

Appendix 2

Investigations, data handling and statistics

■ Science – how is it done?

Biology is a science. It relies on evidence in its attempts to explain and apply ideas about how the natural world functions. People often talk about the ‘**scientific method**’ as if there is a single, unique method of finding out, different from other methods of discovery. In fact there are many successful methods used by scientists. This is clear when we look at the ways in which Charles Darwin, Louis Pasteur and Francis Crick worked, for example. Nevertheless, all science includes some common features. These include:

- observing and measuring
- hypothesizing and predicting
- designing and planning a sequence of observations, investigations or experiments
- carrying out these plans
- recording data
- interpreting results and drawing conclusions
- communicating.

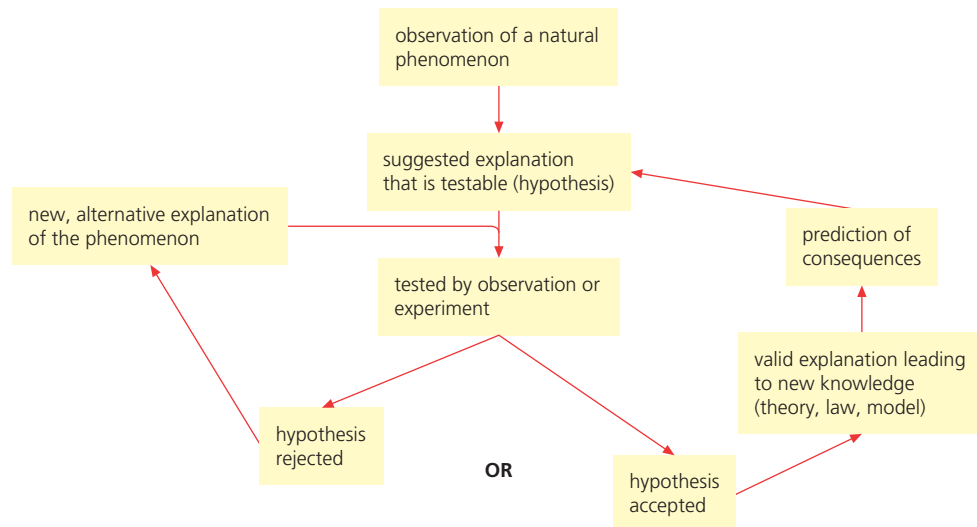
Hypothesis:

A scientific hypothesis is a tentative explanation of a natural phenomenon capable of being tested by observation or experiments.

Central to these is the concept of a **hypothesis**.

Sometime a hypothesis is formulated by examining data or from observations. It is equally likely to arise from an inspired guess or a wild hunch. Whatever the origin of the hypothesis, valid and reliable evidence, or data, is required to support it. This may come from well-planned observations and experiments.

■ **Figure A2.1**
A flow diagram of the processes of science



Additional perspectives

A hypothesis is never really proved but can be disproved

It was Karl Popper, a philosopher of science who showed that a hypothesis is never really proved but can be disproved at any time, by a single contrary observation. He maintained that all explanations of science are of this type. That is, scientific knowledge is always only tentative. It is the best available explanation we can offer at any time. Any explanation may be proved wrong or incomplete, and often is, sooner or later.

■ Obtaining and recording data

Types of data

In practicals and investigations, observations are recorded as data. There are different forms of data, but they are all potentially useful. No one type of data that is relevant to your enquiry is better than any other, providing they have been accurately made and recorded. The type of data we may collect is either qualitative (as words) or quantitative (as numbers).

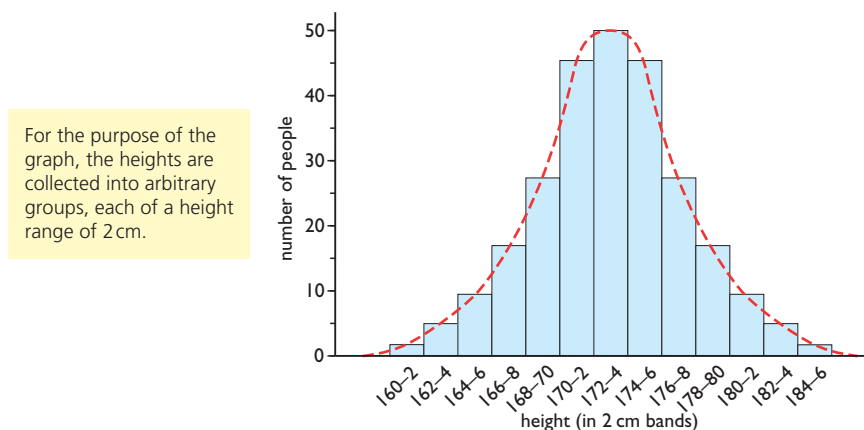
- **Qualitative** (or descriptive) **observations** – An example from classification studies is the colour or shape of the parts of flowers. An example from behaviour studies is the feeding mechanism of honey bees visiting flowers. Qualitative data may be recorded in written observations or notes, by photography or in drawings, for example.
- **Quantitative** (or numerical) **observations** – Examples are the size (length, breadth or area) of the leaves of a plant in shaded and exposed positions, and the pH values of soil samples in different positions.

In Table A2.1 this division is further classified.

■ **Table A2.1**
Categories of qualitative and quantitative data

Type of variable	Type of data
Qualitative	
categoric	nominal, i.e. values or observations belonging to it can be sorted according to category, e.g. colour of flowers, gender
ordered	ordinal, i.e. values that can be placed in an order or rank, the interval between them may not be equal, e.g. opinion judgments – ‘completely agree’, ‘mostly agree’, ‘mostly disagree’, ‘completely disagree’.
Quantitative – illustrated in Figure A2.2	
continuous	interval, that can have any value within a specific range and can be a whole number, a fraction or a decimal. It can be counted, ordered and measured, e.g. body mass, time taken for seeds to germinate after different treatments
discrete	interval, that can have only a limited number of values which are usually whole numbers, e.g. the number of seeds in a bean pod, the number of cells in a hemocytometer grid

■ **Figure A2.2**
Continuous data



Additional perspectives

Handling very large and very small numbers

In science ‘powers of ten’ are used to avoid writing long strings of zeros when recording numbers. For example, the age of the Earth, about 4 500 000 000 years, is written as 4.5×10^9 years. Similarly, a cyanobacterium, a photosynthetic bacterium, may be about 0.000 003 6 metre in diameter, which is written as 3.6×10^{-6} m.

