Scientific Data Analysis

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To my wife Jenny and son Felix for their continued support and encouragement.

About the author

Until his retirement in 2009, Graham Currell was a Principal Lecturer in physics at the University of the West of England, Bristol. During his early career, his particular interest was in the preparation of specialist training programmes to support staff in university science laboratories in Asia, the Middle East, Africa, and Central America but after 2000 he concentrated on the development of data analysis modules and self-study materials for science students, and from 2009 became a part-time research fellow, in which he further explored the development of online learning resources. *Scientific Data Analysis* builds on Graham's previous development of teaching materials for mathematics and statistics, including screen-capture videos in forensic, chemical, biological, and environmental science for the University of the West of England and the Royal Society of Chemistry. The approach reflects his extensive experience of providing tutorial and training support for students and staff carrying out research projects across both the physical and life sciences.

Welcome to *Scientific Data Analysis*

This book was written to satisfy the needs of three main target groups:

- Students following science degrees or masters courses who need support in understanding statistical data analysis, when encountered within taught courses and particularly when applied to their own developing experimental skills.
- University and college science staff wishing to reinforce or extend their own understanding of analytical techniques to help their supervision of student projects, particularly in topics on the border of their own specialisms.
- Teaching staff who are developing courses for science students and wish to structure the curriculum such that it prepares the students for handling the analysis of their own experimental project data.

Students typically learn the techniques of data analysis in two stages:

- Within taught modules during the first and/or second year of undergraduate studies.
- As part of a final year project, when faced with analysing their own experimental data.

This book is divided into two parts to reflect these two different approaches to learning. Part I, 'Understanding the statistics', develops the necessary statistical concepts with the *bottom-up* approach typically used in taught courses, and Part II, 'Analysing experimental data', starts from experimental data, and reviews, *top-down*, the possible techniques that could be used for their analysis.

The content and terminology used in Part I leads into the applications developed in Part II, and, in reverse, the techniques using Minitab and SPSS in Part II are supported through references to the basic statistical concepts in Part I.

For students using this book

If you, as a *first or second year* science student, are in the process of studying the general topic of data analysis, then Part I provides the core statistical concepts, which are developed in this book without complex mathematics but by using statistical models in Excel. The content reflects standard taught courses for science students, but also widens the range of techniques introduced in order to prepare you for the wide variety of different analytical problems encountered in final year projects.

If you, as a *final year* science student, are trying to find an analysis that is applicable to a set of your own experimental data, you should start in Part II by reading Chapter 5, from which you may move to one of Chapters 6 to 9, depending on the type of data involved. The 'Analytical options' feature in each section identifies possible analytical techniques that could be applied to the different types of data, and this approach helps you to select and use possible techniques relevant to your own particular data set. The content in Part II uses references to Part I to reinforce your understanding of the essential statistical concepts.

Video demonstrations

Short video clips are provided throughout the book to demonstrate the analyses using Minitab, SPSS and Excel. Together with the printed instructions, the videos will help you gain confidence in using the software and develop your experience for exploring menu options for other forms of analyses not directly covered in the book. The videos can be accessed directly by scanning the QR code images in the text with your smartphone. Alternatively you can view the videos from the links provided in the Online Resource Centre. Appendix I provides an index of the videos.

Case studies

There are a number of case studies throughout the book, most of which use related examples to develop a specific analytical theme. For example, it can be useful to see how the same or similar data can be analysed in different ways, and the case study links will take you to the relevant sections where the same case study continues. Each case study with more than one appearance starts with an overview which gives an outline of its theme and the locations in which each subsequent step can be found (not necessarily in the linear order of the book). Appendix II provides an index for the case studies.

Analytical software and data files

This book describes the use of **Microsoft Excel 2013**, **Minitab 17**, and **IBM SPSS Statistics 22** for data analysis, giving the required keystrokes in the text and supported by videos accessible directly via the QR codes. The detailed steps described in Excel and SPSS are also the same as those used in the earlier versions of **Excel 2010** and **IBM SPSS Statistics 20** respectively. There are some differences between Minitab versions 16 and 17, many of which make little difference to the keystrokes required, except for the use of regression and the general linear models. Minitab 17 was introduced during the final stages of preparation of the book and the keystrokes and videos were updated to reflect the new software, but the legacy keystrokes and videos for **Minitab 16** are still available in the Online Resource Centre. Some examples in the text were particularly relevant to Minitab 16 and these have been retained but clearly identified as such. Data files for the analyses in the book are also available for downloading from the Online Resource Centre.

Excel has widespread use for data handling in addition to its capabilities for statistical data analysis, and Minitab and SPSS have been chosen to demonstrate the 'next level' of analysis beyond Excel because of their easy-to-use, menu-driven operation. The approach developed using examples in Minitab and SPSS will support a greater understanding for solving problems if the student then moves on to using other packages, e.g. 'R', Prism.

Online Resource Centre

The Online Resource Centre can be found at http://www.oxfordtextbooks.co.uk/orc/currell/, and provides:

- Links to every video in the text, plus additional videos for Minitab 16.
- Download links for the modelling files developed in Excel, together with data files for Excel, Minitab, and SPSS.

In addition there is a 'new content 'section which will be updated regularly to accommodate any new content or videos developed.

For the lecturer: about this book

This book evolved from the experience of working with very many students to support them analysing their own data within final year projects across a range of bioscience, forensic, environmental, and chemistry degree courses. It was instructive to discover the mismatch between the standard learning outcomes of first and second year 'statistics' modules and the practical problems faced by the students when they first encounter their own real world data analysis.

The ability to identify and implement the correct analytical technique requires both an *understanding* of the statistics involved together with the *experience* of a range of possible techniques. Unfortunately, for most science students, there is no simple linear path to achieving this combination, and they only develop a confident understanding of the statistics *after* having the experience of using it themselves to analyse their own real, and often somewhat scrappy, data. This book reflects this dichotomy, in that Part I provides a *bottom-up* review of the underlying statistics relevant to data analysis and Part II allows the student to address analytical problems, *top-down*, starting from the need to analyse typical science project data. A key aim of the book is to prepare the student for using his/her first 'solo' research project as an effective learning experience in data analysis, bringing together understanding of the statistics with its practical applications.

Part I of the book, 'Understanding the statistics', is targeted at the 'taught course' or 'fundamental concepts' phase of learning. The first two chapters develop the basics of experimental uncertainty and statistics within a strong scientific context. They also introduce terminology (e.g. sums of squares, confidence intervals, ANOVA tables) that links directly into the analytical techniques developed in Chapters 3 and 4. The aim of these chapters is then to expose the reader to a *wider range* of possible analytical approaches than is provided by most books concentrating on the standard *t*-test, ANOVA, and chi-squared analyses.

As examples, the first application of the *t*-statistic is not to develop the traditional *t*-test for mean values but to demonstrate its wider use by testing for a difference in the slopes of bacterial growth, and the 'repeated measures' ANOVA is not introduced in a questionnaire but used in a forensic test to differentiate between black inks.

Where it is important to understand the underlying statistics of the techniques, these are developed with a modelling approach using Excel supported by videos, which not only gives a more visual clarity to the analysis but also exposes the reader to wider possibilities in using Excel. With its student-centred approach, this book is an effective text/video resource that provides both content and context for learning the fundamental concepts of data analysis.

Part II, 'Analysing experimental data', is intended to be used mainly in a 'top down' approach to analysing experimental data, starting with Chapter 5 which introduces a phase of reflection to avoid rushing for the first analysis that will produce a (possibly irrelevant) result. The subsequent chapters and sections are then defined by the structure of the particular data set, allowing the student to investigate a wider set of analytical techniques than might have been considered initially.

The book prints keystroke instructions for SPSS and Minitab, together with a discussion of the resultant output, both of which are supported with step-by-step videos. Using this approach, the book provides self-study support for the individual reader, either student or lecturer, and would also be useful within the library of a statistics support centre for science students.

Contents

Part I

Understanding statistics

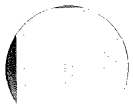
Part I approaches an understanding of analytical techniques in science from a *statistical* perspective. It develops an understanding of how key analytical techniques work and the scientific interpretation of their results. With this approach, it supports first and second year modules in statistical data analysis, but also acts as a reference resource for students subsequently meeting a technique for the first time. The implementation of many of the analytical techniques, developed in Part I, is then described in Part II from a *scientific* perspective using SPSS and Minitab.

Chapter 1. Statistical concepts provides the key topics and statistics that underpin the analytical techniques developed in subsequent chapters. The content reflects the standard elements of an introductory course in statistics, but the approach and terminology is designed to link into later applications.

Chapter 2. Regression analysis builds on the familiar 'best-fit straight line' analysis as an introduction to important analytical techniques. It provides an understanding of its practical implementation in experimental science using Excel, and develops the approach and terminology used in Minitab and SPSS, leading to the introduction of general linear models of analysis.

Chapter 3. Hypothesis testing provides an understanding of the process and significance of hypothesis testing in science, covering a wide range of underlying parametric and nonparametric techniques, from *t*-tests to Monte Carlo re-sampling. The specific concepts are developed, not through extensive statistical theory, but through the use of modelling in Excel, which provides a more relevant perspective for most science students, coupled with (possibly) new skills in Excel.

Chapter 4. Comparing data considers a range of analytical techniques that are often neglected in teaching but do address important questions in science. These relate to the strengths of agreement, association and interaction between the factors and variables in a scientific system.



Statistical concepts

Introduction

This chapter develops the underlying statistical concepts from the perspective of experimental data, emphasizing the link between experimental variability and the role of statistics in quantifying and managing this variability. The topics are introduced at a level suitable for the first year of an undergraduate science course, but developed with an approach which emphasizes the equations and terminologies that are used later in the book.

Section 1.1 introduces the value of visualizing experimental data through a variety of graphs, including the boxplot for raw data and the interval plot for calculated mean values.

Section 1.2 reviews the key terminology used to describe the factors and variables that influence the scientific system being analysed.

Section 1.3 uses the histogram to describe data variations, and introduces important standard distributions.

Section 1.4 discusses the uncertainty and error in measurement and develops the mathematics for combining experimental uncertainties.

Section 1.5 develops the statistics for analysing data samples and their application in the interpretation of experimental results.

Section 1.6 outlines the generic issues associated with hypothesis testing, leading to the relevance of p-values and Types I and II errors.

The following case studies develop the core statistics in this chapter:

Case study: **Blood alcohol / 1. Overview**

This case study is based around the measurement of alcohol (in mg) per 100 ml of blood. Some examples assume that the standard deviation experimental uncertainty is given by $\sigma = 2.0$ mg/100 ml.

1.1.2 / 2. Simple boxplot: Describes the *ranking* of data and the meaning of the simple box and whisker plot.

1.1.3 / 3. Boxplots and interval plots: Compares the presentation of *raw data values* using a boxplot and then *calculates best estimates* for the mean using interval plots.

1.3.1 / 4. Data distribution: Demonstrates the use of a *column* (or bar) graph to record the frequency of recorded values.

1.5.1 / 5. Sample statistics: A sample of five values is used to develop the basic statistics of measurement, including the *standard error* and *confidence interval*.

1.5.3 / 6. Samples and populations: Excel is used to randomly generate samples of five values to demonstrate the relevance of *sample* and *population* measurements.

1.6.2 / 7. Hypothesis tests: Illustrates how the *p-value* is calculated from the tail of the frequency distribution.

3.1.2 / 8. One sample *t*-test: Develops the calculations involved in testing whether a sample mean is *significantly* greater than a target value.

8.1.1 / 9. One sample analysis: Example data for the analysis of a single sample of univariate interval data.

Case study: **Experimental uncertainties / 1. Overview**

This case study links together related issues on managing the errors and uncertainties in experimental data.

1.4.4 / 2. Combining uncertainties: Identifies the basic rules for combining uncertainties.

1.4.4 / 3. Propagation of errors: Uses Excel to lay out a complex calculation.

1.4.6 / 4. DIY dice: Considers combining different types of experimental uncertainty.

5.3.4 / 5. Weighting: Demonstrates the use of 'weighting' to combine values with different uncertainties.

1.1 Data visualization

Data visualization is important at all stages of an experimental investigation. The term *data* is a general term for the recorded values that describe the system that we are investigating, and the term *variable* describes any measured quantity that changes within our experimental system.

After taking a set of readings it is often useful to plot the variable values graphically to get both a mental, and a physical, 'picture' of the raw data. This is also valuable in identifying any gross experimental errors and for picking out data regions that would benefit from more experimental study. When finally reporting the research, it is important to remember that the reader will also want to visualize the raw data to help understand the subsequent analysis.

1.1.1 **Graphical information**

In this book we meet a wide range of different graphs produced in Excel, SPSS, and Minitab, where the visualization of the data is a valuable aid in understanding and interpreting the analysis. Some examples are given in Fig 1.1, reproduced from figures later in the book.

Fig 1.1(a): The most familiar graph is the basic x–y scatterplot in Excel for showing the interrelations between variables. This graph enables us to decide on possible scientific characteristics for analysis by identifying different *slopes* in growth and *differences* between maximum and minimum values. Do not confuse the x–y scatterplot with the 'line' graph in Excel which should only be used if the x-axis has *categorical* data.

Fig 1.1(b): The use of the trendline in Excel, together with calculated uncertainty limits, is used in this example as a visual demonstration of the *confidence interval* involved in extrapolating a best-fit line to intercept the x-axis within a standard additions calculation.

Fig 1.1(c): The Q–Q normality plot of residuals in SPSS highlights deviations from the diagonal line of *normality* showing the skewness and kurtosis in experimental data.

Fig 1.1(d): A frequency column (bar) graph in SPSS records the numbers of data values that fall into specific categories, showing the relative weighting between the categories.

Fig 1.1(e): The Minitab plot of delta deviance against probability shows graphically the reliability with which measured variables can *predict* (diagnose) the binary state of subjects, with points to the bottom left and right indicating accurate predictions into the two possible states.

Fig 1.1(f): The dendrogram in Minitab gives direct visualization of the *similarity* between multiple input variables in anticipation of reducing their number through principal component analysis.

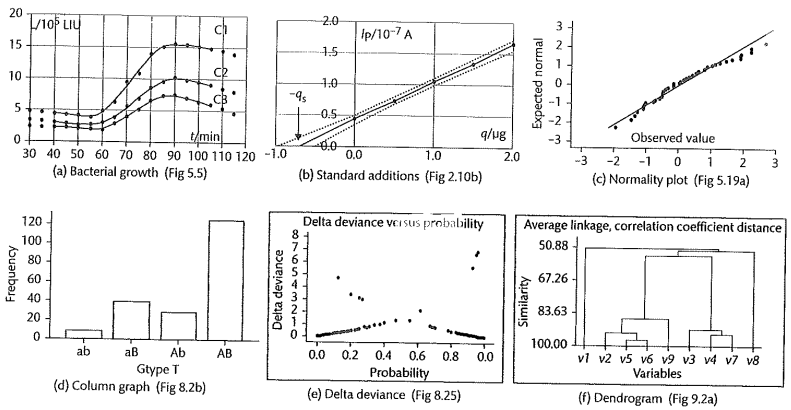


Fig 1.1 Selection of data plots

1.1.2 **Boxplots**

When dealing with repeated measurements, the boxplot shows the *range* of values measured, together with a general indication of their *distribution*. It is a valuable tool, not only in understanding data, but in communicating this understanding in a final report.

Case study: **Blood alcohol / 2. Simple boxplot**

—continued from 1.Introduction, leading to 1.1.3

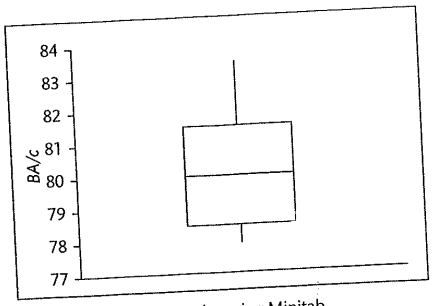
Column A in Fig 1.2(a) gives a set of nine replicate random measurements of alcohol in blood, *BAlc*, in units of mg of alcohol per 100 ml of blood. The data is *ordered* in value in column B and then *ranked* in Column C.

Fig 1.2(b) gives the boxplot for the data set:

- The *centre line* in the box shows the middle value in the data, called the *median*.
- The *ends of the box* show one quarter and three quarters of the way through the data set, and are called the lower and upper *quartiles*. The distance between these is called the *interquartile range, IQR*.
- The *ends of the whiskers* show the maximum and minimum values of the data, except when the data has outliers.

	A	B	C
1	BAlc	Ordered	Ranked
2	83.5	83.5	9
3	80.6	82.4	8
4	79.9	80.6	7
5	78.5	80.2	6
6	78.4	79.9	5
7	80.2	78.7	4
8	78.7	78.5	3
9	82.4	78.4	2
10	77.9	77.9	1

(a) Data values



(b) Boxplot using Minitab

Fig 1.2 Replicate data and its boxplot

An *outlier* is a data value that is more than $1.5 \times IQR$ away from the median value, and is identified as a value that could be checked in case a data transcription or experimental error has been made. Fig 6.24 shows three boxplots on the same graph, identifying three groups within the one data set, including three outliers.

The descriptive values of the boxplot are calculated by first *sorting* the raw data (in column A) into ascending (or descending) order (in column B) and then assigning a *rank position* (in column C).

The *location* of a set of, n, data values is then described by:

Median is the *middle* value in a set of ranked data values and gives the *location* of the data. The Median is the value with the rank $\mathbf{0.50 \times (n+1)}$.
In the example data, the median has the rank $= 0.5 \times (9+1) = 5$.
Median value with rank '5' = 79.9.

Lower quartile, Q_1, is the value *one* quarter of the way from the lowest to the highest value. The lower quartile is the value with the rank $\mathbf{0.25 \times (n+1)}$.
In the example data, the lower quartile value has the rank $= 0.25 \times (9+1) = 2.5$.
Lower quartile value with rank '2.5' is halfway between 78.4 and 78.5 = 78.45.

Upper quartile, Q_3, is the value *three* quarters of the way from the lowest to the highest value.
The upper quartile is the value with the rank $\mathbf{0.75 \times (n+1)}$.

In the example data, the upper quartile value has the rank $= 0.75 \times (9+1) = 7.5$.
Upper quartile value with a rank '7.5' is halfway between 80.6 and 82.4 = 81.5.

The *spread* of nonparametric data is described by:

Interquartile range, *IQR*, is the difference in value between the upper quartile and lower quartile.

$IQR = Q_3 - Q_1 = 3.05$

Total range is the difference in value between the *lowest value* and the *highest value* = 5.6.

The boxplot in Fig 1.2 shows data that is *symmetrical* around the median value. For skewed data, the median line will appear off-centre in the box.

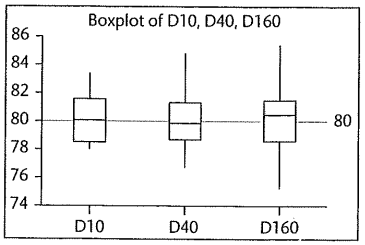
1.1.3 Raw data and calculated values

It is useful to differentiate between describing raw data values and presenting calculated results.

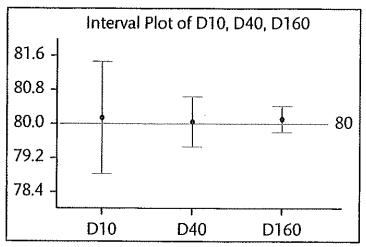
Case study: Blood alcohol / 3. Boxplots and interval plots

—continued from 1.1.2, leading to 1.3.1

The boxplots in Fig 1.3(a) and interval plots in Fig 1.3(b) describe samples of 10, 40, and 160 values selected randomly from a population of possible measurements, *BAlc*, with a mean value of 80 mg/100 ml and a standard deviation of 2.0 mg/100 ml.



(a) Raw data



(b) Calculated results

Fig 1.3 Raw data boxplots (a) and calculated confidence intervals (b) (Minitab)

The boxplots in Fig 1.3(a) describe the *raw data* values, and show similar mean and quartile values defined by their common source population. However, as the sample size increases, the probabilities of observing extreme (maximum and minimum) data values increases, giving longer 'whiskers' for the larger samples.

The interval plots in Fig 1.3(b) show the confidence intervals (1.5.2), which are the *calculated* range for each sample within which you could be 95% confident of finding the true value mean of the source population. The shrinking confidence intervals show that the

precision (1.4.3) of the measurement increases with the square root of the number of measurements according to Eqn 1.21.

1.2 Scientific data

Collection of data in science has developed over many years in many different disciplines, and has resulted in a great variety of styles in which it is classified and described. This section aims to establish some of the categorization and terminology used in this book, and relate these to established descriptors that you will meet elsewhere.

1.2.1 Experimental data

Input and output data

In many of our investigations we are trying to understand the *mechanisms* that might occur within the scientific system. We do this by observing what the system does when it is changed in some way, and consequently the data in a set of experimental results can usually be divided into

- output data that records the response of a system (response or dependent variable) and
- input data that defines specific experimental conditions (independent or factor variable).

For example, in a linear regression calculation (2.1.1) the input (independent) variable is placed on the x-axis and the output (dependent) variable on the y-axis.

However, we need to be careful when categorizing data in this way because we may be measuring two variables that are *both* output data, responding to a separate input variable that is not being recorded. For example, we might observe a correlation (2.1.3) between the height and weight of children plotted on an x–y graph, but both are *output* variables jointly dependent on the age of the child, which is an unrecorded *input* variable. A correlation between observed variables does not necessarily mean that one is a *cause* and the other is an *effect*.

Factors and variables

The term *factor* is used mainly to describe a variable that is known to be a possible *input* to the system. A multifactorial analysis is an analysis where more than one *input* variable contributes to the calculation.

We will also meet references to univariate, bivariate, and multivariate types of data, which can broadly be described as follows.

- Univariate data has one *response* variable, with one or more input variables, e.g. the yield of a chemical reaction related to the time and temperature of the reaction.
- Bivariate data has two variables, often without recording any particular input variable, e.g. observing a relationship between the height and weight of children without recording their age.

Multivariate data occurs in a variety of situations, either as multiple input variables predicting a single output variable, or possibly several variables jointly describing the output state of a system.

1.2.2 Data types

Data can be grouped into five main category types:

Interval (or scale) data are values recorded along a numeric scale, e.g. reading the length along a ruler, or noting an output reading on a *pH* meter. The differences (intervals) on the scale represent *true* measures, e.g. the difference between 40°C and 80°C is twice the temperature difference between 0°C and 20°C. Interval data can be **discrete**, where values are recorded to a limited precision (e.g. rounded to two decimal places) or **continuous**, in which values can be recorded to any possible precision (any number of decimal places).

Nominal data records results that fall *directly* into specific categories, e.g. recording the type of fibre (natural / man-made), the fibre shape (round, cylindrical, bilobal, etc.), or the assessment of image quality (poor / satisfactory / good / excellent).

Binary or digital data is categorical data with only two possible values, e.g. presence or otherwise of delustrants in fibres. Digital data could be described by Yes/No, 0/1, True/False, etc.

Ordinal data is nominal data where the different categories show a *sense of progression* that can be represented by numerical values. For example, the assessment of quality defined by poor, satisfactory, good, excellent, could also be defined by 1, 2, 3, 4. However, the values are not interval data because the difference between excellent (4) and satisfactory (2) *cannot* be assumed to be twice the difference between good (3) and satisfactory (2).

Frequency data is simply the result of counting the number of occurrences of some event, e.g. sightings of a particular animal, or numbers of bacteria on a plate. In some cases, larger frequency values can be treated as interval data, e.g. a density of 3,000 cfu (colony-forming units) of bacteria per millilitre of solution.

We also meet several other data descriptors:

Ratio data is *interval* data where the '0' value equates to a *true zero* in a scientific context. For example, the Kelvin and Celsius temperature scales both have the same *interval* of one degree, but the Kelvin scale is a ratio scale because it has 0°K at the absolute thermodynamic zero, whereas the Celsius scale is not ratio data because it only defines 0°C as the triple point of water. 100°K is twice the thermodynamic temperature of 50°K, but 100°C (373°K) is *not* twice the thermodynamic temperature of 50°C (323°K).

Ranking data are *ordinal* data that describe the *order* in which data can be placed, e.g. the ordinal data values of poor, good, very good, excellent can be described by ranking values, 1, 2, 3, 4. We see in 4.1.2 that ordinal data can be analysed by correlation using ranking values.

Categorical data is data that has been put into specific categories. Nominal data is a natural example of categorical data, but ordinal data with a limited number of values can also be treated as categorical data. Interval data can be grouped into categories by *binning* (8.1.8), and the number (frequencies) of values in each category can be 'counted' using *tabulation* (8.1.7). The analysis of categorical data is developed with chi-squared analysis in Section 3.7.

Normal data is scale data whose randomly selected values occur with characteristic bell-shaped probability distributions (1.3.3).

Nonparametric and parametric tests. A nonparametric test uses only ranked data (ordering of values) and can be used for ordinal or scale data, but a parametric test requires interval data because the differences (intervals) between values are used in the calculations.

The **Likert** scale (after Rensis Likert) is a specific example of ordinal data that is frequently used in questionnaires. Responses are given on a balanced scale of options such as:

Disagree strongly, Disagree, Neutral, Agree, Agree strongly,

which can be scored as –2, –1, 0, +1, +2.

1.2.3 Type and value of data

Finally, in this section, it is important to distinguish between the variable *type* and the *values* that can be used to describe it. The main data types–*interval*, *ordinal*, *binary*, *nominal*, and *frequency*–are clearly defined (1.2.2), but there is some flexibility in how they are described and used, which sometimes leads to confusion and problems with some software analyses.

Table 1.1 Variable types and values

Data type:	Value descriptions:
Interval	Described using *scale* values, e.g. 2.56, 0.037.
Nominal	Described using *text (string)* values, e.g. *blue*, X, 2 (using a numeric character as text). Can be *coded* using *scale integers* for inclusion in a mathematical model (6.4.1).
Ordinal	Described using *text* and/or *scale integer* values, e.g. *agree*, 2, *T3*. When using text, some analyses may require confirmation of the *order* of values, e.g. agree strongly, agree, neutral, disagree, disagree strongly. For some analyses it is necessary to *code* the values using scale integers, e.g. –2, –1, 0, +1, +2 for the Likert scale. Ordinal data can be expressed as *ranked* values: 1, 2, 3, 4, etc.
Binary	Described using *text*, *scale integer*, or *logical* values, e.g. Y/N, 0/1, *True/False*.
Frequency	Described using *scale integer* values, e.g. 3, 56. Larger frequencies may be considered as *continuous* data.

1.3 Data distributions

We saw in 1.1.2 that the location and general spread of a distribution can be described by the median plus interquartile range. We now identify parametric descriptors that allow us to describe the *shape* of a distribution in more detail.

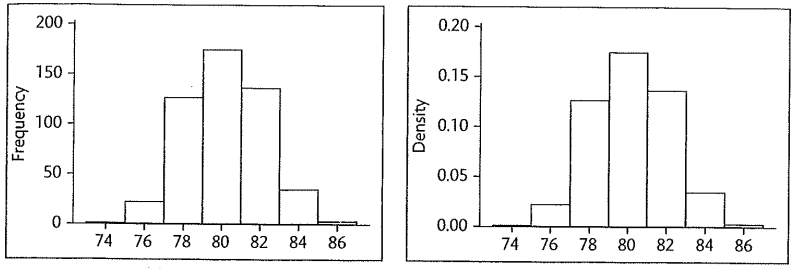
1.3.1 Histogram

A frequency bar (or column) graph (e.g. Fig 1.1(d)) records the numbers of events that fall into specific *categories*. We now develop this into a **histogram** which identifies the categories as ranges of values along a continuous scale axis.

Case study: Blood alcohol / 4. Data distribution

–continued from 1.1.3, leading to 1.5.1

We consider a data set with $N = 500$ replicate blood alcohol, *BAlc*, measurements (in units of mg of alcohol per 100 ml of blood).



(a) Frequency (b) Probability density

Fig 1.4 Histograms (Minitab)

Fig 1.4(a) is a **frequency histogram**, where the vertical *height* of the ith column gives the number, or *frequency*, f_i, of data values within the horizontal range given by the width, Δx_i, of that column, e.g. there are 177 values between 79 and 81.

If a specific value, i, is found to occur with a frequency, f_i, out of a total of N values, then the probability, p_i, of this value occurring could be expressed as

$$p_i = \frac{f_i}{N} \tag{1.1}$$

where N is the sum of all frequency values, given by, $N = \Sigma f_i$.

Fig 1.4(b) shows a **probability density histogram**. The *height* of the ith column is called the *probability density*, pd_i, such that the *area* of each column of the histogram gives the probability, p_i, that a randomly selected data value will occur within the width, Δx_i, of that column.

The *area* of each column equals its height times its width, giving the probability of a value occurring in this range:

$$p_i = pd_i \times \Delta x_i \tag{1.2}$$

The *total* area of the histogram is the probability that *any* value will occur, and is therefore the sum of all individual probabilities, giving a total of 1.0, $\Sigma_i\, p_i = 1.0$.

Taking the central column in Fig 1.4(a), we see that $f_i = 177$, which, using Eqn 1.1, gives a *probability*,

$$p_i = \frac{177}{500} = 0.354$$

Taking the same column in Fig 1.4(b) and using Eqn 1.2, the probability density, $pd_i = 0.177$ and the column width, $\Delta x_i = 2.0$ gives the same probability for category i:

$$p_i = 0.177 \times 2.0 = 0.354$$

1.3.2 Distribution parameters

Starting with a histogram with *broad* columns, as in Fig 1.4(b), we can now develop the histogram in Fig 1.5, where a sample of many thousands of data values has allowed us to reduce the width, and increase the number, of individual columns to the limit when we can just draw a smooth line joining the tops of very many *extremely narrow* columns.

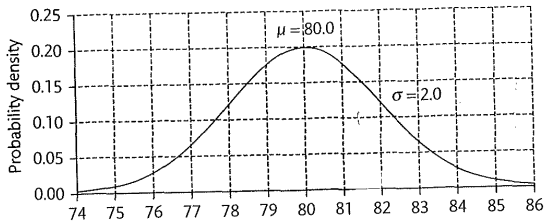


Fig 1.5 Probability density curve

The distribution in Fig 1.5 is an example of a normal distribution (1.3.3), which has an average, or *mean*, value, $\mu = 80$. The spread of a distribution is defined by the *standard deviation* (1.3.2) which, for the distribution in Fig 1.5, has the value, $\sigma = 2.0$.

The total area under the curve equals a total probability of 1.00, and the area of the shaded portion within one standard deviation of the mean (80.0 ± 2.0) is equal to 0.683. From this we can say that the probability that a randomly selected value will fall within one standard deviation of the mean is 0.683 or 68.3%.

In addition to the **median** and **interquartile range** (1.1.2), we can describe a distribution using:

Mean. The mean values, μ for a population and $\bar{x}$ for a sample (1.5.3), are the simple averages of all data values in the distribution.

Mode. The mode is the value which has the greatest frequency of data values, i.e. the position of the *peak* within the distribution. In some cases the distribution may show two or more peaks, in which case it would be referred to as bi- or multi-modal. For example, bimodal distributions can occur in the examination results of a cohort of students which includes two sub-groups with very different abilities.

Standard deviation, σ for a population and s for a sample (1.5.3), measures the spread of the data around the mean value. The calculation of standard deviation is developed in 1.5.1.

Skewness is a measure of the extent to which a given distribution has an *unsymmetrical* shape. Skewness describes whether the given distribution has a shape that, compared to the symmetrical bell-shaped normal distribution, is either

- extended to the **left** (negative skewness), Fig 1.6(a), or
- extended to the **right** (positive skewness), Fig 1.6(b).

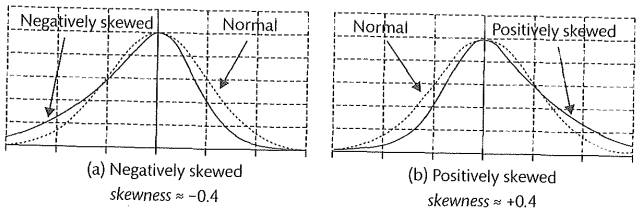


Fig 1.6 Skewness

Kurtosis is a measure of the extent to which the distribution has a central peak that is either

- flatter (platykurtic), Fig 1.7(a), or
- more pointed (leptokurtic), Fig 1.7(b)

than the standard bell-shaped normal distribution (mesokurtic).

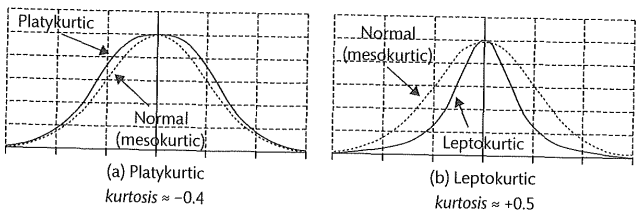


Fig 1.7 Kurtosis

It is important to note that the *uncertainties* in estimating values of skewness and kurtosis are usually large unless the data sample itself is very large.

It is interesting to note that, from a mathematical perspective:

Mean value is calculated by taking the average of the *data values* themselves.

Standard deviation is calculated by taking an 'average' of the *squares* (power of two) of the *deviations* (1.5.1) from the mean value.

Skewness is calculated by taking an 'average' of the *cubes* (power of three) of the *deviations* from the mean value.

Kurtosis is calculated by taking an 'average' of the *fourth power* (power of four) of the *deviations* from the mean value.

1.3.3 Standard distributions

Normal distribution

This is the classic symmetrical bell-shaped curve, with the probability density described by the equation:

$$pd(x) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left\{-\frac{(x-\mu)^2}{2\sigma^2}\right\} \quad (1.3)$$

Fig 1.5 gives an example of the probability density for a normal distribution with mean, $\mu = 80$, and standard deviation, $\sigma = 2.0$.

The *standard normal* distribution, shown in Fig 1.8, is the specific distribution with mean, $\mu = 0.0$, and standard deviation, $\sigma = 1.0$. The value on the 'x-axis' for this specific distribution is usually referred to as the z-value.

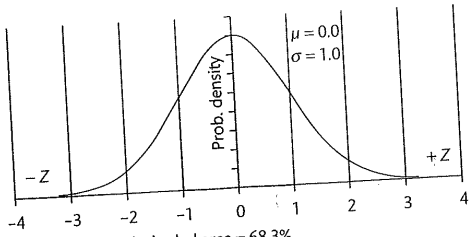


Fig 1.8 Standard normal distribution, with shaded area = 68.3%

The normal distribution is an important element in the analysis of experimental data. The total probability area under the curve = 1.00, but it is also useful to note other specific areas:

Table 1.2 Probability areas under a normal distribution

	Area =	
Within **one** standard deviation, σ, of the mean, from z = −1.0 to z = +1.0	0.683	68.3%
Within **two** standard deviations, σ, of the mean, from z = −2.0 to z = +2.0	0.954	95.4%
Within **three** standard deviations, σ, of the mean, from z = −3.0 to z = +3.0	0.997	99.7%
More than 1.96 standard deviations, σ, from the mean (both sides)	0.05	5.0%
To the right of z = 1.96 × σ (one side)	0.025	2.5%
More than 1.64 standard deviations, σ, from the mean (both sides)	0.10	10.0%
To the right of z = 1.64 × σ (one side)	0.05	5.0%

The mathematical complexity of Eqn 1.3 can be avoided by using functions in Excel for a normal distribution with mean, μ, and standard deviation, σ:

NORM.DIST(x, μ, σ, false) gives the probability density at the point x.

NORM.DIST(x, μ, σ, true) gives the *cumulative* probability from -∞ up to the point x.

NORM.INV(α, μ, σ) gives the value, x, in the distribution such that the cumulative probability up to the value of x is equal to the probability, α.

In many Excel examples developed in this book, we use the function NORM.INV(RAND(), μ, σ) to *select a random value* from a normal distribution with mean, μ, and standard deviation, σ. The RAND() function provides a random value between 0.0 and 1.0, which is used here to provide a random probability for the cumulative probability, α, when selecting the value from the distribution.

Binomial distribution

The binomial distribution gives the probability $p(r)$ of observing r specific outcomes of n trials, where the probability of each r outcome is equal to p.

$$p(r) = {}_nC_r \times p^r \times (1-p)^{(n-r)} \quad (1.4)$$

Fig 1.9 shows three examples, each of which has $n = 20$ trials, but with different individual probabilities, p.

The graph with $p = 0.5$ represents the probability of observing r 'heads' when tossing a balanced coin $n = 20$ times.

The graph with $p = 1/6$ represents the probability of observing r 'sixes' when rolling a six-sided dice.

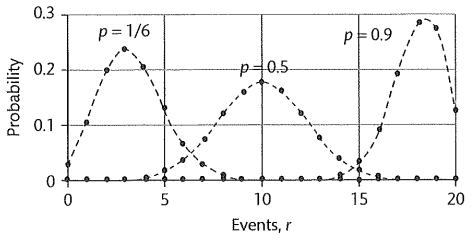


Fig 1.9 Binomial distributions

The distribution of possible values for r will have

Mean value: $$\mu = p \times n \quad (1.5)$$

Standard deviation: $$\sigma = \sqrt{n \times p \times (1-p)} \quad (1.6)$$

The important characteristics of the binomial distribution are that:

- it is defined by the two parameters: n and p.
- the range of values of r is limited between 0 and n.
- it is often skewed, except when $p = 0.5$. When $p < 0.5$ it is positively skewed and negatively skewed when $p > 0.5$.

If $np(1-p) \geq 5$, the distribution can be represented approximately by a normal distribution.

For a binomial distribution with individual probability, p, and total trials, n, the Excel function:

BINOM.DIST(r,n,p, false) gives the probability value for observing *exactly* r outcomes.
BINOM.DIST(r,n,p, true) gives the cumulative probability *up to* r outcomes.

When considering the data as a *proportion*, $P = r / n$:

Mean value of *proportion*:
$$\Pi = \frac{\mu}{n} = \frac{p \times n}{n} = p \tag{1.7}$$

Standard deviation of *proportion*:
$$\sigma(P) = \frac{\sqrt{n \times p \times (1-p)}}{n} = \sqrt{\frac{p \times (1-p)}{n}} \tag{1.8}$$

Poisson distribution

The Poisson distribution is a special case of the binomial distribution when the individual probability, p, is very small, $p << 1.0$. The probability $p(r)$ of observing r specific outcomes, for a distribution with a mean number of outcomes, μ, is given by:

$$p(r) = \frac{e^{-\mu} \times \mu^r}{r!}. \tag{1.9}$$

For example, if the mean number of occurrences of a rare medical condition in a given population is $\mu = 4$, then the probabilities of observing r occurrences is given by the values in Fig 1.10.

