label indicates the two strains of mice involved in the mating. The result of this mating is the F1 generation which is all agouti $aACc$.

A mating set up between brothers and sisters from the F1 generation which are identically heterozygous. It is called an **intercross**. The result is the F2 generation.

The two generation sequence was the classic breeding scheme used by Mendel in the formulation of his laws of heredity.

Colour Distribution in the C57/Bl6 X NOD Intercross

Parents:

C57/Bl6 males		NOD females
Black	×	Albinos
aaCC		AAcc

F1s:

All Agouti
aACc

F2s:

Agouti	Black	Albino
9 (9.02)	3 (3.37)	4 (3.61)
AxCx	aaCx	xxcc
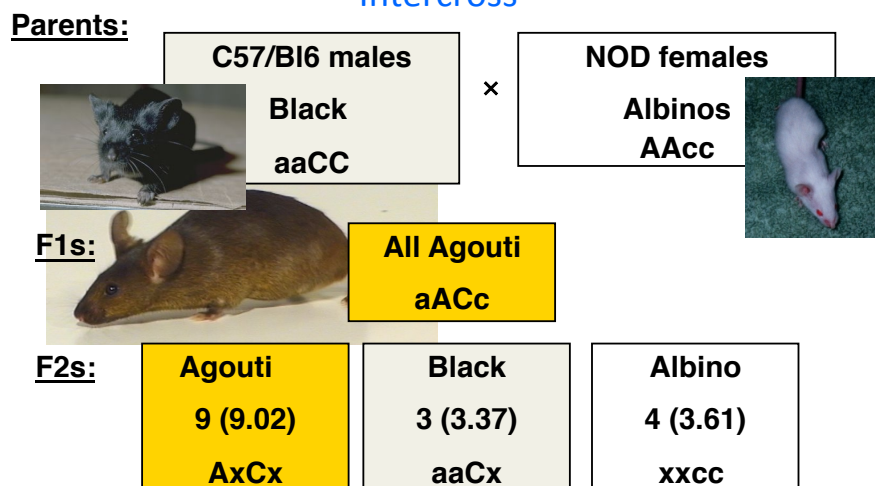
For the F2 generation we have the theoretical ratios of $Agouti : Black : Albino = 9 : 3 : 4$ (see the Punnet diagram below). An experimental ratio was found to be $9.02 : 3.37 : 3.61$.

The punnet diagram below is for the two loci being considered. It shows the resulting phenotypes by shading and the theoretical ratios can be deduced.

	AC	aC	Ac	ac
AC	AACC	AaCC	AACc	AaCc
aC	AaCC	aaCC	AaCc	aaCc
Ac	AACc	AaCc	AAcc	Aacc
ac	AaCc	aaCc	Aacc	aacc

Exercise 10

We consider the F2 generation. Use the shaded punnet diagram to find the probability
of

a of an albino mouse occuring.

b of an agouti or albino mouse .

c of the genotype $AACC$ given agouti phenotype

d of the genotype $aaCc$ given black phenotype.

Exercise 11

Twenty mice from the F2 generation are selected at random. Find the probability of obtaining

- at least one albino mouse

- at least two black mice

- more than 10 agouti mice

What is a Gene map

Gene mapping is the process of establishing the locations of genes on the chromosomes.

The diagram shown below represents a gene map of 150 genetic markers on the 19 non-sex chromosomes (autosomes) for mice. The agouti and albino loci are shown. In the following sections of this document we will discuss how the position of these two loci can be determined. (The unit of map distance shown on the vertical axis is a centiMorgan, cM, this being one-hundredth of Morgan.)

Genetic markers are known sites in the genome that can have different states. Humans and mice all have lots of genetic markers, which is very useful as it turns out.
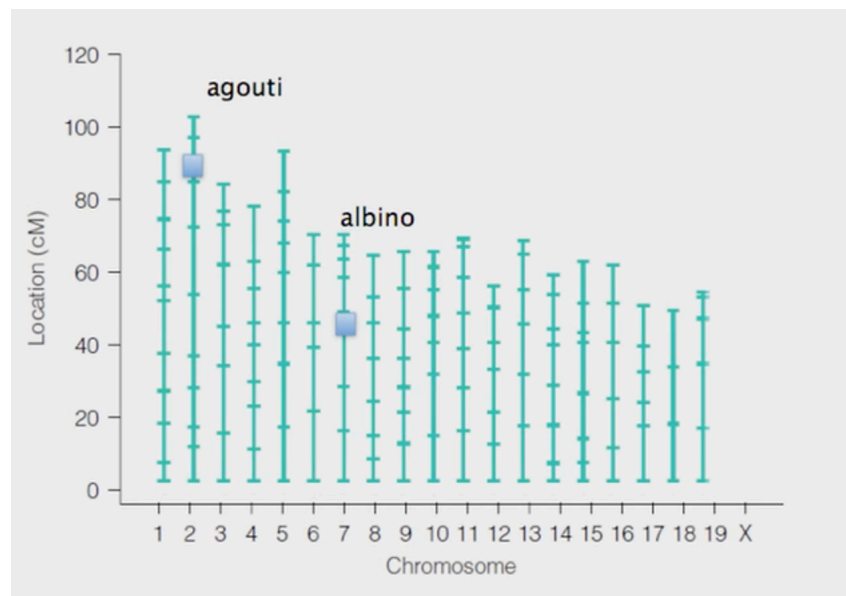
Recombinations

