

King's College London (KQC) University of London

SCIENCE SIMULATIONS LABORATORY

GENETIC MAPPING

STUDENTS' MANUALS (Version 1.02.2003)

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STUDENTS' MANUAL A - MENDELIAN INHERITANCE AND LINKAGE

Although Gregor Mendel (1822-1884) (Figure A1) published the results of his famous experiments on inheritance in peas in 1866, his work remained virtually unknown until 1900 when it was rediscovered simultaneously, and apparently independently, by Hugo de Vries (1848-1935) in Amsterdam, Karl Correns (1864-1933) in Tübingen and Erich von Tschermak (1871-1962) in Vienna. Each repeated Mendel's experiments, confirmed his results and showed that Mendel's so-called 'Laws' were applicable to seed plants in general. In 1902 Lucien Cuenot (1866-1951) and William Bateson (1861-1926) demonstrated that the same principles apply also to animals.



Figure A1 Gregor Mendel

Further experiments during the next few years continued to confirm the validity of Mendel's conclusions. However, in 1906 Bateson and Reginald Punnett (1875-1967) discovered, in the genetics of the sweet pea, a major exception to Mendel's 'Second Law'. This law, known as the 'Law of Independent Assortment' states that:

When two or more pairs of genes segregate simultaneously, the distribution of any pair of them is independent of the others.

It was found that two pairs of alleles, those for flower colour and those for shape, of the pollen grain, did not in fact segregate independently.

Several, rather inadequate, explanations of the anomaly were put forward. It was not until 1910 that Thomas Morgan (1866-1945) brilliantly demonstrated that the exception was the rule, and that the 'Second Law' was the exception! In the process he showed how it was possible, using the data from simple breeding experiments, to map the genes in the chromosomes of animals, plants and (later) micro-organisms. Morgan's student Calvin Bridges (1899-1938) went on to produce detailed genetic maps of the four pairs of chromosomes of the fruitfly, *Drosophila melanogaster*, while other workers mapped the genes of higher plants, such as maize, *Zea mays*, and micro-organisms such as the bacterium, *Escherichia coli*.

Soon after the rediscovery of Mendel's work, the marked similarity between the segregation of Mendelian 'factors' (i.e. genes) and the behaviour of chromosomes during gamete production was noted. Could it be that chromosomes were more or less equivalent to genes?

Morgan pointed out that higher organisms have many thousands of characters, each presumably determined by one or more genes, but the diploid number of chromosomes seldom exceeds one hundred. It follows that each chromosome must bear many different genes and these genes must be 'linked' and therefore have a tendency to be inherited together. The various characters which Mendel examined in peas are determined by genes either on different chromosomes or at least very far apart on the same chromosome. He would not otherwise have obtained the relatively simple 3:1 and 9:3:3:1 F₂ ratios which enabled him to lay the foundations of modern genetics.

STUDENTS' MANUAL B - LINKAGE, CROSS-OVER AND GENETIC MAPPING

The tendency for linked genes to be inherited together is not absolute, owing to the phenomenon of 'crossing-over' or swapping of genes between homologous chromosomes during meiosis as shown in Figure B1. Morgan suggested that the amount of crossing-over (and hence recombination) between a pair of genes in a chromosome is related to the distance between the genes. The greater the distance, the more likely (in general) that a cross-over will occur between them.

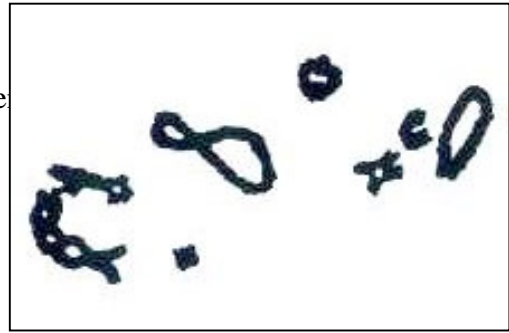


Figure B1 The diplotene stage of meiosis showing 'crossing-over' between homologous chromosomes

Consider a heterozygous individual which is test-crossed (i.e. crossed with a genetically recessive homozygous individual):

$$\begin{array}{ccc}
 \text{female} & & \text{male} \\
 \\
 q \ s \ z & & q \ s \ z \\
 \underline{\quad} \ \underline{\quad} \ \underline{\quad} & \times & \underline{\quad} \ \underline{\quad} \ \underline{\quad} \\
 Q \ S \ Z & & q \ s \ z
 \end{array}$$

where Q/q, S/s and Z/z are the symbols for three genes (in order) in the same chromosome.