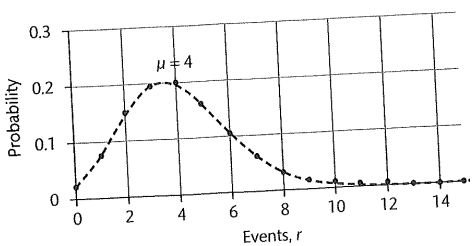


Fig 1.10 Poisson distribution

The important characteristics of the Poisson distribution are as follows.

- There is no theoretical upper limit to r, but the probability for high values becomes vanishingly small.
- The shape of the distribution is defined by the use of a *single* parameter, μ, which is the value of *both* the mean and variance:
  - Mean value = μ.
  - The variance is equal to the mean value. Variance, $\sigma^2 = \mu$.
  - Standard deviation, $\sigma = \sqrt{\mu}$.

For a Poisson distribution with mean, μ, the Excel function:

POISSON.DIST(r, μ, false) gives the probability value for observing *exactly* r outcomes.
POISSON.DIST(r, μ, true) gives the cumulative probability *up to* r outcomes.

It is useful to note that if the mean number of occurrences is N, then the best estimate of the standard deviation uncertainty in that value is $\sqrt{N}$ (1.4.5).

Weibull distribution

The Weibull distribution, given by Eqn 1.10, has a shape that can be adjusted to model different system behaviours for values of $x \geq 0$ (see Fig 1.11). It is often used for time-dependent variations.

$$pd(x) = \frac{k}{\lambda}\left(\frac{x}{\lambda}\right)^{k-1} \times \exp\left\{-\left(\frac{x}{\lambda}\right)^k\right\} \tag{1.10}$$

where

k is the *shape parameter*, and
λ is the *scale parameter* along the x-axis.

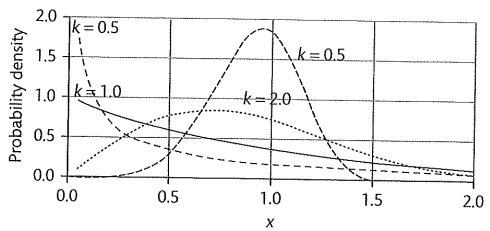


Fig 1.11 Weibull distributions for example values of k with $\lambda = 1.0$

We can interpret the graphs in Fig 1.11 by considering that x represents the time to an event that occurs with a probability, p:

If $k = 1.0$, then the probability, p, is constant in time (e.g. with radioactivity) then the curve is a simple exponential decay which is shown as the solid line.

The curve with $k < 1.0$ represents a situation where the probability, p, decreases with time.

The curves with $k > 1.0$ represent situations where the probability, p, increases with time.

The distribution with $k = 2.0$ produces the Rayleigh distribution which is used to describe the distribution of a value which is the vector combination of two independent normal distributions, e.g. the resultant wind velocity due to two components at right angles to each other.

1.4 Uncertainty and error

When reporting a measured result in science, it is rarely sufficient just to give a value without any indication of the possible uncertainty in that value. Only by combining the best estimates of both the *actual value* and its *uncertainty* can we convey complete information.

We see in 1.5.2 that an effective way of reporting an experimental measurement is given by the *confidence interval*, combining both the best estimate of the unknown value and a calculated value of the possible uncertainty.

1.4.1 Error or uncertainty

In a typical experiment, we aim to arrive at a best estimate of an unknown 'true' value. If we could increase the accuracy of our measurement we would get closer to the unknown 'true' value, but we must accept that there will always be a possible *error* in our result:

Error is the difference between the true value and the best-estimate value.

A key problem is that we never actually know the true value and hence we never truly know the actual magnitude of the error. The best that we can do is to calculate a value for the possible *uncertainty* in our results as the *best-estimate* for that error.

Uncertainty is the best-estimate of possible error.

The term 'error' in statistics normally relates to a statistical 'uncertainty' rather than a *mistake* in the experiment. For example, the standard error of the mean (Eqn 1.21) in a calculated value is a measure of the standard deviation *uncertainty* in that value.

1.4.2 True value

We can identify three main types of 'true' values:

Physical value of a specific characteristic of the system. For example, the *true* concentration of lead in a water sample is a *single* specific value.

Statistical parameter that describes the distribution of values within the system. For example, the weights of all the fish in a tank will have a true *population mean* value, even though no single fish has exactly that weight.

Probability value that describes the probability with which specific events occur. For example, the decay of a radioactive isotope is governed by a true *probability* with which individual nuclei decay. However in this case, we usually prefer to derive a value for the true *half-life* which is the time for half of the nuclei to decay.

1.4.3 Experimental uncertainty

The uncertainty in experimental measurements can then be divided into three corresponding categories:

Measurement uncertainty. The *measurement process* itself will vary slightly between repeated measurements, possible due to small differences in experimental procedures, instrumental responses, human observations, etc. For example, repeated measurements of the calcium content of the same sample of mineral water may give different experimental values.

Subject uncertainty. A *subject* is a representative example of the system being measured, but there will often be differences between subjects. For example, similar plants grown under the same conditions may have a variation in their heights.

Probability uncertainty. There is always an inherent *statistical* uncertainty when the occurrence of an event is governed by random probability.

Whatever the source of uncertainty, it is important that any experiment must be designed both to *counteract* the effects of uncertainty, and also to *quantify* the magnitude of that uncertainty. Many real experiments will be subject to a combination of different types of variation (e.g. DIY dice case study), and good experimental design seeks to minimize and manage uncertainty. For example, if it is known that the uncertainty in a system matches a normal distribution, then the uncertainty can be managed more effectively by using a parametric analysis that assumes normally distributed data.

The behaviour of uncertainties can be further classified as causing either:

Random error: Each subsequent measurement has a random error, whose *magnitude* and *direction* is not related to any other measurement. The **precision** of a measurement is the best estimate for the purely *random error* in a measurement. A measurement with a low random error is said to be a *precise* measurement.

Systematic error: Each subsequent measurement has the *same* recurring error. A systematic error causes the measurement to be *biased*, e.g. when setting the liquid level in a burette, a particular student may always set the meniscus of the liquid a little too low. The term **accuracy** is often used in science to describe the amount of *bias* in a measurement.

We can use Fig 1.12 to illustrate these terms, in which samples, A, B, C, and D (each with five replicates) are taken of the *pH* of the same solution. By looking at the closeness of the values, we can say that samples A and C are more *precise* than B and D. However, we can only know the accuracy of the measurements if we know the true value being measured. If the true value were 9.07 then we could say that samples C and D are more *accurate* than A and B.

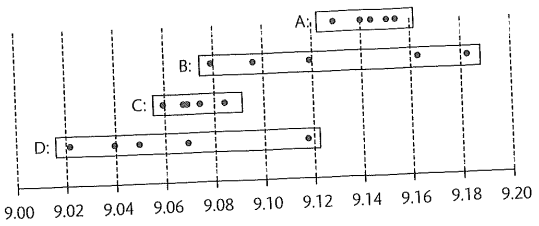


Fig 1.12 Illustration of accuracy and precision

An alternative approach is to use the term **trueness** as the best estimate for the *bias* in a measurement, and then **accuracy** becomes the best estimate for the *overall error* in the final result, and includes both the effects of a lack of precision (due to random errors) and bias (due to systematic errors).

1.4.4 Combining uncertainties

It is common to express the uncertainty (or error) either as an

absolute value, which uses the same units as the value itself, e.g. an uncertainty of 0.3mm in a measurement of a length of 200mm, or as a

- *relative* value, frequently given as a *percentage* uncertainty, e.g. 0.3mm in 200mm is equivalent to a percentage of $100 \times 0.3 / 200 = 0.15\%$.

We will express the *absolute* uncertainty in x as u_x. Typically this is given by the *standard deviation* in the possible values of x. The relative *percentage* uncertainty, $u\%_x$, is then obtained by expressing the uncertainty as a percentage of the value itself:

$$u\%_x = 100 \times \frac{u_x}{x} \qquad \text{or} \qquad u_x = \frac{u\% \times x}{100} \tag{1.11}$$

It is, of course, possible to express a relative uncertainty as a simple ratio without the need to multiply by 100 for the percentage. However, in this book, we will normally use percentage for relative measurement, matching its common use in science.

Case study: Experimental uncertainties / 2. Combining uncertainties

—continued from 1.Introduction, leading to 1.4.4

In this case study, we use the Excel worksheet in Fig 1.13 to model the possible combinations of two variables, X and Y, with true values:

$X = 9.0$ in row 2 with standard deviation uncertainty $u_X = 0.7$, and

$Y = 6.0$ in row 3 with standard deviation uncertainty $u_Y = 0.5$.

The uncertainties in X and Y tell us that, if we were to *randomly repeat the measurements*, we could expect the value of X to be drawn from a normal distribution with a mean of 9.0 and a standard deviation of 0.7, and similarly 6.0 and 0.5 for the value of Y.

We *simulate* these measurements 1,000 times by first generating 1,000 random values of of X and Y in the shaded cells in columns F to ALQ and then we combine X and Y in different ways:

$$X+Y,\ X-Y,\ X \times Y,\ X/Y,\ X^3$$

We derive the *combined* uncertainties in each of these results by calculating the standard deviations of the 1,000 different values.

Combining uncertainties: Excel analysis for Fig 1.13. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

We generate a randomly selected value of X in F2 by using the function:

[F2] = NORM.INV(RAND(), $B2, $C2) = 7.77

We then copy this function to all cells F2:ALQ3 to generate 1,000 random values for both X and Y. The '$' signs lock the columns for the mean and standard deviation values.

	A	B	C	D	E	F	G	ALP	ALQ
1		Value	Uncert, u	%Uncert, u%		1	2	999	1000
2	X =	9.00	0.7	7.78		7.77	8.93	9.38	9.39
3	Y =	6.00	0.5	8.33		5.53	5.92	5.97	6.69
4									
5	$A = X + Y$	15.00	0.861	5.74		13.29	14.84	15.35	15.99
6	$S = X - Y$	3.00	0.847	28.23		2.24	3.01	3.41	2.6
7	$M = X \times Y$	54.00	6.107	11.31		42.92	52.82	55.99	62.19
8	$D = X / Y$	1.50	0.167	11.15		1.41	1.51	1.57	1.39
9	$P = X^3$	729.00	177.199	24.31		468.64	741.1	824.74	802.62

Fig 1.13 Combinations of random uncertainties (Columns H to ALO are 'hidden' in the worksheet)

We use the 1,000 possible values for X and Y in columns F to ALQ to:

add the variables to give, $A = X + Y$, in row 5
subtract Y from X to give, $S = X - Y$ in row 6
multiply them to give, $M = X \times Y$ in row 7
divide X by Y to give, $D = X / Y$ in row 8
raise X to the power of three, $P = X^3$ in row 9

For each of these calculations in columns F to ALQ, the next step is to calculate the respective standard deviation uncertainties of all 1,000 values and record the *combined uncertainties* in column C. For example, the standard deviation uncertainty in $X + Y$ is calculated:

[C5] = STDEV.S(F5:ALQ5)

Column C now contains the experimentally observed uncertainties, u, (in C5, C6, C7, C8, and C9) for each of the different combinations.

Finally, we use Eqn 1.11 to calculate the percentage uncertainties, u%, in D2 and D3 for X and Y:

$u\%_X = 100 \times 0.7 / 9.0 = 7.78\%$
$u\%_Y = 100 \times 0.5 / 6.0 = 8.33\%$

and for each of the experimentally observed uncertainties in D5:D9, e.g.:

[D5] = 100 * C5 / B5

In our model the randomly generated and calculated values, shown in italics, are recalculated every time the F9 button is pressed. Clearly it is unrealistic to use an Excel model like this every time we want to *predict* the uncertainties in calculated values, and we need to know how to *estimate* combined uncertainties from the individual uncertainties, u_X and u_Y.

The **guiding principles** for combining uncertainties are:

- Combine unrelated uncertainties by using the **addition of variances**, which is equivalent to combining standard deviations *squared* in the same way as Pythagoras's equation for the sides of a right-angled triangle.
- For the uncertainty in the **addition** or **subtraction** of values, combine ***absolute*** uncertainties:

$$u_A = u_S = \sqrt{u_X^2 + u_Y^2} \quad (1.12)$$

- Note that the uncertainty variances always *add*, even when taking the difference between values.
- For the uncertainty in the **multiplication** or **division** of values, combine ***percentage*** uncertainties:

$$u\%_M = u\%_D = \sqrt{u\%_X^2 + u\%_Y^2} \quad (1.13)$$

- For the uncertainty in the **power** of a value, multiply the ***percentage*** uncertainty by the value, k, of the power:

$$u\%_P = k \times u\%_X \quad (1.14)$$

Convert between *absolute* and *relative* uncertainties using Eqn 1.11.

We now compare the use of Eqns 1.12 to 1.14 with the results of the Excel model in Fig 1.13, using the values:

$X = 9.0$ with $u_x = 0.7$ and $\%u_x = 7.78\%$, and
$Y = 6.0$ with $u_Y = 0.5$ and $\%u_y = 8.33\%$

Using Eqn 1.12 for the *absolute* uncertainty in **addition** and **subtraction**:

$$u_A = u_S = \sqrt{0.7^2 + 0.5^2} = 0.860$$

which is consistent with the randomly generated values in cells C5 and C6.

Using Eqn 1.13 for the *percentage* uncertainty in **multiplication** and **division**:

$$u\%_M = u\%_D = \sqrt{7.78^2 + 8.33^2} = 11.40$$

which is consistent with the randomly generated values in cells D7 and D8.

Using Eqn 1.14 for the *relative* uncertainty in taking the **power**:

$$u\%_P = 3 \times 7.78 = 23.33$$

which is consistent with the randomly generated value in cell D9.

We can now use the following example to demonstrate the use of an Excel worksheet to lay out the calculations for a multiple combination of errors.

Case study: **Experimental uncertainties / 3. Propagation of errors**

–continued from 1.4.4, leading to 1.4.6

In an experiment to measure the specific heat capacity, c, of a material, a body of mass m is heated electrically using a voltage, V, and current, I, for a time, t. The temperature of the body rises from T_1 to T_2 (C).

The specific heat capacity of the body is given by the equation:

$$c = \frac{I \times V \times t}{m \times (T_2 - T_1)}.$$

The experimental values and standard deviation uncertainties of the variables have been entered into rows 2, 3, 6, 7, 8, and 9 of columns B and D in Fig 1.14:

	A	B	C	D	E	F
1		**Value**	**Units**	**Uncert**		**% Uncert**
2	T_1 =	**40.40**	C	**0.10**		
3	T_2 =	**45.60**	C	**0.10**		
4				↓		
5	$(T_2 - T_1)$ =	5.20	C	0.14	→	2.72
6	I =	10.60	A	0.02	→	0.19
7	V =	12.00	V	0.10	→	0.83
8	t =	120.00	s	1.00	→	0.83
9	m =	0.68	kg	0.01	→	1.47
10						↓
11	c =	4316.74	J kg^{-1}C^{-1}	143.06	←	3.31

Fig 1.14 Propagation of errors

Propagation of errors: Excel analysis for Fig 1.14. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

The best-estimate value for c is calculated in B11 using the given equation:

[B11] = B6 * B7 * B8 / (B9 * (B3 – B2)) = 4316.74 Jkg^{-1}C^{-1}

The equation for c has both multiplication and subtraction calculations, which will involve both the *absolute* and *percentage* uncertainty values. We need to split up the calculation into two main stages, following the steps given by the arrows in the worksheet.

First we calculate the value for $(T_2 - T_1)$ in B5, and then the *absolute* uncertainty of $(T_2 - T_1)$ in D5 by combining the absolute uncertainties in D2 and D3 using Eqn 1.12:

[D5] = SQRT(D2^2 + D3^2)

The rest of the calculation, with multiplication and division, combines the *relative* uncertainties, which are first calculated using Eqn 1.11, e.g.

[F5] = 100 * D5 / B5

The combined *relative* uncertainty in c is calculated in F11 using Eqn 1.13:

[F11] = SQRT(F5^2 + F6^2 + F7^2 + F8^2 + F9^2)

Finally, the *absolute* uncertainty in c is calculated using Eqn 1.11:

[D11] = F11 * B11 / 100 = 143 J kg^{-1}C^{-1}

This gives a final result: c = 4317 (143 sd) J kg^{-1}C^{-1}

1.4.5 **Probability uncertainty**

The probability of an event occurring is given by the binomial distribution (1.3.3), such that, if the probability of an individual occurrence is p, then, out of n trials where the event may, or may not, occur:

Mean number of observed events (Eqn 1.5):

$$\mu = p \times n$$

Standard deviation uncertainty (Eqn 1.6):

$$\sigma = \sqrt{n \times p \times (1-p)}.$$

If the probability of observing an individual event is very small, $p << 1.0$, then the binomial distribution approximates to the Poisson distribution, and the standard deviation becomes:

$$\sigma \approx \sqrt{n \times p} = \sqrt{\mu}.$$

Thus, for an observed count of N, when the individual probability is *small*, the *best estimate* in the uncertainty of that value is given by

$$u = \sqrt{N}. \qquad (1.15)$$

Typical examples include radioactivity, in which the count records the random decay of individual atoms, and the occurrences of a rare, non-communicable disease, observed with a probability given by the Poisson distribution.

1.4.6 **Identifying uncertainties**

An important element of analysis is to separate the different forms of variation and uncertainty that have combined within the experimental data. We see in 3.1.1 how the t-statistic compares an observed value with the uncertainty in the measurement, and in 3.2.2 how an ANOVA (ANalysis Of VAriance) separates variances within the data in order to identify significant factor effects.

A statistical analysis needs to be able to calculate the uncertainties in the data if it is able to provide correct estimations of the overall uncertainty. This is demonstrated in the following case study which analyses a combination of *system* and *probability* uncertainties in two different ways, producing different uncertainty estimations in the final result.

Case study: **Experimental uncertainties / 4. DIY dice**

—continued from 1.4.4, leading to 5.3.4

We wish to measure experimentally the probability of getting a '6' when we roll a die. Instead of using commercial dice, we imagine that we make our own ten dice by cutting ten cubes from a bar of wood with a square cross-section, and writing a '6' on a randomly chosen face of each die. These DIY (do-it-yourself) dice are unlikely to be perfect cubes, and we can expect that we now have a *subject* uncertainty (the different dice), in addition to the inherent *probability* uncertainty. There is no *measurement* uncertainty as we assume that we can tell accurately whether or not we observe a '6'.

In Fig 1.15 we use Excel to simulate DIY dice by *randomly* allocating (in B3:B12) the *probabilities* with which each die records a '6'. These probabilities have been randomly produced from a distribution with the expected mean for a fair die of 1/6 and with a standard deviation of 20% (in B14) to represent the random differences between the dice. With these individual probabilities Excel then uses the binomial distribution to randomly calculate (in C3:C12) the *frequency* of '6s' that each die might record when it is rolled n = 200 times.

DIY dice: Excel analysis for Fig 1.15. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

	A	B	C	D	E
1	**Die**	**Probability**	**Frequency**	**Method**	**Proportion**
2	*i*	*p*	*f*	(2) >	*p*
3	1	0.161	29	/ 200 =	0.145
4	2	0.105	27	/ 200 =	0.135
5	3	0.136	25	/ 200 =	**0.125**
6	4	0.139	32	/ 200 =	**0.160**
7	5	0.150	28	/ 200 =	**0.140**
8	6	0.229	43	/ 200 =	**0.215**
9	7	**0.168**	31	/ 200 =	**0.155**
10	8	**0.156**	25	/ 200 =	**0.125**
11	9	**0.119**	13	/ 200 =	**0.065**
12	10	**0.178**	28	/ 200 =	**0.140**
13			**Method**		↓
14	**Uncert (%) =**	**20**	**(1)**	**Mean =**	**0.1405**
15			↓	**Stdev =**	**0.0371**
16		**Sum =**	**281**	***SE* =**	**0.0117**
17	**Mean proportion =**		**0.1405**	***t* =**	**2.2622**
18	**Uncertainties:**	***Cd* (1) =**	**0.0152**	***Cd* (2) =**	**0.0265**

Fig 1.15 Rolling ten dice 200 times each

We can now use the 'results' to estimate the probability of getting a specific face (e.g. a '6') when we roll a die, and we can do this in two ways.

1. We can add up the total number of '6s' (in C16) from the 200 rolls of every die, giving a total of 281, and then divide by the overall number of rolls (2,000) to get a final proportion:

   [C17] = C16 / 2000 = 0.1405

2. Alternatively, we can calculate the proportion of '6s' for each die, record the results in E3:E12, and then calculate the average of the ten proportions:

[E14]=AVERAGE(E3:E12) = 0.1405

In this particular example, both methods must give the same result because all dice have been rolled the *same number of times*. This is significant because it means that the results of every die (sample) have the same 'importance' when calculating the average of the proportions. See 5.3.4 for the use of *weighting* values when the samples have differing 'importance'.

The important difference between the methods lies in their *estimation of the uncertainty* in the result.

With method 1, the total number of '6s', 281, are counted, and the calculation of the proportion, $P = 0.1405$, is based on $n = 2{,}000$ trials. However, all information about the *sample* variability has been lost. We can use Eqn 1.8 from the binomial distribution in 1.3.3 to estimate the standard deviation uncertainty just due to the inherent *probability* uncertainty:

$$\sigma(P) = \sqrt{\frac{P\times(1-P)}{n}} \Rightarrow \sqrt{\frac{0.1405\times 0.8595}{2000}} \Rightarrow 0.00777$$

This then gives an estimated 95% confidence deviation (1.5.2), $Cd(1) = 1.96 \times 0.00777 = 0.0152$.

We could then conclude that, using method 1, we would be 95% confident that the true probability of recording a '6' was within the range 0.140 ± 0.015.

However, if we use method 2 we see a variation in E3:E12 between the different dice (samples), which will be due to both the *sample* and *probability* uncertainty.
We calculate the standard deviation of these values in E15:

[E15] = STDEV.S(E3:E12) = 0.0371

For a sample size of ten values, this gives an estimated 95% confidence deviation (1.5.2):

$$Cd(2) = 2.26 \times \frac{0.0371}{\sqrt{10}} = 0.0265$$

where 2.26 is the t-value with $df = 10 - 1 = 9$.

We could then conclude that, using method 2, we would be 95% confident that the true probability of recording a '6' was within the range 0.140 ± 0.027.

We know, both from a theoretical expectation and years of experimentation, that the true probability of recording a specific face on a true die will be $1/6 = 0.167$. The uncertainty calculation in method 1 does not take into account the *system* variations due to the different dice, and the confidence range is too narrow and fails to include the true value. However, method 2 takes into account both variations, and the wider confidence range just includes 0.167 as a possible true value.

It is important to ensure that any initial analysis of the data, e.g. taking totals, means, standard deviations, etc. does not 'hide information' and prevent any subsequent analysis of the data being able to calculate the actual experimental variation in the data (5.1.5).

1.5 Sample data

It is important to start by differentiating the use of the word 'sample', as a *statistical* sample of n repeated (replicate) measurements, from the concept of a *chemical* sample, which is a representative amount of material selected for analysis. It is quite common to have a *statistical* sample of several replicate measurements (e.g. calcium content) taken from one *chemical* sample (e.g. of mineral water).

We develop the statistics involved in performing a *statistical* analysis of the repeated measurements in a sample, with the aim of increasing the accuracy of a best-estimate of an unknown true value. This involves calculating the

- *sample mean*, which is the best-estimate for the unknown true value
- *sample standard deviation*, which provides a measure of experimental uncertainty
- *standard error of the mean*, which is a measure of the uncertainty in the sample mean
- *confidence interval* of the mean, which defines a range of possible values within which it is possible to state, with a given confidence, that the true value will lie.

Our initial analysis of the sample statistics assumes that the uncertainty in the measurements is calculated *solely* from the values in the sample. Then, following an outline of the difference between statistical *samples* and *populations*, we then analyse the *scientific* situation where the experimental uncertainty is known using experience from outside of the specific sample of measurements.

1.5.1 Sample statistics

We will develop the key statistics for data samples using a case study of five replicate measurements.

Sample statistics and confidence intervals: Excel analysis for Fig 1.16. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: **Blood alcohol / 5. Sample statistics**

—continued from 1.3.1, leading to 1.5.3

Column B in Fig 1.16 gives five replicate blood alcohol values, x_i, selected randomly from a normal distribution with a mean of $\mu = 80$ and a population standard deviation of $\sigma = 2.0$. We develop the standard error and confidence interval for this sample.

	A	B	C	D	E	F
1	i	x_i	d_i	d_i^2		
2	1	81.4	1.02	1.04	Sample stdev, s =	1.51
3	2	78.8	-1.58	2.50	Standard error of mean, SE =	0.68
4	3	79	-1.38	1.90	t-value =	2.78
5	4	80.4	0.02	0.00	Confidence deviation, Cd =	1.88
6	5	82.3	1.92	3.69	Using CONFIDENCE.T() =	1.88
7						
8	Size =	5	Sum of squares, SS =	9.13	Mean square, MS =	2.28
9	Mean =	80.38	Deg of freedom, df =	4	Sample variance, s^2 =	2.28

Fig 1.16 Statistics of five replicate data values

The separate data values, x_i, are identified by the integer i in column A which runs from 1 to 5.
The sum of values is expressed using capital sigma:

$$\text{Sum of all values} = \sum_i x_i$$

The *mean value*, $\bar{x}$ (x-bar), is calculated as the simple sum divided by the sample size, n:

$$\bar{x} = \frac{\Sigma_i x_i}{n} = \frac{81.4 + 78.8 + 79.0 + 80.4 + 82.3}{5} = \frac{401.9}{5} = 80.38$$

which is calculated directly in cell B9, using the AVERAGE() function in Excel:

[B9] = AVERAGE(B2:B6) = 80.38

The sample mean value, $\bar{x}$ is the *best-estimate* for the true blood alcohol value, μ.

In order to quantify the *spread* of data, we first calculate the deviations, $d_{integer}$, of each data value from the mean value:

$$d_i = x_i - \bar{x}$$

which are calculated in column, C, e.g. [C2] = B2 – B$9 = 1.02.
The dollar sign is used to lock the row value for B9 when this formula is copied down to row 6.
If we added the values of all deviations we would find that

$$\sum_i d_i = d_1 + d_2 + d_3 + d_4 + d_5 = 0$$

This will always be the case as the criterion for the mean value is that the deviations will always sum to zero.

To get a positive measure of the data variability we square each deviation in column D,

e.g. [D2] = C2 ^ 2 = 1.04

and then add all squares to get the total **sum of squares** (SS) of the deviations.

$$SS = \sum_i d_i^2 = \sum_i (x_i - \bar{x})^2 \tag{1.16}$$

calculated in D8 as: [D8] = SUM(D2:D6) = 9.13.

An important factor in many statistical calculations is the **degrees of freedom, *df***, in the calculation. This is related to the number of 'bits of information' in the calculation. We started the analysis with $n = 5$ data values, i.e. with five bits of information. We then used one bit of information to calculate the mean value, $\bar{x}$, of this particular sample. Hence the remaining degrees of freedom are:

$$df = n - 1 \tag{1.17}$$

calculated in D9 as: [D9] = B8 – 1 = 4.

We can now calculate a **mean square** value, *MS*, by dividing the sum of squares by the degrees of freedom, *df*:

$$MS = \frac{SS}{df} \tag{1.18}$$

calculated in F8 as: [F8] = D8 / D9 = 2.28.
For a simple data sample, *MS* is usually called the **sample variance, s^2**:

$$s^2 = MS = \frac{\Sigma_i (x_i - \bar{x})^2}{(n-1)} \tag{1.19}$$

We can also calculate this directly in Excel using the function, VAR.S() in F9:

[F9] = VAR.S(B2:B6) = 2.28

giving the same result as in F8.
The **sample standard deviation, *s***, is the square root of the variance,

$$s = \sqrt{s^2} = \sqrt{\frac{\Sigma_i (x_i - \bar{x})^2}{(n-1)}} \tag{1.20}$$

Using Eqn 1.20:

$$s = \sqrt{s^2} = \sqrt{2.28} = 1.51$$

We can also calculate this directly in Excel using the function STDEV.S() in F2:

[F2] = STDEV.S(B2:B6) = 1.51

The *sample standard deviation* is the best estimate of the uncertainty in a *single* measurement. However, we will normally take the *mean* of n replicate measurements because the average of several values is more likely to be closer to the true value than just a single measurement.

The reduced uncertainty with n measurements is described by the ***standard error of the mean, SE***:

$$SE = \frac{s}{\sqrt{n}} \tag{1.21}$$

We can see that the uncertainty reduces, and the precision increases, in proportion to the *square root* of the number, n, of measurements.

We calculate the standard error, *SE*:

[F3] = F2/SQRT(B8) = 0.68

The units for standard deviation and standard error of the mean are the same as the value itself.

1.5.2 Confidence interval

The confidence interval, *CI*, is a standard way of presenting the uncertainty in our results by stating that we are 95% (for example) confident that the true value being measured lies within a calculated range of values.

The confidence interval, *CI*, is calculated as the range of values defined by the sample mean value, with a confidence deviation *Cd* on either side.

$$CI = \bar{x} \pm Cd$$

Students are sometimes confused between confidence *interval* and the confidence *deviation*, and it is important to check which value is being presented. For example, Excel 2013 uses the function CONFIDENCE.T() to calculate the confidence *deviation*, *Cd*.

The confidence deviation (based on the sample standard deviation, *s*) will depend on:

- the *uncertainty*, which is introduced as the standard error of the mean, *SE*, and
- the *degree of confidence* that we wish to claim for the calculated range, which is introduced by the *t*-value, *t*.

$$Cd(\text{using } s) = t \times SE = t \times \frac{s}{\sqrt{n}} \tag{1.22}$$

The ***t*-value** depends on

- the level of confidence required, typically 95% which is often expressed as a *significance* of 0.05 and
- the degrees of freedom, $df = n - 1$.

For a very large sample, and for a confidence of 95%, the *t*-value becomes equal to the limiting *z*-value (1.5.4) = 1.96. For smaller samples (low values for *n* and *df*), the value of *t* increases to make the confidence deviation greater to allow for the greater uncertainty in the calculated standard deviation.
We calculate the *t*-value directly in Excel using the function, T.INV.2T() in F4:

[F4]= T.INV.2T(0.05, D9) = 2.78

where D9 is the degrees for freedom and '0.05' is the *significance* equivalent to 95% confidence.
We can then calculate the confidence deviation in F5:

[F5] = F4 * F3 = 1.88

It is also possible to use the function CONFIDENCE.T() to calculate the confidence deviation directly from the sample standard deviation (F2) and the sample size (B8):

[F6] = CONFIDENCE.T(0.05, F2, B8) = 1.88

Note that the CONFIDENCE() function does *not* apply to *sample* calculations, as it assumes that the *t*-value is given by the limiting *z*-value for a *population* (1.5.4).
The **confidence interval of the mean** is given by the equation:

$$CI = \bar{x} + Cd = \bar{x} + t \times SE = \bar{x} + \left(t \times \frac{s}{\sqrt{n}} \right) \tag{1.23}$$

The 95% confidence interval, *CI*, for the true blood alcohol in Fig 1.16 is calculated to be:

$CI = 80.38 \pm 1.88$

which, rounded to one decimal place, and including units, becomes:

$CI = 80.4 \pm 1.9$ mg per 100 ml.

For a confidence level of 95%, there is a 95% probability that the true value lies *within* the limits of the confidence interval. In other words, there is only a 5% (= 0.05) probability that we would be wrong if we gave the confidence interval as our *best-estimate* of the true value.

It is important to emphasize that the confidence interval expresses the result of a *specific set of sample values*, and it is *not* a prediction of how the mean values could be expected to vary for *different* samples. We see, in 1.5.3, how the calculated confidence interval, *CI*, varies considerably from sample to sample, but, in 95% of samples, the true value is still found within the confidence interval for that particular sample.

1.5.3 Samples and populations

It is important to understand the difference between data **samples** and **populations**. A 'population' describes *all the measurements* that could be made of a particular variable, but a 'sample' represents a *selection of representative measurements* taken from the population.

For example, if we wish to compare the sizes of fish in two fish farm tanks, we could measure the whole *population* of each tank (every fish), but for economy of effort it might be sufficient, within the required accuracy of comparison, just to take a representative *sample* of a *few* fish from each tank.

As another example, we are familiar with making a few replicate measurements of the absorbance of a chemical solution, such that the few measurements are just a *sample* of the almost unlimited *population* of repeated measurements that could be made.

We can test whether a data set is a *sample* or a *population* by considering the effect of *repeating the process by which the data values were identified*. If repeating the process of identification will always give the same values (e.g. selecting every fish), then the data set is a *population*, but if different random values could occur (e.g. selecting a different sub group) then the set is a *sample*.

A **parameter** is a *variable* that is used to describe some characteristic of a *population*. A parameter is usually given a Greek letter as a symbol, e.g. μ (mu) for population mean and σ (sigma) for population standard deviation.

A **statistic** is a *variable* that is used to describe some characteristic of a *sample*, e.g. $\bar{x}$ for sample mean and *s* for sample standard deviation.

The value of the sample statistic is a *best-estimate* for the true value of the equivalent population parameter. For example, the sample mean, $\bar{x}$, is the best estimate for the true mean, μ, of the population from which it was randomly drawn. Similarly, the sample standard deviation, *s*, is the best estimate for the source population standard deviation, σ.

Different samples from the *same* population will typically give different values for the same statistic (e.g. different *sample* means).

The calculations for mean and standard deviation of both samples and populations are summarized in Table 1.3.

Table 1.3 Means and standard deviations

	Sample statistics		Population parameters	
Mean	$\bar{x} = \frac{\Sigma_i x_i}{n}$		$\mu = \frac{\Sigma_i x_i}{n}$	
Standard deviation	$s = \sqrt{\frac{\Sigma_i (x_i - \bar{x})^2}{(n-1)}}$	(1.24)	$\sigma = \sqrt{\frac{\Sigma_i (x_i - \mu)^2}{n}}$	(1.25)

The calculation is the same for both sample mean, $\bar{x}$, and population mean, μ. However, there is a difference in the calculations for the respective standard deviations, s and μ, in that the degrees of freedom divisor is n for the *population* standard deviation but $n - 1$ for the *sample* standard deviation. The sample calculation loses one degree of freedom, or one bit of information, because of the need to calculate the mean value, $\bar{x}$ for each *specific* sample.

We will see in the next case study that the '$n - 1$' is important because the sample standard deviation is used as an *estimate* of the population standard deviation from which it has been derived, and that, without the use of the '-1' in the denominator, it would tend to underestimate the true population standard deviation.

Case study: **Blood alcohol / 6. Samples and populations**

–continued from 1.5.1, leading to 1.6.2

This case study uses Excel to simulate experimental measurements made to establish the blood alcohol level for a sample where the true value, μ, is 80 mg of alcohol per 100 ml of blood, and the measurement uncertainty is equivalent to a standard deviation, σ, of 2.0 mg/100 ml.

Samples and populations: Excel analysis for Fig 1.17. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

In principle, there is no statistical restriction to the number of replicate measurements that could be made on the same physical sample of blood. Every time we make a measurement we get a potentially new set of sample results.

In the cells D2:D6 (sample 1) in Fig 1.17, a sample of five values is randomly generated from a normal population with $\mu = 80$ and $\sigma = 2.0$, and then a further 1,999 samples are generated in columns E to BYA. This process of data simulation in Excel uses the values of 80 and 2.0 in B2 and B3 respectively, and then the function NORM.INV(RAND(), B2, B3) in every data cell generates values randomly selected on the basis of the defined normal distribution.

	A	B	C	D	E	F	G	BXZ	BYA
1	**Population parameters:**		Sample:	S1	S2	S3	S4	S1999	S2000
2	Mean =	80	(79.996)	*77.9*	*80.3*	*80.5*	*78.6*	*83*	*81.7*
3	StDev =	2	(2.028)	*83.8*	*81.2*	*78.7*	*76.7*	*78.1*	*81.9*
4				*79.4*	*82.9*	*76.9*	*79.4*	*82.9*	*76.5*
5				*81*	*81.4*	*78.5*	*78.1*	*83.7*	*83.8*
6				*80.5*	*82*	*80*	*77.4*	*82.5*	*77.6*
7	**Sample (n = 5) statistics:**	**Averages:**	**Calculations:**						
8	Mean =	*79.996*	Mean =	*80.52*	*81.56*	*78.92*	*78.04*	*82.04*	*80.3*
9	Sample stdev =	*1.912*	Sample stdev =	*2.19*	*0.97*	*1.41*	*1.05*	*2.24*	*3.1*
10	*'Population' stdev* =	~~*1.71*~~	*'Population' stdev* =	~~*1.95*~~	~~*0.86*~~	~~*1.26*~~	~~*0.94*~~	~~*2.01*~~	~~*2.77*~~
11	**Test statistics:**								
12	Standard error (mean) =	*0.855*	Standard error =	*0.98*	*0.43*	*0.63*	*0.47*	*1*	*1.39*
13	True value =	80	*t*-value =	*2.78*	*2.78*	*2.78*	*2.78*	*2.78*	*2.78*
14	**Errors:**		*Cd* (with *s*) =	*2.71*	*1.2*	*1.75*	*1.3*	*2.79*	*3.85*
15	Proportion (using *s*) =	*0.051*	Type I error (with *s*) =	*0*	*1*	*0*	*1*	*0*	*0*
16									
17	**Errors:**		*Cd* (with σ) =	1.75	1.75	1.75	1.75	1.75	1.75
18	Proportion (using σ) =	*0.053*	Type I error (with σ) =	*0*	*0*	*0*	*1*	*1*	*0*

Fig 1.17 Sample statistics (Columns H to BXY are 'hidden' in this worksheet) Numbers in italics show values that change with different randomly generated data sets

The values in cells C2 and C3 record the actual mean (79.996) and standard deviation (2.028) of *all* of the generated values, and can be seen to be consistent with the population values of 80 and 2.0 used to generate them.