A major problem with Mendel's theory was his law of independent segregation of alleles at two or more loci. As mentioned earlier this does not always happen. A measurement of how wrong it was led to the ability to map out exactly where on the chromosome each of its genes might lie.

Consider a double heterozygous father who has genotype $a_1 b_1$ at locus 1 and genotype $a_2 b_2$ at locus 2. If he obtained the pairs $a_1 a_2$ from one parent and $b_1 b_2$ from the other, we call these **parental pairs** and the other two $a_1 b_2$ and $a_2 b_1$ are called **recombinant pairs**.

Recombination may be caused by loci on different chromosomes that sort independently or by a physical crossing over between two loci on the same chromosome, with breakage and exchange of strands of homologous chromosomes paired in meiosis as shown in the diagram and discussed more fully below. Genes which are close together on the same chromosome are rarely separated by a crossover and they often do not segregate independently. It is this relationship between the distance between two genes and the chance that they are transmitted together that allows us to use information about how frequently recombination occurs to estimate the distance beteen two genes. The result of recombination can be illustrated as shown here.
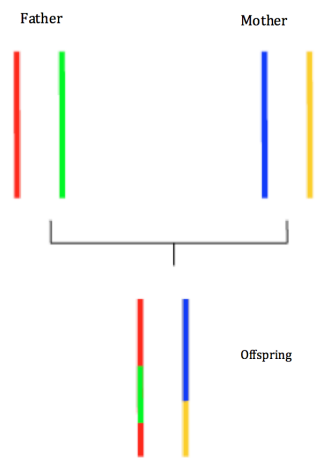

Here is an explanation of meiosis for our purposes. It is from the paper of Bahlo and Speed.

Meiosis

Meiosis is a complex process, but there are only a few features of it we need to know. First, although a father (and mother) passes on to every one of his offspring a copy of each non-sex chromosome and a sex chromosome, these copies are only rarely (perhaps never) precise copies of one of those he received from his (her) parents. Each chromosome passed on by a father in a sperm cell to one of his offspring is a mosaic of his two parental chromosomes, the result of a random process that takes place during meiosis. This random process is repeated independently for each chromosome. The same is the case for mothers and egg cells, although there are important differences in detail that need not concern us here. The mosaic chromosome 1 (for example) that a father passes on to his offspring results from a process whereby his two chromosome 1s are aligned alongside each other and replicated, so that there are now 4 versions of chromosome 1-two copies of his paternal chromosome 1 bundled together and two copies of his mater-