This way of recording numbers is called scientific or **standard notation**. It is used to avoid the errors that are easily made when writing down a large number of zeros. Also, when we need to multiply numbers we can do so by adding powers. Similarly, to divide, the powers are subtracted.

Table A2.2
Powers of ten

Several of the powers of ten have prefixes which are frequently used in biology and are represented by agreed symbols (Table A2.2).

$\times 10^3$	kilo- or k	$\times 10^{-1}$	deci- or d
$\times 10^6$	mega- or M	$\times 10^{-2}$	centi- or c
$\times 10^9$	giga- or G	$\times 10^{-3}$	milli- or m
		$\times 10^{-6}$	micro- or μ
		$\times 10^{-9}$	nano- or n

These letters are written in front of the symbol used to represent the quantity being measured, such as kg, km, mm, nm.

Collecting and recording data

When you plan an investigation or experiment, think carefully about the data you expect to collect. This allows you to prepare a recording sheet with spaces for the information (both qualitative and quantitative). This will show how often you plan to record the data as well as exactly what will be recorded. An example of such a recording sheet is illustrated in Figure A2.3.

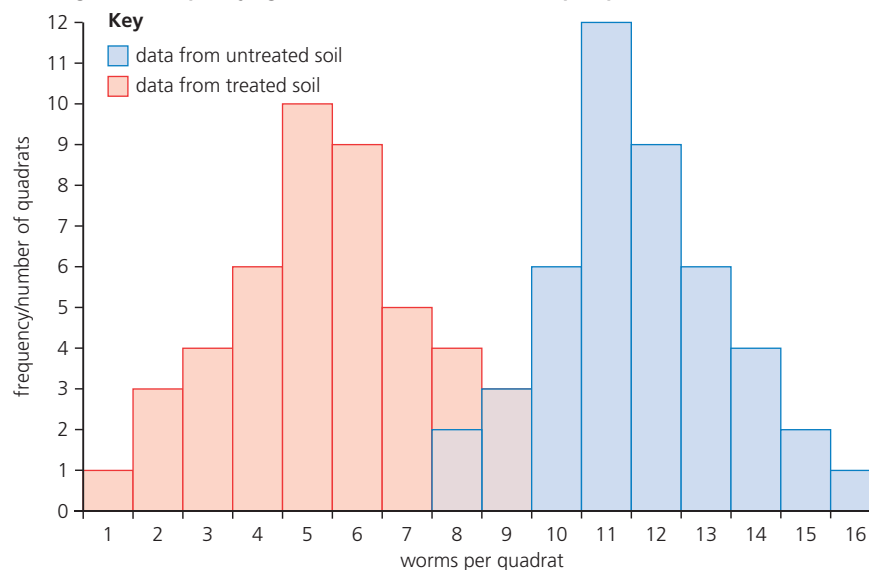
Figure A2.3

An investigation of the effect on earthworm populations of pesticide treatment

experimental results

Quadrats on soil treated with pesticide			Quadrats on untreated soils		
Worms per quadrat	Frequency	Total	Worms per quadrat	Frequency	Total
0		0	7		0
1		1	8		2
2		3	9		3
3		4	10		6
4		6	11		12
5		10	12		9
6		9	13		6
7		5	14		4
8		4	15		2
9		3	16		1
10		0	17		0

histogram of frequency against numbers of earthworms per quadrat



Controlled (standardized) variables:

the variables in an experiment or investigation that are kept the same so they do not influence the measurement of the dependent variable.

Independent variable:

the variable in an experiment or investigation that is manipulated or changed.

Dependent variable:

the variable in an experiment or investigation that is measured.

Reliability:

reliable results are repeatable by the same student and reproducible by others.

Variables

In an investigation or experiment the specific conditions are called variables. Most of the variables are deliberately kept the same, but others vary. In fact there are really three types of variable. We can demonstrate them by referring to an example experiment, such as the investigation of the rate of an enzyme-catalysed reaction, shown in Figure 2.41, page 100.

Remind yourself of this investigation, now.

The variables that are always kept the same in this investigation of the rate of reaction of catalase are the conditions such as temperature, the volumes and concentrations of the reagents, and perhaps the pH (the volume and type of buffer, if used, for example). Variables that are kept constant are called the **controlled** or **standardized variables**.

Meanwhile, in Figure 2.41 you can see that measurements are made at 30 second intervals. So the times that readings are taken are the variable that is manipulated by the experimenter, and is called the **independent variable**. Note that it has been recorded in the table as a list before the experiment was started.

In the experiment, the amount of gas (oxygen) that has collected at 30 second intervals is the variable accurately measured by the experimenter. It is called the **dependent variable** and is recorded in the table. (Sometimes, two dependent variables may be measured. So, for example, in photosynthesis both oxygen production and carbon dioxide intake may be measured.)

In a scientific investigation a **control** is used to ensure that any effects observed are due to changes in values of the independent variable and not some unidentified variable. Thus a control is one value of the independent variable. For example, in the investigation of the action of the enzyme catalase in bringing about the breakdown of hydrogen peroxide, the control involves the use of boiled enzyme, since heat denatures a protein and destroys its catalytic properties (Figure 2.40, page 99).