The 2,000 columns of data between D and BYA each have five randomly selected values in rows 2 to 6, equivalent to a statistical *sample* of size five. Note that each time the F9 key is pressed, Excel generates completely new sets of sample data. Numbers in italics indicate values that can change every time a new data set is created.

For each of the 2,000 samples, we calculate:

- mean values, $\bar{x}$, of every sample in D8 to BYA8:
  e.g. [D8] = AVERAGE(D2:D6)
- sample standard deviations, s, of every sample in D9 to BYA9 based on Eqn 1.24:
  e.g. [D9] = STDEV.S(D2:D6)
- population standard deviations, σ, of every sample in D10 to BYA10 based on Eqn 1.25:
  e.g. [D10] = STDEV.P(D2:D6)

Note that the *population* standard deviation values are recorded with a *strikethrough*, because it would not be usual to calculate this value for a sample, and is only done here for comparison.

The averages of these three statistics for all 2,000 samples are calculated in B8, B9, and B10 respectively.

As expected, the average of all the sample means, [B8] = 79.996, is very close to the true value, 80, of the population. The mean of the sample, $\bar{x}$, gives the *best estimate* of the unknown true value, μ.

We can also see that the *sample* standard deviations (1.912 in B9), calculated using Eqn 1.24, gives a better estimate of the true standard deviation, 2.00, than the *population* standard deviations (1.710 in B10) calculated using Eqn 1.25. This confirms that the sample standard deviation, s, gives the *best estimate* of the unknown true standard deviation, σ, from which the sample has been drawn.

We continue to refer to the Excel worksheet in Fig 1.17 to investigate the use of *standard error* and *confidence interval*.

For each of the 2,000 samples we calculate:

- standard error, *SE* (row 12), based on Eqn 1.21,

  e.g. [D12] = D9 / SQRT(5)
- t-value (row 13) for 95% confidence and $df = 4$ (which is the same for all samples),

  e.g. [D13] = T.INV.2T(0.05, 4) = 2.78
- confidence deviation, *Cd* (row 14), based on Eqn 1.22,

  e.g. [D14] = D13 * D12

Note that the confidence deviation, *Cd*(*s*) in row 14, is based on the *sample* standard deviation, s.

In B12 we calculate the average value of the standard errors from all 2,000 samples, and record a value of 0.855 which is consistent with Eqn 1.21 using the average sample standard deviation of 1.912 in B9:

$$SE = \frac{1.912}{\sqrt{5}} = 0.855$$

We now wish to *test* whether the calculated confidence interval from Eqn 1.23:

$$CI = \bar{x} \pm Cd$$

provides a correct range for finding the true value for 95% of randomly selected values. According to our theory we would expect that the true value will fall *outside* the confidence interval for only 5% of the samples.

The confidence interval prediction will *fail*, causing a Type I error (1.6.3), if the true value, B13, lies *outside* the confidence interval,

i.e. if $80 > \bar{x} + Cd$ or $80 < \bar{x} - Cd$

We use the IF() function in Excel to test whether this may be true:

e.g. [D15] = IF(OR($B13 > D8 + D14, $B13 < D8 − D14), 1, 0)

where $\bar{x}$ is in D8, *Cd* in D14, and the value 80 in B13.

The IF() function returns a '1' in row 15 if the true value, B13, lies *outside* the confidence interval for each of the samples.

We can see that samples 2 and 4 return a '1':

Sample 2: $CI = 81.56 \pm 1.20$, i.e. between 80.36 to 82.76 which does not include 80.

Sample 4: $CI = 78.04 \pm 1.30$, i.e. between 76.74 to 79.34 which does not include 80.

We can calculate the *proportion* of failures in B15 by counting the number of '1s' for all 2,000 samples and dividing by 2,000:

[B15] = SUM(D15:BYA15)/2000 = 0.051.

The sampled data reproduced in Fig 1.17, gives a calculated proportion of errors in B15 of 0.051, which is consistent with the expected value of 0.050 for the error probability. Pressing the F9 key to recalculate new samples produces new values, which continue to be consistent with 0.050.

1.5.4 Known experimental uncertainty

In 1.5.3, the experimental standard deviation uncertainty, s, was calculated *only* from the *sample* measurements. For small samples this means that there is a large uncertainty in the value of s, and hence a large uncertainty in the calculated confidence deviation, *Cd*.

However, there are often practical situations where the true measurement uncertainty, the *population* standard deviation, σ, is already known from previous experience or from a knowledge of the measurement process. In this case, the t-statistic has effectively an infinite number of degrees of freedom and becomes equal to the z-statistic, which for 95% confidence gives $z = 1.96$.

Hence, if we know the true value of the population standard deviation, σ, the confidence deviation becomes:

$$Cd(\text{using } \sigma) = z \times \frac{\sigma}{\sqrt{n}} = z \times SE \qquad (1.26)$$

where $z = 1.96$ for 95% confidence.

Returning to Fig 1.17, we now investigate the implications of knowing the experimental uncertainty *separately* from the sample values.

When the population standard deviation, σ, is already known, the t-value for 95% confidence becomes equal to the z-value of 1.96. In this case study, the known population standard deviation, σ, is taken as the value in B3 = 2.0, and the new confidence deviation is the *same* for all 2,000 samples, calculated in row 17:

Cd(using σ): e.g. [D17] = 1.96 * $B3/SQRT(5) = 1.75

In row 18, we again use the IF() function (see also calculation for row 15) to identify which of these confidence intervals *CI*(using σ) result in an error, and then calculate the overall proportion in B18. The sampled data gives a calculated proportion of errors of 0.053, which is again consistent with the expected value of 0.050.

The *proportion* of errors is actually the same whether we use either *sample* or *population* standard deviations. However, it is not always the same *samples* that record errors. For example, it can be seen that sample 4 gives an error using both methods but not sample 2.

It is now useful to compare the variability of the different samples in Fig 1.17, and in Fig 1.18 we plot the mean values and confidence intervals (using *sample* standard deviations) from 8 of the 2,000 samples.

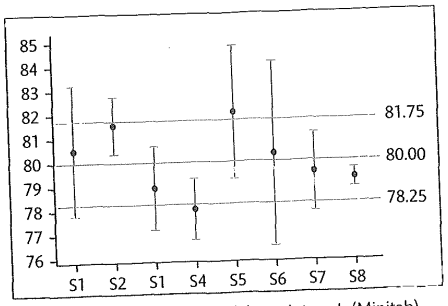


Fig 1.18 Replicate sample confidence intervals (Minitab)

The limits of 78.25 and 81.75, plotted on either side of the true value of 80 in Fig 1.18, are equivalent to the confidence interval, CI(using σ) = 1.75, for a known *population* standard deviation.

Calculations using *sample* standard deviations, s, give errors if the value of 80 lies outside the confidence intervals shown with each data point, i.e. for S2, S4, and S8.

Calculations using a known *population* standard deviation, σ, give errors if the mean value of the data point lies outside the lines drawn at 78.25 and 81.75, i.e. for S4 and S5.

We can see from Fig 1.18 that the conditions giving rise to errors can be different for the two types of calculation, although the overall proportions of error are the same for both at 5%.

1.5.5 **Presenting results**

The Excel model in Fig 1.17 shows that we get a *statistical* error rate of 5% for the confidence interval using either Eqn 1.23 with s or Eqn 1.26 with σ. It is then reasonable to ask whether it makes any difference if we present the 'sample' results when we actually know the true experimental uncertainty.

The apparent equivalence of the two types of analyses relates only to the question: 'Does the confidence interval include the true value?' However, they are not equivalent in the information content that is contained within their calculated confidence intervals. A complete result includes *both* the best-estimate value *and* the uncertainty in that value. The values for the uncertainty estimated solely by the sample values can vary widely, particularly for small samples, and, if we fail to use the known experimental uncertainty, then we would be throwing away information that is essential for the quality of the published result.

Further examples of the use of known experimental uncertainty are given in 2.2.1 (Case study: Spectrophotometer calibration / 4. Calibration result) and 2.2.3.

1.6 Hypothesis tests

Many investigations in science seek to answer a simple 'Yes/No' question.
For example:

- Is a new vaccine effective in preventing an infection?
- Do glass fragments at the scene of a crime come from different sources?
- Does a particular training regime improve athletic performance?

However, most experimental results have associated uncertainty and we cannot be absolutely certain of the correct answer. The 'hypothesis test' in statistics is a well-defined and universally accepted procedure for providing an answer based on agreed levels of confidence.

1.6.1 **Test procedure**

The first step is to define the 'Yes/No' question that we wish to test. The 'Yes' option usually means that we expect to be able to observe some effect or difference in recorded values, whereas the 'No' option shows no effect or difference.

We would like to have a calculation that tells us how confident we can be that 'Yes' is the *correct* answer. Unfortunately, the statistics only tells us how confident we can be that 'No' is *NOT* the correct answer! We often have to infer a *positive scientific* conclusion from a *statistical negative*.

It is then necessary to define the hypotheses of our test:

- Null hypothesis, H_0, as the state where there is no effect or difference, and
- Proposed or alternative hypothesis, H_1, as the state where there is an effect or difference.

Statisticians use the term 'alternative' to differentiate H_1 from the 'null' H_0, but many science students get confused when their 'proposed' *scientific* hypothesis is called a statistical 'alternative'. In this book, we often include the term 'proposed' to identify the scientific objective of the hypothesis test.

H_0 and H_1 should normally be mutually exclusive, such that 'rejecting the null hypothesis' is the same as 'accepting the proposed/alternative hypothesis'.

We must decide on how much confidence we will need to have before deciding that the null hypothesis is not true. This confidence is usually expressed as the *significance level*, α, which is the probability that we would be wrong if we rejected the null hypothesis. For example, the default level of a 95% 'confidence' equates to a significance level (probability of being wrong) equal to 0.05.

After collecting the experimental evidence, we will then either:

- calculate the value of a **test statistic** from the data, and then compare it with a known **critical value** for the statistic to decide whether the evidence suggests that the null hypothesis is unlikely to have produced the observed results. The relevant critical value can be obtained from published tables,

or alternatively, and, more usually, with the use of modern software:

- calculate the probability, ***p*-value**, that the null hypothesis could give the experimental values equal to, or more extreme, than observed. This value is then compared to the **significance level, α**, chosen before the test. The default value for significance in most general analyses is $\alpha = 0.05$.

When using the *p*-value method:

- If $p \leq \alpha$ we state that, on the basis of the experimental evidence, we would reject the null hypothesis and accept that the proposed (alternative) hypothesis is correct.
- If $p > \alpha$ we state that there is insufficient experimental evidence to reject the null hypothesis and that any observed difference/effect could have occurred by chance.

It should be noted that the test cannot prove that the null hypothesis is true, only that there is not enough evidence to claim that it is not true. As our conclusions are based on a statistical calculation, there is always a chance that we could be wrong, and we examine this possibility in 1.6.3.

1.6.2 Hypothesis test and *p*-values

It is important to take care in expressing the scientific objective of your analysis in terms of a hypothesis that can be uniquely tested by experiment. This also requires identifying a measurable value that becomes the *test statistic* to be analysed.

Case study: Blood alcohol / 7. Hypothesis test

—continued from 1.5.3, leading to 3.1.2

A scientific objective is to decide if the blood alcohol level in a sample is above the specific value of $\mu_0 =$ 80 mg of alcohol per 100 ml of blood. In the experiment we make $n = 5$ replicate measurements which give a sample mean of $\bar{x} = 81.6$, which is the *best estimate* of the true blood alcohol value, μ.

In this investigation, we assume that we know from previous experience that the experimental measurement has a standard deviation uncertainty of $\alpha = 2.0$ mg/100 ml.

We need to decide whether it is possible that the true blood alcohol value, μ, in the sample is actually equal to $\mu_0 = 80.0$, or less, and that the measured higher value only occurred due to the statistical variation in the experimental measurements. (Note that in a legal test for driving in the UK with excess alcohol, the actual level of confidence required for a prosecution is considerably higher).

The test statistic in the case study is the measured blood alcohol level, $\bar{x}$ and the scientific objective is to decide whether the true level is above or below (or equal to) 80 mg /100 ml.

The *scientific* null in this case is that μ is less than, or equal to, 80 mg /100 ml, i.e. $\mu \leq \mu_0$. However, the most likely way in which we might conclude, *incorrectly*, that μ is more than 80 is if μ is actually equal to the limit of 80 and experimental *variation* then records a set of unusually high values. Hence we choose to perform the calculation for the *statistical* null at this limit:

Null hypothesis:

H_0: Blood alcohol level equals 80.0 mg/100 ml, $\mu = \mu_0$.

The proposed *scientific* hypothesis, H_1, is that μ is more than 80, but there are two ways of approaching this *statistically*.

The **two-sided** (often called **two-tailed**) approach first tests whether the observed value, $\bar{x}$, is significantly *different* from 80, and then, if it is also greater than 80, we conclude that $\mu > 80$.

The **one-sided** (often called **one-tailed**) approach looks for a difference in a *predetermined* direction. In this case, we test directly whether the observed value, $\bar{x}$ is significantly *greater* than 80 and conclude that $\mu > 80$.

The one-sided approach should only be used if there is a specific reason for testing for an effect in a particular direction *before* any measurements are made. Some researchers advise against using the one-sided approach at all, because it gives the lower *p*-value and may result in a Type I error if used incorrectly. See the example in 3.1.3.

In general then, the proposed or alternative *statistical* hypothesis has two main options depending on the *symmetry* of the question:

Two sided:

H_1: Blood alcohol level does not equal 80.0 mg/100 ml, $\mu \neq \mu_0$.

One sided, depending on the direction of possible effect:

H_1: Blood alcohol level exceeds 80.0 mg/100 ml, $\mu > \mu_0$.

or

H_1: Blood alcohol level is less than 80.0 mg/100 ml, $\mu < \mu_0$.

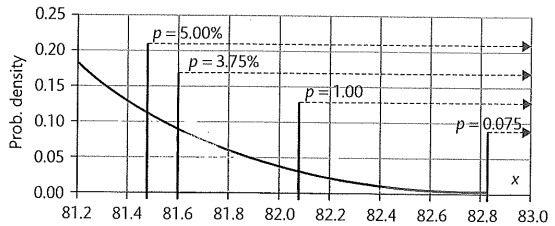


Fig 1.19 One-tailed *p*-values

With a standard deviation, $\sigma = 2.0$, a sample of five values has a standard error of the mean (Eqn 1.21), $SE = 2.0 / \sqrt{5} = 0.894$. We then calculate the probability distribution of measured mean values assuming that the null hypothesis is true, and Fig 1.19 gives the *p*-value probabilities that a randomly selected sample from the distribution (mean = 80, standard deviation = 0.894) could give a measured *mean* value, $\bar{x}$, equal to, or greater than specific values, *x*. The section of the upper tail of this distribution shows that:

Probability of recording a value of 81.47 or greater is 5.0% or 0.05

Probability of recording a value of 81.60 or greater is 3.75% or 0.0375

Probability of recording a value of 82.08 or greater is 1.0% or 0.01

Probability of recording a value of 82.82 or greater is 0.075% or 0.00075

Using the critical value for 5.0%, we could say that we would reject the null hypothesis for any $\bar{x}$ value greater than 81.47 'at a significance level of 0.05'.

Alternatively, for the measured value of $\bar{x} = 81.6$ in the case study example, we see that the p-value = 0.0375, and we reject the null hypothesis at a significance level of $\alpha = 0.05$ because $p < \alpha$.

Fig 1.19 gives the upper tail of the distribution appropriate for the one-tailed hypothesis, but for the two-tailed hypothesis, we must also include the probability area from the *lower* tail of the distribution. Provided that the distribution is *symmetrical*, we are able to calculate:

$$p\text{-value (two-tailed)} = 2 \times p\text{-value (one-tailed)} \quad \text{for symmetric uncertainties.} \tag{1.27}$$

However, this equation is not true for unsymmetrical distributions (3.8.2).

1.6.3 Errors in hypothesis tests

Any scientific investigation is subject to errors and uncertainties, which means that we might pick the *wrong* option when trying to decide whether or not to accept a hypothesis. This gives two different types of **error** that could be made, each with different consequences:

- **Type I Error**: We 'accept H_1' when in fact the null hypothesis is true. We will be claiming to have identified some effect that is not true.

  Type II Error: On the basis of experimental data, we choose to 'NOT accept H_1', when in fact the proposed hypothesis is true. We have failed to identify a real effect. This may be due to poor experimental design or because we did not take enough measurements.

The four possible outcomes from a hypothesis test are illustrated in the Table 1.4. The *columns* give the 'true' situation, and the *rows* give the decision.

Table 1.4 Hypothesis test errors

The decision	**True situation:**	
	H_1 is true:	H_1 is not true:
Accept H_1:	**Correct**	**Type I Error** We claim to have 'discovered 'an effect that is not true.
Do not accept H_1:	**Type II Error** The experimental data was insufficient to detect the effect.	**Correct**

The probability of recording a Type I Error for a specific data set is given by the calculated ***p*-value**, and in general, we define:

Significance Level, α, as the *largest probability of Type I Error* that is acceptable when choosing the proposed hypothesis, H_1.

The default value for general research is $\alpha = 0.05$ (equal to a 5% probability or '1 in 20'). However, other significance levels can be chosen, depending on the *consequences* of a Type I Error.

The power of an experiment is the ability to detect an effect if one exists, i.e. the ability to *avoid* making a Type II Error.

$$\text{Power} = 1 - \beta$$

where β is the probability of a Type II Error.

Although it is possible to calculate the p-value for a given set of data, it is not generally possible to calculate a value for the *power* of the test solely from the data. The power of a particular test can depend on many other factors, and is a main focus for good experiment design.

1.6.4 Bonferroni correction

It is quite common to find that our analyses involve *multiple* hypothesis tests, each giving a separate p-value. For example, we may be *independently* testing several different factors to see if they might have a significant effect on a measured response variable. The problem is that, although the probability of a Type I Error is 0.05 for *one* p-value, the probability of seeing at least one Type I Error increases rapidly when we scan more than one p-value. If we record p-values for ten tests for which the null hypothesis is true then the probability that all ten results *correctly* record $p > 0.05$ is equal to $0.95^{10} = 0.599$, which means that the probability that at least one p-value will give a Type I Error and incorrectly 'discover' a significant effect with $p < 0.05$ will be equal to $1.0 - 0.599 = 0.401$ or about 40%.

A common correction for multiple tests, proposed by Bonferroni, is to make the criteria for rejecting the null hypothesis more difficult by reducing the significance level, α, in proportion to the number of tests performed. For n tests, the null hypothesis will be rejected if:

$$p < \frac{\alpha}{n} \quad \text{(Bonferroni correction).}$$

This effectively reduces the number of Type I Errors, but it becomes rather conservative for large values of n giving Type II Errors, failing to detect some real effects.

Regression analysis

Introduction

This chapter starts with the familiar process of drawing a *best-fit* straight line through a set of experimental data points on an x–y graph, and then derives the statistics in a format that links into the more advanced analyses developed throughout the rest of the book. It concentrates on the practical application of linear regression in science including the derivation of uncertainties and its use for nonlinear data. Finally, the technique of a least squares fit is used as a link into the development of iteration as a method for 'solving' complex mathematical problems. The techniques link into the use of Minitab and SPSS in Part II of the book.

Section 2.1 develops the basic statistics for a least squares regression analysis, deriving the coefficients of the best-fit straight line.

Section 2.2 develops the practical use of linear regression for data analysis including the calculation of derived experimental confidence intervals.

Section 2.3 uses linearization techniques to convert nonlinear data to a straight line relationship for further analysis.

Section 2.4 introduces the process of 'iteration' as a method for finding the best-fit mathematical models using both least squares and maximum likelihood models.

The following case studies develop the core statistics in this chapter:

Case study: **Best-fit straight line / 1. Overview**

This case study develops the statistics and applications related to linear regression, and the use of the best-fit straight line in science.

The basic statistics of 'best-fit' linear regression are developed using Excel in:

2.1.1 / 2. Slope and intercept

2.1.2 / 3. ANOVA table

2.1.3 / 4. Correlation

The use of the straight line as an analytical tool for calculating unknown values, concentrations, etc.:

2.1.4 / 5. Uncertainty in regression

2.2.1 / 6. Confidence interval

2.2.2 / 7. Standard additions

Linear regression is used as an example for the iterative technique using the Excel add-in 'Solver', and demonstrates both the least squares and the maximum likelihood estimation methods of achieving a 'best-fit' result:

2.4.1 / 8. Least squares fit using Solver

2.4.2 / 9. Maximum likelihood using Solver

Case study: **Exponential decay / 1. Overview**

Radioactive decay is used as an example for regression calculations of *exponential* growth and decay.

The technique for the linearization of an exponential curve is developed in:

2.2.4 / 2. Weighted linearization. Uses 'weighting' to reflect the different uncertainties and influences of different data values.

2.3.4 / 3. Linearizing the exponential. Develops the basic technique for linearization and interpreting the results.

Iterative methods for obtaining best-fit lines for nonlinear data are developed in:

2.4.3 / 4. Nonlinear regression using Solver. Uses the iterative analysis of the Excel add-in 'Solver' to solve the problem using different statistical assumptions and to compare their results.

3.4.7 / 5. Generalized linear model. Demonstrates the use of linearization to include the underlying Poisson distribution.

7.2.3 / 6. Nonlinear regression using Minitab and SPSS. Demonstrates the iterative technique in statistical software.

2.1 Regression statistics

Linear regression is the statistical process of fitting a *best-fit* straight line through a set of x–y data points. It is a very common analytical procedure in all areas of science.

In this section, we look specifically at the *statistics* of linear regression. We make the assumption that the experimental *uncertainty* in the measurement process is estimated solely from the data being analysed, which, for small sets of data, significantly increases the overall uncertainty in the final result. In 2.2.3, we introduce the wider *experimental context* for an analysis in which an estimate of experimental uncertainty is available from previous experience or from a knowledge of the measurement process itself.

2.1.1 **Slope and intercept**

The equation of a straight line is often written as

$$y = mx + c \quad \text{or} \quad y = b_0 + bx \tag{2.1}$$

which defines the line using the two parameters: the slope, m or b, and the intercept, c or b_0. The variable, x, is the *independent* variable and is considered to *predict* the value of y, the *dependent* variable. The intercept, c or b_0, is the point that the line crosses the y-axis when $x = 0$.

The form of the equation using the 'b' coefficients is useful for multiple regression (9.1.6) when y depends on several 'x' values, and we can extend the number of 'b' coefficients:

$$y = b_0 + b_A x_A + b_B x_B + \cdots \tag{2.2}$$

However, for simple regression we will normally use the more familiar m and c constants.

Statistics of linear regression: Excel analysis for Fig 2.2. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: **Best-fit straight line / 2. Slope and intercept**

–continued from 2.Introduction, leading to 2.1.2, 2.1.4, and 2.4.1

Fig 2.1(a) presents the x–y data from Fig 2.2 in a scatterplot, together with a best-fit straight line, known as a *trendline* in Excel. The data in the Excel worksheet in Fig 2.2 has five measurements recorded in columns B (*x*-data) and C (*y*-data), with each data pair identified by the label, *i*, in column A.

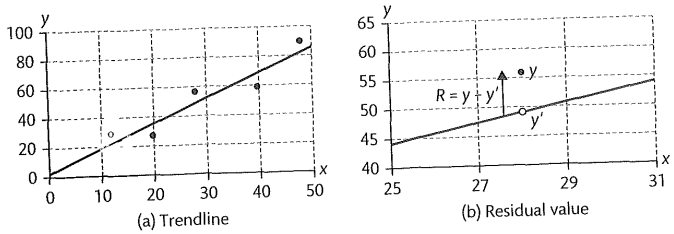


Fig 2.1 Best-fit straight line

	A	B	C	D	E	F	G
1	*i*	*x*	*y*	*y′*	*R*	R^2	
2	1	12	28	22.65	-5.35	28.58	
3	2	20	27	35.90	8.90	79.25	
4	3	28	56	49.15	-6.85	46.92	
5	4	40	59	69.02	10.02	100.45	
6	5	48	89	82.27	-6.73	45.28	
7		Pairs, *n* =	5		ΣR^2 =	300.48	
8		Slope, *m* =	1.656				
9		Intercept, *c* =	2.782				
10		> ANOVA (Analysis of Variance):					
11			*df*	*SS*	*MS*	*F*	*p*
12		Regression	1	2334.32	2334.32	23.31	0.017
13		Residual	3	300.48	100.16		
14		Total	4	2634.80			
15		> Correlation:					
16		Coefficient of determination, r^2 =		0.8860		*t* =	4.828
17		Correlation coefficient, *r* =		0.9413		*p* =	0.017
18		> Uncertainty:					
19		Standard error of regression, SE_{YX} =		10.008			
20		Standard error of slope, SE_{SLOPE} =		0.343			
21		Confidence deviation of slope, Cd_{SLOPE} =		1.092			
22							
23		Standard error of regression, SE_{YX} =		10.008			

Fig 2.2 Statistics of linear regression

The slope and intercept of the best-fit straight line can be calculated directly from the *x*, *y* values, and in Fig 2.2 it is convenient to use the specific functions of SLOPE() and INTERCEPT() in Excel to perform these calculations in cells C8 and C9 respectively:

Slope, *m*: [C8] = SLOPE(C2:C6,B2:B6) = 1.656
Intercept, *c*: [C9] = INTERCEPT(C2:C6,B2:B6) = 2.782

where C2:C6 describes the range of (*y*-) values between C2 and C6 and B2:B6 describes the range of (*x*-) values between B2 and B6.

In column D, we can calculate the *y*-values, *y′*, *on the best-fit line* for each of the *x*-values, using the equation for the *i*th data point:

$$y'_i = mx_i + c_i \tag{2.3}$$

where the '*i*' subscript can take values from one to five referring to the data identifier in column A.

For example, the calculation of the first data point, y'_1:

[D2] = C$8*B2+C$9 = 22.65

The $ sign in front of the rows in C$8 and C$9 *locks* the row values for the slope and intercept when we copy the formula down for the other data points.

The best-fit straight line in Fig 2.1(a) passes through the *y′* values and can be drawn on the graph as a separate line, or directly in Excel using the *trendline* option.

The extent to which the data does not exactly fit the straight line is shown by the *residual* distances between the points and the line in Fig 2.1(b). The standard process of linear regression assumes that the **only uncertainty exists in the *y*-values**, with the *x*-values being known exactly. Hence the uncertainty for the *i*th data point is given by the *vertical* difference, the *residual*, R_i, between the measured *y*-value and the value, y'_i, on the best-fit line.

$$R_i = y_i - y'_i = y_i - (mx_i + c_i) \tag{2.4}$$

In cells E2 to E6 we calculate the residual for each point using Eqn 2.4, e.g.

[E2] = D2 – C2 = –5.35

In cells F2 to F6 we square each of the residuals, e.g.

[F2] = E2^2 = 28.58

Finally, in cell F7 we calculate the sum of squares of all the residuals:

$$SS_{RESID} = \sum R_i^2 \tag{2.5}$$

by using:

SS_{RESID}: [F7] = SUM(F2:F6) = 300.48

The sum of squares of residuals is a measure of the error in the best-fit straight line and is sometimes referred to as SS_{RANDOM} or SS_{ERROR}. See also the 'analysis of variance' (3.2.2).

The best-fit straight line is defined as the line that will give the smallest possible value for SS_{RESID} and, for this reason, the process of deriving the best-fit line is often called the 'method of least squares, LS'.

The calculations of SLOPE() and INTERCEPT() in cells C8 and C9 provide the values for the best-fit straight line which has the minimum value of SS_{RESID}.

In 2.4.1 we use this case study to show that it is also possible to use a process of iteration, using the Excel add-in 'Solver', to adjust values of m and c through an *iterative* process until a *minimum* value is reached for SS_{RESID}, producing the same values for m and c as calculated in Fig 2.2. It is also useful to see that the alternative method of 'maximum likelihood estimation' (MLE) (2.4.2) will also derive the same values for m and c.

The calculation of the slope and intercept for the best-fit line has produced a mathematical *regression model* that represents our experimental data. We will see (in Section 3.4) the use of more complex mathematical models to represent other forms of experimental data.

Minitab and SPSS produce results for linear regression in similar formats (e.g. Fig 2.3) to that in Fig 2.2:

Minitab > Stat > Regression > Regression > Fit Regression Model ... Response: y
Continuous predictors: x
→ Output: Fig 2.3

SPSS > Analyze > Regression > Linear...
Dependent: y
Independent(s): x
→ Output: Gives the same values as in Fig 2.3

In Fig 2.3, the slope, $m = 1.656$, is given by the *Coef* of 'x' and the intercept, $c = 2.78$, by the value of 'constant'. The confidence deviations, Cd, in the slope, m, and intercept, c, can be calculated as the respective values for *SE Coef* multiplied by the appropriates t-value for the degrees of freedom, $n - 2$ (see Eqn 2.16).

More advanced use of Minitab and SPSS for regression can be found in 3.4.3 and 9.1.6.

```
The regression equation is
y = 2.8 + 1.66 x

Predictor     Coef  SE Coef     T      P
Constant      2.78    11.10  0.25  0.818
x           1.6560   0.3430  4.83  0.017

S = 10.0080   R-Sq = 88.6%   R-Sq(adj) = 84.8%

Analysis of Variance

Source           DF      SS      MS      F      P
Regression        1  2334.3  2334.3  23.31  0.017
Residual Error    3   300.5   100.2
Total             4  2634.8
```

Fig 2.3 Extract from linear regression output using Minitab

2.1.2 ANOVA table

We can now extend the basic regression calculations to derive other relevant statistics that will relate to questions and topics elsewhere. These statistics are based on how much the observed data deviates from the regression model. We start with a set of statistics which is often referred to as an analysis of variance (or ANOVA) table (3.2.3). In this, the variability in the data is described by the sums of squares terms that we first met in Section 1.5.

Using Eqn 1.16, the total variability in the y-values can be described by the total *sum of the squares* of the deviations from the mean value, $\bar{y}$:

$$SS_{TOT} = \sum(y_i - \bar{y})^2 \tag{2.6}$$

We have also seen (Eqn 1.19) that the sample variance in the y-values is given by

$$s_y^2 = \frac{\sum(y_i - \bar{y})^2}{df} \tag{2.7}$$

where the degrees of freedom, $df = n - 1$.

Case study: Best-fit straight line / 3. ANOVA table

–continued from 2.1.1, leading to 2.1.3

Referring to the data in Fig 2.2, we develop the ANOVA results table in B10:G14.

Combining Eqns 2.6 and 2.7 we can derive

$$SS_{TOT} = s_y^2 \times df \tag{2.8}$$

which is calculated in D14 using:

SS_{TOT}: [D14] = VAR(C2:C6) * (C7 – 1) = 2634.80

The total variability, SS_{TOT}, in the data equals the variability described by the regression model, SS_{REG}, plus the remaining variability described by the residuals, SS_{RESID}:

$$SS_{TOT} = SS_{REG} + SS_{RESID} \tag{2.9}$$

We copy the value of SS_{RESID} from F7 to D13, and as we already know SS_{TOT} and SS_{RESID} we can calculate SS_{REG} in cell D12 by simple subtraction,

SS_{REG}: [D12] = D14 – D13 = 2334.32

Now that we have the three sums of squares we can calculate the *mean square values*, MS_{REG}, and MS_{RESID} in E12 and E13 using the general Eqn 1.18:

$$MS = \frac{SS}{df}$$

The degrees of freedom, df, for the y-value calculations can be understood as follows:

- SS_{TOT} has $df = n - 1$ because one degree of freedom has been used in the prior calculation of $\bar{y}$.

- SS_{RESID} has df = n – 2 because two degrees of freedom have been used in the prior calculation of slope and intercept.
- SS_{REG} then has the remaining degree of freedom, $df = 1$.

We can now use the *F*-statistic (3.2.1) to calculate the ratio of the variance explained by the regression model divided by the variance due to residual experimental variations.

$$F = \frac{MS_{REG}}{MS_{RESID}} \tag{2.10}$$

This value is calculated in F12:

F_{STAT}: [F12] = E12 / E13 = 23.31

A large value for the *F*-statistic implies that the variation described by the straight line is more than just random variations, and that the best-fit straight line has a slope that is significantly different from zero. It is possible to calculate the *p*-value in cell G12 for the *F*-test using the Excel function F.DIST.RT() with the relevant degrees of freedom C12 and C13 for the numerator and denominator

p-value: [G12] = F.DIST.RT(F12, C12, C13) = 0.017

The *p*-value is the probability that you would be wrong if you stated that the slope of the best-fit line was not zero.

2.1.3 Correlation

In this section we derive the statistics of correlation, which is a measure of the extent to which the variation in the *y*-values can be *predicted* by the variation in the *x*-values (and vice versa).

The coefficient of determination, r^2, is a measure of how much the variation in the *y*-values is explained by the regression model, and is given by the fraction, from 0.0 to 1.0, of the sum of squares variation explained by the regression:

$$r^2 = \frac{SS_{REG}}{SS_{TOT}}$$

which can be rearranged to give:

$$r^2 = \frac{SS_{REG}}{SS_{TOT}} \Rightarrow \frac{SS_{TOT} - SS_{RESID}}{SS_{TOT}} \Rightarrow 1 - \frac{SS_{RESID}}{SS_{TOT}} \tag{2.11}$$

The square root of the coefficient of determination is, for the simple straight line, called the Pearson's product moment correlation coefficient, *r*:

$$r = \sqrt{1 - \frac{SS_{RESID}}{SS_{TOT}}} \tag{2.12}$$

We also calculate the correlation coefficient in a different way in 4.1.1, which provides a different approach to the same statistics.

Case study: Best-fit straight line / 4. Correlation

–continued from 2.1.1

Referring to the data in Fig 2.2, we develop the correlation results in B15:G17.

The coefficient of determination is calculated in E16 using Eqn 2.11:

Coefficient of determination, r^2: [E16] = 1 – D13 / D14 = 0.8860

Values of r^2 are often given when a statistical analysis fits a mathematical model to a set of data points, and are used as a measure of 'goodness of fit' of the model for the given data (4.4.1). For example, good calibration lines might expect to have values for r^2 of at least 0.999. It is also important to note the use of an 'adjusted r^2' which takes into account the degrees of freedom of the data in the calculation (2.1.5).

The magnitude of the correlation coefficient is calculated in E17 using Eqn 2.12:

Correlation coefficient, *r*: [E17] = SQRT(1 – D13 / D14) = 0.9413

The sign (+ or –) of the correlation coefficient is the same as the *sign* of the slope, but the magnitude is **not related** to the *magnitude* of the slope.

It is also possible to get this result directly in Excel by simply using the function CORREL() or PEARSON().

We can use the correlation coefficient, *r*, as the relevant statistic in a hypothesis test for *linear correlation*, i.e. testing that the best-fit straight line is not zero. We can either compare the value with tables of critical values or calculate the *p*-value. In calculating the *p*-value for the significance test for correlation we first calculate a value for an effective *t*-statistic for the correlation with $n - 2$ degrees of freedom:

$$t_S = r \times \sqrt{\frac{n-2}{1-r^2}} \tag{2.13}$$

which we have calculated in G16:

t-statistic, t_S: [G16] = E17 * SQRT((C7 – 2) / (1 – E17^2)) = 4.828

The equivalent *p*-value is then calculated in G17 using the T.DIST.2T() function with $n - 2$ degrees of freedom:

p-value: [G17] = T.DIST.2T(G16, C7 – 2) = 0.017

We can see that the two *p*-value calculations in G12 and G17 give exactly the same value, which was to be expected as they both relate to a test for a non-zero best-fit slope.

As with the ANOVA calculations, the *p*-value is only of relevance when *testing* whether or not a non-zero linear relation might exist for the given data points. However, when we are using linear regression to produce a *calibration* line, we would be primarily concerned with *how close* the coefficient of determination, r^2, is to 1.00 (4.4.1).

Minitab and SPSS produce the same results in Fig 2.4 as above for the correlation coefficient and *p*-value:

Minitab > Stat > Basic Statistics > Correlation...
Variables: *yx*
☑ **Display p-values**
→ Output: Gives the same values as in Fig 2.4

SPSS > Analyze > Correlate > Bivariate...
Variables: *yx*
☑ **Pearson**
→ Output: Fig 2.4

Correlations

		x	y
x	Pearson Correlation	1	.941*
	Sig. (2-tailed)		.017
	N	5	5
y	Pearson Correlation	.941*	1
	Sig. (2-tailed)	.017	
	N	5	5

*. Correlation is significant at the 0.05 level (2-tailed).

Fig 2.4 Pearson's correlation in SPSS

2.1.4 Regression uncertainties

When using statistics for deriving scientific results, it is also essential to derive the uncertainties in those results. The key statistic for the uncertainty in regression is given by the *standard error of regression*:

$$SE_{REG} = \sqrt{\frac{\sum R_i^2}{n-2}} = \sqrt{\frac{SS_{RESID}}{n-2}} \qquad (2.14)$$

This value is effectively a best estimate for the standard deviation of the *individual* data values around their 'true' values in the linear model.

Case study: Best-fit straight line / 5. Uncertainty in regression

–continued from 2.1.1, leading to 2.2.1

Referring to the data in Fig 2.2, we develop the uncertainty results in B18:E21.

The standard error of regression can be calculated directly in E19 from the x–y data in columns B and C using the Excel function, STEYX():

Standard error of regression: [E19] = STEYX(C2:C6, B2:B6) = 10.008.

The standard error of the *slope* can then be calculated as:

$$SE_{SLOPE} = \frac{SE_{REG}}{\sqrt{\sum (x_i - \bar{x})^2}} \Rightarrow \frac{SE_{REG}}{\sqrt{s_x^2 \times (n-1)}} \qquad (2.15)$$

which is calculated in E20:

Standard error of slope: [E20] = E19 / (SQRT(VAR(B2:B6) * (C7 – 1))) = 0.343

The 95% confidence deviation (1.5.2) in slope can then be calculated by multiplying by the relevant t-value for 95% with degrees of freedom, $df = n - 2$:

$$Cd_{SLOPE,95\%} = t_{95\%,n-2} \times SE_{SLOPE} \qquad (2.16)$$

which is calculated in E21 using:

95% Confidence deviation in slope, *Cd*: [E21] = T.INV.2T(0.05, C7 – 2) * E20 = 1.092

This calculation gives the **95% confidence interval** for the slope (to 1 dp) as:

$m = 1.7 \pm 1.1$

This possible range for the slope does not include $m = 0$, showing that the slope is significantly different from zero, which is consistent with the correlation and ANOVA results of $p = 0.017$.