nal chromosome 1 bundled together, as illustrated in the figure. The two chromosomes in a bundle, which are just copies of one another, are called 'sisters.'
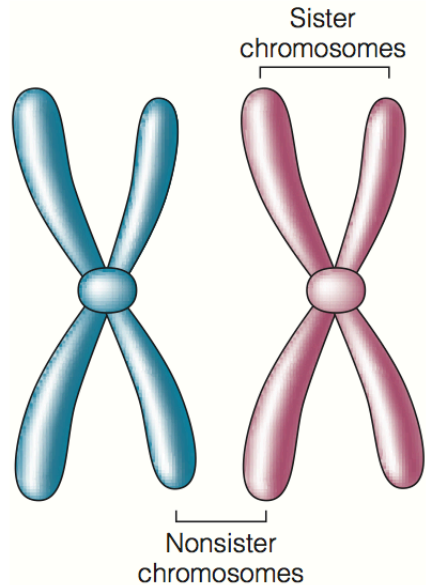
These chromosome pairs line up with each other gene by gene. The adjacent non-sister chromosomes twist around each other and then break and exchange segments, resulting in new allele combinations. That is, rather than the alleles from each parent staying together, new combinations are formed, as shown in the figures below.
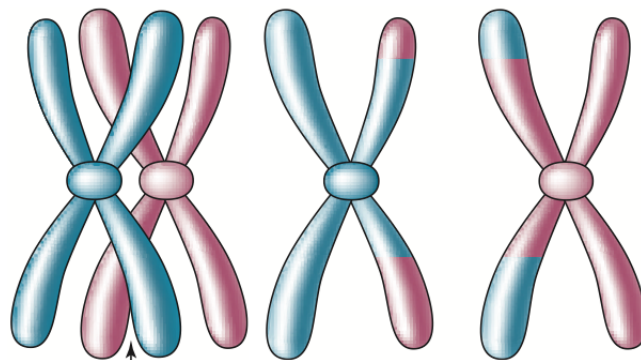
At the end of this process, there are now four versions of chromosome 1- two that were inherited from a parent (called the parental) and two that are a combination of paternal and maternal alleles (called recombinations). These four versions of chromosome 1 now separate, and each is passed to a sperm cell. Thus, a sperm cell might contain a parental or a recombination version of chromosome 1. The positions of the break points are 'random,' and this entire process appears to take place independently for the different chromosomes. With this background, we can now begin to understand the meaning of the

term linkage. For two loci nearby on the same chromosome, there will be a tendency for the alleles from one parent to be transmitted together to a sperm cell. In fact, the closer the loci are to each other on the chromosome, the more likely it will be that these alleles are transmitted together. All of this is a clear violation of MendelâĂŹs fourth postulate. On the other hand, when two loci are on different chromosomes, or far apart on the same, long chromosome, the joint probabilities of co-segregation of the four combinations of alleles turn out to be close to $\frac{1}{4}$ all around. When two loci are on different chromosomes the choice of transmitting one allele or another at one locus is independent of the choice of allele at the other locus because meiosis treats different chromosomes independently. When the loci are well separated on the same chromosome, a similar conclusion holds approximately, as multiple breakage and rejoining events effectively randomize the chromosomal segment between the loci. It is this relationship between the distance between two genes and the chance that they are transmitted together that allows us to use information about how frequently recombination occurs to estimate the distance between two genes. If recombination occurs as often as $\frac{1}{2}$ the time, it is an indication that the two genes under study are either located on different chromosomes or that they are very far apart on the same chromosome. If recombination rarely occurs, it is an indication that the two genes are close together on the same chromosome.

The recombination factor

In the following the recombination factor r between locus 1 and locus 2 is simply the fraction of recombinant pairs passed onto offspring.

For two loci that independently segregate $r = \frac{1}{2}$. This happens if the loci of genes are a long way apart on the same chrosome or are on different chromosomes.

The table below gives the two way tables of their genotypes, where we use the symbols A, B, and H for the genotypes at both loci. Here:

- A means homozygous for the albino strain allele
- B means homozygous for the black strain allele
- H means heterozygous for these two alleles.

We comment that the following material is again based on an article by Bahlo and Speed. The data comes from the associated experiment.

Locus 2

	A	H	B	Total
A	26	10	0	36
Locus 1 H	10	46	9	65
B	0	5	23	28
Total	36	61	32	129

Recall that this intercross resulted from crossing the progeny from the cross of two inbred strains. If we denote the albino-strain alleles by a_1 and a_2 and the black-strain alleles by b_1 and b_2, then all the off- spring of the first mating received pairs $a_1 a_2$ on one chromosome and $b_1 b_2$ on the other from their inbred parents. An *H* parent will (produce the pair $a_1 a_2$ with probability $\dfrac{1-r}{2}$, and similarly for $b_1 b_2$, while the pairs $a_1 b_2$ and $b_1 a_2$ are transmitted with probability $\dfrac{r}{2}$. Where do the factors of $\dfrac{1}{2}$ come from? To see this, recall that each recombination event involves two non-sister chromosome pairs breaking and rejoining differently, but the sperm or egg receives just one of these, chosen at random. Thus if r is the probability of recombination, $\dfrac{r}{2}$ is the probability of an offspring receiving a particular one of the two recombinant chromosomes. We present the result of this in the following table.