Errors and the need for replicates

Errors and mistakes may occur in experiments and investigations, just as in any other human activity. You cannot have complete confidence about the accuracy of every individual observation you make. For example, an incorrect amount of reagent may accidentally be measured out and the mistake not noticed. Alternatively, a stop watch may be misread or a reading from an instrument may be inaccurately taken on some occasion. Meanwhile, you need confidence your results are accurate.

Consequently, the experiment should be repeated to provide another set of readings at the same time intervals. If any of the measurements taken are wildly different you would be expected to further repeat the experiment. Ideally, a minimum of three sets of measurements is required for minimum **reliability**.

Repetitions of an experiment are called **replicates**. So, in a class practical experiment carried out by you and your peers, individual results may be pooled together as replicate readings. Replicate readings with similar results give confidence in the conclusions drawn.

■ Presenting data

Firstly, you must select from the data what is important. For example, it may be appropriate to round numbers in order to avoid giving data to a greater level of accuracy than the measurements warrant. Then you need to display the important data using a visual summary, making their importance clear. This might involve graphs, bar charts, histograms, scatter graphs or pie charts, for example.

Graphs

Graphs show relationships or trends between two variables. It is conventional to plot the dependent variable (the variable being measured) on the y (vertical) axis and the independent variable (the variable altered by the experimenter) on the x (horizontal) axis.

Both axes must be labelled and the units indicated. Points (a dot within a circle or a cross, for example) must be plotted with a sharp pencil. The points should be joined by a smooth curve only if you are confident that it indicates the likely points of intermediate readings. If you are not confident of this, connect points with a straight line. If more than one line is plotted, then the plot points and lines must be different and be clearly labelled. If the two plots have different vertical axes, then the scale of the axes should be placed on either side of the graph.

The term *graph* applies to the whole graphic representation. The line on the graph showing a relationship, whether straight or curved, is referred to as a *curve*. Examples of graphs are seen throughout this book.

- 1 The following results were obtained in an investigation of the effects of pre-incubation of starch and amylase solutions at different temperatures on the subsequent hydrolysis of the starch to sugar.

Temp/°C	10	20	30	35	40	45	50	55	60
Time/s	100	58	30	21	15	11	19	46	100

Plot a graph of these results, applying the rules and conventions detailed here. Check your work against the model graph provided at the end of this Appendix.

Bar charts

Bar charts are used to show relationships between the independent variable (on the x axis) and the dependent variable (on the y axis) when the independent is categoric and the dependent variable is continuous, such as the range of tree species found in woods. There should be small gaps between the lines or bars used, which should be of equal width, and typically presented in order of magnitude (Figure A2.4A).

Histograms

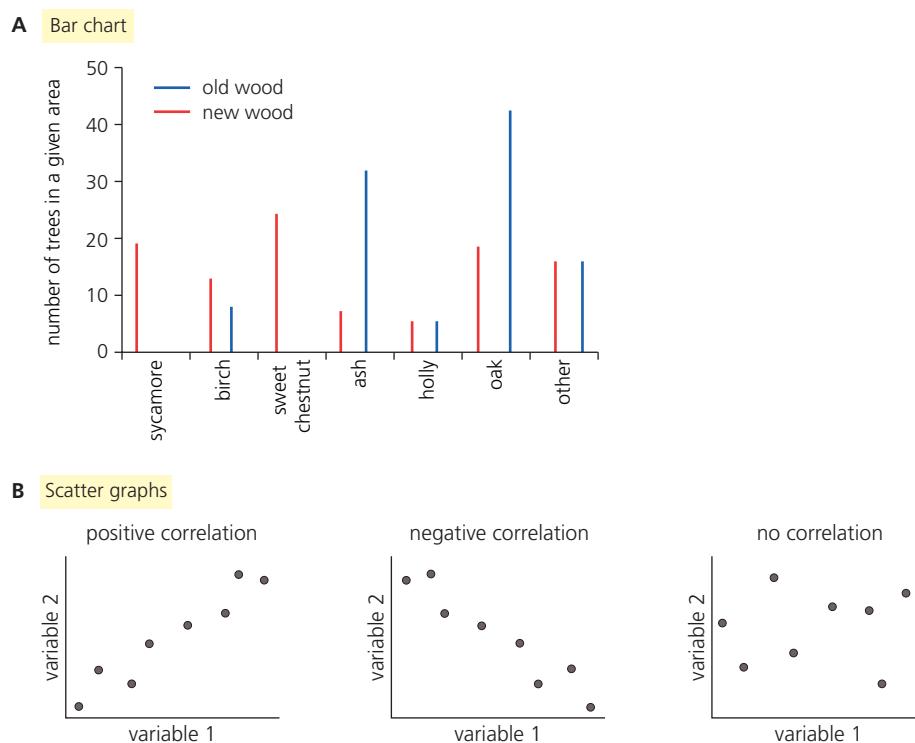
Histograms are useful to display continuous data. They show the variations in a sample of repeated measurements. The x axis represents the variation in the repeated measurements. The y axis is the frequency or number in each class. Normally the blocks are drawn touching. There should be an informative title (Figure A2.3).

Scatter graphs

We construct a scatter graph to investigate whether there may be a relationship between two variables. If one variable increases, does the other also increase? This is a common situation in biology, for example, the question of whether there is a correlation between blood pressure and heart rate, or smoking and heart disease, or river flow rate and diversity of aquatic non-vertebrates. The first step in investigating a correlation is to plot a scatter graph of one variable against another. The shape of the scatter graph indicates the type of correlation. By 'correlation' we mean a mutual relation between two (or more) things, or an interdependence of variable quantities. If both variable increase together then there is a **positive correlation**; if one variable decreases when the other increases then there is a **negative correlation**. The closer the data points come to laying on a straight line, the closer the relationship; the more scattered the data points the less close the relationship. If the scatter graph has random points then there is no correlation (Figure A2.4B).

It is important to realize that a correlation between two variables does *not* necessarily mean that the variable are **causally linked**. So, having applied a statistical test that indicates the possibility of a correlation, we have to go on to investigate the mechanisms of the linkage, if there is one.