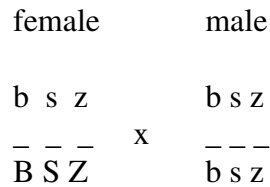
The male is homozygous recessive for all three genes and so crossing-over will have no effect on the genotypes of his gametes. The phenotypes of the offspring from this cross will therefore correspond to the maternal gamete genotypes:

Parental genotypes	Gamete genotypes
$ \begin{array}{l} q \ s \ z \\ \underline{\quad} \ \underline{\quad} \ \underline{\quad} \\ Q \ S \ Z \end{array} $	$ \begin{array}{l} \dots\dots\dots \\ qsz, QSZ, Qsz, qSZ, qSz, QsZ, QSz, qsZ \end{array} $
$ \begin{array}{l} q \ s \ z \\ \underline{\quad} \ \underline{\quad} \ \underline{\quad} \\ q \ s \ z \end{array} $	$ \begin{array}{l} \dots\dots\dots \\ qsz \end{array} $

If a cross-over involving these genes occurs during gamete formation it could be between Q and S, or between S and Z. It is also possible for two crossovers to occur simultaneously, one between Q and S, and another between S and Z. This latter, so-called double cross-over, being a combination of two relatively unlikely events, occurs less frequently than either of the two single cross-overs.

STUDENTS' MANUAL C - SOME EXAMPLES OF GENETIC MAPPING

Consider a test-cross involving the genes B, S and Z. These genes are all in the same chromosome, but their order is unknown:



The distribution of offspring phenotypes from the cross is:

bsz	105)	BsZ	26)
) 172) 58
BSZ	67)	bSz	32)
BSz	8)	Bsz	64)
) 18) 152
bsZ	10)	bSZ	88)

The total number of offspring in this example is 400.

We have seen that the relatively rare double crossovers result in the smallest pair of phenotypic classes. In this case the smallest classes are BSz and bsZ. This means that the order of the genes must be BZS, because only in this order will double cross-overs (i.e. between B and Z, and between Z and S) result in the phenotypes BzS (i.e. BSz) and bZs (i.e. bsZ). Notice that the recessive allele of the middle gene (z) is the one isolated from the recessive allele of the other two genes (b and s) in the double cross-over classes.

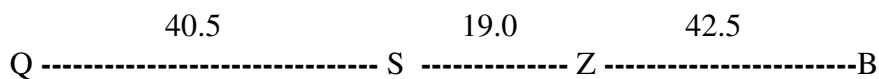
$$\text{Percent crossovers between B and Z} = \frac{152 + 18}{400} \times 100 = 42.5\%$$

$$\text{Percent crossovers between Z and S} = \frac{58 + 18}{400} \times 100 = 19.0\%$$

The genetic map of this part of the chromosome is thus:



By matching up such partial genetic maps, rather like doing a linear jig-saw puzzle, it is possible to piece together the linkage group:



Data from test-crosses involving other genes (D, E, G, N and so on.....) enable the investigator to fill in further details and eventually construct a picture of the entire linkage group.

Consider another test-cross:

female		male
e s z		e s z
— — —	x	— — —
E S Z		e s z

Here is the distribution of offspring phenotypes from the test-cross:

esz	31	EsZ	6
ESZ	34	eSz	8
ESz	3	Esz	8
esZ	2	eSZ	8

- C1 *What is the order of these genes?*
- C2 *What distances separate adjacent genes in this group? Draw a map of the linkage group ESZ and fit this map into that of the linkage group as it has been developed.*
- C3 *Consider an organism whose genotype is*

$$\begin{array}{c} d \ g \ n \\ \overline{\quad} \ \overline{\quad} \ \overline{\quad} \\ \overline{D} \ \overline{G} \ \overline{N} \end{array}$$

What would be the genotypes of gametes produced in a meiotic division when two cross-overs occur, both between the genes D and G? Would two cross-overs between D and G be detectable?