Male

	.	$a_1 a_2$	$a_1 b_2$	$b_1 a_2$	$b_1 b_2$
	$a_1 a_2$	$\dfrac{(1-r)^2}{4}$	$\dfrac{r(1-r)}{4}$	$\dfrac{r(1-r)}{4}$	$\dfrac{(1-r)^2}{4}$
Female	$a_1 b_2$	$\dfrac{r(1-r)}{4}$	$\dfrac{r^2}{4}$	$\dfrac{r^2}{4}$	$\dfrac{r(1-r)}{4}$
	$b_1 a_2$	$\dfrac{r(1-r)}{4}$	$\dfrac{r^2}{4}$	$\dfrac{r^2}{4}$	$\dfrac{r(1-r)}{4}$
	$b_1 b_2$	$\dfrac{(1-r)^2}{4}$	$\dfrac{r(1-r)}{4}$	$\dfrac{r(1-r)}{4}$	$\dfrac{(1-r)^2}{4}$

The sum of the probabilities in the table is one.

Terms in this table can now be summed to allow us to build up the table of probabilities for the nine different two-locus genotypes.

Three of the summations are done here and the remaining summations are left to the reader.

- we observe A at locus 1 and H at locus 2 if and only if the transmitted male and female combinations are $a_1 a_2$ and $a_1 b_2$ or $a_1 b_2$ and $a_1 a_2$, leading to a combined probability of $\dfrac{r(1-r)}{4} + \dfrac{r(1-r)}{4} = \dfrac{2r(1-r)}{4}$

- we observe A at locus 1 and A at locus 2 if and only if the transmitted male and female combination is and $a_1 a_2$ leading to a combined probability of $\dfrac{(1-r)^2}{4}$

- we observe H at locus 1 and H at locus 2 if and only if the transmitted male and female combination is and $a_1 a_2$ and $b_1 b_2$ or $b_1 b_2$ and $a_1 a_2$ or $a_1 b_2$ and $b_1 a_2$ or $b_1 a_2$ and $a_1 b_2$ leading to a combined probability of

$$\frac{(1-r)^2}{4} + \frac{(1-r)^2}{4} + \frac{r^2}{4} + \frac{r^2}{4} = \frac{2(r^2 + (1-r)^2)}{4}$$

These results are collected in the table below.

Locus 1

		A	H	B
Locus 2	A	$\dfrac{(1-r)^2}{4}$	$\dfrac{2r(1-r)}{4}$	$\dfrac{r^2}{4}$
	H	$\dfrac{2r(1-r)}{4}$	$\dfrac{2[r^2 + (1-r)^2]}{4}$	$\dfrac{2r(1-r)}{4}$
	B	$\dfrac{r^2}{4}$	$\dfrac{2r(1-r)}{4}$	$\dfrac{(1-r)^2}{4}$

The multinomial distribution function gives:

$$Pr(X_1 = 26, X_2 = 10, \cdots) = \frac{129!}{26! \times 10! \times 0! \times \cdots \times 5! \times 23!} \left(\frac{(1-r)^2}{4}\right)^{26} \times \left(\frac{2r(1-r)}{4}\right)^{10} \times \left(\frac{r^2}{4}\right)^{0} \times \cdots \times \left(\frac{(1-r)^2}{4}\right)^{23}$$

For $r = \dfrac{1}{2}$ this table of probabilities looks as shown.

Locus 1

		A	H	B
Locus 2	A	$\dfrac{1}{16}$	$\dfrac{1}{8}$	$\dfrac{1}{16}$
	H	$\dfrac{1}{8}$	$\dfrac{1}{4}$	$\dfrac{1}{8}$
	B	$\dfrac{1}{16}$	$\dfrac{1}{8}$	$\dfrac{1}{16}$

In this case the multinomial distribution function gives: :

$$Pr(X_1 = 26, X_2 = 10, \cdots) = \frac{129!}{26! \times 10! \times 0! \times \cdots \times 5! \times 23!} \left(\frac{1}{2^6}\right)^{26} \times \left(\frac{1}{2^4}\right)^{10} \times \left(\frac{1}{2^5}\right)^{0} \times \cdots \times \left(\frac{1}{2^4}\right)^{23}$$

Likelihood function

Each value of r results in a function

$$L(r) = \left(\frac{(1-r)^2}{4}\right)^{26} \times \left(\frac{2r(1-r)}{4}\right)^{10} \times \left(\frac{r^2}{4}\right)^0 \times \cdot \times \left(\frac{(1-r)^2}{4}\right)^{23}$$

This expression is called the lilelihood function, $L(r)$.

We are interested in the value of r for which the observed data recorded in the table above is most likely to occur. To do this we maximise $L(r)$ with respect to r.