■ **Figure A2.4**
Bar charts and
scatter graphs



Pie charts

Pie charts are best used for showing relative proportions, for example Figure 5.13, page 224.

■ Statistical checks on data

Statistical tests should be used when you are not sure about the numerical relationships your data indicates. The application of simple statistical tests is described below. Many calculators are programmed to carry out statistical tests. Microcomputers will run spreadsheet programs with statistical tests programmed in. Dedicated statistical software is available too.

The normal distribution

Data obtained from biological experiments may show a 'normal distribution'. By this we mean that when the frequency of particular classes of measurements (such as the number of humans at any particular height) is plotted against the classes of measurements (their different heights arranged in ascending order), a symmetrical bell-shaped curve is obtained (Figure A2.5).

With normal distributions, two issues may arise.

1 At what value do the readings cluster?

This can be expressed as:

- the average or arithmetic **mean**, calculated by dividing the sum of the individual values by the number of values obtained

$$\text{The formula for the arithmetic mean: } \bar{x} = \frac{\sum x}{n}$$

where \bar{x} = arithmetic mean

$\sum x$ = the sum of all the measurements

n = the total number of measurements.

- the **mode**, the most frequent value in a set of values
- the **median**, the middle value in a set of values arranged in ascending order.

2 How spread out are the readings?

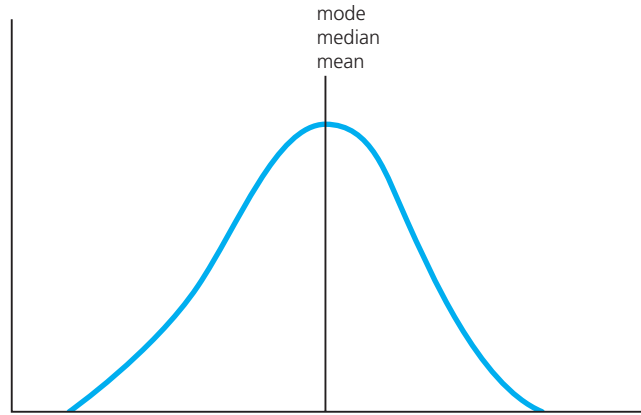
This can be expressed as the **standard deviation** (s) from the mean. This is a measure of the variation from the mean of a set of values.

Standard deviation: the spread of a set of data from the mean of the sample is a measure of the variability of a population from a sample. A small standard deviation indicates that the data is more reliable.

■ **Figure A2.5**
Frequency distributions
of statistical and
skewed data

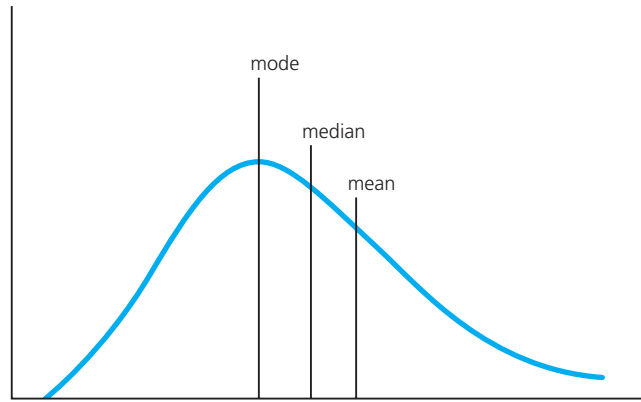
Normal distribution curve
Most biological data shows variability, but with values grouped symmetrically around a central value.

Here the mode, median and mean coincide.



Skewed distribution
Values reduce in frequency more rapidly on one side of the most frequently obtained value than the other.

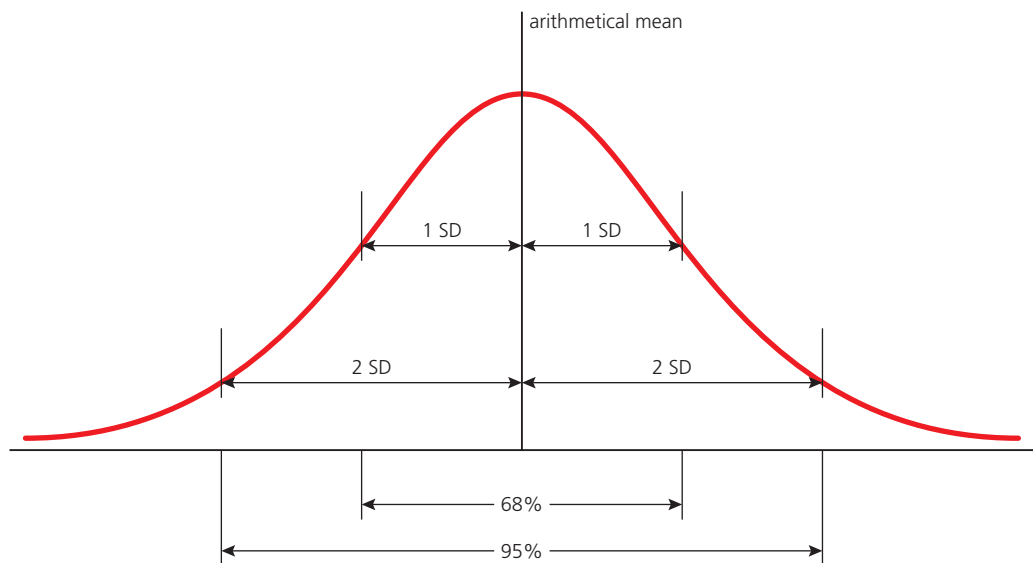
Here the difference between the mean and mode is a measurement of 'skewness' of the data.



- A small standard deviation indicates that the data is clustered closely around the mean value.
- A large standard deviation indicates a wider spread around the mean.