2.1.5 Quality of fit

An important use of linear regression is for the calibration of experimental measurements using *known* samples to enable the analysis of *unknown* samples.

Case study: Spectrophotometer calibration / 3. Linearity range

–continued from 7.1.5, leading to 2.2.1

The use of a calibration line in spectrophotometry is also introduced in 7.1.1 as *related* data between absorbance and concentration, and in 7.1.5 we use the results from Minitab and SPSS to estimate the confidence in interval of an unknown concentration. We consider here the *linearity* of calibration data and, in 2.2.1, the calculation of the *confidence interval* of an unknown value using Excel.

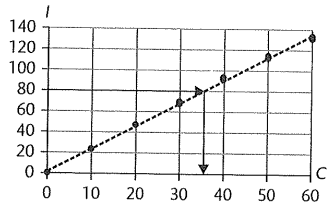
The data in Fig 2.5 gives the calibration data for an ICP-OES (inductively coupled plasma optical emission spectrometer), with the values of the emission intensity, I, for solutions of standard concentrations (in arbitrary units). In the interest of space, all intensity values in this case study have been divided by 1,000 and rounded to one decimal place.

The analytical region of interest for experimental measurements is for concentrations between $C = 0$ and $C = 40$. We also know from *previous experience* that measurements of I have a standard deviation uncertainty of about $\sigma = 0.7$.

Three replicate measurements of an *unknown* solution give an average value, $I_S = 79.2$, and we wish to calculate the best-estimate value for the concentration, C_S, of this unknown solution. However, we first assess the quality of the calibration line here by checking its linearity using residuals, and then complete the analysis in 2.2.1.

C	I_1	I_2	I_3	s
0	0	0	0	0.00
10	22.9	22.3	23.2	0.46
20	45.3	46.7	46.4	0.74
30	69	69.5	67.8	0.87
40	91.2	90.9	92.5	0.85
50	114.9	111.2	112.8	1.86
60	131.5	129.9	132.8	1.45

(a) Calibration data



(b) Calibration graph

Fig 2.5 Calibration data and graph for spectrophotometric calibration

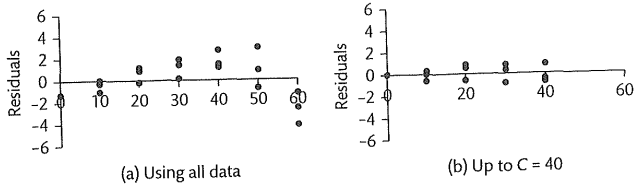
We shall see in 2.2.1 that the value of $I_S = 79.2$ gives an equivalent value of $C_S = 34.6$, as shown by the arrows in the calibration graph in Fig 2.5(b). However, we are particularly concerned here with the 'quality of fit' of the calibration line.

Visually the data points in Fig 2.5(b) appear to sit on a straight line through the origin. However, we check linearity by plotting the *residuals* against C, using

Excel > Data Analysis > Regression

☑-Residual plots

to give Fig 2.6(a). Residual plots are also available from the regression options in Minitab and SPSS.



(a) Using all data (b) Up to C = 40

Fig 2.6 Residuals around best-fit straight lines

In Fig 2.6(a) we see that there is a curvature of the line, with the upper points curving downwards. This is a common behaviour in an instrumental response which often shows decreasing sensitivity with higher concentration values.

As the principal range of interest is between 0 and 40, we investigate whether we should fit the calibration line just through these points, and we compare the statistics of the two options in Table 2.1.

Table 2.1 compares the statistics of fitting a straight line, (a) through all data points and (b) just through points up to $C = 40$. The important value is the coefficient of determination, r^2, with $r^2 = 0.9997$ for points just up to $C = 40$ which shows a better fit than for the whole range, $r^2 = 0.9986$. However, care should be taken when limiting the number of points for a best-fit line, as the fit always becomes 'better' as 'unhelpful' points are rejected. The *adjusted* r^2 value takes the change in the degrees of freedom into account, but still shows improvement for (b). Note that the p-values are both far less than 0.05 and

Table 2.1 Comparison of best-fit statistics for quality of fit

	Fit all data points:	Fit points up to C = 40:
Residual plots -	See Fig 2.6(a)	See Fig 2.6(b)
Coefficient of determination, r^2 =	0.9986	0.9997
Adjusted, r^2 =	0.9985	0.9997
Standard error of regression, SE_{REG} =	1.77	0.61
p-value for regression =	1.93×10^{-28}	3.20×10^{-24}

are of little practical value here as we are not testing whether or not there is a non-zero best-fit line.

The calculated standard error of regression, SE_{REG}, (2.1.4) is the best estimate of the experimental uncertainty in each data point, and the value of $SE_{REG} = 0.61$ for the lower 'straight line' section is consistent with our previous knowledge of the experimental standard deviation, $\sigma = 0.7$. The higher value of 1.77 for the whole data set includes the additional deviations due to the upper curvature. The analysis here has been simplified by assuming equal uncertainties for all values of I, but this does not affect the identification of the curvature in the data.

The statistical analysis of regression and the residuals indicates that it is appropriate to use the best-fit straight line just up to $C = 40$. This case study is continued in 2.2.1, where we calculate the confidence interval for the concentration of an unknown solution, for which three measurements give an average intensity value of $I_S = 79.2$.

2.2 Experimental uncertainties

Introduction

The standard error of regression, SE_{REG}, gives the best estimate of the *experimental uncertainty* around a best-fit straight line when based on the *regression data itself*, and in 2.1.4 we derived the confidence interval for the *slope* of the line. In 2.2.1 we derive the confidence interval in using a straight line for calibration and in 2.2.2 consider exact intercept values when *interpolating* or *extrapolating* the best-fit line.

In 2.2.3 we consider the experimental situation where the actual experimental uncertainty is already *known* from previous measurement, and finally in 2.2.4, we review the regression calculation in the situation where the experimental uncertainty is not the same for all points on the regression line.

The use of Minitab and SPSS for these calculations is introduced in 7.1.5.

2.2.1 Calibration uncertainty

We often use linear regression in science to provide a calibration line from which we calculate specific values using the simple straight line equation. We now introduce methods for calculating the uncertainty in those interpolated, or extrapolated, values.

Calibration uncertainty 1: Excel analysis for Fig 2.7. See also 7.1.5. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: Best-fit straight line / 6. Confidence interval

--continued from 2.1.4, leading to 2.2.2

We consider the simple example of the calibration of a spectrophotometric measurement. In the Excel worksheet given in Fig 2.7, the absorbances, A (y-values), are entered in B4:B7 for four standard samples with known concentrations, C (x-values) in A4:A7. These values are also plotted, together with a 'best-fit' calibration line, in Fig 2.8 (a) and (b).

The aim of the analysis is to calculate the concentration, x_S, of an unknown solution for which three replicate measurements have recorded an average absorbance of $y_S = 0.63$.

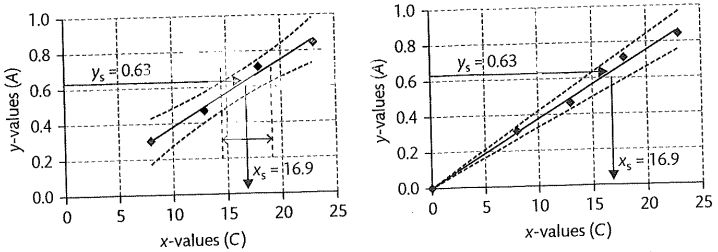
	A	B	C	D	E	F	G	H	I	J
1	**95% *CI* for the *x* value of an intercept on a free-fit (*m* and *c*) calibration line**									
2	**Data:**			**Calculations:**		**Uncertainties:**			**y_S with *k* replicates**	**y_S exact**
3	***x***	***y***		***n* =**	4					
4	8.00	0.31		**Slope, *m* =**	0.037	**Uncertainty, u_x =**			0.746	0.496
5	13.00	0.46		**Intercept, *c* =**	0.010	**u_x (central) =**			0.737	0.482
6	18.00	0.71				*** $t_{n-2,95\%}$ =**			4.303	4.303
7	23.00	0.84		**y_S =**	0.63	**Cd_x =**			3.210	2.136
8				***k* =**	3	**Confidence interval (95%):**				
9				**x_S =**	16.859	**CI_x =**	16.86	±	3.21	2.14
10				*** SE_{REG} =**	0.035					
11				**Mean of *y* =**	0.6	**{NB: For 99% confidence, change 0.05**				
12				**Variance, s_x^2 =**	41.67	**to 0.01 in the *t*-value in I6 and/or J6}**				

Fig 2.7 Confidence intervals for intercept on a 'free fit' calibration line (calculations for column J appear in 2.2.2)

We make the assumption (Beer–Lambert law) that, over our range of measurements, the relationship between y (A) and x (C) is linear.

A linear regression analysis now gives us two alternatives in calculating the slope, m, and intercept, c, of the calibration line:

1. Without any knowledge of the behaviour of the line beyond the range of measurement values we must allow a free fit for *both* slope and intercept, Fig 2.8(a), or,
2. Based on the science, we may be able to assume that the calibration line must pass through the origin of the graph, which then *only* allows uncertainty in the best-fit slope, Fig 2.8(b).



(a) Free-fit calibration

(b) Forced through origin

Fig 2.8 Fitting a calibration line to four absorbance values

In Fig 2.8 (a) and (b), the dashed lines in the diagrams show the ranges within which it is possible, given the variability of the data points, to be 95% confident of drawing the position of the 'best-fit' calibration line.

In a typical scenario, we might then make k replicate readings of the absorbance of an *unknown* solution giving an average or *mean* value of y_S. We can then derive a best-estimate value for the unknown concentration, x_S, by graphical construction as represented by the arrows in the graphs. The uncertainty, u_x, in x_S is partly generated by the range of possible x-values within the dashed lines at the y-value = y_S (shown in Fig 2.6(a)), *together with* possible uncertainty in the measured value of y_S itself.

We now develop the statistics, plus the equations in Excel, that can be used to calculate the uncertainty, u_x, in the best estimate value for x_S. In Fig 2.7 we calculate the slope, m, in cell E4 using the function SLOPE() for a free fit straight line or LINEST() if we wish the line to pass through the origin of the graph. The intercept, c, is calculated in E5 by the function INTERCEPT().

We enter the mean value, y_S, in E7, based on k measurements entered in E8, and then calculate the unknown concentration, x_S, by using the rearranged straight line equation:

$$x_S = \frac{y_S - c}{m} \tag{2.17}$$

which is calculated in E9 as: [E9] = (E7 – E5) / E4 = 16.859.

Having calculated the best-estimate of the unknown, x_S, value, the next step is to calculate the estimate for the experimental uncertainty, u_x, in that value for a *free-fit straight line*.

$$u_x = \frac{SE_{yx}}{m} \times \sqrt{\left\{\frac{1}{k} + \frac{1}{n} + \frac{(y_S - \bar{y})^2}{m^2 \times (n-1) \times s_x^2}\right\}} \tag{2.18}$$

The standard uncertainty, u_x, derived in the Eqns 2.18 and 2.19, arises from two sources:

Vertical uncertainty in the true value of y_S, based on k replicate measurements.

Horizontal uncertainty in the interception of the calibration line.

In the derivation of the formula for u_x, we start with the standard error of regression, SE_{REG}, calculated in E10 with the function STEYX(), which gives the measure of uncertainty in the *vertical* y-direction.

The uncertainty in the *horizontal* x-direction is then given approximately by SE_{REG} / m, where m is the slope of the line. For example, for a slope, $m = 1$ at an angle of 45°, the uncertainties will be the same in both directions, but, if the slope, m, becomes *less*, the horizontal uncertainty will become *larger*.

The next factors in the equation relate to the number of replicate measurements, k, of the unknown sample and the number, n, of data points in the calibration line, which both reduce the uncertainty in proportion to their square roots (Eqn 1.21). It is assumed that the uncertainty in each y_S-value is the same as in each calibration value.

The last term in the square root of Eqn 2.18 is the factor that is responsible for the 'opening-up' of the uncertainty range at both ends of the calibration line in Fig 2.8(a).

This term becomes *zero* (and disappears) when y_S equals the mean y-value ($\bar{y}$) of the points used to generate the best-fit line, i.e. when the measured value falls in the 'centre' of the calibration values. The separate values within this term are calculated in the worksheet as follows:

$\bar{y}$ is the mean of all the y values: [E11] = AVERAGE(B4:B7) = 0.6

s_x^2 is the sample variance of x values: [E12] = VAR.S(A4:A7) = 41.67

The free-fit calculation for u_x in cell I4 using Eqn 2.18 is then given by:

[I4] = (E10 / E4) * SQRT(1/E8 + 1/E3 + (E7 − E11)^2 / (E4^2 * (E3 − 1) * E12)) = 0.746.

In a well-designed experiment, we would want the measured value, y_S, to fall close to the centre of the calibration line, i.e. close to the mean, $\bar{y}$, of the calibration y-values. In this case, the term $(y_S - \bar{y})$ is close to zero and can be ignored, giving an approximate equation, calculated in cell I5, for the *central* region of the free fit calibration line:

$$u_x \approx \frac{SE_{REG}}{m} \times \sqrt{\left\{\frac{1}{k} + \frac{1}{n}\right\}} \qquad (2.19)$$

which is calculated in I5 as:

[I5] = (E10 / E4) * SQRT(1/E8 + 1/E3) = 0.737

The scientific situation sometimes requires the best-fit straight line to pass through the origin of the graph, as in Fig 2.8(b). In this case, Eqn 2.18 for the uncertainty, u_x, would become, for a *line passing through the origin*:

$$u_x = \frac{SE_{yx}}{m} \times \sqrt{\left\{\frac{1}{k} + \frac{1}{n} + \frac{(y_S)^2}{m^2 \times (n-1) \times s_x^2}\right\}} \qquad (2.20)$$

The confidence deviation, Cd_x, is calculated in I7 using:

$$Cd_x = t_{n-2}\ u_x \qquad (2.21)$$

where the t-value, for degrees of freedom, $df = n - 2$, and with the appropriate level of confidence (typically 95% or 0.05 significance), is calculated in I6 using the equation:

[I6] = T.INV.2T(0.05, E3 − 2) = 4.303

The final result for x_S is the given as the confidence interval, CI_x

$$CI_x = x_S \pm Cd_x \qquad (2.22)$$

In the example given in Fig 2.7, the analysis shows that the 95% confidence interval using the *free-fit* uncertainty for the best estimate concentration is

$CI = 16.86 \pm 3.21$

which gives the range from 13.7 to 20.1, rounded to 1 dp.

Case study: **Spectrophotometer calibration / 4. Calibration result**

– continued from 2.1.5

We now wish to calculate the best-estimate value for the concentration, C_S, of an unknown solution, based on the calibration data for the spectrophotometer in Fig 2.5(a), where the known experimental uncertainty is given by $\sigma = 0.7$. Three replicate measurements of an unknown solution give an average value, $I_S = 79.2$.

We enter the data from Fig 2.5(a) into an Excel worksheet calculation similar to that in Fig 2.7, and the equation of the calibration line is calculated to be:

$I = 2.29 \times C + 0.04$

The confidence interval, *based solely on the calibration line data*, is then calculated to be:

$CI = 34.56 \pm 0.39$

However, we are also told that the experimental standard deviation is known to be $\sigma = 0.7$. If we replace the standard error of regression, SE_{REG}, in E10 with this value and substitute 1.96 for the t-value we would get a more reliable confidence interval which *includes the known experimental uncertainty* (2.2.3):

$CI = 34.56 \pm 0.41$

2.2.2 **Exact *x*/*y* intercepts**

The calculation in 2.2.1, and specifically Eqn 2.18, assumes that there is experimental uncertainty in the y-value, y_s, which is calculated as the mean of k measurements.

We now consider the situation where the y-value is known *exactly*, which removes the '$1/k$' uncertainty in the vertical direction, and we can remove the $1/k$ term from Eqn 2.18 to give:

$$u_x = \frac{SE_{yx}}{m} \times \sqrt{\left\{\frac{1}{n} + \frac{(y_S - \bar{y})^2}{m^2 \times (n-1) \times s_x^2}\right\}} \qquad (2.23)$$

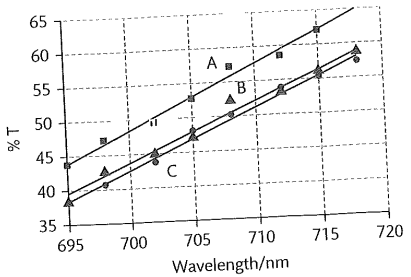
The calculations for this are performed in column J of the worksheet in Fig 2.7.

Case study: **Ink analysis / 3. Exact y-intercept**

–continued from 5.2.3, leading to 3.6.2 and 3.3.3

In this case study, continued from 5.2.3 and Fig 5.6, we now want to calculate the confidence intervals for the wavelengths at which each of the transmission spectra for three inks in Fig 2.9(a) cross the horizontal 50%T line.

Exact x/y intercepts: Excel analysis for Figs 2.9(b) and 2.10(a). Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/



(a) Data plot

	A	B	C	D
1		%T (A)	%T (B)	%T (C)
2	Slope, m =	0.91	0.86	0.85
3	Intercept, c =	-590.41	-555.97	-554.96
4	$\%T$ =	50.00	50.00	50.00
5	λ =	701.59	707.27	708.32
6	SE =	0.82	1.03	0.71
7	n =	8	8	8
8	u_λ =	0.38	0.43	0.30
9	t =	2.45	2.45	2.45
10	Cd =	0.93	1.04	0.74
11	95% CI Min	700.66	706.22	707.58
12	95% CI Max	702.53	708.31	709.06

(b) Excel calculations

Fig 2.9 Confidence intervals for the long-wavelength transmission cut-off of black inks

Fig 2.9(b) shows the calculation steps for each of the three lines, calculating the best-estimate intercept wavelengths, λ, in row 5. The 50%T line represents an *exact* y-intercept, and we use Eqn 2.23 to calculate the uncertainty, u_λ, in row 8. The calculations of the confidence deviations in row 10 give the following results for the long wavelength cut-off, λ_{50}, for the three inks:

	A	B	C
95% confidence intervals:	701.6 ± 0.9	707.3 ± 1.0	708.3 ± 0.7

Line *A* is clearly different from *B* and *C*, but we would need to perform a two sample *t*-test (3.1.3) to decide whether the apparent difference between *B* and *C* is significant.

Further analyses:

In 3.3.3 we use an ANCOVA (ANalysis of COVAriance) to test for a *vertical* difference between *A*, *B*, and *C*.

In 3.6.2 we use repeated measures to test for *vertical* differences between *A*, *B*, and *C*.

Case study: **Best-fit straight line / 7. Standard additions**

–continued from 2.2.1

Using anodic stripping voltammetry, a solution containing an unknown quantity, q_S, of lead gives an analytical response, I_P, in Fig 2.10(a) which we plot on the *y*-axis in Fig 2.10(b). Additional known amounts, q, of lead are added and plotted on the *x*-axis, with the increasing analytical response on the *y*-axis.

We now extrapolate the best-fit straight line for the data back to its intercept with the horizontal q-axis, when $I_P = 0$. If we assume a linear response between I_P and q, then the intercept will occur at a value, $q = -q_S$, allowing us to calculate the value for the lead, q_S, in the original solution.

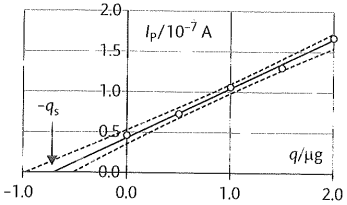
The method of standard additions is a particularly relevant example for two reasons:

It is a further example of using an exact value of *y*, but, more importantly

it demonstrates the increased uncertainty of extrapolating the calibration line beyond the range of calibration data.

	A	B
1	I_P	q
2	0.0	0.45
3	0.5	0.72
4	1.0	1.06
5	1.5	1.30
6	2.0	1.68

(a) Data values



(b) Trendline with 95% prediction limits

Fig 2.10 Method of standard additions

As the I_P-value for the intercept is known exactly (= 0), we use Eqn 2.23 where there is no $1/k$ term in the equation.

In this analysis we are *extrapolating* beyond the range of the calibration data, and the intercept, y_S-value, is a long way from the mean value, $\bar{y}$, of the calibration values. This gives a large value for the $(y_S - \bar{y})^2$ term in Eqn 2.23, with a considerably increased uncertainty, which can be seen in the spreading 95% prediction limits drawn on either side of the trendline.

The calculation gives a confidence interval for the intercept when $I = 0$ as -0.71 ± 0.22, which then directly gives the confidence interval for q_S:

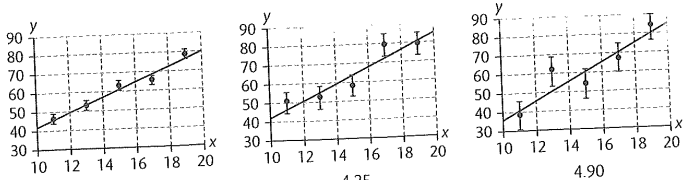
$q_S = 0.71 \pm 0.22$ µg.

The graph shows how an apparently reasonable set of calibration points can still result in a large uncertainty in the intercept with the axis. In the calculation we see that the confidence deviation when $I_P = 0$ is ±0.22 whereas in the *central* region of the calibration data the confidence deviation would be only ±0.08. This increased uncertainty in the final result is due to the extrapolation of the line beyond the data points, in which the second term in the square root now becomes the major factor. The most effective way of reducing this uncertainty is by increasing the variance, s_x^2, of the values along the *x*-axis, i.e. by making measurements over a larger range of *x*-values (provided that we are confident that the response remains linear).

2.2.3 **Known uncertainty**

In the regression analysis, developed in Section 2.1, the random uncertainty in experimental values is estimated *solely* from the sample data itself on the basis of how much the data deviates from the best-fit straight line, and it is recorded as the standard error or regression, SE_{REG}.

We now consider the situation where the experimental uncertainty for the measurement is *already known*, either from previous experience or from an estimation based on



	(a)	(b)	(c)
Slope, m =	3.80	4.25	4.90
Intercept, c =	4.00	-0.15	-12.7
SE_{REG} =	2.61	5.85	7.80
Cd (slope) =	1.31	2.94	3.92
CI (slope) =	3.8 ± 1.3	4.3 ± 2.9	4.9 ± 3.9

Fig 2.11 Randomly selected sample data

the practical steps in the experimental process. In statistical terms we can now assume that population standard deviation, σ, *is a known value.* We can compare this with the situation discussed in 1.5.4.

The problem of relying *solely on sample data* is illustrated in Fig 2.11 by randomly selecting three sets of five values from a population of simulated data where the true y-values are given by

$$y = 4.0 \times x$$

and where the known standard deviation uncertainty in the y-values is given by $\sigma = 5.0$. This relatively large uncertainty has been used to improve the visualization of the differences.

The three sets of results, (a), (b), and (c), in Fig 2.11 are representative, but not extreme, examples of randomly selected experimental data sets. The lengths of the error bars drawn on either side of the data points are equal to the standard error, SE_{REG}, calculated from the data itself and represent the experimental uncertainty estimated *solely* from the five data points.

We see that, if the points happen, *by chance,* to lie in a near straight line, then the resultant residual values are small and the calculation interprets this as low overall uncertainty, e.g. in graph (a), $SE_{REG} = 2.61$. However, where the random points are more spread out away from a straight line, then this is interpreted as a large uncertainty, e.g. in graph (c), $SE_{REG} = 7.80$.

Relying on the random spread of just five data points is clearly not an accurate method of estimating experimental uncertainty, particularly when the slope and intercept of the best-fit line adjusts to minimize these deviations.

If you know *beforehand* the true standard deviation, σ, of the experimental uncertainty then you can compare your value of σ with the calculated value, SE_{REG}. If SE_{REG} appears to be much smaller than a known uncertainty, σ, then you should check that the separate measurements are truly random, but if it is much larger, then you should check for additional variations in the data (e.g. nonlinearity in 2.1.5).

If you are convinced that your value of σ is a valid description of the experimental uncertainty, then you can replace SE_{REG} *with* σ in the regression calculations, and Eqn 2.15 for the standard error of the slope of the straight line now becomes:

$$SE_{SLOPE} = \frac{\sigma}{\sqrt{\sum (x_i - \bar{x})^2}} \Rightarrow \frac{\sigma}{\sqrt{s_x^2 \times (n-1)}} \qquad (2.24)$$

In the above example, the variance of the x-values (11, 13, 15, 17, 19) is $s_x^2 = 10.0$, which gives

$$SE_{SLOPE} = \frac{5.0}{\sqrt{10 \times 4}} = 0.7906$$

As we are now using the 'population' value for standard error rather than an estimated value, we can use the z-value (1.5.4) of 1.96 *instead of the t-value for (n – 2) degrees of freedom,* which further improves the precision of the result.

Confidence deviation of slope for a *known* standard deviation is:

$$Cd_{SLOPE,95\%} = 1.96 \times SE_{SLOPE}$$

which gives $Cd_{SLOPE,95\%} = 1.55$ for our example data.

The confidence intervals, CI, for the slopes estimated from the three replicate sets of data will now be:

A: 3.8 ± 1.6 B: 4.3 ± 1.6 C: 4.9 ± 1.6

The use of prior knowledge of experimental uncertainty provides a better overall *scientific* result.

2.2.4 Weighting uncertainties

In the regression analyses performed in previous units, we have assumed that all the data points have the same random uncertainty, i.e. they are all equally weighted in importance. However, it is not unusual to find that the uncertainty varies between data points.

It is possible to take account of these variations by 'weighting' the importance of each data value. If we can represent the uncertainty in a value by its standard deviation, u, (and variance, u^2) then the weighting factor, w, for each point is given by the proportionality relationship:

$$w \propto \frac{1}{u^2} \qquad (2.25)$$

Case study: Exponential decay / 2. Weighted linearization

–continued from 2.Introduction and 2.3.4, leading to 2.4.3, 3.4.7, and 7.2.3

Fig 2.12 (same data as Fig 2.16) gives radioactive counts, N_t, in column B for times, t, in column A. We analyse this data in a variety of ways throughout the book, and in this analysis we wish to take into account the varying uncertainties in the data when we linearize the relationship (see 2.3.4) by plotting the logarithm, ln (N_t), against t, to obtain a straight line.

Weighting data: Excel analysis for Fig 2.12. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

	A	B	C	D	E	F
1	*t*	*N*	ln(*N*)	√*N*	*u*	$w = 1/u^2$
2	0	97	4.57	9.85	0.10	96.33
3	1	63	4.14	7.94	0.13	62.33
4	2	36	3.58	6.00	0.17	35.33
5	3	25	3.22	5.00	0.20	24.33
6	4	15	2.71	3.87	0.26	14.33
7	5	6	1.79	2.45	0.43	5.32

Fig 2.12 Weighting exponential decay values

N_t is expected to follow Eqn 2.34:

$$N_t = N_0 \times \exp(-0.693 \times t/T_{1/2})$$

where $T_{1/2}$ is the half-life and N_0 is the count rate measured when $t = 0$.
We see in 2.3.4 that we can *linearize* this equation by taking natural logarithms to get:

$$\ln(N_t) = \ln(N_0) - (0.693/T_{1/2}) \times t$$

such that the slope of a best-fit straight line of $\ln(N_t)$ against t will be $m = -0.693 / T_{1/2}$.
The values of $\ln(N_t)$ against t are plotted in Fig 2.13(a).

Initially we will assume that the uncertainties in the $\ln(N_t)$ values are all the same. If we now use the techniques of 2.1.4 to perform a linear regression on these points by calculation (or possibly a best-fit by eye), we get a 95% confidence range for the slopes of possible best-fit lines as

Slope assuming *equal uncertainties*: $m = -0.53 \pm 0.11$.

This would translate approximately into a 95% confidence interval for the half-life, $T_{1/2} \approx 1.31 \pm 0.28$.

However, the simplistic assumption that there is the same uncertainty in each point is far from true in this case, and we will now investigate how the varying uncertainty can affect the results.

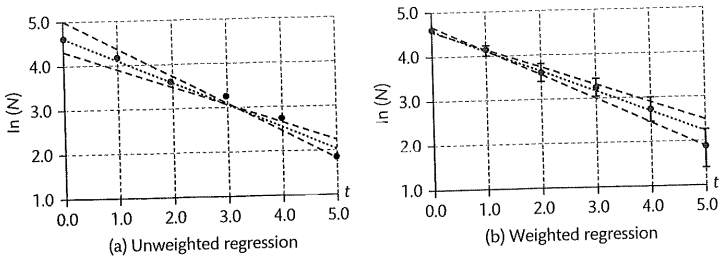


Fig 2.13 Taking uncertainties into account

We have two factors affecting the final uncertainties:

- The uncertainty in a radioactivity count of N_t is equal to $\sqrt{N_t}$ (Eqn 1.15).
- The act of taking the log of N_t will further transform the relative uncertainties of different values.

In order to derive the uncertainty estimates, u_t, at each time, we first calculate the uncertainties, $\sqrt{N_t}$, in column D for each value of N_t. e.g.

[D2] = SQRT(B2) = 9.85.

We now need to estimate how the uncertainty $\sqrt{N_t}$ translates into the uncertainty, u_t, when we take the value of $\ln(N_t)$. A simple way of approximating this in Excel is to take logs of the *extreme values* $N_t + \sqrt{N_t}$ and $N_t - \sqrt{N_t}$ and use half the difference:

$$u_t = 0.5 \times \{\ln(N_t + \sqrt{N_t}) - \ln(N_t - \sqrt{N_t})\}$$

which we calculate in column E, e.g.

[E2] = 0.5 * (LN(B2+D2) – LN(B2–D2)) = 0.10.

The data values together with u_t plotted as error bars are shown in Fig 2.13(b). We can see that the process of linearization has dramatically increased the uncertainty in points with *lower* values of N.

We also see that the point at $t = 0$ now has relatively little uncertainty which constrains the possible spread of the best-fit lines as they pass through this point.

The simplest method of deriving *weightings* for the experimental results is to estimate the relative weighting, w, in column F using Eqn 2.25 and the uncertainties in column E, e.g.

[F2] = 1/E2^2.

Using the weighted linear regression in SPSS or Minitab:

Minitab > Stat > Regression > Regression > Fit Regression Model...
Response: *LnN*
Continuous predictors: *t*
> Options...: Weights: *w*
☑ Output: Gives the same values as in Fig 2.14

SPSS > Analyze > Regression > Linear...
Dependent: *LnN*
Independent(s): *t*
WLS Weights: *w*
☑ Output: Fig 2.14

Coefficients[a,b]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	4.591	.047		98.151	.000
	t	-.487	.026	-.994	-18.894	.000

a. Dependent Variable: LnN.
b. Weighted Least Squares Regression - Weighted by w

Fig 2.14 Weighted least squares regression using SPSS

The intercept in Fig 2.14 gives $\ln(N_0) = 4.591$ from which we can derive $N_0 = 98.6$.
The slope of –0.487 with a standard error of 0.026 multiplied by the t-value of 2.78, gives the 95% confidence range for the best-fit slope:

Slope using data weighting, $m = -0.49 \pm 0.07$

This would translate approximately into a 95% confidence interval for the half-life, $T_{1/2} \approx 1.42 \pm 0.21$.

In Fig 2.13 (a) and (b), we see that the data point at $t = 5.0$ causes a significant difference between the two calculations. In the *un-weighted* regression, it exerts more influence than its uncertainty should allow and forces the best-fit line into a steeper slope. Also, the reduced relative uncertainty of the point $t = 0$ in the *weighted* regression restricts the range of possible slopes that can be drawn, resulting in a more precise estimation of the half-life.

2.3 Linearization techniques

We have introduced, in 2.1 and 2.2, the statistical tools that are available to analyse data that can be expected to have a *linear* relationship. However, if we now have *nonlinear* data, then we have two broad options:

- Use a specific analytical technique to fit a nonlinear model (e.g. a polynomial) that reflects the expected system behaviour. These techniques are introduced in Section 7.2.
- Use a mathematical transformation to *linearize* the data so that it can be treated as a straight line, and then use the familiar straight line techniques.

These *linearization* methods use two main approaches:

Change of variable.

Taking logarithms of both sides of an equation to handle exponential and power equations.

We start with the basic 'change of variable' technique in 2.3.1, and then review some of the key properties of logarithms and exponential relationships in 2.3.2 and 2.3.3, before developing their use in the linearization of exponential equations in 2.3.4 and power relationships in 2.3.5. Finally, we introduce combined examples in 2.3.6, with a final warning about the effect of linearization on the uncertainty values of the different data points.

2.3.1 Change of variable

If we have a nonlinear relationship between variables p and q, then we can seek to rearrange the equation into the form of a straight line equation:

$$f(p) = m \times f(q) + c$$
$$\downarrow \qquad \downarrow$$
$$y = m \times x + c$$

where $f(p)$ and $f(q)$ could be nonlinear functions of p and q respectively.

If we plot the *values* of $f(p)$ on the y-axis and the *values* $f(q)$ on the x-axis, then we can measure the values of m and c in the equation from the slope and intercept of the best-fit straight line.

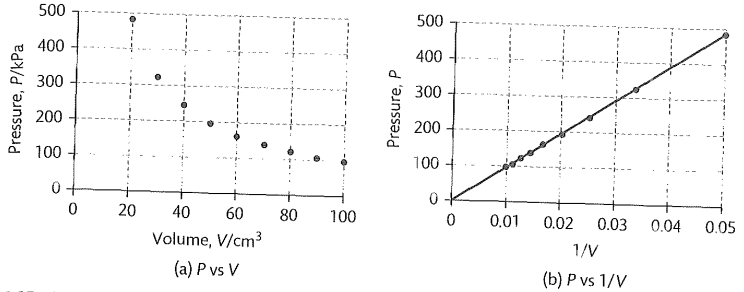


Fig 2.15 Linearization of P vs $1/V$

We can demonstrate this technique using the simple example of the ideal gas equation, where the pressure, P, volume, V, and absolute temperature, T, of n moles of an 'ideal' gas are given by:

$$P = n\frac{RT}{V}$$

and R is the gas constant = 8.31 $JK^{-1}mol^{-1}$.

Plotting the values of P and V for a fixed quantity of gas at a constant temperature, $T = 293K$, gives the nonlinear curve in Fig 2.15(a). We can use this data to calculate the quantity of gas in moles.

We can rearrange the equation to give

$$P = nRT\left(\frac{1}{V}\right) + 0$$
$$\downarrow \quad \uparrow \quad \downarrow \quad \uparrow$$
$$y = m \times x + c$$

which is in the form of a straight line, *provided* that we plot P on the y-axis and the values of the function, $1/V$, on the x-axis. In this case, the slope and intercept of the resulting straight line in Fig 2.15(b) are given by:

Slope: $m = nRT$
Intercept: $c = 0$

Using the values from Fig 2.15(b), the calculated slope is $m = 9.74 \times 10^3$, with pressure measured in kPa and the volume in cm^3. Converting the units of pressure to Pascals, Pa, and $1/V$ from cm^{-3} to m^{-3}, the slope becomes

$$m = 9.74 \times 10^3 \times 10^3 \times 10^{-6} = 9.74$$

which allows us to calculate the number of moles, n, of the gas:

$$n = m / (RT) = 9.74 / (8.31 \times 273) = 0.004 \text{ mol}$$

Note that it is important to be careful to use the correct *units* when interpreting the results.

2.3.2 Using logarithms

Logarithms can be defined as the *inverse operation* of a power. If y is equal to b raised to a power, x, then, reversing the process, x is equal to the logarithm to base b of y, which can be written as:

$$\text{If } y = b^x \quad \text{then} \quad x = \log_b(y) \tag{2.26}$$

Although it is possible to use any value for the base, b, we generally use either logs to base 10 or logs to base e, where e is Euler's constant = 2.71828....

Logs to base 10 are convenient in that they relate to the standard decimal system with powers of 10.

- $\log_{10}(x)$ is normally abbreviated as $\log(x)$.

Logs to base e are also used extensively because e has a unique property in rate of change equations. The rate of change of e^x with x simply equals e^x: $\frac{d}{dx}(e^x) = e^x$.

These are also often referred to as natural or Naperian logarithms.

- $\log_e(x)$ is normally abbreviated as $\ln(x)$. Note that this is $\ln(x)$ *not* $\text{in}(x)$.

The inverse definition of logarithms leads to the important relationships:

$$\ln(e) = 1 \quad \text{and} \quad \log(10) = 1 \tag{2.27}$$

A key effect of taking logs of an equation is that it **moves any power** (e.g. B in the equation below) **onto the equation line**:

$$\ln(A^B) = B \times \ln(A) \quad \text{and} \quad \log(A^B) = B \times \log(A) \tag{2.28}$$

which gives important relationships when there are powers of e or 10:

$$\ln(e^B) = B \times \ln(e) = B \quad \text{and} \quad \log(10^B) = B \times \log(10) = B \tag{2.29}$$

Another important property of logs in our calculations is that 'the logarithm of a *product* becomes the *sum* of the individual logarithms':

$$\ln(A \times B) = \ln(A) + \ln(B) \quad \text{and} \quad \log(A \times B) = \log(A) + \log(B) \tag{2.30}$$

and 'the logarithm of a *ratio* is the *difference* between the logarithms of the numerator and denominator':

$$\ln(A / B) = \ln(A) - \ln(B) \quad \text{and} \quad \log(A / B) = \log(A) - \log(B) \tag{2.31}$$

In respect of linearizing equations, we will investigate the effect of taking logarithms of two main types of equation:

Exponential growth and decay with a *variable* within a power (see 2.3.4):

$$N_t = N_0 \times e^{kt}$$

Equations with an unknown *constant* power (see 2.3.5):

$$E = A \times T^B$$

where B is a constant with an unknown value.

It would also be possible to linearize the gas laws equation, $P = nRT/V$, by taking logarithms of both sides of the equation, giving

$$\ln(P) = \ln(nRT) - \ln(V)$$

and then use the intercept of the straight line, with slope of -1, to calculate the value of nRT.

2.3.3 Exponential relationships

Exponential relationships occur in many branches of science, and their treatment has developed in different ways. However, it is possible to interpret their behaviour using the general equation:

$$N_t = N_0 \times e^{kt} \tag{2.32}$$

For example, the elimination of a drug of concentration, C, from the body in a time, t, can be described in pharmokinetics by

$$C_t = C_0 \times e^{-Kt}$$

where K (= $-k$ above) is the elimination constant.