LOD function

To investigate whether two loci on the same chrosome we are investigating are linked statististicians look at the LOD function. The LOD function for our situation is

$$LOD = \log_{10}\left[\frac{L(\hat{r})}{L(1/2)}\right]$$

where $\hat{r}$ is the maximum likelihood estimator of r. When $\hat{r}$ is near $\frac{1}{2}$ we can see that LOD is near 0 and that it will be large when it is unlikely that $r = \frac{1}{2}$ We briefly discuss how the LOD function can be implemented in the section below.

Linkage groups

We recall that a marker is a DNA sequence with a known physical location on a chromosome. In our case there are 156 markers. Concerning the use of the LOD function I directly quote from Bahlo and Speed. 'Normal statistical practice would have us determine a cutoff for the test that would limit the chance of a type 1 error under the null hypothesis, that is, the chance of rejecting the hypothesis that $r = \dfrac{1}{2}$ when it is in fact correct. This practice is rarely adopted in genetics, where tradition there dictates the use of more stringent thresholds that take into account the multiple testing common with linkage mapping. In genetics, we rarely carry out a single test of the kind just described; rather, many similar tests are conducted with the same data, some of which are correlated, and so new methods for controlling the overall error rate have evolved. For example, to create a genetic map of markers from a cross like ours, we would begin by computing all pairwise recombination fractions and the associated LOD scores. In our case, this would be $\frac{156 \times 155}{2} = 12{,}090$ calculations. We would then set a LOD cutoff, for example, 3, and a recombination fraction cut-off, for example 0.3, and declare as linked any pair of markers whose recombination fraction was less than 0.3 and whose LOD exceeds 3, or is connected by a sequences of marker pairs each satisfying this threshold.

We could then use these results to group the markers, putting markers that were thought to be linked into the same group. '

These groups are called **linkage groups**. Once these linkage groups are found the next step would be to order loci in each linkage group. In the diagram below you will see that there are on average 7-8 markers per chromosomes. To compare these might involve doing 8! = 40320 eight-locus calculations.

The mapped locations of the markers is shown in the diagram below. The 19 autosomes and the X chromosome is shown.
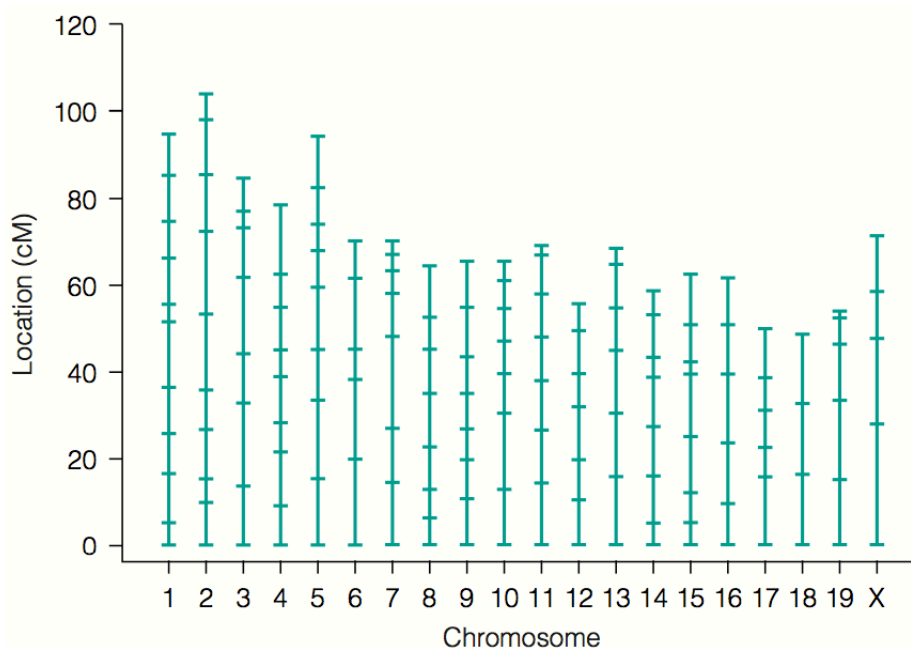
Mapping the albino trait

Now that the markers are mapped, we next describe how to map the *albino* locus. In the following we work with a model connecting phenotype with genotype. The albino trait is easily seen to segregate as a a recessive gene. We postulate the existence of a locus having a recessive and dominant allele, with the recessive case leading to the albino phenotype. The analyis seeks markers closely linked to the albino locus and so we calculate the LOD score for each marker in relation to this trait. Here we demonstrate the process with three markers, one on chromosome 12, another on chromosome 2 and a third on chromosome 7. The first is unlinked to either trait, the second closely linked to the agouti locus and the third closely linked to the albino locus.