Once obtained, the value may be applied to the normal distribution curve (Figure A2.6). Note that 68% of the data occurs within one standard deviation of the mean and more than 95% of the data occurs within two standard deviations of the mean. So, a small standard deviation indicates the observations (the values) differ very little from the mean. Standard deviation is also a measure of reliability, so a small standard deviation indicates reliability.

■ **Figure A2.6**
The normal
distribution and
its SD



Calculating standard deviation

The standard deviation (s) is calculated in five steps.

- 1 First, calculate the mean, \bar{x} .
- 2 Calculate the deviation of each value from the mean: $x - \bar{x}$.
- 3 Square the deviations: $(x - \bar{x})^2$.
- 4 Add the squared deviations: $\Sigma(x - \bar{x})^2$.
- 5 Finally, divide by the number of values (n).

An example

An ecologist investigated the reproductive capacity of two species of a common grassland flower *Ranunculus acris* and *R. repens*. The latter species spreads vegetatively via strong and persistent underground stems. Would the use of vegetative reproduction result in fewer fruits from sexual reproduction compared to the other species that reproduces only by fruit production?

Using comparable sized plants growing under similar conditions in the same soil, the numbers of fruits formed in 100 flowers of each species were counted and recorded. The results are given in Figure A2.7 and calculations of the standard deviations are shown in Figure A2.8. Note that you are not expected to know the formula for calculating standard deviation. The purpose of presenting the steps in the calculation is to take away the mystery of a calculation normally carried out by a scientific calculator or programmed spreadsheet.

■ **Figure A2.7**
Data on fruit
production in two
species of *Ranunculus*

Number of fruits	Frequency	
	<i>R. repens</i>	<i>R. acris</i>
15	1	0
16	1	0
17	1	0
18	2	1
19	4	1
20	4	1
21	8	1
22	7	1
23	9	3
24	10	4
25	16	4
26	9	5
27	10	5
28	4	6
29	5	8
30	3	14
31	1	12
32	1	10
33	2	7
34	1	3
35	1	2
36	0	3
37	0	2
38	0	3
39	0	2
40	0	2
41	0	0

Interpreting standard deviation

We have noted that a low value for the standard deviation indicates that the observations differ very little from the mean and that a high values for the standard deviation indicates a wider spread around the mean.

Consequently, the standard deviation can be used to help to decide whether the differences between the two related means are significant or not, such as those shown in Figure A2.3 for example. If the standard deviations are much larger than the difference between the means, then the differences in the means are highly unlikely to be significant. On the other hand, when the standard deviations are much smaller than the differences between the means, then the differences between the means is almost certainly significant.

fruit production in *Ranunculus acris*

Values obtained in ascending order x	Frequency f	fx	Deviation of x from the mean $(x - \bar{x}) [= d]$	d^2	fd^2
17	0				
18	1	18	-12	144	144
19	1	19	-11	121	121
20	1	20	-10	100	100
21	1	21	-9	81	81
22	1	22	-8	64	64
23	3	69	-7	49	147
24	4	96	-6	36	144
25	4	100	-5	25	100
26	5	130	-4	16	80
27	5	135	-3	9	45
28	6	168	-2	4	24
29	8	232	-1	1	8
30	14	420	0	0	0
31	12	372	1	1	12
32	10	320	2	4	40
33	7	231	3	9	63
34	3	102	4	16	48
35	2	70	5	25	50
36	3	108	6	36	108
37	2	74	7	49	98
38	3	114	8	64	192
39	2	78	9	81	162
40	2	80	10	100	200
41	0				
$\Sigma f = 100$		$\Sigma fx = 2999$	$\Sigma fd^2 = 2031$		

$$\text{Mean of data} = \frac{\Sigma fx}{\Sigma f} = \frac{2999}{100} = 29.99$$

$$\text{SD} = \sqrt{\frac{\Sigma fd^2}{\Sigma f - 1}} = \sqrt{\frac{2031}{99}} = \sqrt{20.51} = 4.53$$

Thus the mean of the sample *Ranunculus acris* = 29.99, and the SD = 4.53.

 fruit production in *Ranunculus repens*

Values obtained in ascending order x	Frequency f	fx	Deviation of x from the mean $(x - \bar{x}) [= d]$	d^2	fd^2
14	0				
15	1	16	-9	81	81
16	1	17	-8	64	64
17	1	36	-7	49	98
18	2	76	-6	36	144
19	4	80	-5	25	100
20	4	168	-4	16	128
21	8	154	-3	9	63
22	7	207	-2	4	36
23	9	240	-1	1	10
24	10	400	0	0	0
25	16	234	1	1	9
26	9	270	2	4	40
27	10	112	3	9	36
28	4	145	4	16	80
29	5	90	5	25	75
30	3	31	6	36	36
31	1	32	7	49	49
32	1	66	8	64	128
33	2	34	9	81	81
34	1	35	10	100	100
35	1	36	11	121	121
36	0				
$\Sigma f = 100$		$\Sigma fx = 2479$	$\Sigma fd^2 = 1479$		

$$\text{Mean of data} = \frac{\Sigma fx}{\Sigma f} = \frac{2479}{100} = 24.79$$

$$\text{SD} = \sqrt{\frac{\Sigma fd^2}{\Sigma f - 1}} = \sqrt{\frac{1479}{99}} = \sqrt{14.93} = 3.86$$

Thus the mean of the sample *Ranunculus repens* = 24.79, and the SD = 3.86.

■ **Figure A2.8** Calculating the means and standard deviations of the data in Figure A2.7

Standard error:

an estimate of the reliability of the mean of a population sample. A small standard error indicates that the mean value is close to the actual mean of the population.

Standard error

The **standard error** (S_M) represents how well the sample mean approximates the population mean. The larger the sample, the smaller the standard error, and the closer the sample mean approximates the population mean. The standard error is obtained by dividing the standard deviation, s , by the square root of n , the sample size.

$$\text{Standard error, } S_M = \text{standard deviation, } \frac{s}{\sqrt{n}}$$

When graphs are presented showing mean values, error bars are added to each value plotted to demonstrate the deviation of the sample from the true population mean. Error bars ($\pm S_M$) extend above and below the points plotted on a graph to show this variability.