- C4 *What effect would such an occurrence have upon the accuracy of the genetic map of these genes?*
- C5 *What phrases would be more appropriate than 'number of cross-overs' and 'percent cross-overs' as used up till now?*
- C6 *What, if any, special significance has a map distance of 50 map units?*

STUDENTS' MANUAL D – THE COMPUTER PROGRAM GENETIC MAPPING

The computer program *Genetic Mapping* was written to give you an opportunity to reinforce your understanding of these ideas by putting into practice the theory of genetic mapping of chromosomes by the triple test-cross procedure. *Genetic Mapping* models inheritance in a hypothetical diploid species which has a single chromosome or linkage group of ten genes whose symbols are A, C, H, J, K, M, P, R, T and Y. This is not, however, the order of the genes. It is your task to establish their arrangement in the linkage group. *Genetic Mapping* will effect a series of genetic crosses, as specified by you, and display the results for any combination of three genes from the above list.

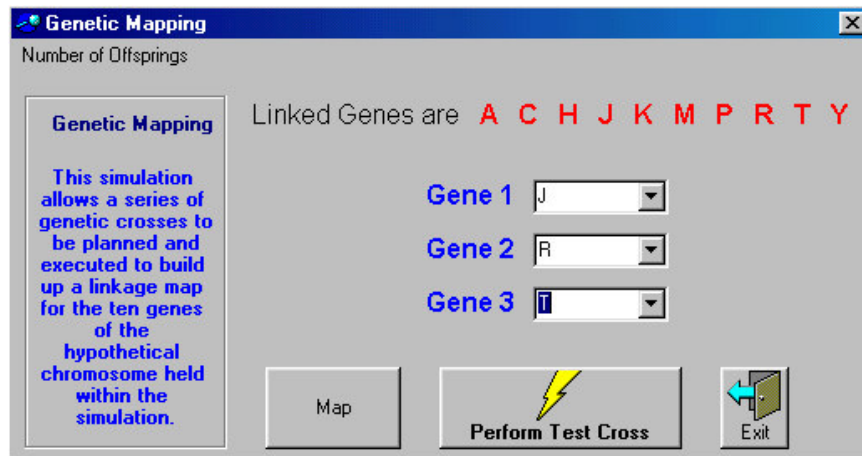


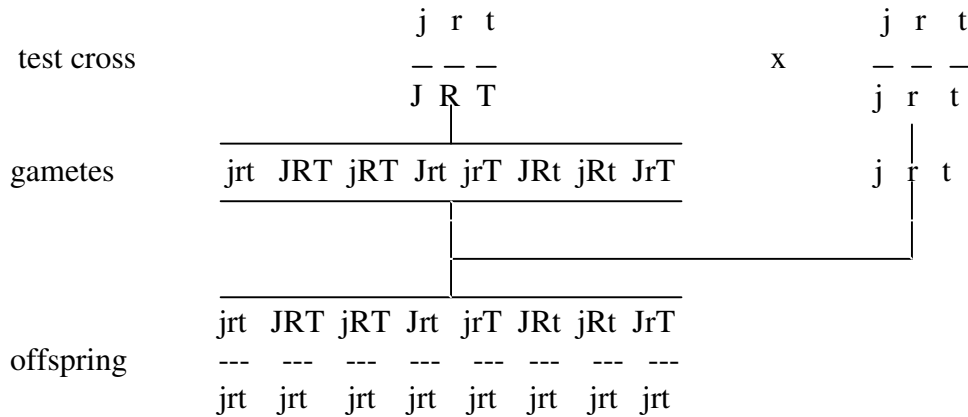
Figure D1 Screen printout from *Genetic Mapping* showing the specification of a cross

To specify your first genetic cross, select the three genes to be involved in a triple test-cross as shown in Figure D1.

When the genes are specified the program brings into 'existence' a strain of the species which is homozygous recessive for those genes and another strain which is homozygous dominant. The two strains are crossed, resulting in a heterozygous F1 generation. Suppose the genes J, R and T are chosen as in the example in Figure D1.

parents	j r t	x	J R T
	— — —		— — —
	j r t		J R T
gametes	j r t		J R T
	— — —		— — —
	j r t		J R T
	— — —		— — —
	j r t		J R T

An F1 organism is then test-crossed. Test-crossing involves mating an organism with a homozygous recessive strain (which, in this instance, is equivalent to a back-cross with the homozygous recessive parent):



The phenotypes (not the genotypes) of one hundred offspring of this test-cross are displayed already classified and counted after pressing the *Process* button as shown in Figure D2.