Chromosome 12

Coat	A	H	B	Total
Agouti	19	35	18	72
Black	8	18	3	29
Albino	9	12	7	28
Total	36	65	28	129

Chromosome 2

Coat	A	H	B	Total
Agouti	24	46	2	72
Black	0	1	28	29
Albino	5	14	6	25
Total	29	61	36	126

Chromosome 7

Coat	A	H	B	Total
Agouti	3	47	19	69
Black	0	19	10	29
Albino	21	1	0	22
Total	24	67	29	120

Here is the table introduced on page 24

Locus 1

		A	H	B
Locus 2	A	$\dfrac{(1-r)^2}{4}$	$\dfrac{2r(1-r)}{4}$	$\dfrac{r^2}{4}$
	H	$\dfrac{2r(1-r)}{4}$	$\dfrac{2[r^2+(1-r)^2]}{4}$	$\dfrac{2r(1-r)}{4}$
	B	$\dfrac{r^2}{4}$	$\dfrac{2r(1-r)}{4}$	$\dfrac{(1-r)^2}{4}$

We can identify the *A* row with the albino geotype and aggregate the *H* and *B* rows into the full colour genotype to form the table below.

Locus

| *Colour*	A	H	B
Albino	$\dfrac{(1-r)^2}{4}$	$\dfrac{2r(1-r)}{4}$	$\dfrac{r^2}{4}$
Full colour	$\dfrac{1}{4} - \dfrac{(1-r)^2}{4}$	$\dfrac{1}{2} - \dfrac{2r(1-r)}{4}$	$\dfrac{(1-r^2)}{4}$

We can also collapse the data tables above to two rows: albino and full colour. This is done here for chromosome 7.

Chromosome 7

Coat	A	H	B	Total
Albino	21	1	0	22
Full Colour	3	66	29	98
Total	24	67	29	120

For Chromosome 7 we have the following likelihood function Each value of r results in a probability:

$$L(r) = \left(\frac{(1-r)^2}{4}\right)^{21} \times \left(\frac{2r(1-r)}{4}\right)^1 \times \left(\frac{r^2}{4}\right)^0 \times \left(\frac{1}{4} - \frac{(1-r)^2}{4}\right)^3 \times \left(\frac{1}{2} - \frac{2r(1-r)}{4}\right)^{66} \times \left(\frac{(1-r^2)}{4}\right)^{29}$$

A section of the graph of $L(r)$ against r is shown for a suitable domain.
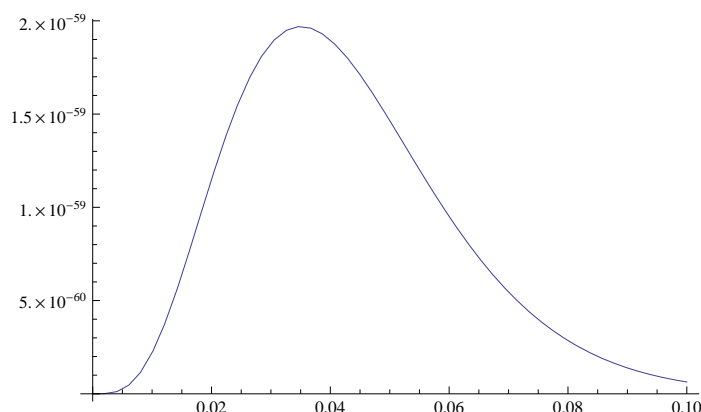

Using Mathematica it is found that the maximum occurs when $r = 0.350621...$ and the corresponding value of the LOD function is 24.0879.

In our present context, with 152 markers, what is known as a genomewide scan was carried out; that is, the LOD score was computed at every marker locus. This is shown in