We see below how a number of context-specific exponential equations are related to Eqn 2.32 by using specific expressions for k to fit the context of the problem.

Generation time, T_G, (or doubling time) is the time a population takes to double in number:

$$N_t = N_0 \times 2^{\left(t/T_G\right)}$$

For example, when $t = T_G$, the above equation gives $N_t = 2 \times N_0$.

As we know that $2.0 = e^{0.693}$ (because $\ln(2) = 0.693...$), we can substitute for '2' in the above equation to derive:

$$N_t = N_0 \times e^{0.693\left(t/T_G\right)} \tag{2.33}$$

which matches the *general* equation, with

$$k = 0.693 / T_G \quad \text{and} \quad T_G = 0.693 / k$$

Radioactivity half-life, $T_{1/2}$, is the time during which the radioactivity falls to one half of its value:

$$A_t = A_0 \times 0.5^{\left(t/T_{1/2}\right)}$$

For example, when $t = T_{1/2}$, the above equation gives $A_t = 0.5 \times A_0$.

As we know that $0.5 = e^{-0.693}$ (because $\ln(0.5) = -0.693...$), we can substitute for '0.5' in the above equation to derive:

$$A_t = A_0 \times e^{-0.693\left(t/T_{1/2}\right)} \tag{2.34}$$

which matches the *general* equation, with

$$k = -0.693/T_{1/2} \quad \text{and} \quad T_{1/2} = -0.693/k$$

Decimal reduction time, T_D, is the time a population takes to fall to 10% of the initial value:

$$N_t = N_0 \times 10^{-\left(t/T_D\right)}$$

For example, when $t = T_D$, the above equation gives $N_t = N_0/10$.
As we know that $10 = e^{2.30}$ (because $\ln(10) = 2.30...$), we can substitute for '10' in the above equation to derive:

$$N_t = N_0 \times e^{-2.30\left(t/T_D\right)} \tag{2.35}$$

which matches the general equation, with

$$k = -2.30/T_D \quad \text{and} \quad T_D = -2.30/k$$

Time constant, τ, is the time during which a value falls to $1/e = 36.8\%$ of its initial value:

$$V_t = V_0 \times e^{-\left(t/\tau\right)} \tag{2.36}$$

which matches the general equation, with

$$k = -1/\tau \quad \text{and} \quad \tau = -1/k.$$

2.3.4 Linearizing the exponential

We can now linearize the general exponential equation and derive the implications for the different *context* equations. Starting with Eqn 2.32:

$$N_t = N_0 \times e^{kt}$$

we choose to take natural logs (to base e) of both sides because the base in this equation is e.

Taking natural logs of both sides:

$$\ln(N_t) = \ln(N_0 \times e^{kT})$$

We then use the properties of the logarithms, from Eqns 2.30 and 2.28, to develop the right-hand side of the equation.

Log of a product is the sum of the logs:

$$\ln(N_t) = \ln(N_0 \times e^{kt}) = \ln(N_0) + \ln(e^{kt})$$

Eqn 2.28 gives $\ln(e^{kT}) = kT$:

$$\ln(N_t) = \ln(N_0) + \ln(e^{kt}) = \ln(N_0) + kt$$

With some rearrangement, we can write:

$$\ln(N_t) = k \times t + \ln(N_0)$$
$$\searrow \quad \downarrow \quad \downarrow \quad \swarrow$$
$$y = m \times x + c \tag{2.37}$$

which compares directly with the straight line equation, provided that we plot values of $\ln(N_t)$ on the y-axis and values of t on the x-axis.

We would then expect that the slope and intercept of this plot would be given by:

Slope: $m = k$

Intercept: $c = \ln(N_0)$ from which we can 'reverse' the logarithm to give, $N_0 = e^c$.

We can now interpret the slope of the equation using the values of k from the different scientific contexts discussed above.

Generation time, $T_G = 0.693 / m$

Radioactivity half-life, $T_{1/2} = -0.693 / m$

Decimal reduction time, $T_D = -2.30 / m$

Time constant, $\tau = -1 / m$

Case study: Exponential decay / 3. Linearizing the exponential

–continued from 2.Introduction, leading to 2.2.4, 2.4.3, 3.4.7, and 7.2.3.

Fig 2.16 gives radioactive counts, N_t, in column B for times, t, in column A. We aim to calculate the half-life of the radioactive decay using a linearization technique.

	A	B	C	D	E	F
1	***t***	***N***	**ln(*N*)**		**Slope, *m* =**	**-0.531**
2	0	97	4.57		**Intercept, *c* =**	**4.664**
3	1	63	4.14			
4	2	36	3.58		**$T_{1/2}$ =**	**1.305**
5	3	25	3.22		**N_0 =**	**106.1**
6	4	15	2.71			
7	5	6	1.79			

Fig 2.16 Radioactive decay data

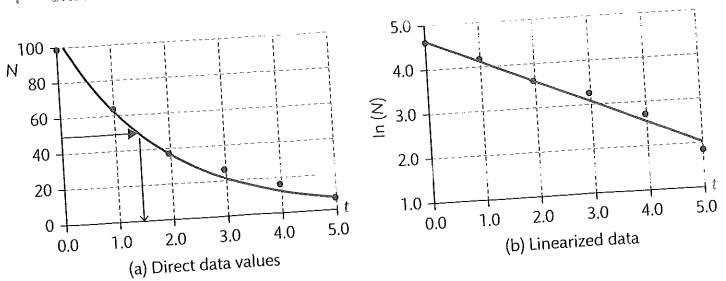


Fig 2.17 Linearization of radioactive decay

The exponential radioactive decay of N_t with time, t, is recorded in Fig 2.17(a), and, by eye, we could *estimate* that the time taken for the radiation to fall to one half of its initial value is about 1.4 s.

In the process of linearization, we first calculate the natural log, $\ln(N_t)$, of each value of N_t in column C, e.g.

[C2] = LN(B2)

If we then plot $\ln(N_t)$ against t in Fig 2.17(b), we see that we have an approximately linear relationship, which we can analyse by calculating the slope and intercept of the best-fit straight line:

Slope, m: [F1] = SLOPE(C2:C7, A2:A7) = −0.531

Intercept, c: [F2] = INTERCEPT(C2:C7, A2:A7) = 4.664

The half-life, $T_{1/2}$, is then related to m by the equation (calculated in F4):

$T_{1/2} = -0.693/m$: [F4] = −0.693 / F1 = 1.31

The value of N_0 can be derived from the equation $c = \ln(N_0)$ from which we can calculate:

$N_0 = e^c$: [F5] = EXP(F2) = 106.1

In this calculation, we obtained the values $N_0 = 106.1$ and $m = -0.531$, having assumed that the

random uncertainties follow a normal distribution, and

the uncertainties are the same for each *transformed* value, $\ln(N)$.

However, neither is true because the inherent uncertainty in N is proportional to $\sqrt{N}$ based on a Poisson distribution for random events and the process of taking the logarithm will also result in different absolute uncertainties. In 2.2.4 we use a process of data *weighting* to allow for the varying uncertainty, giving:

$N_0 = 98.6$ and $m = -0.487$

In 2.4.3 we use a process of iterative analysis to develop other possible models of analysis for nonlinear regression, and these methods are repeated using the generalized linear model in 3.4.7.

A normal distribution that assumes equal variance for the *untransformed* data points, gives

$N_0 = 97.9$ and $m\,(k) = -0.476$

Assuming *correctly* that the uncertainties in distribution and magnitude are given by the Poisson distribution gives

$N_0 = 99.4$ and $m\,(k) = -0.494$

2.3.5 Unknown power

As an example of an equation with an unknown constant power, we use a simple power equation relating variables E and T with constants A and B:

$$E = A \times T^B$$

where B is a power with an unknown value.

Again we take logs of both sides. In this case there is no preference for using either ln or log.

$$\ln(E) = \ln(A) + B\ln(T)$$

which can be rearranged to compare this directly with the straight line equation:

$$\begin{array}{c} \ln(E) = B \times \ln(T) + \ln(A) \\ \searrow \quad \downarrow \quad \downarrow \quad \nearrow \\ y = m \times x + c \end{array} \qquad (2.38)$$

We can see that the equation will act as a straight line provided that we treat $\ln(E)$ as the y-variable and $\ln(T)$ as the x-variable.

In this case, we would expect that the slope and intercept of this plot would be given by:

Slope: $m = B$

Intercept: $c = \ln(A)$, giving $A = e^c$

See 7.2.4 for an example of this type of calculation using the 'Rowing' case study.

2.3.6 Combined linearization

Some examples require combinations of the above processes. For example, the calculation of the activation energy, E, in the Arrhenius equation

$$k = A\exp\left(-\frac{E}{RT}\right)$$

(where k and T are the variables, R is the gas constant, A is an unknown constant) would require a plot of $\ln(k)$ against $(1/T)$, based on the rearranged equation

$$\ln(k) = \ln(A) - \left(\frac{E}{R}\right) \times \left(\frac{1}{T}\right)$$

with the slope of linear regression, $m = -E/R$.

For the Michaelis–Menten equation, which gives the initial velocity of an enzyme reaction, v, as a function of the substrate concentration, S,

$$v = \frac{v_{max} S}{K_M + S}$$

(where K_M is the Michaelis–Menten constant and v_{max} would be the maximum reaction velocity for large values of S) it is possible to rearrange the equation to give:

$$\frac{1}{v} = \left(\frac{K_M}{v_{max}}\right) \times \frac{1}{S} + \frac{1}{v_{max}}$$

In this case, a plot of $1/v$ against $1/S$ gives $1/v_{max}$ as the intercept, and then, using the slope, (K_M/v_{max}), it is possible to calculate the value of K_M.

2.3.7 Error warning

When using a linearization technique for nonlinear data, the transformation process will also act on the errors/uncertainties in the data points, and this can have the effect of distorting the *importance* of some of the points in the final regression process, resulting in slope and intercept errors. For example, refer to the linearization of an exponential decay using *weighting* in 2.2.4 and an *iterative* analysis in 2.4.3.

2.4 Iteration using Solver

Solver is an add-in in Excel which uses a process of *iteration* to fit a mathematical model to the supplied data within defined constraints. If, for example, we wish to calculate the *coefficients*, m and c, of a best-fit straight line, $y = mx + c$, the iteration:

- starts with initial *guesses* for the values for these coefficients
- calculates the value of an overall *test statistic* (e.g. sum of residuals) that is to be used to measure the goodness of fit
- uses specific algorithms *step-by-step* to change the coefficients in directions that should improve the goodness of fit
- repeats the steps until the test statistic gets *very close to* a desired (e.g. minimum) value.

Although this is not an exact *calculation* of the coefficients, the repetitive power and accuracy of modern computing can produce very accurate *estimated* coefficients.

In 2.4.1 we demonstrate this use of Solver for linear regression using the sum of the squares of the residuals (2.1.1) as the test statistic to be minimized, and in 2.4.2 we demonstrate the use of maximum likelihood estimation, MLE, for the same data. In 2.4.3 we use the flexibility of Solver to investigate how the underlying experimental uncertainty can be modelled within nonlinear regression.

2.4.1 Operation of Solver

We use the example of linear regression to demonstrate the operation of Solver through the identification of a best-fit straight line by minimizing the sum of squares of the residuals.

Case study: Best-fit straight line / 8. Least squares fit using Solver

–continued from 2.1.1, leading to 2.4.2

Fig 2.18 reproduces data from Fig 2.2, and presents the x-y values for five measurements recorded in columns B (x-data) and C (y-data), with each data pair identified by the label, i, in column A. We wish to calculate the coefficients of the best-fit straight line using the method of 'least squares'.

Using Solver: Excel analysis for Fig 2.18. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

	A	B	C	D	E	F	G	H
1	*i*	*x*	*y*	*y'*	*R*	R^2	*MLE*	*Probability*
2	1	12	28	0.00	-28.00	784.00		0.00507
3	2	20	27	0.00	-27.00	729.00		0.00509
4	3	28	56	0.00	-56.00	3136.00		0.00407
5	4	40	59	0.00	-59.00	3481.00		0.00394
6	5	48	89	0.00	-89.00	7921.00		0.00260
7		Pairs, n =	5		SE_{RESID}, ΣR^2 =	16051.00	Combined:	1.08E-12
8		Slope, m =	0.000		SE_{REG} =	73.15		$\times 10^6$
9		Intercept, c =	0.000				Objective:	1.08E-06

Fig 2.18 Starting values for a least squares fit using Solver (calculations in column H are developed in 2.4.2)

The calculations of residuals, R, and the sum of squares of residuals, $SS_{RESID} = \Sigma R^2$ in Fig 2.18 are explained in 2.1.1. In this calculation, the *starting* values for the slope, m, and intercept, c, of a best-fit straight line are entered into cells C8 and C9, with example values of 0.0 for both. Using these values, the predicted values for y' are calculated in column D, from which the values of R, R^2, and $SS_{RESID} = 16{,}051$ are then calculated.

The process of linear regression seeks to arrive at values of m and c that give the lowest possible value for SS_{RESID}. In 2.1.1 we used the functions, SLOPE and INTERCEPT, to calculate these values *directly*, obtaining values $m = 1.656$ and $c = 2.782$, respectively. We now demonstrate the use of Solver to arrive at the same values through the process of *iteration*.

Fig 2.19 Section of the Solver dialogue window in Excel

In the Solver dialogue window in Fig 2.19, we enter F7 containing SS_{RESID} as the 'objective', and check the ◉ **Min** option that directs the algorithm to find a *minimum* value for the 'objective' value. We then identify the cells C8 and C9 as the values of m and c that can be changed to arrive at this minimum. Solver then goes through an iterative process of changing C8 and C9, until it settles on values where any further change will begin to *increase* F7. In this example, Solver arrives at the values $m = 1.656$, $c = 2.782$, and $SS_{RESID} = 300.48$, which agree with the direct calculations in 2.1.1.

There are situations where Solver fails to end with the required answer. For complex mathematical models, it is possible that there are 'local' minima in the values of the 'objective' variable. This is similar to local dips in the side of a hill as it slopes down towards the bottom of a valley, and rain can be trapped in the local dips and fail to reach the very lowest point. It is also possible that there is a local maximum between the starting point and the true minimum in another 'valley'. If there is a possibility of such local problems, then it can be useful to try starting the iteration from a different point, e.g. with different values of C8 and C9. It is also important to check that the 'objective' values being calculated are not so *small* that no iteration occurs, and in 2.4.2 we multiply the objective by 10^6 to ensure that it does not fall below the resolution limits of Excel.

With any iteration, it is necessary to establish rules by which the process can decide when it should stop, and these involve setting minimum change levels. However, for most operations the default settings in Solver work well.

2.4.2 Maximum likelihood estimation

Maximum likelihood estimation, MLE, is an alternative to the least squares, LS, method for calculating a best-fit model to a set of data. It works by first calculating the probabilities with which the observed set of experimental values would occur for different possible models. For example, in the case of linear regression, each *model* is a specific set of values of slope, m, and intercept, c, giving a possible 'true' straight line, and, for each of these models, the process calculates the *relative probability* of the observed experimental values occurring *by chance*. The iteration then changes model values of m and c to find the model which gives the *maximum* probability for the observed data, and these values become the 'best-fit' result.

Case study: Best-fit straight line / 9. Maximum likelihood using Solver

–continued from 2.4.1

We use the same data as in Fig 2.18 (taken from Fig 2.2) which presents the x-y data for five measurements recorded in columns B (x-data) and C (y-data), with each data pair identified by the label, *i*, in column A. We wish to calculate the coefficients of the best-fit straight line using the 'maximum likelihood estimation' method.

The calculations of residuals, R, and the sum of squares of residuals, $SS_{RESID} = \Sigma R^2$, for this data are explained in 2.1.1 and 2.4.1. In addition, the standard error, SE, which is the *estimated* standard deviation uncertainty in each data point, is calculated in F8 from the value of ΣR^2 in F7 using Eqn 2.14:

Standard error, SE: [F8] = SQRT(F7 / (C7 – 2))

The *target* values for the slope, m, and intercept, c, of a best-fit straight line are entered into cells C8 and C9, starting with values of 0.0 for both, and then the predicted values, y', are calculated using these values of m and c.

We assume that the random experimental uncertainty is given by the *normal* distribution, and the key statistic is the *probability* with which each *observed* value of y in column D would be randomly selected from a normal distribution with mean, y', in column C and standard deviation, SE, in F8. For example, for the first data value:

[H2] = NORM.DIST(*D2*, C2, F$8, FALSE)

The *combined relative probability* for all five data points is then calculated by *multiplying* all the individual probabilities together in H7:

[H7] = PRODUCT(H2:H6) = 1.08×10^{-12}

It is this value that the maximum likelihood process seeks to *maximize*, but the iteration process in Solver does not work with such low values, and we choose to multiply by a factor of 10^6 to produce a more realistic 'objective' value in H9:

[H9] = H7 * 10^6 = 1.08×10^{-6}

Running the Solver iteration, we now seek to maximize H9 by checking the ◉ **Max** option to change the values of C8 and C9. The result is the same as in 2.4.1 with the values $m = 1.656$, $c = 2.782$.

The underlying reason for the MLE model and the LS models giving the same result in this particular calculation is that they *both* rely on the *normal distribution* in their calculations. In 2.4.3 we can now see how the maximum likelihood model can be used for data that does not follow the normal distribution.

2.4.3 Nonlinear regression

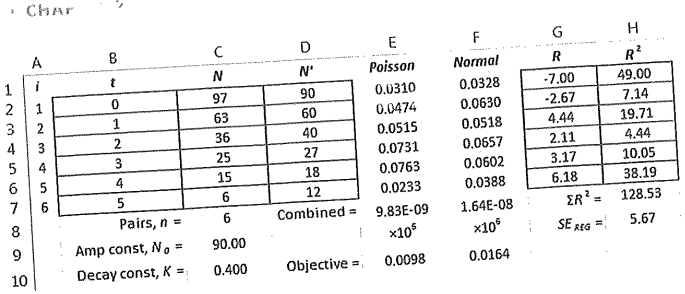
By using maximum likelihood estimation in Solver, we can unpick the various elements in modelling regression. It is useful to use the MLE method to compare the situations when the underlying uncertainty in the data is due to a Poisson distribution and not a normal distribution, and we use the example of radioactive decay.

Nonlinear regression: Excel analysis for Fig 2.20. See also 7.2.3. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: Exponential decay / 4. Nonlinear regression using Solver

–continued from 2.3.4, leading to 3.4.7 and 7.2.3

Fig 2.20 presents the same *x*-*y* data as Fig 2.16 with six measurements of radioactive decay recorded in columns B (*x*-data) and C (*y*-data), with each data pair identified by the label, *i*, in column A. We wish to calculate the best-fit coefficients for an exponential decay model.



	A	B	C	D	E	F	G	H
1	*i*	*t*	*N*	*N'*	*Poisson*	*Normal*	*R*	R^2
2	1	0	97	90	0.0310	0.0328	-7.00	49.00
3	2	1	63	60	0.0474	0.0630	-2.67	7.14
4	3	2	36	40	0.0515	0.0518	4.44	19.71
5	4	3	25	27	0.0731	0.0657	2.11	4.44
6	5	4	15	18	0.0763	0.0602	3.17	10.05
7	6	5	6	12	0.0233	0.0388	6.18	38.19
8		Pairs, *n* =	6	Combined =	9.83E-09	1.64E-08	ΣR^2 =	128.53
9		Amp const, N_0 =	90.00		$\times 10^6$	$\times 10^5$	SE_{REG} =	5.67
10		Decay const, *K* =	0.400	Objective =	0.0098	0.0164		

Fig 2.20 Data for nonlinear regression using Solver

Fig 2.20 gives the results of a measurement of radioactive decay, with experimental data of N counts as a function of time, t. The predicted counts, N', in column D are calculated using the mathematical model:

$$N' = N_0 \times e^{-Kt}$$

where N_0 is the amplitude constant in C9 and K is the decay constant in C10, with $K = -k$ in Eqn 2.32.

The calculations for the iterative analysis are developed in 2.4.1 and 2.4.2. We start with initial values of N_0 and K equal to 90 and 0.4 respectively, which give the predicted values of N' in the table.

We now use two different statistical assumptions for the uncertainty distributions in the data:

- **Poisson distribution** for randomly generated frequency values, which gives a standard deviation (1.3.3) equal to the square root of the value of the theoretical count, $\sqrt{N'}$.
- **Normal distribution**, which, in this example, is assumed to have the same standard deviation for all data values, equal to standard error of regression in H9, calculated from the squares of the residuals.

For the **Poisson** distribution, the probability for each of the observed counts, N, being observed, given the corresponding theoretical value, N', is calculated in column E, e.g. for data, $i = 1$:

[E2] = POISSON.DIST(C2, D2, FALSE) = 0.0310

As with the calculation in 2.4.2, the product of all the probabilities is calculated in E8 and then multiplied by a factor of 10^6 to give a realistic 'objective' probability in E10 for entry into Solver.

Solver can then be used to change the values of N_0 and K to obtain a *maximum* value for the 'objective' in E10. This results in the best-fit values, using the Poisson error distribution:

$N_0 = 99.41$ and $K = 0.494$ giving $k = -0.494$

For the **normal** distribution, the probability for each of the observed counts, N, being observed, given the corresponding theoretical value, N', is calculated in column F, e.g. for data, $i = 1$:

[F2] = NORM.DIST(D2, C2, H$9, FALSE) = 0.0328

where H9 is the estimated standard deviation in the experimental data.

Solver can then be used to change the values of N_0 and K to obtain a *maximum* value for the 'objective' in F10. This results in the best-fit values, using the normal error distribution:

$N_0 = 97.86$ and $K = 0.476$ giving $k = -0.476$

We can also use the same worksheet to perform the *least squares* analysis by using Solver to change the values of N_0 and K to obtain a *minimum* value for the sum of squares of residuals in H8. As expected, this results in the same best-fit values as above when using the normal error distribution.

This case study example is also analysed in 2.3.4, providing a review of the different options for performing nonlinear regression for an exponential decay.

Hypothesis testing

Introduction

The hypothesis test is a key element in the scientific method, in which a proposed hypothesis is tested experimentally by measuring the value of a *test statistic* and then using a statistical analysis to calculate the probability that the observed value could have occurred by chance. If the probability is low, typically less than one in twenty, then it may be *reasonably* safe to assume that an underlying scientific effect might be responsible. The decision logic has been introduced in Section 1.6, including the p-value, significance level, α, and the possibilities of Type I and Type II errors.

In this chapter we introduce different forms of hypothesis testing and analysis, both as a basis for analyses in later chapters and as a perspective of the variety of possible methods. The 'theoretical' basis is developed mainly through modelling with Excel and without difficult statistics, but provides the understanding necessary for the implementation of these tests using Minitab and SPSS in Part II.

Section 3.1 develops the 't' and 'z' statistics for testing differences in mean values, including the family of standard t-tests.

Section 3.2 develops the F-statistic that is at the heart of the 'analysis of variance' approach to testing and introduces the basic ANOVA.

Section 3.3 further extends the ANOVA concept to include the simultaneous testing for the effects of multiple factors.

Section 3.4 reviews the overlapping analyses of t-tests, linear regression, and ANOVAs to develop the concept of general linear models.

Section 3.5 introduces nonparametric testing with the example of the Mann–Whitney test and with reference to the range of alternative techniques.

Section 3.6 considers the additional power of making repeated measurements, either with two measurements for paired tests or more for a 'repeated measures' analysis.

Section 3.7 develops the 'chi-squared' approach to testing the frequencies with which observations fall into different categories.

Section 3.8 then considers the situation with binary outcomes of just two categories leading to tests for proportions.

Section 3.9 uses repetitive modelling in Excel to give an introduction to the developing field of 'resampling' for the calculation of p-values.

3.1 t-tests and z-tests

The t-test, developed by William Gosset under the pen name 'Student', is usually the first statistical test presented to science students, for testing the difference between the mean values of two data samples. In this section we start with the underlying principle of the test, demonstrating its applicability to other problems, before reviewing the 'named' t-tests. We also consider the z-test (3.1.5) which allows for the fact that we sometimes know the *experimental uncertainty* in a measurement beforehand and do not need to rely solely on the sample data to estimate uncertainty as is the case with the t-test.

3.1.1 General principle of hypothesis testing

In one of the most common types of hypothesis test (Section 1.6), we record an *observed* (*non-zero*) value for a variable, but we wish to test whether

the *true* value is *zero* and that the observed value is just due to random experimental variations, or

the *true* value is really *not* zero.

The test calculates a *statistic* which is the ratio between the observed *value* and the *uncertainty* in that measured value, defined as the *standard error*, *SE*:

$$t_S \text{ (or } z_S) = \frac{\text{Observed value}}{\text{Standard error, } SE}. \tag{3.1}$$

When the uncertainty is estimated from the data itself, this ratio is the t-statistic, t_S, but when the *SE* is already known accurately (e.g. by many previous measurements) then it becomes the z-statistic, z_S (1.5.4).

We first met the standard error in the calculation for the confidence interval (1.5.2) of a single replicate measurement, but we also meet calculations for standard error occurring for other characteristic values, e.g. the slope of a graph in 2.1.4.

If the *observed value* in Eqn 3.1 is *much* larger (either positive or negative) than the *standard error*, *SE*, giving a large t_S (or z_S) ratio, then we could be confident in deciding that the true value was not zero and did not occur just by random chance. However, for less extreme values, we use a set of *critical values* for t_S (and z_S) to act as reference values to help us make this decision.

Using a *critical value*, t_C, we would decide that the observed value was significant, and did not occur by chance, if

$t_S \geq t_C$ for positive values of t_S or if

$t_S \leq -t_C$ for negative values of t_S.

The value of t_C can be found from published tables or by using the function T.INV.2T(α, *df*) in Excel, which depends on

the confidence that we want in our decision, which is defined by the significance level, α, (1.6.1) and

- the degrees of freedom, *df*, (1.5.1) which increases with the number of measurements made to calculate the standard error.

If we were to make a large number of replicate measurements (i.e. giving a large value of *df*), then we could estimate the experimental uncertainty quite accurately. Reversing this logic, if we already know the uncertainty, then this information would be equivalent to a very large value of *df*. Hence, the *t*-value decreases with larger values of *df* and becomes equal to the *z*-value for 'infinite' *df*. The *z*-value for 95% confidence is $z_C = 1.96$, and can usually be taken as 2.00 for most practical purposes.

The basic test, using the *t*-statistic, is at the core of a wide range of tests for specific variables (e.g. *t*-test in 3.1.2), but we can also use Eqn 3.1 directly. For example, the analysis of residuals for *species* given in Table 5.3 in 5.4.7 records a *skewness* value of 1.064 with a standard error, *SE*, of 0.269. As the observed value (which would be zero for a normal distribution) is greater than $\pm 2 \times SE$, then we decide that there is evidence of *significant* skewness in the data.

Difference in slopes: Excel analysis for Fig 3.1. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: **Bacterial growth / 2. Difference in slopes using *t*-test**

–continued from 5.2.2 (overview), leading to 3.4.3

Using data from the graph in Fig 5.5, we wish to test whether the rates of growth of the bacterial populations are *different* for different cleaner concentrations, *C2* and *C3*, between the times $t = 60$ and 85. The values of *C2* and *C3*, measured in luminescence units, are reproduced in an Excel worksheet in Fig 3.1.

	A	B	C	D	E	F
1	**Data:**			**Calculation:**	**C3**	**C2**
2	**Time / *t***	**C3**	**C2**	**Slope =**	**0.2277**	**0.2784**
3	**60**	1.76	2.98	*n* =	6	**6**
4	**65**	2.78	3.68	SE(reg) =	0.1753	**0.2952**
5	**70**	3.93	5.54	SE(slope) =	0.0084	**0.0141**
6	75	**5.06**	6.8	Difference =		**0.0507**
7	80	**6.55**	8.55	SE(difference) =		**0.0164**
8	85	**7.24**	9.55	***t*-stat =**		**3.0914**
9				***df* =**		**8**
10				***p* =**		**0.0149**

Fig 3.1 Case study: Bacterial growth / 2. Difference in slopes

The null hypothesis for testing whether the two slopes are equal is:

H_0: Difference between the slopes of the two lines, *C2* and *C3*, is zero.

We start by calculating the slopes (E2 and F2), the standard errors of regression (E4 and F4), and standard errors of the slopes (E5 and F5) using the methods introduced in 2.1.4, e.g.:

Slope: [E2] = SLOPE(B3:B8, $A3:$A8)
Standard error of regression: [E4] = STEYX(B3:B8, $A3:$A8)
Standard error of the slope: [E5] = E4 / SQRT(VAR.S($A3:$A8) * (E3-1))

We calculate the *observed value* in this analysis, which is the *difference* between the slopes:

Difference in slopes: [F6] = F2 – E2 = 0.0507

and the *standard error* of the difference is calculated by combining the two *SE* uncertainties, using Eqn 1.12 from 1.4.4:

Standard error of difference: [F7] = SQRT(E5^2 + F5^2) = 0.0164

We wish to test whether the *difference* in slopes, measured in F6, is significantly different from zero, and hence we calculate the *t*-statistic in Eqn 3.1 as the ratio of F6 divided by F7:

$$t_S = \frac{\text{Observed value}}{\text{Standard error, } SE}: \qquad [F8] = \frac{F6}{F7} = 3.09$$

The degrees of freedom for this calculation is derived from the sample sizes of the two data sets, and is given by: $df = n_1 + n_2 - 4$:

Degrees of freedom, *df*: [F9] = E3 + F3 – 4 = 8

The two-tailed *p*-value can then be calculated:

p-value: [F10] = T.DIST.2T(F8, F9) = 0.0149

As $p = 0.0149$ is less than 0.05 we conclude that there *is* a significant difference between the two slopes. We see that this agrees with the *p*-value that we calculate in 3.4.3 when using general regression with the factors: *Time*, *Conc* and *Time*Conc*.

3.1.2 One sample *t*-test

In a one sample *t*-test, the *n* replicate measurements of a variable with a true value, μ, give an observed sample mean, $\bar{x}$. The aim of the test is to assess whether the unknown *true* value, μ, differs from a *specified* value, μ_0.

We have already met the confidence interval (1.5.2) of a simple measurement where $\bar{x}$ is the best estimate of an unknown true value, μ,

$$CI(\mu) = \bar{x} \pm Cd \Rightarrow \bar{x} \pm (t \times SE) \Rightarrow \bar{x} \pm \left\{ t \times {}^{s}/_{\sqrt{n}} \right\} \qquad (3.2)$$

In this equation, the *t*-value is used to calculate the critical limit within which we have a chosen level of confidence of finding the true value, μ. The *t*-test uses the same critical level statistics but expressed in a different way.

In the one sample *t*-test, the best estimate of the true value is the sample mean, $\bar{x}$, and the 'observed value' in Eqn. 3.1 is the difference between the sample mean and the specified value $= \bar{x} - \mu_0$. The standard error in this 'value' is the standard error in $\bar{x}$, $SE = s/\sqrt{n}$ (Eqn 1.21).

The relevant statistic becomes:

$$t_S = \frac{\text{Observed value}}{\text{Standard error}} = \frac{(\bar{x} - \mu_0)}{SE} = \frac{(\bar{x} - \mu_0)}{s/\sqrt{n}} \qquad (3.3)$$

The null hypothesis of the test is that there is no difference between μ and μ_0,

$H_0: \mu = \mu_0$

The proposed, or alternative hypotheses, could be

- for a two-sided or two-tailed test, $H_1: \mu \neq \mu_0$
- for a one-sided or one-tailed test, $H_1: \mu > \mu_0$ or $H_1: \mu < \mu_0$

The critical values, t_C, can be obtained from published tables using degrees of freedom given by:

$$df = n - 1 \tag{3.4}$$

The reason for the '−1' is that one bit of information has been used in the calculation of the sample mean value.

Case study: **Blood alcohol / 8. One sample *t*-test**

—continued from 1.6.2, leading to 8.1.1

The data in cells A2:A6 in Fig 3.2 show five replicate measurements of alcohol level (mg/100 ml) in a blood sample. We wish to test whether the true value, μ, of alcohol in the sample is greater than $\mu_0 =$ 80 mg/100 ml. Note that in this calculation (unlike in 1.6.2) we do not assume any previous knowledge of the measurement uncertainty.

The hypotheses are:

Null hypotheses, H_0: The true value, $\mu = 80$ mg/100 ml.

Alternative/proposed hypothesis, H_1: The true value, $\mu > 80$ mg/100 ml.

The specified value of 80 is entered into cell C5.

	A	B	C	D	E
1	Data:	Statistics:		Critical values:	
2	83.8	Mean =	82.82	1-tail, t_c =	2.13
3	81.2	St dev, s =	2.54	2-tail, t_c =	2.78
4	84.8	St Error, SE =	1.14	p-values:	
5	85.1	Test value =	80	1-tail, p =	0.034
6	79.2	t-statistic, t_s =	2.48	2-tail, p =	0.068

Fig 3.2 Case study: Blood alcohol / 8. One sample *t*-test

We calculate the relevant sample statistics for Eqn 3.3.

Mean: [C2] = AVERAGE(A2:A6) = 82.82

Standard deviation: [C3] = STDEV.S(A2:A6) = 2.54

Standard error (Eqn 1.21): [C4] = C3/SQRT(5) = 1.14

The relevant test statistic, using Eqn 3.3, is:

t-statistic: [C6] = (C2 − C5)/C4 = 2.48

The critical t-values are derived for a significance of 0.05, using degrees of freedom (Eqn 3.4), $df = n - 1 = 4$:

One-tailed critical value: [E2] = −T.INV(0.05, 4) = 2.13

(The reason for the negative sign is that the t-value is calculated from the left-hand tail area, and an area of 0.05 gives a *negative* value for t)

Two-tailed critical value: [E3] = T.INV.2T(0.05, 4) = 2.78

The p-values are then calculated directly from the t-statistic and degrees of freedom:

One-tailed p-value: [E5] = T.DIST.RT(C6, 4) = 0.034

(calculates just the area of the right-hand tail, as in Fig 1.19)

Two-tailed p-value: [E6] = T.DIST.2T(C6, 4) = 0.068

(calculates the area of both tails of the distribution.)

We see that, for a one-tailed test, $t_S > t_C$, suggesting that there is a significant difference, which agrees with the p-value, $p = 0.034 < 0.05$, but that, for the two-tailed test, $t_S < t_C$, suggesting that the apparent difference could have occurred by chance, which agrees with the p-value, $p = 0.068 > 0.05$. We can note that, for this symmetrical calculation, the two-tailed p-value is twice the one-tailed p-value as given in Eqn 1.27.

The *original* question was whether the blood alcohol level was *greater* than 80, and it is therefore acceptable to use the one-tailed test result. It would not be acceptable to choose the significant one-tailed test *after* seeing the results.

See 6.1.4 for the use of Minitab and SPSS for a one sample t-test.

3.1.3 **Two-sample *t*-test**

We can develop a similar test for a possible difference between the true means (μ_A and μ_B) of two samples, based on the best estimate values $\bar{x}_A$ and $\bar{x}_B$ from sample sizes of n_A and n_B, with standard deviations s_A and s_B.

This test is also called the independent samples t-test because each data value is measured as an *unrelated* measurement *independently* of any other value. We consider the possibility of *related* measurements in the paired t-test in 3.6.1.

We compare the difference in the two sample means with the standard error in this difference.

$$t_S = \frac{\text{Difference}}{SE(\text{Difference})} = \frac{(\bar{x}_A - \bar{x}_B)}{s' \times \sqrt{\frac{1}{n_A} + \frac{1}{n_B}}} \tag{3.5}$$

The standard error is calculated using a *pooled* standard deviation, s', which is a weighted 'average' of the two *sample* standard deviations, s_A and s_B. This calculation assumes that the two samples are drawn from *populations* with the same standard deviation, which is then estimated by using s'.

$$s' = \sqrt{\frac{(n_A - 1) \times s_A^2 + (n_B - 1) \times s_B^2}{n_A + n_B - 2}} \tag{3.6}$$

Note that we always combine uncertainties by combining *variances* (1.4.4).
The degrees of freedom for this test is given by:

$$df = n_A + n_B - 2 \tag{3.7}$$

Two bits of information have already been used in calculating the two sample mean values.

Two sample *t*-test and *F*-test: Excel analysis for Fig 3.3. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: River *pH* / 2. Two sample *t*-test and *F*-test

–continued from 6.2.1 (overview), leading to 3.2.2 (to 3.6.1 for the paired *t*-test and to 3.5.1 for the Mann–Whitney test)

The values in B2:E2 and B3:E3 in Fig 3.3 record four replicate *pH* values taken from each of two rivers, *A* and *B*. We wish to test for a difference between the true mean values for each river μ_A and μ_B respectively.
(The *F*-statistic referred to in L5 is developed in 3.2.1.)

The hypotheses for testing whether there is a difference in the *pH* values of the two rivers, *A* and *B*, become:

Null hypothesis, $H_0: \mu_A = \mu_B$
Alternative/proposed hypothesis, $H_1: \mu_A \neq \mu_B$

	A	B	C	D	E	F	G	H	I	J	K	L
1							**Mean, x**	**StDev, s**	**Size, n**		*df*	**Variance**
2	A	7.56	7.52	7.7	**7.61**		7.60	0.0776	4		3	**6.0**3E-03
3	B	7.47	7.4	7.49	**7.55**		7.48	0.0618	4		3	**3.8**2E-03
4												
5				Pooled **stdev**, s' =				0.0702			***F* statistic**, F =	1.58
6					***t*-statistic**, t_s =		2.42				***p*-value** =	0.36
7	***df*** =	6			***t*-critical (95%)**, t =		2.45					
8					***p*-value** =		**0.0520**	T.TEST() =	0.0520			

Fig 3.3 Case study: River *pH* / 2. Two sample *t*-test and *F*-test (3.2.1)

In this calculation we make the assumptions that:

- the four recorded values for each river are independent *replicate* measurements.
- the population of measurements for each river follow a normal distribution. In this example, given the relatively small variations of the replicate results and no reason to anticipate non-normality, we are probably safe to assume a normal distribution (Section 5.4).
- the population standard deviations (and variances) for each river are *equal* (homoscedasticity). We will see in Section 3.2.1 that the *p*-value given in L6 of the worksheet shows that there is no significant difference between the variances.