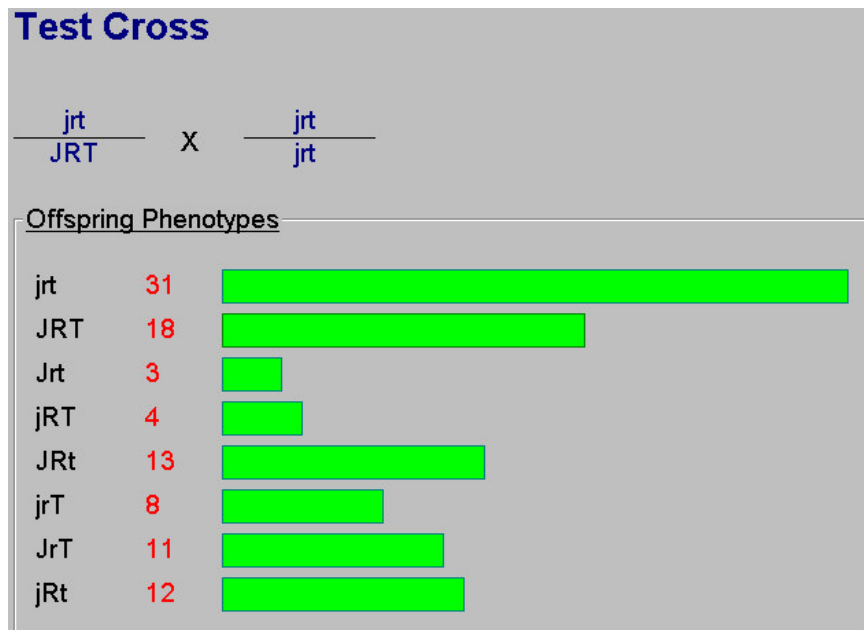


Figure D2 Screen Printout from *Genetic Mapping* showing the offspring phenotypes from the cross specified in Figure D1.

D1 Why, do you suppose, has this simulation been designed to produce 100 test-cross progeny in the first instance?

By performing two simple calculations a map of the genes J, R and T can be constructed.

D2 What, according to the results of this c-roes (Figure D2), is the arrangement of the genes J, R and T in the linkage group?

After the first test cross you can select different combinations of genes and carry out further breeding 'experiments' until sufficient information has been accumulated to draw a complete map of the linkage group. A cross involving the genes C, J and T is illustrated in Figure D3.

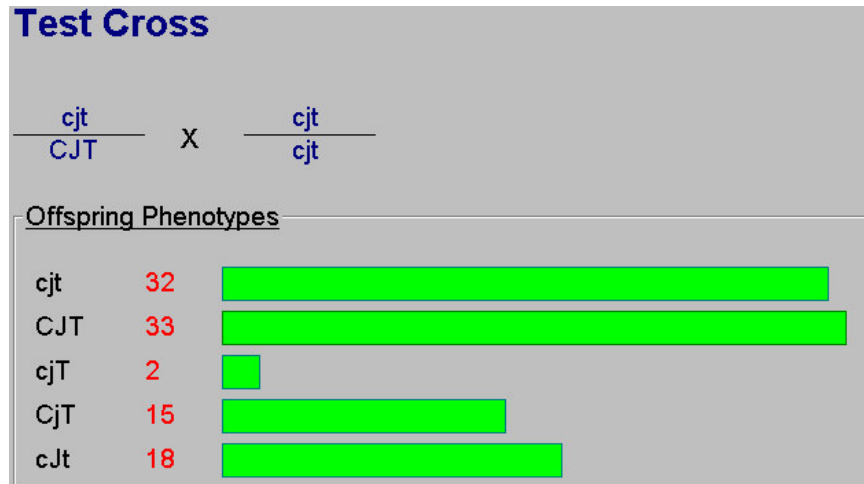
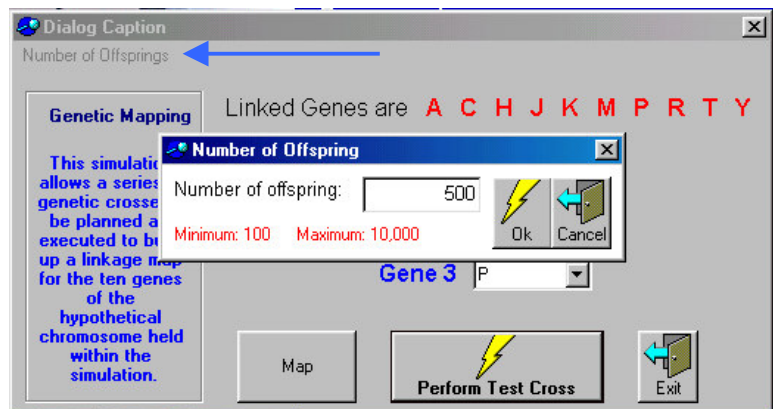