the graph below.. The result is striking and clearcut: a single high peak above 20 on chromosome 7 at the locus corresponding to the counts in the table. It turns out that this is marker is just 6 cM from the albino locus. Since most mouse chromosomes have length of the order of 1 Morgan, or less, a distance of 6 cM is quite small.
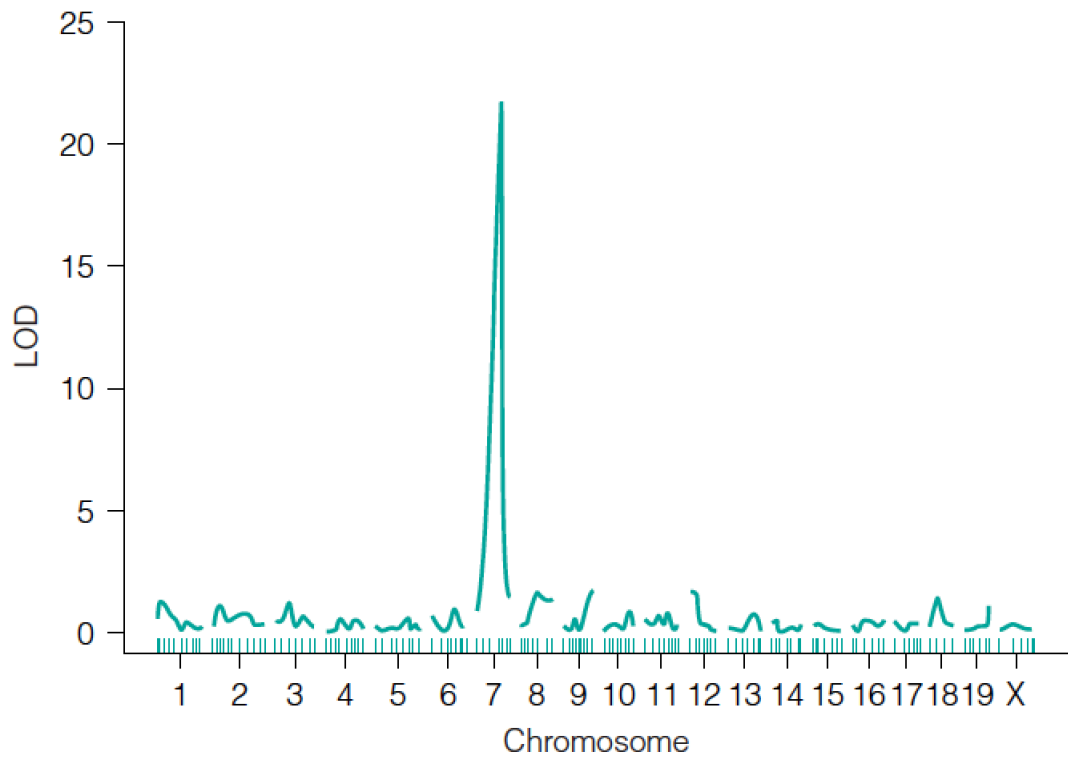
Answers to exercises

Exercise 1

a Probability of *yyww* given yellow and wrinkled = $\dfrac{1}{3}$

b Probability of *yyww* given yellow = $\dfrac{1}{12}$

c Probability of *yyww* given yellow = $\dfrac{1}{4}$

d Probability of *yyww* given yellow = $\dfrac{1}{4}$

Exercise 2

a Probability of at least 5 seeds being green = 0.5852 (Binomial with 20 trials, Probability of green = $\dfrac{1}{4}$

b Probability of at least 5 seeds being green and round = 0.3164 (Binomial with 20 trials, Probability of green and round = $\dfrac{3}{16}$

c Probability of less than 10 seeds being yellow and round = 0.0039 (Binomial with 20 trials, Probability of yellow and round = $\dfrac{3}{4}$

Exercise 3

We have $P = 0.64$, $2Q = 0.32$ and $R = 0.04$. $P' = (P + Q)^2 = 0.8^2 = 0.64 = P$ $2Q' = 2(P + Q)(Q + R) = 2(0.8)(0.2) = 0.32 = 2Q$ $R' = (Q + R)^2 = 0.2^2 = 0.04 = R$

Note that $Q^2 = 0.2056$ and $PR = 0.64 \times 0.04 = 0.0256$

Exercise 4

a The proportion of $A_1 A_2$ is 18

Let p be the proportion of the A_1 allele and q the proportion of the A_2 allele.

We have $2pq = 0.18$ and thus $pq = 0.09$. Also $p + q = 1$.

$$pq = 0.09$$

$$p(1 - p) = 0.09$$

$$p - p^2 - 0.09 = 0$$

$$(p - 0.1)(p - 0.9) = 0$$

$$p = 0.1 \quad \text{or} \quad p = 0.9$$

The proportion of allele A_1 is 0.1 or 0.9.

b   The proportion of genotype $A_1 A_!$ is 0.01 or 0.81.

c   The proportion of allele A_2 is 0.9 or 0.1.

d   The proportion of genotype $A_2 A_2$ is 0.81 or 0.01.