Using the Excel worksheet in Fig 3.3, we calculate the sample mean values $\bar{x}_A$ and $\bar{x}_B$ in G2 and G3 and their sample standard deviations s_A and s_B in H2 and H3.

The pooled standard deviation s', is calculated using Eqn 3.6 in H5:

s': [H5] = SQRT(((I2 – 1) * H2² + (I3 – 1) * H3²) / (I2 + I3 – 2)) = 0.0702

The *t*-statistic is calculated using Eqn 3.5 in G6:

t_S: [G6] = (G2 – G3) / (H5 * SQRT(1/I2 + 1/I3)) = 2.42

The degrees of freedom are calculated using Eqn 3.7 in B7:

df: [B7] = I2 + I3 – 2 = 6

The critical *t*-value (two-tailed) is calculated in G7:

t_C: [G7] = T.INV.2T(0.05, B7) = 2.45

The *p*-value (two-tailed) is calculated in G8:

p: [G8] = T.DIST.2T(G6, B7) = 0.0520

We can also calculate the *p*-value (two-tailed) *directly* from the *raw data* using the T.TEST() function in I8:

p: [I8] = T.TEST(B2:E2, B3:E3, 2, 2) = 0.0520

where the first '2' in the argument identifies a two-tailed test and the second '2' is for data with equal variance. We see that this agrees with the value calculated in G8.

We can base our conclusion for this hypothesis test on either of the comparisons:

- The *t*-statistic is less than the critical value: $t_S = 2.42 < t_C = 2.45$.
- The *p*-value is greater than the default significance level: $p = 0.052 > \alpha = 0.05$.

Both comparisons show that there is not enough evidence to reject the null hypothesis, and we conclude that the apparent difference could have occurred by chance.

If the *original* hypothesis had been to test whether $\mu_A > \mu_B$, we would need to calculate the one-tailed *p*-value. In this analysis the data uncertainties are symmetrical, and hence the *p*-value for a one-tailed test would be half of the two-tailed test, giving $p = 0.026$ (Eqn 1.27). For the one-tailed hypothesis, we would conclude that the *pH* of river A was indeed greater than that of river B, but it would be incorrect to decide to use the one-tailed hypothesis *after* you have seen the fact that it would suggest a significant effect in a specific direction.

In this case study, the two samples happened to be of the same size ($n = 4$), but the *t*-test can be applied in exactly the same way for samples of different sizes.

The calculation for the *t*-test assumes that the data is derived from populations with normal distributions. If the data is not normally distributed, then, in principle, it is necessary

either to transform the data to a near normal distribution (5.4.7), or to use the equivalent non-parametric test: the Mann–Whitney test (3.5.1). However, the *t*-test is *robust* for minor deviations from normality, and this means that it will tend to give the correct conclusion even if the distributions are not exactly normal. It is most likely to fail if a distribution is significantly *skewed*, i.e. with a long tail.

See 6.2.5 for the use of Minitab and SPSS for the two (independent) sample *t*-test.

3.1.4 **Unequal variances**

The standard calculation for the two sample *t*-test assumes *homoscedasticity* or *homogeneity of variance*, i.e. the two populations have the same variances (and standard deviations). However we may wish to perform the *t*-test on samples that are drawn from populations with *different* standard deviations. In this case, a modified *t*-statistic by B L Welch uses both standard deviations separately and introduces a non-integer value for the degrees of freedom. With its increased complexity, Welch's modified test is normally performed in software.

In principle, we should perform a hypothesis test for the equality of variance (*F*-test or Levene's test, 3.2.1) to check whether it is necessary to use Welch's modified test. However, the variance test can be unreliable for *small* samples, and, given the fact that the standard *t*-test is *robust* in accepting such differences, we often only use a variance test when the *scientific* conditions for the two samples suggest that the variances could be different (see Section 5.4).

It is possible to test for a difference in variance using the *F*-test in Excel (3.2.1). SPSS automatically carries out Levene's test for variance when requested to perform the two samples *t*-test, and also gives the results to both types of *t*-test (6.2.5). Minitab performs either *t*-test (6.2.5) and has a separate menu option that reports the results of both the *F*-test and Levene's test (6.2.4).

3.1.5 ***z*-tests**

Statistical analyses using *t*-values do not use any *external* information about experimental variability, and calculate the uncertainty just from the sample data. For small samples, this estimation is itself subject to increased error and the relevant *t*-value automatically increases (via the degrees of freedom) to accommodate this wider uncertainty range. However, in many routine laboratory analyses the experimental uncertainty is actually known, either from extended previous experience or by a review of the inherent variability in the measurement process itself.

We saw in 3.1.1 that, if the uncertainty is already known, we replace the *t*-value with the equivalent value of *z* for the relevant level of confidence. The *t*-test then becomes a *z*-test, with

$z_C = 1.64$ for a one-sided test with 95% confidence, and

$z_C = 1.96$ for a two-sided test with 95% confidence

We can see the increased analytical *power* achieved through knowing the experimental uncertainty by reference to the 'Blood alcohol' case study. In 3.1.2, the one-sided *t*-test with a mean value of 82.82 for five data values gives $p = 0.034$, but by using the data in Fig 1.19 with a known standard deviation uncertainty of 2.0 we get a more significant result with $p = 0.00075$ for the same sample mean of 82.82.

3.2 Analysis of variance

As its name suggests, the analysis of variance technique tests for significant differences by analysing variances within the data. The key statistic in this process is the sum of squares, *SS*, (1.5.1) which measures the overall variation in a data set. We can then calculate the mean square, *MS*, for that data by dividing by the degrees of freedom (Eqn. 1.18):

$$MS = \frac{SS}{df}$$

The value of the mean square, *MS*, for a *single data set* is equal to the *variance* of the data.

We start by introducing the *F*-test, which is the key statistical test at the heart of the general analysis of variance (ANOVA) series of techniques.

3.2.1 ***F*-test**

The ***F*-test** tests whether there is a significant difference between the variances, s_A^2 and s_B^2, of two data samples of size n_A and n_B. We calculate the *F* statistic:

$$F_S = \frac{s_A^2}{s_B^2} \tag{3.8}$$

which has degrees of freedom (1.5.1) given by,

$$df_A = n_A - 1 \text{ and } df_B = n_B - 1 \tag{3.9}$$

The null hypothesis is that both samples have the same variance, but we would accept the one-tailed proposed hypothesis, that s_A^2 was greater than s_B^2 if

$$F_S \geqslant F_C$$

where F_C is the critical value available from tables or by using F.INV.RT(α, df_A, df_B).

Referring to Fig 3.3, the variances s_A^2 and s_B^2 are calculated in L2 and L3 by simply squaring the standard deviations in H2 and H3, and the *F*-statistic, $F = 1.58$, in L5 is derived using Eqn 3.8. The degrees of freedom for both samples in K2 and K3 are derived from the sample sizes using Eqn 3.9.

We calculate the *p*-value in L6 using the function: F.DIST.RT(F_S, df_A, df_B):

p-value: p = F.DIST.RT(L5, K2, K3) = 0.36

Since $p > 0.05$, we conclude that there is no evidence of a difference in variance, and the observed difference could have occurred by random chance.

Levine's test is also a test for a difference in variance, but it is a distribution-free test that does not assume the normal distribution. It is not used in the ANOVA calculations, but it is used in SPSS for testing for equality of variances between samples (6.2.5).

3.2.2 Basic principle of ANOVA calculations

The family of 'analysis of variance' calculations (ANOVAs) start by identifying the different sources of variation within the data (often referred to as partitioning the variance). The variations in a simple ANOVA are combined using the *sum of squares*:

$$SS_{TOTAL} = SS_{RANDOM} + SS_{FACTOR} \tag{3.10}$$

For example, the *factor* being tested could be a variation *between* the mean values of two samples and the *random* variations would then be due to the experimental uncertainty *within* each sample.

Taking the degrees of freedom of each component into account, we can calculate (Eqn 1.18) the *mean square* components:

MS_{RANDOM} is the variance due only to the random experimental uncertainty, and

MS_{FACTOR} is the additional variance due to the factor that is being tested.

The ANOVA process compares these *mean square* values, and if MS_{FACTOR} is much greater than MS_{RANDOM} then it would be clear that the factor must be having an effect. We use the *F*-test to test whether MS_{FACTOR} is *significantly* greater than MS_{RANDOM}:

$$F = \frac{MS_{FACTOR}}{MS_{RANDOM}} \tag{3.11}$$

MS_{RANDOM} often appears as MS_{ERROR} in ANOVA results tables.

We will use the same example as for the two sample *t*-test to illustrate a simple ANOVA calculation.

Analysis of variance: Excel analysis for Fig 3.4. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: River *pH* / 3. ANOVA calculations

—continued from 3.1.3, leading to 3.4.2

In Fig 3.4, the *pH* values of two rivers, *A* and *B*, are recorded in B2:E2 and B3:E3 respectively, and we wish to test if there is a significant difference in their mean values.

	A	B	C	D	E	F	G	H
1							Mean	Variance
2	A	7.56	7.52	7.7	7.61		7.60	0.0060
3	B	7.47	7.4	7.49	7.55		7.48	0.0038
4	Sample size, *n* =	4		Factor levels, *k* =	2			
5	MS(between) =	0.0288		Variance of sample means, *VSM* =			0.0072	
6	MS(within) =	0.0049		Mean of sample variances, *MSV* =				0.0049
7	*df* (between) =	1						
8	*df* (within) =	6		*F* =	5.85	*p* =	0.0520	

Fig 3.4 Case study: River *pH* / 3. ANOVA calculations

The first step is to calculate the *experimental* variability separately *within* each set of replicate measurements, recording the variance values in H2 and H3, and then calculate the mean of these sample *variances*, *MSV*, in H6. This value will not be affected by any differences *between* the samples.

Mean of the sample variances, *MSV*: [H6] = AVERAGE(H2:H3) = 0.0049

This gives directly the mean square (within), MS_W, in B6, which is the best estimate of the *random* variance in the data, and is not affected by the difference between the samples.

$MS_W = MSV = 0.0049$

The second step is to include the variability due to the *factor*, which, in this case, has resulted in the difference in the two mean values in G2 and G3. We calculate the *variance* of these sample mean values, *VSM*, in G5.

Variance of the sample means, VSM: [G5] = VAR.S(G2:G3) = 0.0072

The factor variance due to the difference between the means is then given by mean square (between), MS_B, which is calculated in B5 as

$MS_B = VSM \times n = 0.0288$.

The *n* term reverses the reduction in uncertainty that had occurred when taking the mean of *n* values in each sample (Eqn 1.21).

The terms 'within' and 'between' are convenient here because they refer directly to calculations *within* and *between* the samples, and the terminology also appears elsewhere in understanding other data analysis techniques (e.g. 3.6.2). We can also relate these terms respectively to the 'random' and 'factor' terms in the ANOVA calculation:

$MS_W = MS_{RANDOM}$
$MS_B = MS_{FACTOR}$.

The *F*-test is then used to test whether MS_B is significantly greater than MS_W. If it is, then this would show that the factor being tested was also significant. We test for any significant difference by calculating the *F*-statistic in E8.

$F_s = \frac{MS_B}{MS_W}$: [E8] = B5 / B6 = 5.85

We then calculate the associated *p*-value, with degrees of freedom in B7 and B8, using the function

p-value: [G8] = F.DIST.RT(E8, B7, B8) = 0.052

We see that this gives the same value as the *t*-test in 3.1.3. There is not enough evidence at a significance of 0.05 for a difference between the *pH* values of the two rivers.

3.2.3 One-way ANOVA

In 3.2.2 we developed the working principle of the ANOVA using the same data as for the two sample *t*-test in 3.1.3. In practice however, ANOVA calculations are normally performed

using a dedicated statistics software package, and, for comparison, Fig 3.5 gives the output that would be obtained using statistical software (in this example, Minitab) for the same data. The use of SPSS and Minitab for ANOVA analyses is given in Section 6.3.

Source	DF	SS	MS	F	P
River	1	0.02880	0.02880	5.85	0.052
Error	6	0.02955	0.00492		
Total	7	0.05835			

Fig 3.5 ANOVA results (Minitab) for the data in Fig 3.4

In addition to the same *MS* values, *F*-statistic, and *p*-value, the typical ANOVA calculation also presents the *SS* values, from which the mean squares, *MS*, are then derived by dividing by the relevant degrees of freedom (Eqn 1.18). Note that the addition of sums of squares agree with Eqn 3.10:

$$0.05835 = 0.02955 + 0.02880.$$

This introductory example was effectively testing for the difference between two *levels* of one factor, where the factor was the *choice* of water sample, but, for just two levels, there was no advantage in using an ANOVA over the simple *t*-test. The real value of the ANOVA procedure is that, unlike the *t*-test, it can be extended to test for a difference between *three or more* levels of the factor, by simply including the variations from all levels in the calculation of *MS*(between).

One-way ANOVA: Minitab and SPSS analyses leading to Fig 3.8. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: **Catalyst / 1. One-way ANOVA (overview)**

–leading to 3.3.1

Fig 3.6 gives the percentage yields from a chemical reaction using three catalysts, *C1*, *C2*, and *C3*. The measurements are repeated over four days, *D1*, *D2*, *D3*, and *D4*, and we wish to test whether there is a significant difference in yield due to the choice of catalyst.

3.3.1 / 2. Two-way ANOVA: Develops the analysis to include the effect of multiple factors.

3.3.2 / 3. Interactions: Develops the two-way ANOVA to include an interaction between factors.

	A	B	C	D	E
1		D1	D2	D3	D4
2	C1	79	78	82	77
3	C2	74	76	71	72
4	C3	78	75	75	81

Fig 3.6 Percentage yields using catalyst, *C1*, *C2*, and *C3* on four days *D1*, *D2*, *D3*, and *D4*

In this initial calculation, we will treat the yields on the four days as *replicate* measurements for each catalyst, giving three samples of four replicates each. This data is said to be *balanced* because all three levels have the same number of data values.

The first step is to put the data into the *column* format that is expected in both Minitab and SPSS for unrelated samples:

Yield%	Catalyst	Day	CatN
79	C1	D1	1
74	C2	D1	2
78	C3	D1	3
78	C1	D2	1
76	C2	D2	2
75	C3	D2	3
82	C1	D3	1
71	C2	D3	2
75	C3	D3	3
77	C1	D4	1
72	C2	D4	2
81	C3	D4	3

Fig 3.7 Stacked data from Fig 3.6

In this format, every variable and factor is entered into its own column. Each data value is identified as a separate record on a unique row, and the entries in the other columns provide the information related to that particular data value.

In this analysis, the *factor* that is being tested is the choice of catalyst, with *C1*, *C2*, and *C3*, being the three *levels*. The variable *CatN* has been added to express the factor levels in numeric form, as this is required by SPSS for the basic analysis used in Fig 3.8(b). The days on which specific measurements were made are also recorded, but in this analysis they are considered to be replicate measurements for each catalyst.

The ANOVA tests the null hypothesis:

H_0: The sample means for all catalysts are equal, $\mu_{C1} = \mu_{C2} = \mu_{C3}$.

The calculation requires that the data values are collected *independently*, with the random variations following a *normal distribution*, and with *equal variance* (homoscedasticity) for the values recorded under different experimental conditions. For data recorded as a proportion or percentage, we could consider transforming the data with an 'arcsin($\sqrt{P}$)' transformation (5.4.7). However, in this example the variations in the values are small compared to the difference from either end (0% or 100%) of the data range and such a transformation is unlikely to be necessary.

Source	DF	SS	MS	F	P
Catalyst	2	69.50	34.75	5.85	0.024
Error	9	53.50	5.94		
Total	11	123.00			

(a) Minitab

Yield

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	69.500	2	34.750	5.85	.024
Within Groups	53.500	9	5.944		
Total	123.000	11			

(b) SPSS

Fig 3.8 One-way ANOVA results using the data in Fig 3.7

The ANOVA result tables using basic one-way analyses of variance in Minitab and SPSS are given in Fig 3.8. The factor variance is identified as the 'catalyst' for Minitab and 'between groups' for SPSS, and the random variance as 'error' and 'within groups' respectively. In both cases, the relevant *F*-statistic is calculated as 5.85 which gives a *p*-value = 0.024.

The *total* degrees of freedom for the ANOVA is equal to the number of data values (initial bits of information) minus one, giving 11. The degrees of freedom for the *factor*

is equal to $n - 1$ where n is the number of levels in the factor. In this case, there are three catalysts which gives three levels and two degrees of freedom for the *catalyst* factor. The difference between these values then gives the degrees of freedom, 9, for the random *error* variance.

Since $p < 0.05$, we reject the null hypothesis and conclude that there is a difference between the mean values of *at least one pair* of samples. The ANOVA procedure is able to detect whether a difference occurs between several samples, but it does not identify which level is different from which other level(s). The procedures for finding *where* the differences lie is dealt with in 3.2.4 under post hoc tests.

It is reasonable to ask whether using an ANOVA to identify a difference between several samples has any advantage over using *multiple* t-tests to test for significant differences between each *pair* of samples separately. In fact, there are two problems with using multiple tests to *identify* differences:

- The numbers of pairs to be tested would increase rapidly with more samples, e.g. just four samples would require six possible tests.
- The probability of a Type I error (typically the default 0.05) occurs for *every* t-test, giving an increasing probability of an error in *at least one* of the tests as the number of tests increases, whereas the error probability for the *single* calculation ANOVA remains at the default 0.05.

The problem of interpreting multiple p-values may be addressed by using the Bonferroni correction (1.6.4) which modifies the required significance level, reducing the Type I error probability:

$$\text{Bonferroni significance} = \alpha / n$$

where n is the number of multiple tests.

However, given the problems with multiple t-tests, it is far simpler just to use a single ANOVA.

The uses of Minitab and SPSS for ANOVA calculations are developed in 6.3.5, where we see that it is possible to perform ANOVA calculations using the general linear model (Section 3.4) which gives greater flexibility to the analysis.

3.2.4 **Post hoc comparison tests**

The ANOVA analysis detects whether there is a significant difference between one or more of the sample mean values, but it does not identify which sample(s) may be different from the others.

There exist a range of procedures called *post hoc* (from Latin 'after this') tests that can be used to identify where any differences lie, *after* the ANOVA has confirmed that they do exist. These tests are also called *comparison* tests. A common test is the Tukey test which compares each pair of samples to identify differences. Although this sounds like multiple t-tests, the Tukey procedure uses variance data from *all* the samples in assessing the differences between pairs and is less likely to make errors than using multiple t-tests.


```
Grouping Information Using Tukey Method and 95.0% Confidence
Catalyst  N   Mean  Grouping
C1        4  79.00  A
C3        4  77.25  A B
C2        4  73.25    B
Means that do not share a letter are significantly different.

Tukey 95.0% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Catalyst

Catalyst = C1  subtracted from:
Catalyst   Lower  Center   Upper  --------+---------+---------+--------
C2       -10.57  -5.750  -0.9347  (-------*-------)
C3        -6.57  -1.750   3.0653        (-------*-------)
                                  --------+---------+---------+--------
                                      -6.0       0.0       6.0

Catalyst = C2  subtracted from:
Catalyst    Lower  Center  Upper  --------+---------+---------+--------
C3        -0.8153   4.000  8.815                  (-------*-------)
                                  --------+---------+---------+--------
                                      -6.0       0.0       6.0
```

Fig 3.9 Tukey test output (Minitab 16) for the data in Fig 3.7 (Minitab 17 provides the same information in a rearranged format)

The Minitab 16 output from a Tukey test for the data in Fig 3.7 (ignoring the effect of different days) is given in Fig 3.9. The 'grouping information' section shows that the catalysts *C1* and *C2* are significantly different from each other because *C1* is just in group A and *C2* is just in group B, but *C3* appears in both groups A and B and is therefore not significantly different from *C1* or *C2*. The 'pairwise comparison' sections show the *confidence intervals* of the *differences* between catalysts both numerically and also graphically within the brackets. The fact that the confidence interval for the difference between *C1* and *C2*, from −10.57 to −0.9347, does not include 0 shows that this is a significant difference. However, the confidence interval of *C1* compared with *C3 overlaps* 0 and hence shows no significant difference. The third plot of *C2* compared with *C3* shows that the confidence interval of the difference also *overlaps* 0, and thus there is no significant difference between these catalysts. Minitab 17 uses a graphical plot to display the confidence intervals.

Multiple Comparisons

Dependent Variable: Yield
Tukey HSD

(I) CatN	(J) CatN	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	5.75000*	1.72401	.021	.9365	10.5635
	3.00	1.75000	1.72401	.586	-3.0635	6.5635
2.00	1.00	-5.75000*	1.72401	.021	-10.5635	-.9365
	3.00	-4.00000	1.72401	.104	-8.8135	.8135
3.00	1.00	-1.75000	1.72401	.586	-6.5635	3.0635
	2.00	4.00000	1.72401	.104	-.8135	8.8135

*. The mean difference is significant at the 0.05 level.

Fig 3.10 Tukey test output (SPSS) for the data in Fig 3.7

The equivalent output from SPSS is given in Fig 3.10, where we see the same conclusions. The three catalysts have been coded by scale values 1, 2, and 3 respectively, and the same confidence intervals have been calculated between pairs of samples. SPSS also calculates a p-value for the significance of the difference between each pair, with:

1.00 and 2.00 (i.e. between *C1* and *C2*) $p = 0.021$ indicating a *significant* difference

1.00 and 3.00 (i.e. between *C1* and *C3*) $p = 0.586$ indicating *no* significant difference

2.00 and 3.00 (i.e. between *C2* and *C3*) $p = 0.104$ indicating *no* significant difference

which is in agreement with the result from Minitab.

There is a wide range of possible post hoc tests. Most will set a significance level, sometimes called the *family rate* that takes into account the 'family' of multiple tests being made, i.e. the maximum probability of one Type I error in *all* of the comparisons. See, for comparison, consideration of the Bonferroni correction in 1.6.4.

Tests available in Minitab and/or SPSS include:

Table 3.1 Post hoc tests

Tukey HSD	Compares all samples pairwise. It is a common default choice. HSD (honestly significantly different).
Fisher LSD	Compares all samples pairwise. Uses *individual* error rate which is the maximum probability of a Type I error in *every* comparison. LSD (least significant difference).
Dunnett	Compares each sample with one *control* sample which needs to be identified.
Hsu's MCB	Compares each sample with the sample which has either the highest or lowest mean (to be selected). MCB (multiple comparisons with the best).
Scheffe	Allows all possible combinations of sample means to be tested, and tends to be more conservative.
Bonferroni	Based on multiple t-test but with adjusted significance level.
Sidak	As for Bonferroni, but producing tighter bounds for the confidence intervals.

3.3 Multiple factors ANOVA

We saw in Section 3.2 the use of a one-way ANOVA to perform a one factor, multi-level, analysis, but we now extend the concept of the ANOVA to test more than one factor. Each factor will have two or more levels, and each combination of different levels is sometimes called a *treatment*. The ANOVA is said to be balanced if every treatment has the same number of replicate measurements. In this section, we are only considering *univariate* data, i.e. we are measuring the *same* output variable for different factor levels.

3.3.1 Two-way ANOVA

We start with a two-way ANOVA by illustrating a variant of the analysis performed in 3.2.3.

Case study: Catalyst / 2. Two-way ANOVA

–continued from 3.2.3, leading to 3.3.2

Fig 3.11 gives similar data to that in Fig 3.6, with the percentage yields from a chemical reaction, but using three *new* catalysts, *C4*, *C5*, and *C6*. The measurements are repeated over four days, *D1*, *D2*, *D3*, and *D4*. Again, we wish to test whether there is a significant difference in yield due to the choice of catalyst.

	A	B	C	D	E
1		D1	D2	D3	D4
2	C4	76	77	74	74
3	C5	75	74	72	71
4	C6	77	75	75	72

Fig 3.11 Reaction yields as functions of *catalyst* and *day*

Multi-factorial ANOVA (Minitab): Analysis for data in Figs 3.11 and Fig 3.15. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Using a *one*-way ANOVA to test for differences between the new catalysts, *C4*, *C5*, and *C6* in Fig 3.11, gives the results in Fig 3.12 with $p = 0.236$, which suggests that there is no significant difference.

```
Source     DF     SS     MS     F      P
Catalyst    2  11.17   5.58  1.70  0.236
Error       9  29.50   3.28
Total      11  40.67
S = 1.810    R-Sq = 27.46%    R-Sq(adj) = 11.34%
```

Fig 3.12 One-way ANOVA output (Minitab) for the data in Fig 3.11

Multi-factorial ANOVA (SPSS): Analysis for data in Figs 3.11 and Fig 3.15. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

However, if we now plot the individual yields for each of the three catalysts for the four days we see a pattern emerging in Fig 3.13 (see 6.4.3 for deriving this 'interaction' plot).

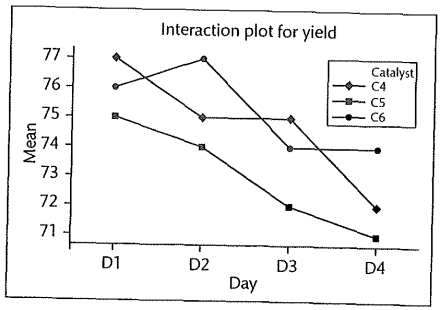


Fig 3.13 Interaction plot (Minitab 16) for the data in Fig 3.11

There appears to be a general downward trend in the yield over the four days. This may, for example, be due to the degradation over time of chemical reagents that were produced at the beginning of the investigation.

From a statistical perspective, the one-way ANOVA is unable to tell the difference between the *systematic* changes over the four days and possible *random* variations, and can only interpret the changes as an increased *uncertainty* in the data giving $SS_{ERROR} = 29.5$. With this apparent increase in uncertainty, the calculated F-statistic will be reduced, making it less likely that any differences between the catalysts can be seen as significant.

However, we can identify the effect of the different days as a *second* factor in the analysis, and perform a *two*-way ANOVA, giving the results in Fig 3.14 which reports p-values for both of the *Catalyst* and *Day* factors. For the analysis of multiple factors we use the general linear model options for performing ANOVAs, as described in Sections 3.4 and 6.4.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Catalyst	2	11.1667	11.1667	5.5833	6.93	0.028
Day	3	24.6667	24.6667	8.2222	10.21	0.009
Error	6	4.8333	4.8333	0.8056		
Total	11	40.6667				

S = 0.897527 R-Sq = 88.11% R-Sq(adj) = 78.21%

Fig 3.14 Two-way GLM/ANOVA output (Minitab) for the data in Fig 3.11

The *Day* factor has $p = 0.009$ showing that *Day* is indeed a significant effect, and we also see that the ANOVA has been able to separate the variance due to the *Day* from the *Error*, reducing the remaining random uncertainty, from $SS_{ERROR} = 29.5$ in Fig 3.12 to $SS_{ERROR} = 4.8$ in Fig 3.14. The goodness of fit of the model (4.4.1) is also shown to increase from R^2(adj) from 11.3% to 78.2%. With this decreased uncertainty and improvement in fit, the analysis is now able to confirm, with $p = 0.028$, that there is a significant difference between the different levels of *Catalyst*.

3.3.2 Interactions between the different factors

We can take the complexity of the ANOVA one stage further by looking at possible *interactions* between the different factors.

Case study: **Catalyst / 3. Interactions**

–continued from 3.3.1

In a further investigation, Fig 3.15 gives the yields of two *new* catalysts *C6* and *C7*, measured at three increasing temperatures, *T1*, *T2*, and *T3*. It is important to note that, in this data set, there are two *replicate* measurements made at each combination of factor levels, giving *two rows of data* for each catalyst.

	A	B	C	D
1		T1	T2	T3
2	C6	77	73	74
3	C6	74	75	78
4	C7	69	74	78
5	C7	70	76	78

Fig 3.15 Reaction yields as functions of *catalyst* and *temperature*

Performing a two-way ANOVA calculation with just the *catalyst* and *temperature* as possible factors gives the results in Fig 3.16, which appear to show that neither the choice of catalyst or the temperature have any significant effect on the yield of the reaction.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Catalyst	1	3.000	3.000	3.000	0.44	0.527
Temp	2	40.667	40.667	20.333	2.96	0.109
Error	8	55.000	55.000	6.875		
Total	11	98.667				

S = 2.62202 R-Sq = 44.26% R-Sq(adj) = 23.35%

Fig 3.16 Two-way GLM/ANOVA output (Minitab 16) for the data in Fig 3.15

However, it is again useful to present the data visually, as in Fig 3.17, where we have plotted the *mean of each pair* of replicate yields against the increasing temperatures. The points from the different catalysts are linked for clarity.

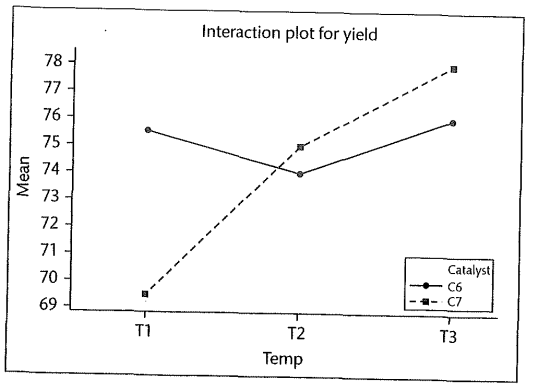


Fig 3.17 Interaction plot (Minitab 16) for the data in Fig 3.15

From the graph it appears that there may be a difference between the ways in which the *level* of temperature influences the *effect* of each catalyst. The yield for *C6* appears to be unchanged with temperature, whereas the yield for *C7* increases with temperature. This interlinked behaviour is described as an *interaction* between the two factors.

We can include the interaction as an additional factor to be tested in the ANOVA, giving the output in Fig 3.18 for Minitab and Fig 3.19 for SPSS, both using the general linear model analyses.

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Catalyst	1	3.000	3.000	3.000	1.06	0.343
Temp	2	40.667	40.667	20.333	7.18	0.026
Catalyst*Temp	2	38.000	38.000	19.000	6.71	0.030
Error	6	17.000	17.000	2.833		
Total	11	98.667				

S = 1.68325 R-Sq = 82.77% R-Sq(adj) = 68.41%

Fig 3.18 Two-way GLM/ANOVA output (Minitab) with *interaction* for the data in Fig 3.15

The important conclusion is that the *interaction* between catalyst and temperature is significant, with $p = 0.030$. In this respect, *both* catalyst and temperature are significant factors in the overall model, even though the p-value for the 'average' catalyst effect is greater than 0.05. By including interaction as an additional factor, the R^2(adj) value has increased from 23.35% to 68.41% demonstrating the better overall fit of the model to the experimental values.

Dependent Variable: Yield

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	81.667[a]	5	16.333	5.765	.027
Intercept	66901.333	1	66901.333	23612.235	.000
Catalyst	3.000	1	3.000	1.059	.343
Temp	40.667	2	20.333	7.176	.026
Catalyst * Temp	38.000	2	19.000	6.706	.030
Error	17.000	6	2.833		
Total	67000.000	12			
Corrected Total	98.667	11			

a. R Squared = .828 (Adjusted R Squared = .684)

Fig 3.19 Two-way GLM/ANOVA output (SPSS) with *interaction* for the data in Fig 3.15

An *interaction* is frequently labelled as product of the two (or more) factors, e.g. *Catalyst*Temp*. The use of the *product* in labelling an interaction also reflects the way in which an interaction can be modelled in a GLM of the system (Eqn 3.12).

It is important to note that an ANOVA can only identify an *interaction* if the data includes *replicate measurements* under the same combinations of factor levels. The ANOVA needs to estimate the *experimental uncertainty* before it can separate the interaction from experimental error. Comparing the two sets of results, we can see that, in Fig 3.16, the variance due to the interaction is 'hidden' within the *Error* term with $SS_{ERROR} = 55.0$, but in Figs 3.18 and 3.19 we have a partitioning of this variance to give $SS_{INTERACTION} = 38.0$ plus $SS_{ERROR} = 17.0$.

3.3.3 **Analysis of Covariance, ANCOVA**

The factors considered in previous sections each have a *limited* number of levels, typically defined by the experiment, e.g. three levels of temperature in Fig 3.15. We now introduce a factor that is a *continuous* variable whose value we do not necessarily control in the selection of our subjects for analysis, but which might show some correlation with the measured variable. This is referred to as a *covariate*.

ANCOVA
Excel and Minitab analyses for Fig 3.20. See also 6.4.8. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

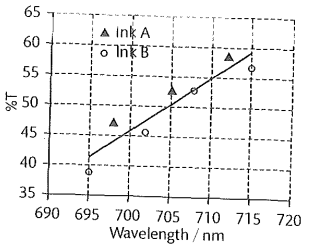
Case study: **Ink analysis / 5. ANCOVA analysis 1**

–continued from 5.1.6 (overview), 5.2.3, and 3.6.2

The data in Fig 3.20(a), plotted in Fig 3.20(b), shows the percentage transmission, *%T*, for two inks *A* and *B* measured at *different* wavelengths, and we wish to test for a *vertical* difference in *%T* between the lines. For a related problem in 3.6.2, we are able to use a 'repeated measures' analysis which requires that measurements are made for each line at the *same* wavelengths. This is not the case here, but we will assume that the *%T* values for each line varies *linearly* with wavelength, as appears in Fig 3.20(b).

	A	B	C	D	E	F	G
1	Ink	Wavelength	%T	Best-fit	Residuals		
2							
3	A	698.00	47.16	43.98	3.18	Best-fit line:	
4	A	705.00	52.84	50.21	2.63	Slope =	0.89
5	A	712.00	58.63	56.45	2.18	Intercept =	-577.95
6	B	695.00	38.48	41.30	-2.82		
7	B	702.00	45.37	47.54	-2.17		
8	B	708.00	52.56	52.89	-0.33		
9	B	715.00	56.45	59.12	-2.67		
10	Two-Sample t-test results:						
11		p =	0.432	p =	0.001		

(a) Excel calculations



(b) Plotting data from (a)

Fig 3.20 Case study: Ink analysis / 5. ANCOVA analysis

We wish to test for a difference in %T between lines *A* and *B*, but a two sample *t*-test based on the values in column C gives $p = 0.432$ (in C11) which fails to identify a significant difference. However, in Fig 3.20(b), we can see that there may be a difference between the *A* and *B* samples in that the *A* points tend to be more to the *top left* of the graph and the *B* points to the *bottom right*. However, the *t*-test *only* aims to test for a difference between the two groups *in the vertical direction*, and the effect of the wavelength covariate has been to spread out both samples vertically, giving the appearance of a greater uncertainty.

In the analysis of covariance, ANCOVA, we first use a *linear regression analysis* to calculate the slope (G4) and intercept (G5) values for the best-fit straight line that relates *%T* to wavelength. We then calculate the points on the best-fit line (column D) for each wavelength, giving the trendline in Fig 3.20(b). Then, in column E, we take the differences between the values in columns C and D to get the *residuals* for each wavelength. These residuals are plotted in Fig 3.21, where we can see that there is a clear difference in vertical *residuals* between lines *A* and *B*. If we now perform a two sample *t*-test between the residual values we get $p = 0.001$ (E11), reporting a highly significant difference between the two lines.

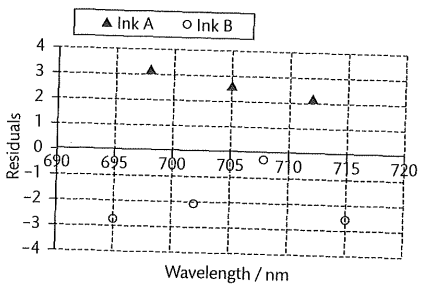


Fig 3.21 Residual values from Fig 3.20(b)

For comparison, we use the GLM/ANCOVA analysis on the three lines *A*, *B*, and *C*, using the data from Fig 3.40(a):

Minitab

Minitab > Stat > ANOVA > General Linear Model > Fit General Linear Model...
Reponses: *%T* **Factors:** *Ink* **Covariates:** *Wavelength*
→ Output: Similar to Fig 3.22 (a)

and then for the post hoc tests and data plots:

Minitab > Stat > ANOVA > General Linear Model > Comparisons...
Response: *%T* **Type of comparison:** ∇ ***Pairwise***
Select a post hoc test (3.2.4), e.g. ☑-*Tukey*
Choose terms for comparison: Double click on *ink* to see: C Ink
> Graphs... ☑-Interval plot for difference in means
> Results...
☑-Grouping information
☑-Tests and confidence intervals
→ Output: Same information as in Fig 3.22 (b)

```
Source       DF   Seq SS   Adj SS   Adj MS        F      P
Wavelength    1  1064.36  1064.36  1064.36  1469.07  0.000
Ink           2   169.96   169.96    84.98   117.29  0.000
Error        20    14.49    14.49     0.72
Total        23  1248.82
S = 0.851185   R-Sq = 98.84%   R-Sq(adj) = 98.67%

Term           Coef  SE Coef       T      P
Constant    -567.11    16.12  -35.17  0.000
Wavelength  0.87455  0.02282   38.33  0.000
```

(a) Factor significance

```
All Pairwise Comparisons among Levels of Ink
Ink = A  subtracted from:
Ink   Lower  Center  Upper  ------+---------+---------+---------+
B    -6.222  -5.145  -4.068       (----*----)
C    -7.116  -6.039  -4.961  (----*----)
                             ------+---------+---------+---------+
                                -6.0      -4.0      -2.0       0.0

Ink = B  subtracted from:
Ink   Lower  Center  Upper  ------+---------+---------+---------+
C    -1.971  -0.8938  0.1836                      (----*----)
                             ------+---------+---------+---------+
                                -6.0      -4.0      -2.0       0.0
```

(b) Post hoc (Tukey) test output

Fig 3.22 ANCOVA output for data from Fig 3.40(a) (Minitab 16) (Minitab 17 uses a graphical plot to display the confidence intervals)

The p-values in the ANCOVA test output in Fig 3.22(a) confirm that the wavelength effect is significant and also that there is a significant difference somewhere between the inks. It also reports the linear regression calculation to find the best-fit straight line for the three lines A, B, and C, giving slope, $m = 0.875$ and intercept, $c = -567$, which closely matches the values of 0.89 and −578 that we obtained above using reduced data for just lines A and B.