Figure D3 Screen printout from *Genetic Mapping* showing another cross.

D3 What is the order of the genes C, J and T? Phenotype classes which happen to contain no organisms are not displayed and so the smallest classes may not necessarily be the obvious ones.

You can specify the number of offspring from 100 to 10000. If this is not set, 100 is assumed.



STUDENTS' MANUAL E - MAPPING THE LINKAGE GROUP

The task of mapping the linkage group is best tackled in two stages with somewhat different goals:

- 1 Establish, beyond doubt, the order of the genes.
- 2 Produce a map with the greatest degree of accuracy, by performing crosses involving adjacent genes.

E1 Why is it suggested that crosses involving adjacent genes should result in a more accurate map t crosses involving randomly picked genes?

You should tote that chance plays a very important part in this computer simulation, as it does in nature, and that repetition of a particular cross may not result in the same frequencies of phenotypes. For accurate mapping of genes you may need to duplicate some crosses, particularly those involving closely linked genes.

So, your task is to map the entire linkage group of this hypothetical species.

E2 What is the genetic map of the linkage group?

With **Genetic Mapping**, unlike in experiments with living organisms, you can compare your map with the actual ' chromosome' inside the computer program. When you have constructed your genetic map, ask your teacher how it can be compared with the so-called ' cytological' map.

E3 How well does you--.genetic map compare with the ' cytological' map? Is the order of the genes the same in each? Are the relative distances between the genes the same or are there lengths of chromosome subject to particular distortion?

E4 In the light of this Comparison, do you think that Morgan' s suggestion, that the amount of crossin-over between a pair of linked genes is related to their separation, is valid?

Figure E1 shows the genetic map of part of one of the chromosomes of the fruit fly, *Drosophila melanogaster*. It was compiled by similar methods to those you have used with *Genetic Mapping*.

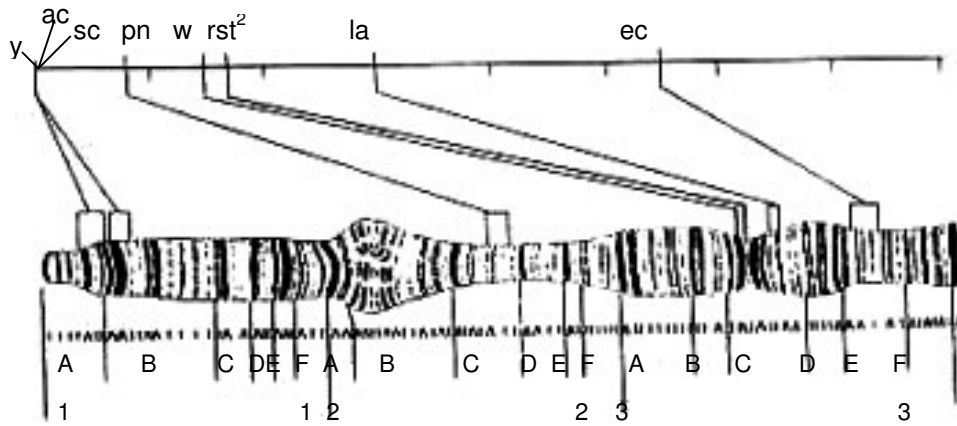


Figure E1

Part of a giant chromosome from the salivary gland of the fruit fly, *Drosophila melanogaster*. The reference system below was produced by C. Bridges and the letters above mark areas known to be associated with certain characteristics, e.g. "y" is the gene responsible for yellow body colour.