Exercise 5

Probability of type O blood is $\dfrac{1}{4}$

Exercise 6

- The individuals with type A blood are homozygous $I^A I^A$

- The individuals with type AB blood are heterozygous $I^A I^B$

- The individuals with type B blood are homozygous $I^B I^B$

$$\text{Frequency of } I^A = \frac{2 \times (\text{number of } I^A I^A) + ((\text{number of } I^A I^B)}{2\,(\text{total number of individuals})}.$$

$$= \frac{2 \times 200 + 75}{2 \times (200 + 75 + 25)}$$

$$= \frac{475}{600}$$

$$= \frac{19}{24}$$

$$\approx 0.792$$

$$\text{Frequency of } I^B = \frac{125}{600}$$

$$= \frac{5}{24}$$

$$\approx 0.208.$$

Exercise 7

Phenotype	Genotype	Probabilty
A	$I^A I^A$	p_1^2
B	$I^B I^B$	p_2^2
O	$I^O I^O$	p_3^2
AB	$I^A I^B$	$2 p_1 p_2$
A	$I^A I^O$	$2 p_1 p_3$
B	$I^B I^O$	$2 p_2 p_3$

$$Pr(A) = p_1^2 + 2p_1 p_3$$

$$Pr(B) = p_2^2 + 2p_2 p_3$$

$$Pr(AB) = 2p_1 p_2$$

$$Pr(O) = p_3^2$$

Exercise 8

$$p_1^2 + 2p_1 p_3 = 0.378 \tag{1}$$

$$p_2^2 + 2p_2 p_3 = 0.14 \tag{2}$$

$$2p_1 p_2 = 0.045 \tag{3}$$

$$p_3^2 = 0.437 \tag{4}$$

From (4) $p_3 \approx 0.6611$

From (1) $p_1 \approx 0.2417$

From (2) $p_2 \approx 0.0985$

Exercise 9

Blood Type	O	A	B	AB
Frequency	0.441	0.435	0.090	0.034

Let p_1, p_2 and p_3 be the frequency of the alleles I^A, I^B and I^O respectively.

Then $p_3^2 = 0.441$. Hence $p_3 = 0.6641$

$$p_1^2 + 2p_1 p_3 = 0.435 \qquad (1)$$

$$p_2^2 + 2p_2 p_3 = 0.09 \qquad (2)$$

$$2p_1 p_2 = 0.034 \qquad (3)$$

From (1), $p_1 = 0.2719$

From (2) $p_2 = 0.0646$

Exercise 10

a The probabability of albino $= \dfrac{1}{4}$.

b The probabability of albino or agouti $= \dfrac{13}{16}$.

c The probability of the genotype $AACC$ given agouti $= \dfrac{1}{9}$.

d The probability of the genotype $aaCc$ given agouti $= \dfrac{1}{3}$.

Exercise 11

a Probability of at least 1 albino mouse

$= 1 -$ probability of no albinos

$= 1 - \left(\dfrac{3}{4}\right)^{20}$

$= 0.9968.$

b Probability of at least two black mice $= 0.9117 \qquad$ (Binomial, $n = 20$ and $p = \dfrac{3}{20}$)

c Probability of more than 10 agouti mice $= 0.2493 \qquad$ (Binomial, $n = 20$ and $p = \dfrac{9}{20}$)

Links and References

Links

http://www.mendelweb.org

MendelWeb is an educational resource for teachers and students interested in the origins of classical genetics, introductory data analysis, elementary plant science, and the history and literature of science. Constructed around Gregor Mendel's 1865 paper "Versuche uber Pflanzen-Hybriden" and a revised version of the English translation by C.T. Druery and William Bateson, "Experiments in Plant Hybridization",

http://www.genome.gov/Glossary/

The National Human Genome Research Institute (NHGRI) created the Talking Glossary of Genetic Terms to help everyone understand the terms and concepts used in genetic research. In addition to definitions, specialists in the field of genetics share their descriptions of terms, and many terms include images, animation and links to related terms.

http://www.mendel-museum.com

Masaryk University Mendel Museum Augustinian Abbey in Old Brno, Brno, Czech Republic

References

Melanie Bahlo and Terry Speed, *HOW MANY GENES? Mapping Mouse Traits,* Statistics: A Guide to the Unknown, chapter 17, Duxbury Press, 4th edition, in partnership with the American Statistical Association,(2005).

Ewens W.J. *Mathematical Population Genetics (2nd Edition).* Springer-Verlag, New York,(2004).

G.H. Hardy, *Mendelian Proportions in a Mixed Population,* (Letter to Science, 1908) Science N. S. Vol. XXVIII:49-50, (July 10 1908).

0   1   2   3   4   5   6   7   8   9   10   11   12