The t -test

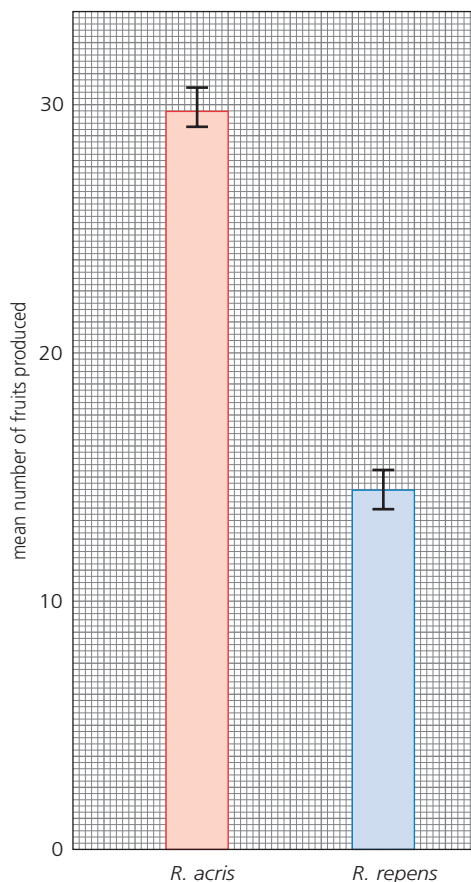
Statistical tests using standard deviation or standard error typically compare large, randomly selected representative sample of normally distributed data. In practice it is often the case that data can only be obtained from quite small samples. The t -test may be applied to sample sizes of more than 5 and less than 30 taken from normally distributed data. It provides a way of

■ **Figure A2.9**
Standard error and error bars

Calculating the standard errors (S_M):

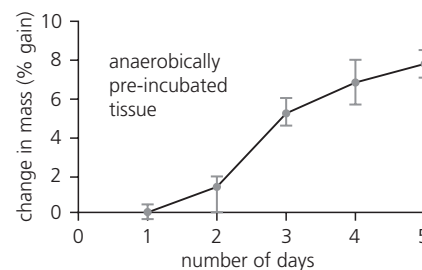
	Standard deviation, s	$\frac{s}{\sqrt{n}}$	Standard error, S_M
<i>R. acris</i>	4.53	$\frac{4.53}{10}$	0.453
<i>R. repens</i>	3.86	$\frac{3.86}{10}$	0.386

Adding standard errors to a display of the means:



An example of the addition of error bars to a graph:

In an experiment on the effect of anaerobic pre-treatment of tissue discs on their subsequent change in mass, samples of 10 thin discs of plant tissue were used. (Thin cut discs allow all cells in a sample to receive more or less identical conditions.) The results of this enquiry indicated that anaerobic pre-treatment of the discs leads to a gain in mass. Error bars have been added to the curve in this graph.



measuring the overlap between two sets of data – a large value of t indicates little overlap and makes it highly likely there is a significant difference between the two data sets. The following example illustrates the method of the t -test; however, you should note that you are not expected to calculate values of t .

Additional perspectives

The null hypothesis

Statistical tests are hypothesis-testing statistics. They test a mathematical statement called the **null hypothesis**. Where we are comparing data the null hypothesis states that there is no difference between the sets of data. When we are looking for an association it states that there is no association.

The outcome of a statistical test is a probability that the null hypothesis is true. A probability (known as the p value) varies from 0 (impossible) to 1 (certain). Since the p values are small, they are given as a percentage (0 to 100%) to avoid possible confusion with small numbers. The lower the probability, the less likely it is that the null hypothesis is true.

Now the t -test makes comparison between means of data to test for significant differences between the samples. For the t -test the null hypothesis is 'There is no difference between the means'. By convention in biology, if the probability is greater than 0.05 (5%) then the null hypothesis is accepted. However, if the probability is 0.05 or less ($p < 0.05$), then the null hypothesis is rejected. This implies the event is predicted to happen by chance less than once in twenty times. So the difference is judged to be **significant**.

Applying the *t*-test

An ecologist was investigating woodland microhabitats, contrasting the communities in a shaded position with those in full sunlight. One of the plants was ivy (*Hedera helix*), but relatively few occurred at the locations under investigation. The issue arose: were the leaves in the shade actually larger than those in the sunlight?

Leaf widths were measured but, because the size of the leaves varied with the position on the plant, only the fourth leaf from each stem tip was measured. The results from the plants available are shown in Table A2.3.

■ **Table A2.3**
Widths of leaves of
Hedera helix in sun
and shade

Size-class/mm	Widths of leaves from plants in sun (a)	Widths of leaves from plants in shade (b)
20–24	24	
25–29	26, 26	26
30–34	30, 31, 31, 32, 32, 33	33, 34
35–39	37, 38	35, 35, 36, 36, 36, 37
40–44	43	41, 42
45–49		45

The steps of the *t*-test

- 1 The null hypothesis assumes the difference under investigation has arisen by chance. That is, there is no difference in width between leaves from plants growing in sun and shade. The role of this statistical test is to determine whether to accept or reject the null hypothesis. If it is rejected in this case, we can have confidence that the difference in the leaf sizes of the two samples is statistically significant.
- 2 Next, check that the data is normally distributed. This is done by arranging the data for the two samples as in Table A2.3 (and plotting a histogram, if necessary).
- 3 **You are not expected to calculate values of *t*.** This is a statistic which, if required can be found by using a scientific or statistics calculator or by means of a spreadsheet incorporating formulae.