From the Tukey test results in Fig 3.22(b), we see that neither of the confidence intervals (given by the bracketed ranges) for the differences between A and B and A and C overlap 0, so we conclude that there is a significant difference between A and both B and C. This agrees with the Excel result obtained for A and B in Fig 3.20(a). However, the confidence interval, −1.97 to 0.184, for the difference between B and C does overlap 0, which means that we are unable to detect the difference between B and C.

The analysis of this data using SPSS is carried out in 6.4.8.

3.4 General linear model

The general linear model (GLM) uses the techniques of linear regression to develop a mathematical model to describe the factor effects, interactions, and uncertainties in a system. The historical advantage of the ANOVA was perhaps that it gave a more intuitive understanding of the analysis and that small problems could be analysed by hand. However, with modern software the GLM provides a more comprehensive analytical approach that includes regression, ANOVAs, and t-tests.

The GLM develops a regression analysis, as in Chapter 2, and assumes that the dependent variable is derived from a normal distribution with a constant variance, which is consistent with a wide range of other familiar techniques, e.g. t-test, ANOVA, linear regression. Not only does it duplicate these standard analyses, but it also provides a greater flexibility in their implementation for different analytical problems. The generalized linear model (3.4.7) provides more flexible options permitting a choice in the underlying distribution and integrated transformations.

3.4.1 General linear model

We saw in (2.1.1) that the behaviour of y as a simple function of x can be written as

$$y = mx + c \text{ or } y = b_0 + bx.$$

If the variation of y is dependent on more factor variables, x, then we can express it using a linear combination of x_A, x_B, etc.:

$$y = b_0 + b_A x_A + b_B x_B + b_{AB} x_A x_B + b_C x_C \ldots \tag{3.12}$$

where the $x_A x_B$ term represents an *interaction* (3.3.2) between the factors x_A and x_B.

It is also possible to introduce a *power* term into the analysis by using 'interaction' terms through products of the *same* variable: $x_A^2 = x_A\, x_A$ and $x_B^3 = x_B\, x_B\, x_B$, etc.

We can investigate the effect of the interaction term if we choose example values for the coefficients, $b_0 = 0.0$, $b_A = 0.5$, $b_B = 0.5$ and $b_{AB} = 4.0$, producing the equation:

$$y = 0.5\ x_A + 0.5\ x_B + 4.0\ x_A x_B.$$

We calculate the observed values of y for values of $x_A = 0.5$ and 2.0 and $x_B = 0.5$ and 3.0, giving:

Table 3.2 Interaction calculation

	$x_B = 0.5$	$x_B = 3.0$
$x_A = 0.5$	$y = 0.25 + 0.25 + 1.0 = 1.5$	$y = 0.25 + 1.5 + 6.0 = 7.75$
$x_A = 2.0$	$y = 1.0 + 0.25 + 4.0 = 5.25$	$y = 1.0 + 1.5 + 24.0 = 26.5$

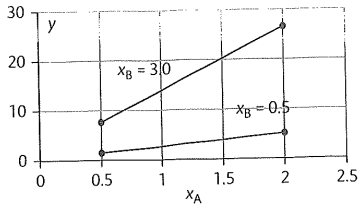


Fig 3.23 Graphical illustration of an interaction

We can plot the values from Table 3.2 using the graph in Fig 3.23, in which we see that, due to the interaction term, the two lines have different *slopes*, and the *effect* of x_A on y is *dependent* on the value of x_B.

3.4.2 GLM, ANOVA, and the *t*-test

It is useful to use a simple example to demonstrate how the linear regression approach can provide the same analytical results as the familiar *t*-test.

General linear model: Excel analysis for Fig 3.24. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: River *pH* / 4. GLM, ANOVA, and the *t*-test

—continued from 3.1.3 and 3.2.2

The independent samples of *pH* data for two rivers, *A* and *B*, were introduced in 3.1.3, and are reproduced here in Fig 3.24. A *t*-test and ANOVA calculation (3.2.2) gave the same *p*-value = 0.052 for a significant difference in their mean values. We now use a linear regression analysis to retest for this difference.

For consistency with linear regression in Chapter 2, we identify the measured *pH* values in Fig 3.24 as the '*y*' response values, and identify the two levels, *A* and *B*, with dummy '*x*' values, 1 and 2. We can now plot the data on the *x*–*y* graph in Fig 3.25.

	A	B	C	D	E	F	G	H	I
1		'x'	*pH*						
2	A	1	7.56	*n* =	8				
3	A	1	7.52	*SE* =	0.0702				
4	A	1	7.7						
5	A	1	7.61		*SS*	*df*	*MS*	*F*	*p*
6	B	2	7.47	**Regression (B):**	0.0288	1	0.0288	**5.85**	**0.052**
7	B	2	7.4	**Residual (W):**	0.0296	6	0.0049		
8	B	2	7.49	**Total:**	0.0584	7			
9	B	2	7.55						

Fig 3.24 Case study: River *pH* / 4. GLM, ANOVA, and the *t*-test

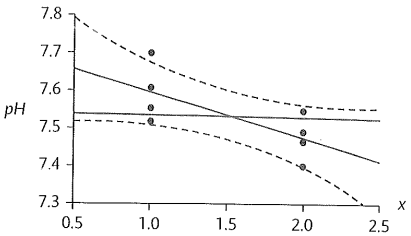


Fig 3.25 *pH* values for two rivers, coded as '1' and '2'

A hypothesis test for a significant *difference* between the mean values of the data at $x = 1$ and $x = 2$ becomes equivalent to showing that the line between their true mean values will *not* have a zero slope. Hence the null hypothesis for this test becomes:

H_o: The linear relationship between *pH* and '*x*' has a *zero* slope.

Using the techniques in Section 2.2, we can draw the 95% confidence limits, given as dashed lines in Fig 3.25, within which possible best-fit lines may lie. We see immediately that it is just possible, within 95% confidence, to draw a horizontal best-fit line that does have a *zero slope* at a constant value of about 6.53 *pH*. We cannot therefore reject the null hypothesis, and this implies that we cannot be confident at 0.05 significance that there is a *difference* between the samples *A* and *B*. This agrees with the *t*-test conclusion in 3.1.3.

Using the same techniques from 2.1.2 and 2.1.4, we derive the values for a hypothesis test:

SE_{REG}:	[E3] = STEYX(C2:C9, B2:B9)	= 0.0702	
SS_{RESID}:	[E7] = E3^2*(E2 – 2)	= 0.0296	(using Eqn 2.14)
SS_{TOT}:	[E8] = VAR.S(C2:C9)*(E2 – 1)	= 0.0584	(using Eqn 2.8)
$SS_{REGRESS}$:	[E6] = E8 – E7	= 0.0288	(using Eqn 2.9)

where the *factor* in the analysis is the regression fit of the data to a straight line and the *random* uncertainty is given by the residual values.

Dividing by the relevant degrees of freedom (Eqn 1.18), we can calculate the *MS* values, 0.0288 in G6 and 0.0049 in G7, and then obtain the *F*-statistic in H6 using Eqn 3.8:

$$F = \frac{MS_{REGRESS}}{MS_{RESID}} = \frac{0.0288}{0.0049} = 5.85$$

The *p*-value for a non-zero slope can then be calculated:

p-value: [I6] = F.DIST.RT(H6, F6, F7) = 0.052

This is exactly the same value as calculated using the two sample *t*-test (3.1.3) and ANOVA (3.2.2). Hence, we see the parallel between the techniques of linear regression and the analysis of variance.

Historically, statistical packages have grouped their menu options based on specific *tests*, but the use of GLM calculations has created overlaps between these tests, resulting in different

approaches to analysis. In Minitab 16, GLM options are available under both *ANOVA* and *Regression* headings. In SPSS v20 the individual tests can still be accessed through the legacy dialogs, but the main menu option under *Analyze* is *General Linear Model* followed by the identification of the data structure (univariate, multivariate, repeated measures) before presenting a range of possible analyses.

The practical use of the general linear model to conduct ANOVA 'style' *factor* analyses is developed in Chapter 6. Section 3.4.3 uses the general regression option for analysing a 'regression' problem.

3.4.3 General regression

The following case study gives a further example of the overlap, from a *regression* perspective, between regression and ANOVA techniques.

General regression (Minitab): Analysis for Fig 3.26 data. See also 7.2.5. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: Bacterial growth / 3. Difference in slopes as an interaction

—continued from 3.1.1

In 3.1.1 we used a *t*-statistic analysis which identified a difference between the *slopes* of two bacterial growth curves with a *p*-value = 0.0149. We now analyse the same data, reproduced in Fig 3.26, using a general regression model.

We wish to test whether there is a difference in the slopes of the two (nearly) straight lines, *C3* and *C2*, in Fig 5.5, over the *t*-range from 60 to 85. The same data from Fig 3.1 is reproduced in Fig 3.26, but with the output variable, *Data*, entered into the single column, B.

	A	B	C	D	E
1	*Time*	*Data*	*Conc*	*ConcN*	*Interact*
2	**60**	1.76	C3	**1**	60
3	**65**	2.78	C3	**1**	65
4	**70**	3.93	C3	**1**	**70**
5	**75**	5.06	C3	**1**	**75**
6	**80**	6.55	**C3**	1	**80**
7	**85**	7.24	**C3**	1	**85**
8	**60**	**2.98**	**C2**	**2**	**120**
9	**65**	**3.68**	**C2**	**2**	**130**
10	**70**	**5.54**	**C2**	**2**	**140**
11	**75**	**6.8**	**C2**	**2**	**150**
12	**80**	**8.55**	**C2**	**2**	**160**
13	**85**	**9.55**	**C2**	**2**	**170**

Fig 3.26 Bacterial growth as a function of time under difference conditions, *C3* and *C2*

A general linear model gives *Data* as the linear function:

$$Data = b_0 + b_1 \times Time + b_2 \times Conc + b_{12} \times Time \times Conc$$

The 'average' slope of the two lines with time will be given by the coefficient, b_1, but the *difference* in slope between the two lines will be generated by the *interaction* term, b_{12}.

The null hypothesis for the test of a difference between the two slopes is then:

H_0: The interaction term, b_{12}, is zero.

It is important to note that the concentrations are only identified here as *nominal* values, *C3* and *C2*, but this is not a problem as we do not need to calculate a numeric *value* for b_{12}. We only need to test whether it is zero or not.

Minitab

Minitab > Stat > Regression > Regression > Fit Regression Model...
Responses: *Data* **Continuous predictors:** *Time* **Categorical predictors:** *Conc*
> Model: Highlight *Time* and *Conc* in **Terms in the model,** then
Cross predictors and terms in the model - Add
Delete any cross terms that are not required.
The terms in the model should now include *Time, Conc, Time*Conc*
→ Output: similar to Fig 3.27

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	64.5545	64.5545	21.5182	365.105	0.000000
Time	1	56.0205	56.0205	56.0205	950.517	0.000000
Conc	1	7.9707	0.1723	0.1723	2.924	0.125661
Time*Conc	1	0.5632	0.5632	0.5632	9.557	0.014858
Error	8	0.4715	0.4715	0.0589		
Total	11	65.0260				

Fig 3.27 General regression calculation for data from Fig 3.26 (Minitab 16)

This gives $p = 0.0149$ for the interaction term which agrees with the calculation in 3.1.1, again showing that there is a significant difference between the two slopes.

As a matter of interest, the difference, for *Conc*, between the 'sum of squares' values *seqSS* = 7.907 and *adjSS* = 0.1723 occurs because *seqSS* is calculated *before* the interaction term is taken into account, and the variance contribution then becomes less *after* including the interaction (*adjSS*). This topic is discussed in 3.4.5.

It is also useful to note that, if we were using a standard ANOVA calculation, it would not be possible to analyse for an *interaction* term, because this data does not include any replicate measurements from which the ANOVA could assess the experimental uncertainty (3.3.2). However, the GLM regression calculation is able to assess this uncertainty by measuring the *residual values* between the data values and the best-fit linear model.

It is possible to derive the same results by performing a standard regression calculation in Minitab 16, but to do this it is necessary to code the concentrations with the dummy *numeric* variable, *ConcN*, in column D, and introduce a new dummy variable, *Interact*, in column E whose value is simply the product *Time*ConcN*.

Minitab 16

Minitab> Stat > Regression > Regression...
Response: *Data*
Predictors: *Time ConcN Interact*
→ Output: Fig 3.28

```
The regression equation is
Data = - 9.90 + 0.177 Time - 2.05 ConcN + 0.0507 Interact

Predictor      Coef  SE Coef      T      P
Constant     -9.903    1.895  -5.23  0.001
Time        0.17691  0.02595   6.82  0.000
ConcN        -2.049    1.198  -1.71  0.126
Interact    0.05074  0.01641   3.09  0.015
```

Fig 3.28 Standard regression calculation in Minitab 16 for data from Fig 3.26

The values of the coefficients, b_0, b_1, b_2, and b_{12}, in the regression equation are not numerically relevant because they depend on our choice of *dummy* variables for the concentration. However, the important value is $p = 0.015$ for the *Interact* term, which again agrees with the other hypothesis tests.

3.4.4 Fixed and random factors

With the increased flexibility in the GLM analyses, we need to be more aware of exactly how the statistical analysis matches the objectives of the scientific investigation, and we start by looking at the difference between *fixed* and *random* factors.

Using GLM in software, we are able to define which of the input factors might be 'random' as opposed to 'fixed'. The important difference is the scope of any conclusions that can be drawn from the analysis.

A **fixed factor** has specific levels defined by the experiment, e.g. choice of water samples when measuring *pH*, and, for the fixed factor, the conclusion is relevant *only* for the levels chosen from that factor. For example, when testing two water samples, a conclusion of 'no significant difference' only applies to those samples, and cannot be extended to any other water samples.

A **random factor** has its levels selected at random from a *population* of possible levels, and a conclusion of 'no significant difference' then applies to the whole population of levels.

3.4.5 Sequential and adjusted sums of squares

The concept of sum of squares, *SS*, was introduced in 2.1.2 and in 3.2.2 for the different factors in an ANOVA. An important feature of attempting to fit a *number* of factors into a mathematical model is that it is possible to consider different *sequences* with which the different factors are introduced into the model.

Sequential sum of squares, *seqSS*, for a given factor, describes the amount of variation that is explained by that factor after the *previous* terms have been taken into account. This value could also include some variations that might be explained by a new factor that is to be introduced *later*.

Adjusted sum of squares, *adjSS*, for a given factor describes the amount of variation that is explained by that factor after *all other* terms have been taken into account. Unlike *seqSS*, the *adjSS* term will not include any variations that can be explained by a factor that is to be introduced later (e.g. in Fig 3.27). The *adjSS* value can be interpreted as indicating how much *new information* is provided by this factor separately from other factors.

In the following examples we see how the *adjSS* value calculated for a factor can be less than the *seqSS* for the same factor, because a *later* factor in the sequence explains some of the variation that was previously included in the *seqSS* value. We also see that, for the *last* factor in the model, the *adjSS* value will equal the *seqSS* value because the *previous* terms are the same as *all other* terms in the model.

A choice can be made as to whether to use the *seqSS* or *adjSS* values to calculate the *MS* values in the *p*-value calculations. The **Type I sum of squares** calculation uses *seqSS* but the normally default **Type III sum of squares** calculation uses *adjSS* values.

Case study: Correlated variables / 2. Sums of squares

—continued from 4.1.4

The data in Fig 3.29 presents an output variable, *R*, measured together with two input variables, *v1* and *v2*, reproduced from Fig 4.7(a). An additional variable, *v3*, has been added, which is the same as *v2* except for two small changes in rows 3 and 5, and this effect is discussed in 3.4.6. The discussion in 4.1.4 differentiates between bivariate and partial correlation for related variables, and we now investigate the effect of these relationships between input variables when developing a regression model. The analysis in 3.4.6 demonstrates the difference between 'pure error' and 'lack of fit'.

	A	B	C	D
1	*R*	*v1*	*v2*	*v3*
2	10.00	2.24	0.94	0.94
3	12.30	2.39	1.11	1.12
4	9.60	1.71	0.76	0.76
5	8.00	1.85	0.89	0.91
6	9.80	1.65	0.85	0.85
7	9.60	2.18	1.12	1.12
8	8.20	2.08	0.81	0.81
9	9.50	2.30	0.91	0.91
10	7.40	1.69	0.72	0.72
11	11.90	2.50	1.25	1.25

Fig 3.29 Response variable, *R*, with possible predictor variables, *v1*, *v2*, and *v3*

We start with a general regression analysis of *R* against *v1*, with the result in Fig 3.30.

```
Source       DF   Seq SS   Adj SS   Adj MS       F          P
Regression    1  10.1809  10.1809  10.1809  6.80989  0.0311508
  v1          1  10.1809  10.1809  10.1809  6.80989  0.0311508
Error         8  11.9601  11.9601   1.4950
Total         9  22.1410
S = 1.22271       R-Sq = 45.98%       R-Sq(adj) = 39.23%
```

Fig 3.30 Regression analysis of *R* vs *v1*

The analysis shows an apparent 'dependence' on *v1*, with $p = 0.031$. However, in Fig 3.31 we include *v2* in the analysis.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	2	13.6980	13.6980	6.84901	5.67845	0.034241
v1	1	10.1809	0.0890	0.08904	0.07382	0.793695
v2	1	3.5171	3.5171	3.51714	2.91603	0.131461
Error	7	8.4430	8.4430	1.20614		
Total	9	22.1410				

S = 1.09824 R-Sq = 61.87% R-Sq(adj) = 50.97%

Fig 3.31 Regression analysis of *R* vs *v1* and *v2*

The regression model in Fig 3.31 is again significant with $p = 0.034$, and we can see the increased 'fit' with the data through the increase in the R^2(adj) from 39.23% to 50.97%.

Notice the dramatic effect of introducing the *v2* term on the *SS* values for *v1*. Before considering the effect of *v2*, we have *seqSS*(*v1*) = 10.1809 which would include the variations due to the *following v2* term, but after including the effect of *v2*, the value becomes *adjSS*(*v1*) = 0.0890. This suggests that it is *v2* that is responsible for much of the variance in the data.

However, why has the *p*-value increased (from 0.031 to 0.034) when we have introduced an important term, which we might expect to reduce the *p*-value? The answer is that by including another factor, we increase the probability (*p*-value) of obtaining the observed data through random chance when using *two* variables rather than just one.

Examining the data in Fig 3.31, we might also ask whether there is an apparent contradiction in that, although the overall regression is significant, the *p*-values for both *v1* and *v2* are greater than 0.05. The answer is that the analysis is using the Type III method where the *individual p*-values are based on the *adjSS* values, which provide the significance of the individual factor *after* the effect of the other factors have been taken into account. We saw in the previous analysis that *v1 alone* already provides evidence of a significant effect, so that the *additional* information from *v2* is then less significant with $p = 0.131$, and hence there is no contradiction, and similarly for *v1*. The individual *p*-values for *v1* and *v2* correspond to their individual *partial correlations* (4.1.4) with *R*, which measure the remaining variation after the other factors have been taken into account.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	2	13.6980	13.6980	6.84901	5.67845	0.034241
v2	1	13.6090	3.5171	3.51714	2.91603	0.131461
v1	1	0.0890	0.0890	0.08904	0.07382	0.793695
Error	7	8.4430	8.4430	1.20614		
Total	9	22.1410				

S = 1.09824 R-Sq = 61.87% R-Sq(adj) = 50.97%

Fig 3.32 Changing the order of *v1* and *v2*

If we now consider that *v2* might be more relevant than *v1*, and *change the order* in which the model is developed, we find that the conclusions in Fig 3.32 are the same as the previous analysis. The order of *v1* and *v2* does not make any difference to the *p*-values, because, using the Type III method, the *p*-values are calculated using *adjSS* values which are independent of factor order.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	1	13.6090	13.6090	13.6090	12.7604	0.0072707
v2	1	13.6090	13.6090	13.6090	12.7604	0.0072707
Error	8	8.5320	8.5320	1.0665		
Total	9	22.1410				

S = 1.03272 R-Sq = 61.47% R-Sq(adj) = 56.65

Fig 3.33 Regression analysis of *R* vs *v2*

Finally, with the belief that *v1* is not an important factor, we *remove* it from the analysis, and we now see, in Fig 3.33, a very significant effect. The use of the single factor, *v2*, in our model has considerably reduced the likelihood of the observed data occurring *by chance* to $p = 0.0073$. You should also note that the degrees of freedom, *df*, for regression equals the number of factors involved, and the reduction from two to one similarly affects the calculated *p*-value.

3.4.6 Lack of fit and error

It is possible to illustrate a further aspect of using the general linear model. In Fig 3.29 the data set *v3* is the same as *v2*, *except* that the value in row 3 is changed from 1.11 to 1.12 and the value in the row 5 from 0.89 to 0.91.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	1	13.4215	13.4215	13.4215	12.3140	0.007972
v3	1	13.4215	13.4215	13.4215	12.3140	0.007972
Error	8	8.7195	8.7195	1.0899		
Lack-of-Fit	6	3.9495	3.9495	0.6582	0.2760	0.907072
Pure Error	2	4.7700	4.7700	2.3850		
Total	9	22.1410				

S = 1.04400 R-Sq = 60.62% R-Sq(adj) = 55.70%

Fig 3.34 Regression analysis of *R* vs *v3*

Performing the general regression analysis for the single factor *v3* we get the results in Fig 3.34. The small change in the *v2* data has made only a minimal difference in the *p*-value results compared with Fig 3.33. However, there are now *two pairs* of *replicate* measurements in the data which have the same *v3* values of 1.12 and 0.91. By using these replicate values, the analysis is able to make an estimate of the inherent *experimental uncertainty* in the measurements, and this variation is calculated in the output as the 'pure error'. Then, by removing 'pure error' from the total error variations, the analysis can attribute the remaining variation to a 'lack of fit' between the calculated model and the experimental data.

3.4.7 Generalized linear model

The general linear model considered so far is based on the assumption that the data is normally distributed and that the underlying link between input and output data is a linear response. The general*ized* linear model (GsdLM) takes the analysis further to include systems that do not necessarily satisfy those two assumptions, and thereby seeks to encompass a wider range of analyses within one unified approach.

The GsdLM analysis starts by specifying the *three main elements* of the system being analysed:

- The experimental **uncertainty distribution** which describes the inherent random distribution of experimental data, e.g. normal, Poisson.
- A **link function** which compensates for nonlinear behaviour, either as a result of a theoretical relationship in the system (e.g. using a log function to linearize an

exponential, 2.3.4) or in the type of variables being analysed (e.g. using the logit function for a binary or ordinal variable, 8.3.4).

- Identifying the types of input variable(s) as **factor(s)** and/or **covariate(s)**.

Although the details of the process are beyond the scope of this book, we can demonstrate the use of the GsdLM by analysing a simple exponential radioactive decay.

Generalized linear model (Poisson loglinear): SPSS analysis leading to Fig 3.35. See also 6.4.7. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: Exponential decay / 5. Generalized linear model

–continued from 2.3.4 and 2.4.3, leading to 7.2.3

The linearization of radioactive decay to calculate half-life was described in 2.3.4, based on the data in Fig 2.16. We now use the *GsdLM* to repeat the analysis.

The counts, N_t, in radioactivity as a function of time, t, are given by the exponential decay:

$$N_t = N_0 \times e^{kt}$$

with $k = -0.693/T_{1/2}$, where $T_{1/2}$ is the half-life.
By taking logarithms of both sides of the equation we get:

$$\ln(N_t) = \ln(N_0) + k \times t$$

To calculate $T_{1/2}$ we need to measure the slope in the straight line defined by the covariates $\ln(N_t)$ and t. The *link function* between N_t and t is therefore a logarithm.
The *uncertainty* in radioactivity is based on the very low random probability of each atom decaying, and therefore follows a Poisson distribution (1.3.3).
We can see how this is implemented in SPSS using the data from Fig 2.16:

SPSS > Analyze > Generalized Linear Models > Generalized Linear Models...
Type of Model: Either select a standard option: ⊙-***Poisson loglinear***
or: ⊙-***Custom*** and select ***Poisson*** distribution and ***Log*** link function
Response: Dependent variable: *N*
Predictors: Select *t* as a ***Covariate***
Model: Specify t for the ***Model***
→ Output: Fig 3.35

Parameter Estimates

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	4.599	.0859	4.431	4.768	2863.529	1	.000
t	-.494	.0460	-.584	-.403	115.002	1	.000
(Scale)	1[a]						

Dependent Variable: N
Model: (Intercept), t
a. Fixed at the displayed value.

Fig 3.35 *SPSS* output using the GsdLM

The output in Fig 3.35 confirms that the model fits the data with p-values less than 0.0005, and provides best estimate values for the coefficients, B, of the equation:

$$\ln(N_t) = B_{Intercept} + B_t \times t = 4.599 - 0.494 \times t$$

We can then derive values for N_0 and k:

$\ln(N_0) = 4.599$ from which we can derive $N_0 = 99.4$, and

$k = -0.494$.

These results agree with the result obtained in 2.4.3 using Solver with the Poisson distribution.
We could repeat the analysis (incorrectly) using the normal distribution of uncertainty with the log link function and obtain:

$\ln(N_0) = 4.584$ from which we can derive $N_0 = 97.9$, and $k = -0.476$

which also agrees with the similar analysis in 2.4.3.

3.5 Nonparametric analyses

Nonparametric tests do not make any assumptions about the distribution of the random variations in the data. They use the values only to establish their relative *rank order*, with the magnitude of the *difference* between adjacent values having no significance. As these differences are not relevant, the calculations are not affected if particular sections of data are more or less 'spread out' in value. They are independent of the 'shape' of the distribution of values, and are therefore called 'distribution-free' tests.

We normally need to use nonparametric tests if we have

continuous data that does not have a known (e.g. normal, Poisson) distribution

ordinal data (e.g. Likert scale data) or

data that has already been given ranked values.

The common nonparametric tests are based on similar principles, in which a test statistic is derived from the distribution of ranks, and is then either compared with a critical value or used to calculate a p-value. There is no need to describe the process underlying each test, and we will just consider the example of the Mann–Whitney test which is the nonparametric equivalent of the independent samples t-test. Section 3.5.2 reviews other nonparametric tests in relation to their parametric equivalents.

3.5.1 Mann–Whitney example

The Mann–Whitney test is a hypothesis test for a difference between the *medians* of two independent samples. The typical calculation first works out the *rank values* of all of the data, and then derives a test statistic that describes the distribution of these ranks between the samples. Then, assuming that the null hypothesis were true, it calculates the probability of that distribution, or one more extreme, occurring by chance. As with other tests, the final

stage can be completed either by comparing the calculated test statistic with a critical value or, in modern software, by calculating a *p*-value directly.

Case study: River *pH* / 5. Mann–Whitney test

–continued from 3.1.3, leading to 3.9.2

Fig 3.36 gives the *pH* (in row 2) from two samples relating to two rivers, *A* and *B*, identified in row 1.

For the nonparametric test we test for a difference in the *median* values, given the null hypothesis:

H_0: The two population medians are equal, $m_A = m_B$.

	A	B	C	D	E	F	G	H	I	J	K
1	River		*A*	*A*	*A*	*A*		*B*	*B*	*B*	*B*
2	Data / *pH*		6.56	6.52	6.7	6.61		6.47	6.4	6.49	6.55
3	Rank		3	5	1	2		7	8	6	4
4					W_A =	11				W_B =	25
5			n_A =	4	U_A =	1		n_B =	4	U_B =	15

Fig 3.36 Case study: River *pH* / 5. Mann–Whitney test

The procedure is to rank all of the data values across *both* samples in row 3. It does not matter whether we rank from 'high to low' or 'low to high' as long as we are consistent. When ranking *negative* values, it is useful to interpret the statement that '*a* is greater than *b*' as actually stating that '*a* is more *positive* than *b*', e.g. $-3 > -4$ is interpreted as '–3 is *more positive* than –4'. Where two or more data have the same values, the *average* rank should be returned for each of the possible values.

In Fig 3.36, we calculate the rank of each value in row 3 out of all the values from C2 to K2, using the function (for example):

[C3] = RANK.AVG(C2, $C2:$K2).

The '$' signs lock the column values when we copy this function to other cells in row 3 to generate the other ranks in C3 to K3.

We then calculate the rank totals, W_A, and W_B, in F4 and K4, for *each* sample,

$W_A = 3+5+1+2 = 11$
$W_B = 7+8+6+4 = 25.$

The two possible *extreme* situations for two samples each with four values would be:

- If the two sample median values were *very different* with *no overlap* in sample values, then W_A would be equal to 10 (=1+2+3+4) and W_B equal to 26 (=5+6+7+8), or vice versa.
- If the median values were the *same* (null hypothesis), then W_A and W_B would become closer in value.

We see in 3.9.2 how we can use the Monte Carlo method to test whether the *W* values are more extreme than would be expected by chance, and calculate a *p*-value. However, when using the critical value approach we need to derive a new statistic, U_i, for each sample, *i*, (where *i* is *A* or *B*):

$$U_i = W_i - n_i(n_i + 1) / 2 \tag{3.13}$$

which give values for each sample of

$U_A = W_A - n_A(n_A + 1) / 2 = 11 - 4 \times 5 / 2 = 1$
$U_B = W_B - n_B(n_B + 1) / 2 = 25 - 4 \times 5 / 2 = 15.$

These values can then be compared with a table of critical values, U_C, where we **reject the null hypothesis** if either:

$U_A \leq U_C$ or $U_B \leq U_C$.

In our example, for two samples each of size four, the critical values are $U_C = 0$ for two-tailed test and $U_C = 1$ for a one-tailed test. For a *two-tailed test*, neither U_A nor U_B are less than or equal to $U_C = 0$, hence we *do not reject* the null hypothesis. However, if we had originally decided to perform a *one-tailed test*, $U_C = 1$, and, because $U_A = U_C$, we would accept that the median for river *A* was a significantly higher than for river *B*. These conclusions are consistent with the parametric *t*-test result in 3.1.3.

3.5.2 Nonparametric and parametric test equivalents

Table 3.3 gives the nonparametric alternatives equivalent to the most common parametric tests, together with links for examples of their operation.

Table 3.3 Parametric and nonparametric test equivalents

Parametric test	Link	Nonparametric test	Link
One sample *t*-test (mean)	6.1.4	One sample Wilcoxon test (median)	6.1.5
Two sample *t*-test (means)	6.2.5	Mann–Whitney test (medians)	6.2.6
Paired *t*-test (means)	6.2.7	Paired Wilcoxon test (medians)	6.2.8
1-way ANOVA (means)	6.3.5	Kruskal–Wallis test (medians)	6.3.7
2-way ANOVA (means)	6.4.4	Friedman test (medians)	6.4.6
Pearson's *r* (linear correlation)	7.1.4	Spearman's *p* (monotonic correlation)	7.1.4
F-test (variance)	6.2.4	Levene's test (variance)	6.2.4

The following tests use *ranking* as a means of quantifying the relationships within the data set.

Wilcoxon signed rank test (6.1.5) is the nonparametric equivalent of the **one sample *t*-test**, and tests whether the sample has been drawn from a population that has a *median* value that is different from a specific value, m_0. The calculation derives a test statistic, *W*, based on the *ranking* of values on either side of the test value, m_0.

Wilcoxon paired test (6.2.8) is the nonparametric equivalent of the **paired *t*-test**, and tests whether there is a significant difference between the median values of two *related* samples.

Kruskal–Wallis test (6.3.7) is the nonparametric equivalent of the **one-way ANOVA**, in that it tests for a difference between the median values of k-samples (where $k > 2$). All data values are given their rank value, and the test develops a test statistic, H, which is a measure of how *unevenly* the ranked values are spread between the samples. A large H value indicates a difference between the median values of the samples, and can be compared with a critical value using the chi-squared distribution.

	A	B	C	D	E
1		D1	D2	D3	D4
2	C4	76	77	74	74
3	C5	75	74	72	71
4	C6	77	75	75	72

Fig 3.37 Same data as Fig 3.11 for a two-way analysis of catalyst yields

Friedman test (6.4.6) is the nonparametric equivalent of the **two-way ANOVA**, in that the analysis of the effect of one factor is tested while 'blocking' the effect of the other. For the data in Fig 3.37, a Friedman test would be testing for a difference in median values due to the *day* factor, *D1*, *D2*, *D3*, and *D4*, while taking into account (blocking) the catalyst levels *C4*, *C5*, and *C6*. The test for the significance of the *catalyst* factor would require the data to be *transposed* with the columns defined by the catalyst and the rows by the day.

The next tests use *binomial probability* as a basis for their analysis.

Sign test is another nonparametric equivalent of the **one sample *t*-test**, but, unlike the Wilcoxon test, it only *counts* the numbers of values in the sample on either side of the test value, m_0, and does not rank the differences. It is therefore less powerful than the Wilcoxon test.

Runs test (6.1.6) is a simple test for the randomness with which values above and below a particular value appear in a data set, and can be used as a check on the expected randomness of experimental data.

Fisher's exact test (4.2.3) tests the numbers of events falling into *two categories* (i.e. proportions) with the frequencies that might be expected by chance.

Some measures of relative relationship uses *concordant and discordant data pairs* (4.3.5). For example, **Kendall's test for concordance** (4.4.3) can be used to test for the *agreement* between several variables.

The other major family of nonparametric analyses (developed in Section 3.7) is based on the **chi-squared *probability distribution***. These analyse categorical data and have no parametric equivalents.

3.6 Repeated measurements

An important option in experimental design is called 'repeated measures' and involves making repeated measurements of the *same subject*, but under different conditions. A common example is where the same test is repeated 'before' and 'after' an intervention on each one of a number of different subjects, and consequently this experimental arrangement is often called a 'within subject' design. The term 'subject' follows the common use of this type of analysis in questionnaires asking related questions on human 'subjects'. However, in general, the term 'subject' only represents a unique 'link' that identifies related data values, and can easily represent an inanimate connection, e.g. the specific *pH* meters in the case study in 3.6.1 below.

When the linked measurements are between just *two* samples, the analysis is called a 'paired' test. Where repeated measures *within* subjects are used together with tests *between* subjects it is called a *mixed* design.

3.6.1 Paired samples

Pairing between two data samples occurs when each data value in one sample shares a unique 'link' with one data value in the other sample. In this section, we will develop the *parametric* analysis of the paired *t*-test for testing for a difference in mean values. The *non-parametric* equivalent is the paired Wilcoxon test for a difference in median values. How to use Minitab and SPSS for the two tests is given in 6.2.7 and 6.2.8 respectively.

Case study: River *pH* / 6. Paired *t*-test

—continued from 3.1.3

We saw in 3.1.3 that when using the independent samples *t*-test for four *pH* measurements from two rivers, *A* and *B*, there was no significant difference between the mean *pH* values. However, we now include the additional information that four *pH* meters, *M1*, *M2*, *M3*, and *M4*, were used, with each meter making one measurement of each river. Hence each pair of data values are now linked by a unique meter, as in rows 2 to 5 in Fig 3.38.

	A	B	C	D	E	F	G	H	I
1		A		B		Diff, *d*		Size	4
2	M1:	6.56	-	6.47	=	0.09		*df* =	3
3	M2:	6.52	-	6.4	=	0.12			
4	M3:	6.7	-	6.49	=	0.21		t-statistic, t_s =	3.70
5	M4:	6.61	-	6.55	=	0.06		t-critical (95%), t =	3.18
6								p-value =	0.034
7				Mean		0.120			
8				StDev		0.065		TTEST() =	0.034

Fig 3.38 Case study: River *pH* / 6. Paired *t*-test

In terms of experiment design we call this a 'repeated measures' design or a 'within subjects' design, where the different 'subjects' are identified as the different *pH* meters, and the paired values are 'related'.

To analyse the data, it is appropriate to take the differences, 'within subjects', between the values of each linked pair, calculated as *Diff*, *d*, in column F. The sample mean and standard deviation of these differences are calculated in F7 and F8 respectively. If there is no difference in the true mean values of *A* and *B* then we would expect that the true mean value, $\bar{d}$, of *Diff* would be zero.

Hence the *paired* test has now become a *one sample* t-test (3.1.2), testing whether the mean of *Diff* is significantly different from zero. We can derive the relevant t-statistic in I4 using Eqn 3.3:

$$t_s = \frac{(\bar{d} - 0)}{s/\sqrt{n}}: \qquad [I4] = F7/(F8/SQRT(I2))$$

The degrees of freedom for the test are given by Eqn 3.4, $df = n - 1$, and calculated in [I2]

df: [I2] = I1 − 1 = 3

We then calculate the p-value (two-tailed) from the t-statistic:

p-value: [I6] = T.DIST.2T(I4, I2) = 0.034

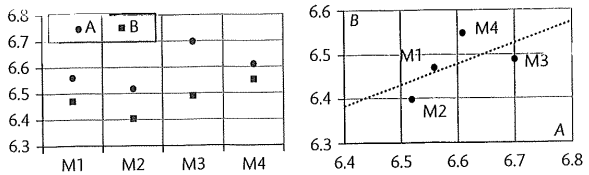
or, from the raw data directly:

p-value: [I8] = T.TEST(B2:B5, D2:D5, 2,1) = 0.034

where the '2' in the argument identifies a two-tailed test and the '1' is for paired data.

With $p = 0.034 < 0.05$, this paired test shows a significant difference between the *pH* values of the two rivers, whereas the unrelated t-test in 3.1.3 failed to detect the difference.

We can use graphical representations of the data in Fig 3.39 to see why the *paired* t-test identified a difference that was missed by the *independent* samples t-test. Fig 3.39(a) is drawn using a 'line graph' in Excel to plot each data value against the meter used, and it can be seen that there is a pattern of variation between the different meters, with M2 giving lower readings than the other meters. This may be due to poor initial calibration of the meters, but, whatever the physical cause, there is a *bias* (systematic error) between the meters, which is ignored by the *independent* samples t-test and appears as increased random uncertainty in the data. The independent samples t-test is then unable to identify the difference in mean values in the presence of the greater apparent random uncertainty. The *paired* t-test performs the difference calculation 'within' each pair of meter readings where the random variation 'between' the meters can have no effect.



(a) *Differences* between individual meters

(b) *Correlation between meters*

Fig 3.39 Identifying bias between *pH* meters

It is also useful to plot each *pair* of meter readings from the two rivers on an x–y graph, as in Fig 3.39(b) where each dot represents a separate *pH* meter. Any *bias* between the meters will result in higher reading meters towards the top right and the lower reading meters towards the bottom left of the graph, and we would expect to see a positive slope for a best-fit straight line. In this case, a test for correlation between the data values gives $r = 0.588$ which does show a degree of positive correlation, although it is not statistically significant with $p = 0.412$.