Actually, the formula for the *t*-test for unmatched samples (data sets a versus b) is:

$$t = \frac{\bar{x}_a - \bar{x}_b}{\sqrt{\left(\frac{s_a^2}{n_a} + \frac{s_b^2}{n_b}\right)}}$$

where:

\bar{x}_a = the mean of data set a

\bar{x}_b = the mean of data set b

s_a^2 = the standard deviation of data set a, squared

s_b^2 = the standard deviation of data set b, squared

n_a = the number of data items in set a

n_b = the number of data items in set b

- 4 Once a value of *t* has been calculated (the value of *t* in this case is 2.10) we need to consult a table of critical values for the *t*-test, once we have determined the degrees of freedom (df) for the two samples, using the formula:

$$\begin{aligned} df &= (\text{total number of values in both samples}) - 2 \\ &= (n_a - 1) + (n_b - 1) \end{aligned}$$

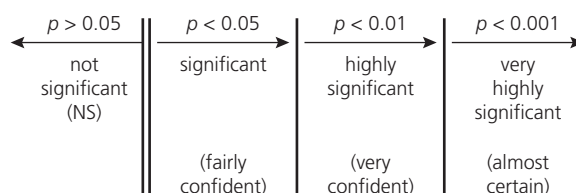
In this case, there are $11 + 11 = 22$ degrees of freedom.

- 5 A table of critical values for the *t*-test is given in Figure A2.10. Look down the column of significance levels (*p*) at the 0.05 level until you reach the line corresponding to 22 degrees of freedom. You will see that in this case, $p = 2.08$.

- 6 Since the calculated value of t (2.10) exceeds this critical value (2.08) at the 0.05 level of significance, it indicates that there is a lower than 0.05 probability (5%) that the difference between the two means is solely due to chance. Therefore, we can reject the null hypothesis, and conclude **the difference between the two samples is significant**. (For the experimenter, the significance of this statistic suggests there is a reason for the difference in the means. This can be further investigated and perhaps a fresh hypothesis proposed.)

■ **Figure A2.10**
Critical values for
the t -test

Degrees of freedom (df)	decreasing value of p →			
	p values			
	0.10	0.05	0.01	0.001
1	6.31	12.71	63.66	636.60
2	2.92	4.30	9.92	31.60
3	2.35	3.18	5.84	12.92
4	2.13	2.78	4.60	8.61
5	2.02	2.57	4.03	6.87
6	1.94	2.45	3.71	5.96
7	1.89	2.36	3.50	5.41
8	1.86	2.31	3.36	5.04
9	1.83	2.26	3.25	4.78
10	1.81	2.23	3.17	4.59
12	1.78	2.18	3.05	4.32
14	1.76	2.15	2.98	4.14
16	1.75	2.12	2.92	4.02
18	1.73	2.10	2.88	3.92
20	1.72	2.09	2.85	3.85
22	1.72	2.08	2.82	3.79
24	1.71	2.06	2.80	3.74
26	1.71	2.06	2.78	3.71
28	1.70	2.05	2.76	3.67
30	1.70	2.04	2.75	3.65
40	1.68	2.02	2.70	3.55
60	1.67	2.00	2.66	3.46
120	1.66	1.98	2.62	3.37
∞	1.64	1.96	2.58	3.29



The chi-squared test

χ is the Greek letter *chi*. We have previously introduced and applied the **chi-squared** (χ^2) test to discover whether observed numerical results differ from the expected numerical result (pages 188–191 and Table 10.5, page 426). It tests for ‘goodness of fit’ between an observed distribution and a theoretical one. It allowed us to test whether the observed results obtained from the dihybrid cross between *Drosophila* of normal flies (wild type) with flies homozygous for vestigial wing and ebony body differ significantly from the expected outcome.

Turn back to page 426 and refresh your memory of the formula for the chi-squared statistic and how it is applied. Note that in the chi-squared test, the null hypothesis is ‘Observed frequencies equal expected frequencies’. The chi-squared test is also applied in ecology for looking at the differences in distribution of organisms in different habitats.

In summary

We have now introduced the four tests that you may need to be able to apply. The formulae for all four are given in Figure A2.11.

You do not need to memorize the formulae or the meaning of their symbols. However, you may need to use them in practical work:

- to calculate a standard deviation
- to put error bars on graphs
- to test for a significant difference between the means of two small samples
- to perform a chi-squared test on suitable data.

To do this you will have access to the formulae, the meaning of the symbols, a *t*-table and a chi-squared table. Rather than carry out all the steps of a test in an examination, you may be given partly completed calculations to finish. Consequently, it is helpful to be fully acquainted with the use of an approved electronic calculator and have used it to become familiar with each of the four tests.

■ **Figure A2.11**
Statistical tests and
their symbols

standard deviation	$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$	
<i>t</i> -test	$t = \frac{ \bar{x}_1 - \bar{x}_2 }{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$	$v = n_1 + n_2 - 2$
χ^2 test	$\chi^2 = \sum \frac{(O - E)^2}{E}$	$v = c - 1$
standard error	$S_M = \frac{s}{\sqrt{n}}$	

Key to symbols

s = standard deviation	\bar{x} = mean	S_M = standard error	c = number of classes
Σ = 'sum of'	n = sample size (number of observations)	O = observed 'value'	
x = observation	v = degrees of freedom	E = expected 'value'	

■ Linear magnification and actual sizes of images and objects

1 Calculating linear magnification of drawings and photographs

Example 1

In a TEM of a red blood cell, the diameter of the image of the cell was 45 μm , and the actual size of the cell was 7.5 μm . What is the magnification of this TEM?

Magnification is the number of times larger an image is compared to its real size.

$$\text{magnification} = \frac{\text{observed size of the image}}{\text{actual size}}$$

First, convert the observed size in mm to μm .

$$45 \times 1000 \mu\text{m} = 45\,000 \mu\text{m}$$

Then,

$$\text{magnification} = \frac{45\,000}{7.5} = 6000$$

The 'x' symbol means 'times', so the magnification is recorded as $\times 6000$.

Example 2 – to try

In a TEM of a mitochondrion, the length of the image of the organelle was 20 mm and the actual size was 5.0 μm . What is the magnification of this TEM?

2 Calculating the actual size of an organism or structure from a given scale bar**Example 1**

In an interpretive drawing of *Amoeba* of image length 105 mm, the scale bar of length 22 mm represents 0.1 mm. What is the actual length of the *Amoeba*?

The scale bar represents 0.1 mm = 100 μm .

So the actual length of the *Amoeba* is

$$\frac{105}{22} \times 100 \mu\text{m} = 477 \mu\text{m}$$

(approximately 480 μm)

Example 2 – to try

In a TEM of a chloroplast of image length 111 mm, the scale bar of length 10 mm represents 8 μm . What is the actual length of the chloroplast?

3 Determining the length of an appropriate scale bar for a magnified image**Example 1**

In an SEM, a mitochondrion of actual length 9 μm was magnified to a length of 45 mm.

Calculate how long a scale bar representing 5 μm would have to be on this SEM.

Here, 9 μm is equivalent to 45 mm.

So, 5 μm is represented by

$$\frac{45 \times 5}{9} \text{ or } 25 \text{ mm}$$

A scale bar representing 5 μm would be 25 mm in length.

Example 2 – to try

In a TEM of an animal cell, the nucleus of diameter 6 μm was magnified to a diameter of 135 mm.

Calculate how long a scale bar representing 1 μm would have to be on this TEM.

4 Calculating the sizes of specimens from magnified drawings and photographs**Example 1**

In a photomicrograph showing a human cheek cell, the image had been magnified $\times 800$. What is the actual length of this cell?

First, measure the length of the image of the cell in the photomicrograph in mm, using a ruler. It is 90 mm long at its maximum length.

Next, convert this length to μm .

$$90 \text{ mm} = 90 \times 1000 \mu\text{m} = 90\,000 \mu\text{m}$$

Then,

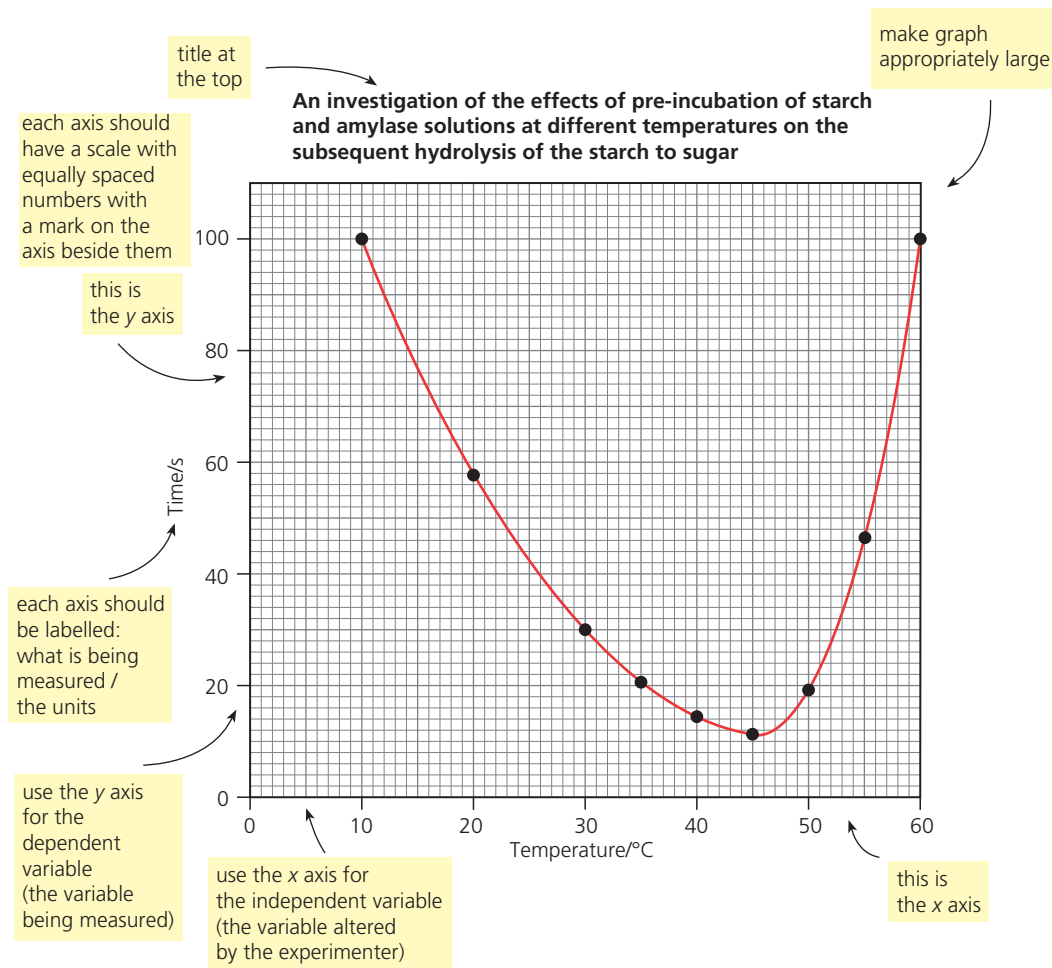
$$\begin{aligned} \text{actual size} &= \frac{\text{image size}}{\text{magnification}} = \frac{90\,000}{800} \mu\text{m} \\ &= 112.5 \mu\text{m} \end{aligned}$$

Example 2 – to try

In a photomicrograph of a green plant cell, the image had been magnified $\times 400$. The image of the cell was 94 mm in length.

What is the actual size of this cell?

Answer to question 1 (page 5)



Finally decide where the curve should go:

A line that goes through as many points as possible is a **line of best fit** – curved or straight. In this graph you would draw the *same* line if the results had been (for example):

Temperature/°C	10	20	30	35	40	45	50	55	60
Time/s	100	61	28	23	14	11	22	43	100

Use a line connecting all the points where you are not sure what the overall relationship between the variables is. For example