3.6.2 Repeated measures

When the number of 'linked' samples exceeds two it becomes a *repeated measures* (within subjects) design. As an example, we use the 'Ink analysis' case study data to test for a difference between three inks: *A*, *B*, and *C*.

Case study: Ink analysis / 4. Repeated measures

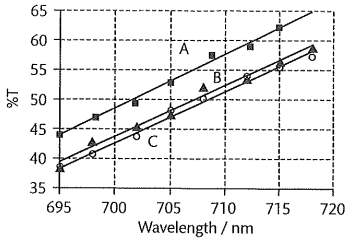
—continued from 5.1.6 and 5.2.3, leading to 3.3.3

The data in Fig 3.40(a) gives the percentage transmission, *%T*, for three inks as a function of wavelength, and we wish to test whether there is a significant difference between them. We can use a repeated measures analysis because there is a unique wavelength link between measurements made with each ink.

Repeated measures 1: SPSS analysis leading to Figs 3.41, 3.42, and 3.43. See also 6.3.8. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

	Wavelength	A	B	C
1	695.00	44.03	38.48	38.47
2	698.00	47.16	42.93	40.93
3	702.00	49.45	45.37	43.86
4	705.00	52.84	47.49	48.05
5	708.00	57.27	52.56	50.23
6	712.00	58.63	53.42	53.95
7	715.00	62.04	56.45	55.50
8	718.00	65.33	58.89	57.45

(a) SPSS data



(b) *%T vs wavelength*

Fig 3.40 'Repeated' *%T* values for three inks at each of eight wavelengths

We analyse this data using the *parametric* 'repeated measures' analysis in SPSS, and in Minitab we can treat the 'within-subjects' factor and 'between-subjects' factor as factors in a two-way ANOVA. The *nonparametric* analysis of repeated measures uses the Friedman test introduced in 6.4.6.

SPSS

SPSS > Analyze > General Linear Model > Repeated Measures...
Within Subjects Factor Name: *Inks* (we enter a name to describe the 'repeated' factor)
Number of levels: 3 (corresponding to the three inks)
> Add
> Define: Transfer *A*, *B*, and *C* to **Within Subjects Variables (Inks)**
> Model... ◉-Custom Transfer *Inks* **to Within Subjects Model**

> **Options...** Transfer *Inks* **to Display Means for**
☑**-Compare Main Effects**
Confidence Interval Adjustment: Select Bonferroni or Sidak
→ Output: Figs 3.41, 3.42, and 3.43

As with the two sample example, the analysis works by taking differences between the possible pairs of each subject, and an important requirement is that the variances of these values should be the same between all possible pairs. A test for this equality of variance, albeit with some limitations for both large and small samples, is Mauchly's test of sphericity. SPSS performs this test as a default (Fig 3.41), and also gives values for epsilon, which is a multiplication factor that can be applied to the degrees of freedom for the *F*-test if the sphericity condition is not met.

We see in Fig 3.41 that Mauchly's test of sphericity gives, $p = 0.571$, which allows us to assume that the equality of variance condition has been met. SPSS then gives *p*-values in Fig 3.42 for significant differences in the sample mean value, both on the assumption that the sphericity condition is met and also for other corrected tests if it is not: Greenhouse–Geisser, Huynh–Feldt, and Lower-bound (most conservative).

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Inks	.830	1.121	2	.571	.854	1.000	.500

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept
Within Subjects Design: Inks

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Fig 3.41 Test for equality of variance in a repeated measures analysis

This is a 'mixed' design which tests for differences both between wavelengths and between inks. The analysis gives $p = 0.000$ (not given in figures here) for the 'between-subjects' test confirming that there is a significant difference in *%T* for different *wavelengths*. However, we wish to test, 'within-subjects', for a difference between the *inks*, *A*, *B*, and *C*, and this is reported in Fig 3.42 showing a significant difference between at least two of the inks.

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Inks	Sphericity Assumed	169.964	2	84.982	164.845	.000
	Greenhouse-Geisser	169.964	1.709	99.461	164.845	.000
	Huynh-Feldt	169.964	2.000	84.982	164.845	.000
	Lower-bound	169.964	1.000	169.964	164.845	.000

Fig 3.42 Significance results for the 'within subjects' test

Although not required in this analysis, we can see that the corrected tests in Fig 3.42 use the same *F*-statistic, 164.8, but the degrees of freedom are adjusted by the value of epsilon. Note for example that the corrected degree of freedom for the Greenhouse–Geisser test is equal to the initial degree of freedom multiplied by the relevant epsilon value, 0.854, from Fig 3.41:

$2 \times 0.854 = 1.709$ (within rounding errors)

The direct post hoc option is for locating differences between 'between-subjects' factors. However, we wish to locate the difference 'within-subject', i.e. between *A*, *B*, and *C*, and we use the 'compare main effects' for inks under options as a means for applying a post hoc test (LSD, Bonferroni, or Sidak) to the repeated measurements. Using the Bonferroni test we get the comparisons in Fig 3.43.

Pairwise Comparisons

Measure: MEASURE_1

(I) Inks	(J) Inks	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	5.145*	.275	.000	4.284	6.006
	3	6.039*	.390	.000	4.819	7.258
2	1	-5.145*	.275	.000	-6.006	-4.284
	3	.894	.398	.179	-.352	2.140
3	1	-6.039*	.390	.000	-7.258	-4.819
	2	-.894	.398	.179	-2.140	.352

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Fig 3.43 Post hoc tests for 'within-subjects'

In Fig 3.43 the inks, *A*, *B*, and *C*, are labelled as 1, 2, and 3, corresponding to the order of entry into the software, and the Bonferroni comparison then identifies significant differences ($p = 0.000$) between *A* and *B* and between *A* and *C*, but not ($p = 0.179$) between *B* and *C*, which is consistent with the results in 3.3.3.

3.7 Chi-squared analyses

The chi-squared, χ^2, test is a hypothesis test that compares frequencies with which 'events' are *observed* to occur in different 'categories' with the frequencies that could have been *expected* based on the science involved. The experimental data can appear first as a simple list of individual observations or events, and the process of adding up the *numbers* of observations/events that fall into specific categories is called 'tabulation' (putting the data into tables). We deal with tabulation and cross-tabulation in 8.1.7 and 8.2.4 respectively, but in this section we just analyse the resultant frequency values.

The principle calculation for the chi-squared statistic, χ^2, uses Pearson's formula introduced in Eqn 3.19, but can also use the Yates continuity correction in Eqn 3.21 or the likelihood ratio in Eqn 3.22.

3.7.1 Tabulated data

Figs 3.44 and 3.45 show the two main forms of tabulated data, in which the data values are frequencies (simple counts) recorded as integer values.

	A	B	C	D
1	Category, *i*	1	2	3
2	Observed, *O*	23	26	11
3	Expected, *E*	15	30	15

Fig 3.44 one-way frequency table

	A	B	C	D
1	Gender \ Subject	*Science*	*English*	*History*
2	*M*	11	18	17
3	*F*	18	20	6

Fig 3.45 two-way contingency table

For the one-way table in Fig 3.44, the chi-squared test compares the *observed* distribution of frequencies in row 2 with a distribution of *expected* frequencies in row 3, and tests whether any observed difference between the distributions could have occurred by chance, or whether the difference is statistically significant. This is often called a 'goodness of fit' test.

In the two-way 'contingency' table in Fig 3.45, the rows and columns are identified by different levels of two factors: *Gender* and *Subject*. The chi-squared analysis compares the *distributions* of frequencies between different rows and between different columns, and tests whether these differences are statistically significant. If, for example, the distribution *across the columns* changes significantly between the rows, then there is said to be an *association* between the two factors. Such an association between the factors would also cause a difference in the distribution *down the rows* for different columns.

The chi-squared test, applied to a contingency table, is a hypothesis test which identifies whether any apparent association between the two factors could have occurred by chance, and is a *symmetric* measure (4.3.2) in that it identifies a relationship between the two factors, but does not identify one as being dependent on the other. In Sections 4.2 and 4.3, we consider a range of other statistics that can be used to measure the *strength* of association.

3.7.2 One-way 'goodness of fit'

We can illustrate the principle of the chi-squared, χ^2, test by using the simple one-way table of observed frequencies, O, and expected frequencies, E, in Fig 3.44.

The null hypothesis for the test is:

H_0: The distribution of observed values is the same as the expected ratios (in this case given directly by expected values).

The relevant formula for the Pearson's chi-squared statistic is:

$$\chi^2 = \sum_i \frac{(O_i - E_i)^2}{E_i} \tag{3.14}$$

This formula is essentially a set of instructions which says that for each observed value, i, divide the square of the difference between the observed and expected value by the expected value, and then add up these results for all the values, i. For the example data, this gives:

$$\chi^2 = \frac{(23-15)^2}{15} + \frac{(26-30)^2}{30} + \frac{(11-15)^2}{15} = 4.27 + 0.53 + 1.07 = 5.87$$

It is clear that, as the difference between observed and expected frequencies increases, then the value of χ^2 will also increase. However, we would expect that, just by random chance, there will be differences and χ^2 will not be zero. As with other test statistics, we can use tables of critical values, χ^2_C, to decide how large χ^2 must be before we decide that the difference is significant. We accept that the difference is significant and reject the null hypothesis if:

$$\chi^2 \geq \chi^2_C$$

The significance of the calculated χ^2 value also depends on the degrees of freedom, df, which, for the one-way test is given by:

$$df = n - 1 \tag{3.15}$$

where n is the number of categories.

For the example with $n = 3$, $df = 2$, the critical chi-squared value, $\chi^2_C = 5.99$. Since $\chi^2 < \chi^2_C$ there is not enough evidence to reject the null hypothesis and we conclude that the observed distribution is not significantly different from the expected values.

In practice, it is usually necessary to calculate the expected frequencies based on expected ratios or probabilities, as is demonstrated in the following case study.

Case study: Chi-squared / 2. One-way 'goodness of fit' test

—continued from 8.1.1 (overview), leading to 3.9.3

It is expected that genotypes *AB*, *Ab*, *aB*, and *ab* will be observed in the ratio 9:3:3:1.

In an experimental measurement, the number of genotypes observed in these categories were 125, 28, 39, and 8 respectively. We wish to test whether the observed frequencies show a significant deviation from the expected ratios.

Chi-squared 'goodness of fit': Excel analysis for Fig 3.46. See also 8.1.5. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

The null hypothesis for this test is:

H_O: The observed distribution of frequencies occurred randomly with probabilities based on the given theoretical ratios.

	A	B	C	D	E	F	G
1	Category	Expected	Expected	Observed	Expected	Difference	Chi-squared
2		ratios	proportions	numbers	numbers		
3	*i*	R_i	P_i	O_i	E_i	$O_i - E_i$	$(O_i - E_i)^2 / E_i$
4	1 (AB)	9	0.5625	125	112.5	12.5	1.39
5	2 (Ab)	3	0.1875	28	37.5	-9.5	2.41
6	3 (aB)	3	0.1875	39	37.5	1.5	0.06
7	4 (ab)	1	0.0625	8	12.5	-4.5	1.62
8	$\Sigma_i R_i =$	16	$N = \Sigma_i O_i =$	200		$\chi^2 =$	5.48
9						$\alpha =$	0.050
10	$p =$	0.140	=CHISQ.DIST.RT()			$df =$	3
11	$p =$	0.140	=CHISQ.TEST()			$\chi^2_c =$	7.81

Fig 3.46 Case study: Chi-squared / 2. One-way 'goodness of fit' test

In Fig 3.46, the four categories are identified by the values of i in A4:A7 with the observed frequencies, O_i in D4:D7 and the expected ratios, R_i, in B4:B7. The chi-squared calculation works by comparing the observed frequencies, O_i, for each category i, with the frequencies, E_i, that would be observed if all the observations were distributed *exactly* according to the expected ratios.

We start by converting expected *ratios*, R_i, into expected *proportions*, P_i, by first calculating the sum of all ratios in B8

[B8] = SUM(B4:B7) = 16

and then, for each category, i, using the equation:

$$P_i = \frac{R_i}{\sum_i R_i} \tag{3.16}$$

e.g. [C4] = B4 / B$8 = 9 / 16 = 0.5625 or directly [C4] = B4 / SUM(B$4:B$7)

The expected *frequency* for each category is then calculated by *multiplying* the total number of observed events, N, (calculated in D8) by the expected proportion, P_i, for that category:

$$E_i = N \times P_i \tag{3.17}$$

e.g. [E4] = SUM(D$4:D$7) * C4 = 200 × 0.5625 = 112.5

Note that the theoretical expected values can have *non-integer* values, whereas all observed frequencies must have integer values.

Now that we have the observed and expected frequencies for each category, we can use the Pearson's formula (Eqn 3.14) for the chi-squared statistic:

$$\chi^2 = \sum_i \frac{(O_i - E_i)^2}{E_i}$$

The formula is essentially a set of instructions, which are implemented as below:

1. For each category, i, calculate $O_i - E_i$ in column F:
   e.g. [F4] = D4 – E4 = 12.5
2. Square this value and divide by E_i in column G:
   e.g. [G4] = F4^2 / E4 = 1.39
3. Take the sum of the values for all categories, i, to calculate the chi-squared value:
   [G8] = SUM(G4:G7) = 5.48

The degrees of freedom, calculated from Eqn 3.15, are $df = 4 - 1 = 3$, which is entered into G10.

The critical chi-squared value for a specific significance and degrees of freedom can be found in look-up tables, or it can be calculated using the function, CHISQ.INV.RT(), with the significance level, $\alpha = 0.05$ (in G9). This is the value in the chi-squared distribution which defines the upper, or right-hand, 5% tail of the distribution:

[G11] = CHISQ.INV.RT(G9, G10) = 7.81

As the experimental value of $\chi^2 = 5.48$ is less than 7.81, we decide that there is not enough evidence to indicate that the distribution is significantly different from the null hypothesis.

We can also calculate a p-value using Excel (or other software), using the function, CHISQ.DIST.RT(), based on the values of χ^2 and df,

[B10] = CHISQ.DIST.RT(G8, G10) = 0.140

or the function, CHISQ.TEST(), to calculate directly from the observed and expected values.

[B11] = CHISQ.TEST(D4:D7, E4:E7) = 0.140

The fact that the value of $p = 0.140$, calculated in B10 and B11, is greater than the significance level of 0.05 is consistent with the decision that there is not enough evidence that the distribution is significantly different from the expected ratios.

3.7.3 Low value of chi-squared

We are almost always looking for the chi-squared statistic being *greater* than a critical value to show that there is a variation in the data that is greater than would be expected by chance. However, it is useful to note that it also possible to test whether the observed variations are significantly *less* than would be expected by random chance. This would be done by calculating the *left-hand* critical value of the distribution using the function CHISQ.INV(). For example, for $\alpha = 0.05$ and $df = 3$, χ^2 would have to be less than the critical value of 0.35 to show a significant *lack* of random variation. Such a situation might point towards a problem with the data collection, possibly with the data values not being truly independent.

3.7.4 Contingency table

The other main use of the chi-squared calculation is in the analysis of a contingency table, and in this section we develop the basic test for an *association* between factors. Further analyses are presented in Section 4.2, and the analysis of contingency tables using Minitab and SPSS is then developed in Section 8.2.

A typical contingency table is defined in two dimensions by two factors. Each individual observation can be 'placed' in one of the cells defined by the two axes, and the total *number* in each cell is recorded as a 'frequency'.

Case study: Association / 2. Contingency table

–continued from 8.2.1

In this example, a total of 90 children, 46 boys and 44 girls, each identify their favourite subject, *Science*, *English*, or *History*, resulting in the numbers given in Fig 3.47.

The null hypothesis for the test is:

H_0: The distribution of choices for boys is the same as for girls.

Contingency table test for association: Excel analysis for Fig 3.47. See also 8.2.4. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

	A		B	C	D	E	F
1	Observed values:						
2	Gender \ Subject		*Science*	*English*	*History*	Totals	
3		*M*	**11**	**18**	**17**		46 = R_1
4		*F*	**18**	**20**	**6**		44 = R_2
5	Totals		29	38	23		90 = T
6			= C_1	= C_2	= C_3		
7	Expected values:						
8	Gender \ Subject		*Science*	*English*	*History*	Totals	
9		*M*	**14.82**	**19.42**	**11.76**		46 = R_1
10		*F*	**14.18**	**18.58**	**11.24**		44 = R_2
11	Totals		29	38	23		90 = T
12			= C_1	= C_2	= C_3		
13	Chi-squared values:						
14	Gender \ Subject		*Science*	*English*	*History*		
15		*M*	0.986	**0.104**	**2.340**		
16		*F*	1.030	**0.109**	**2.446**		
17					Sum =	7.015	
18	*p*-value (CHISQ.DIST.RT) =		0.030		*df* =	2	
19	*p*-value (CHISQ.TEST) =		0.030		Critical value =	5.991	

Fig 3.47 Contingency table / 2. Tests for association

The observed data values are in shaded cells B3:D4, and the first step is to calculate the total frequencies for each row, R_1 and R_2, for each column, C_1, C_2, and C_3, and the total $T = 90$.

The column totals, C_1:C_2:C_3, give the *average* ratios with which the events are distributed between the columns. If there is no difference between the rows, then we could expect the events in each row to be distributed in this same proportion.

If we wish to calculate the expected frequencies in the first row in the table, we need to distribute the R_1 events (= 46) in the ratio C_1:C_2:C_3, where $C_1+C_2+C_3 = T$. This is similar to the calculation that we performed in the one-way table in 3.7.2 using Eqn 3.16,

$$E_{1j} = R_1 \times \frac{C_j}{T} = \frac{R_1 \times C_j}{T}$$

In general, for row i, we can write

$$E_{ij} = \frac{R_i \times C_j}{T} \tag{3.18}$$

We use Eqn 3.18 to calculate the expected values in the shaded cells, B9:D10. For example, the expected value, E_{11}, in B9 is calculated:

$$E_{11} = \frac{46 \times 29}{90}: \qquad [B9] = \$E3 * B\$5 / \$E\$5 = 14.82$$

Note that the theoretical *expected* values can be noninteger. The use of the dollar signs in the Excel expression allows us to copy the same formula into all cells in B9:D10, because $E locks the reference to column E (for the row totals) and $5 locks the reference to row 5 (for the column totals).

We next calculate the contributions $(O - E) / E$ in shaded cells B15:D16:

e.g. [B15] = (B3 – B9) ^ 2/B9

The calculated chi-squared contributions are now summed over all rows, i, and columns, j:

$$\chi^2 = \sum ij \frac{(O_{ij} - E_{ij})^2}{E_{ij}} \tag{3.19}$$

In our analysis we need to calculate, in E17, the sum of all values from B15:D16:

Chi-squared, χ^2: [E17] = SUM(B15 : D16) = 7.015

The critical chi-squared value will depend on the degrees of freedom for a contingency table, which are given by

$$df = (r - 1)(c - 1) \tag{3.20}$$

where r and c are the numbers of rows and columns respectively.

In our example, $df = (2 - 1)(3 - 1) = 2$, which is entered into E18.

The critical chi-squared value for a specific significance and degrees of freedom can be found in look-up tables, or it can be calculated using the function, CHISQ.INV.RT(α,df), with the significance level, $\alpha = 0.05$. This is the value in the chi-squared distribution which defines the upper, or right-hand, 5% tail of the distribution:

[E19] = CHISQ.INV.RT(0.05, E18) = 5.991

As the experimental value of $\chi^2 = 7.02$ is greater than 5.99, we decide that there is a significant association between the choice of subjects and the gender of the child for the population from which the 90 children were a representative random sample.

We can also calculate a p-value using Excel, using the function, CHISQ.DIST.RT(), based on the values of χ^2 and df,

[B18] = CHISQ.DIST.RT(E17, E18) = 0.030

or the function, CHISQ.TEST(), to calculate directly from the observed and expected values,

[B19] = CHISQ.TEST(B3:D4, B9:D10) = 0.030

The fact that the value of $p = 0.030$, calculated in B18 and B19, is less than the significance level of 0.05 is consistent with the above decision that there is a significant association between the choice of subjects and the gender of the child.

3.7.5 Yates continuity correction

We need to note that there is a specific issue when using the standard chi-squared formula in Eqn 3.19, for problems where the degrees of freedom, df, = 1. Due to the fact that we are comparing a chi-squared value based on integer values with a continuous distribution, the

standard calculation tends to *overestimate* the value of χ^2, which means it is more likely to produce Type I errors in borderline cases. This can be corrected by using the Yates continuity correction which modifies the formula slightly:

$$\chi^2 = \sum_{ij} \frac{\{|(O_{ij} - E_{ij})| - 0.5\}^2}{E_{ij}} \quad (3.21)$$

This formula states that for every category cell we must take the *positive* (or absolute) value of the difference between O and E before subtracting 0.5 and then squaring the result and dividing by E.

The need for this correction occurs for 2×2 contingency tables, which have just two levels for each factor and hence degrees of freedom, $df = 1$. However, the problem is equivalent to testing for a difference between two *proportions*, and it is also possible to use other tests including Fisher's exact test. See Sections 3.8 and 4.2.3.

3.7.6 Likelihood ratio

An alternative method of calculating a chi-squared value is based on comparing the likelihoods (probabilities) of obtaining the observed distribution of frequencies under the two possible hypotheses set for the test. This likelihood ratio is calculated as:

$$LR = 2\sum_{ij} O_{ij} \times ln(\frac{O_{ij}}{E_{ij}}) \quad (3.22)$$

The statistic can also be described as G or G^2 and the test as a G-test.

The calculated value for the likelihood ratio chi-squared is usually slightly larger than the Pearson's chi-squared, χ^2, but they become closer for larger sample sizes.

3.7.7 Sample size limitations

An important limitation with the standard chi-squared test occurs with a limited number of observations and/or a large number of categories, resulting in low expected frequencies giving unreliable statistical conclusions. Cochran's criterion for the minimum reliable sample size is that all of the cells must have *expected* frequencies of at least one and at least 80% of the cells must have *expected* frequencies of five or over. Note that that the criterion refers to *expected* frequencies and it is possible that some *observed* frequencies could even be 0.

A common reason for low expected frequencies is that too many category levels have been included in the model for the amount of available experimental data. The options available when the minimum criteria are not met are:

- Collect more experimental data, although this is not always possible.
- Combine category levels (8.2.7).
- Use a resampling technique (3.9.3, 8.2.7).

3.8 Frequency and proportions

When recording frequencies in just two categories we are actual measuring *proportions*. There are different methods for testing proportions, and we will develop the use of:

- binomial theory
- normal approximation of the binomial theory
- chi-squared analysis of a 2×2 contingency table.

3.8.1 Probability distribution

In arriving at a frequency proportion we would normally count the number of measurements or observations that fall into each of two categories, and, for convenience, we can define the two categories here as 'Y' and 'N'. In statistics terminology, each observation is often called a *trial*, and a specific outcome, e.g. 'Y', an *event*. We can define the individual event probability, p, as:

p = probability that a *single randomly* chosen observation will give the outcome Y.

As there are just two options, we can use binomial probability theory from 1.3.3 to calculate the probability $p(r)$ that, out of n observations (trials), there will be r events in the 'Y' category:

Eqn 1.4: $p(r) = {}_n C_r \times p^r \times (1-p)^{(n-r)}$
Eqn 1.5: Population mean, $\mu = p \times n$
Eqn 1.6: Population standard deviation, $\sigma = \sqrt{\{np(1-p)\}}$

Given that the **proportion** of r events is defined by

$$P = \frac{r}{n}$$

we can derive expressions for the true value, Π (Greek capital *pi*), and standard deviation, σ_P, for the *proportion* in the population:

$$\textit{Proportion} \text{ true value } \Pi = \frac{\mu}{n} = p \quad (3.23)$$

$$\textit{Proportion} \text{ standard deviation } \sigma_P = \frac{\sigma}{n} = \frac{\sqrt{\{np(1-p)\}}}{n} = \sqrt{\frac{p(1-p)}{n}} = \sqrt{\frac{\Pi(1-\Pi)}{n}} \quad (3.24)$$

3.8.2 One proportion test

In a one proportion hypothesis test we have a sample of values which give an experimentally observed proportion, P, which is a *best estimate* of the true population proportion, Π. We wish to test whether the true proportion is equal to a specific test proportion, Π_0, which gives the null hypothesis:

H_0: $\Pi = \Pi_0$

It is possible to

- use the binomial theory to perform an 'exact' calculation of the p-value
- use the normal distribution approximation to calculate a confidence interval and perform a z-test
- use a chi-squared test for a 'goodness of fit' for the two frequencies compared to the test ratios.

These three approaches are demonstrated using the 'Frogs' case study.

One proportion: Excel and Minitab analysis for Fig 3.48. See also 6.1.7. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study **Frogs / 2. One proportion test**

—continued from 6.1.7 (overview), leading to 3.8.3

The aim of the investigation is to test whether the proportion of female frogs in a given large lake is *greater than* 0.6. Randomly selecting a sample of 50 it is found that 37 are female, and we wish to test whether this proportion, $P = 37/50 = 0.74$, is significantly greater than the expected proportion of 0.6.

We start with the null hypothesis:

H_0: The true proportion of female frogs, $\Pi = \Pi_0 = 0.60$

Binomial test

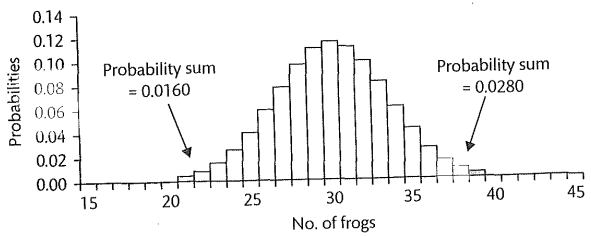


Fig 3.48 Binomial probabilities

Using the binomial equation (Eqn 1.4) for a random sample of 50 frogs ($n = 50$ trials), we can calculate the probabilities of observing r females, given that the probability of each individual being female, $p = 0.060$. The distribution of these probabilities is given in Fig 3.48, and we see that the probability of observing 37 or more female frogs is obtained simply by adding the individual shaded probabilities on the right, $p(r \geq 37) = 0.028$.

This is the probability, given that the *null hypothesis is true*, that we will observe 37 or more female frogs by chance. This is then the p-value for the one-tailed test:

p-value (1-tailed) = 0.028

Since p-value = 0.028 < 0.05, we would reject the null hypothesis and conclude that the female proportion was indeed greater than 0.6.

We can now also consider the calculation of the two-tailed p-value, but the distribution in Fig 3.48 is not exactly symmetrical, and the equation (Eqn 1.27) stating that the two-tailed p-value is twice the one-tailed value does not apply in this case. We see in Fig 3.48 that the two tails include '37 and above' with the probability of 0.028 and '22 and below' with the probability of 0.016, giving

p-value (2-tailed) = 0.028 + 0.016 = 0.044

which would show a significant difference between the observed proportion and 0.6.

Using a normal distribution approximation

We saw in 1.3.3 that the normal distribution can be used as an approximation for the binomial distribution, provided that $np(1-p) \geq 5$. In this case, $n = 50$ and the experimental value is $P = 0.74$, such that $nP(1-P) = 9.6$ which suggests that it may be a reasonable approximation.

Using the test value, the proportion standard deviation for the null hypothesis becomes

$$\sigma_P = \sqrt{\frac{0.60(1-0.60)}{50}} = 0.0693$$

As we only make one measurement of the proportion, the standard error in the measurement is the same as the standard deviation (Eqn 1.21), and we can derive an expression for the z-statistic:

$$z = \frac{P - \Pi_0}{\sigma_P} = \frac{0.74 - 0.60}{0.0693} = \frac{0.14}{0.0693} = 2.02$$

The critical value for a one-tailed test is 1.64, and since 2.02 > 1.64 we conclude that the measured proportion is significantly greater than 0.6. The test would also be significant for a two-tailed hypothesis where the critical value would be 1.96.

We can also derive an estimate for the confidence interval (Eqn 1.23) for the true proportion, by using the normal approximation. The experimentally measured proportion, P, is the best estimate for the true proportion, and using σ_P as the standard error and the t-value given by $z = 1.96$, we get:

$$CI = P \pm 1.96 \times \sqrt{\frac{P \times (1-P)}{n}} = 0.74 \pm 1.96 \times \sqrt{\frac{0.74 \times (1-0.74)}{50}} = 0.74 \pm 0.12$$

This is a symmetrical confidence interval, from 0.62 to 0.86, which is an *approximation* to the true confidence interval. This calculation puts the test value of 0.6 just outside lower limit of the confidence interval, again showing a significant difference.

Using Minitab to perform the same calculations (the use of SPSS is given in 6.1.7):

Minitab

Minitab > Stat > Basic Statistics > 1 Proportion...

▼ **Summarized data** (for directly entering observed frequencies)

Number of Events: 37 **Number of Trials:** 50
☑**-Perform hypothesis test**
Hypothesized proportion: 0.6
> Options... Under options we can choose one- or two-tailed tests and whether to use the normal distribution approximation
→ Output: Fig 3.49

Fig 3.49 gives the result for a one-tailed binomial test and a two-tailed test based on the normal distribution, recoding the same values as calculated above.

```
Test of p = 0.6 vs p > 0.6

                                    95% Lower      Exact
Sample   X   N   Sample p            Bound      P-Value
1       37  50   0.740000         0.618736        0.028
```

(a) One-sided binomial test

```
Test of p = 0.6 vs p not = 0.6

Sample   X   N   Sample p              95% CI              Z-Value   P-Value
1       37  50   0.740000   (0.618419, 0.861581)             2.02     0.043

Using the normal approximation.
```

(b) Two-sided test using the normal approximation

Fig 3.49 One proportion test (Minitab)

Performing a chi-squared test

We can perform a one-way chi-squared test with observed values of 37:13 for female and male frogs to be compared with an expected ratio of 0.6:0.4. The expected ratio of 0.6:0.4 for a total of 50 trials becomes equal to expected frequencies of 30:20, which are given as the one-way table of frequencies in Fig 3.50(a).

	A	B	C
1		*F*	*M*
2	**Observed**	**37**	**13**
3	Expected	**30**	20

(a) One-way table of frequencies

	A	B	C
1		*F*	*M*
2	**Lake 1**	**37**	**13**
3	**Lake 2**	**30**	**20**

(b) Contingency table of frequencies (see 3.8.3)

Fig 3.50 One and two proportion test data

Using the Yates continuity correction (Eqn 3.21), we can calculate the chi-squared value

$$\chi^2 = \frac{(37-30-0.5)^2}{30} + \frac{(13-20-0.5)^2}{20} = \frac{42.25}{30} + \frac{42.25}{20} = 3.52$$

As the chi-squared value of 3.52 is less than the critical value, 3.84, for $df = 1$, this test is unable to detect a significant difference between the observed proportion of 0.74 and 0.60 when based on 50 trials.

Reviewing the three different tests, we see that they all gave different results, although both the binomial test and the normal approximation z-test identified a significant difference, while the chi-squared test failed to detect a difference.

3.8.3 Two proportions test

Case study: Frogs / 3. Two proportions test

—continued form 3.8.2

In 3.8.2, we compared the proportion of frogs, 37/50, to a *specific* test proportion of 0.6, but we now consider comparing the results of 37 out of 50 for one lake with the *experimental* results of 30 out of 50 for a second lake. We can express this situation using the *contingency* table in Fig 3.50(b).

Two proportions: Minitab analysis leading to Fig 3.51. See also 6.2.9. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Although the two tables in Fig 3.50 have the same *values*, they represent different experimental situations. The second row in Fig 3.50(a) gives the *specific expected* values calculated for the one proportion calculation, but the second row in Fig 3.50(b) gives the *observed experimental* values for the second lake in a test between *two* proportions.

In a two proportion analysis we compare two pairs of experimentally measured frequencies from two data samples. This gives us the 2 × 2 contingency table, in which we test whether the distribution of frequencies (the proportion) in one row is significantly different from the distribution (the proportion) in the other row.

H_0: The proportions are the same for different rows and for different columns.

It is possible to use a chi-squared test with the Yates continuity correction, but the Fisher exact test for the 2×2 table is usually the preferred analysis as it is an exact test based on the binomial distribution.

It is also possible to use the normal approximation to the binomial distribution to test for a difference between proportions, using the test statistic:

$$z = \frac{P_A - P_B}{\sqrt{P'(1-P')\times(1/n_A + 1/n_B)}}$$

where P' is a pooled proportion for the two samples

$$P' = \frac{n_A P_A + n_B P_B}{n_A + n_B}$$

Using Minitab for a two proportions test:

Minitab

Minitab > Stat > Basic Statistics > 2 Proportions...
▼ **Summarized data** (for directly entering observed frequencies)
First: **Events** 37 **Trials** 50
Second: **Events** 30 **Trials** 50
→ Output: Fig 3.51

The output in Fig 3.51 gives $p = 0.132$ for the normal approximation and $p = 0.202$ for the Fisher's exact binomial test which both report that there is not enough evidence to claim a significant difference. The reason that the one proportion test for the data in Fig 3.50(a) finds

a significant difference but the two proportion test for the same data in Fig 3.50(b) does not is that the two proportion test compares two *experimental* values both with inherent uncertainties, whereas, in the one proportion calculation, the test proportion of 30/50 is an *exact* value.

```
Difference = p (1) - p (2)
Estimate for difference:  0.14
95% CI for difference:  (-0.0422661, 0.322266)
Test for difference = 0 (vs not = 0):  Z = 1.51  P-Value = 0.132

Fisher's exact test: P-Value = 0.202
```

Fig 3.51 Two proportion test (Minitab)

The use of SPSS for a two proportion test is given in 6.2.9, and, if we use SPSS for the above calculation, it gives $p = 0.202$ for the Yates corrected chi-squared test as well as $p = 0.202$ for the two-tailed Fisher's exact test.

3.9 Resampling techniques

There is a developing range of techniques (e.g. *Monte Carlo*, *bootstrapping*) which use the experimental results observed for a system as a basis for *randomly* regenerating possible system values (typically 10,000 times), and then by analysing the *distribution* of these values it is possible to derive estimates of *p*-values. We can only give an introduction to the techniques here, starting with the general approach and then with examples of possible applications.

We will use the two sample 'River *pH*' case study to illustrate the method for calculating *p*-values for the *t*-test and Mann–Whitney test, and we will use the 'Chi-squared' case study to illustrate the calculation for a goodness of fit test. This allows us to compare the Monte Carlo results with those obtained by other methods. However, the real value of the technique is for analyses when alternative methods are not available, for example using the Monte Carlo method when low expected frequency values make the chi-squared analysis unreliable (8.2.7).

3.9.1 General approach to resampling

The basic procedure has the following steps:

Step 1. An initial analysis of the experimental data calculates the value of a relevant test statistic, e.g. *t*-statistic for a two sample *t*-test, *W*-statistic for a Mann–Whitney test, or the probabilities in a chi-squared test (3.7.2).

Step 2. A mathematical model is then developed, based on the null hypotheses, but including random variations in values. The resampling process then randomly generates a large number (e.g. 10,000) of possible values in the model.

Step 3. The value of the relevant test statistic is calculated for every generated data set.

Step 4. The final step calculates the *proportion* of resample data sets that give a test statistic value that is equal to, or more extreme (i.e. further from the null hypothesis) than the test statistic calculated from the *experimental* data. This proportion gives the *probability* that the null hypothesis could give an experimental result at least as extreme as the values observed—this is the *p*-value.

Because of the inherent randomness of the resampling process, there is also an inherent uncertainty in the calculated *p*-value. For this reason, the result is expressed as a *confidence interval* for the *p*-value.

3.9.2 *t*-test and Mann–Whitney test

Case study: River *pH* / 7. Monte Carlo analysis

—continued from 3.1.3 and 3.5.1

Four replicate *pH* measurements have been made of the water in each of two rivers, *A* and *B*, with the intention of performing a hypothesis test for a difference in the *pH* of the two rivers. The data is given in Fig 3.3.

Resampling *t*-test and Mann–Whitney test: Excel analysis for Fig 3.52. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Step 1

The *pH* values already appear in Fig 3.3 and initial calculations have derived the *t*-test statistic, $t_s = 2.42$, and the pooled standard deviation for the two samples, $s' = 0.0702$, in 3.1.3, and the Mann–Whitney upper rank total, $W_U = 25$, in 3.5.1. These values have been entered into the Excel worksheet in Fig 3.52 in cells B4, D2, and B17 respectively.

Step 2

We develop a mathematical model of two samples with four values each. The first sample *A* is in cells F2:F5 and sample *B* in cells F6:F9. The null hypothesis assumes that there is no difference between the means of the populations from which the samples are drawn, and we will generate both samples with a mean value = 0. We assume that the two samples are drawn from populations which have the same standard deviation, and for this we use the pooled value, $s' = 0.0702$, in D2. In fact we could use any value for standard deviation in this calculation, but it is useful to use a model which matches the observed data.

To generate the random data values we use the Excel function in F2:

[F2] = NORM.INV(RAND(),0,D2)

which randomly selects a value from the normal distribution with mean of 0 and standard deviation given by the value in D2. This function may then be copied to other cells, F3:F9, to generate other randomly selected values for samples *A* and *B*. The '$' signs lock the reference to the row and column of cell D2.

We have now generated four random values for each of samples *A* and *B* in column F. The next step is to generate a total of 10,000 pairs of samples, all with independently selected data values. We do this by simply copying the formulae in F2:F9 to column NTU, which produces a total of 10,000 randomly generated data sets that are all based on the same model.

	A	B	C	D	E	F	G	H	NTT	NTU
1	*t*-test:				Rivers	1	2	3	9999	10000
2	Pooled stdev =			0.0702	A	0.007	0.125	0.176	0.079	-0.031
3					A	0.009	-0.038	0.043	0.080	0.040
4	*t*-statistic =	2.42			A	0.073	0.024	0.022	0.090	0.120
5					A	0.053	-0.085	-0.001	-0.054	0.091
6	Proportion =	0.0527			B	-0.029	-0.009	0.010	-0.057	0.082
7	StDev =	0.0022			B	0.013	-0.031	-0.097	0.030	-0.070
8					B	0.063	0.004	-0.074	0.004	0.055
9	*p*-range =	0.048	to	0.057	B	-0.043	0.158	-0.048	0.001	-0.053
10										
11					Pooled st dev =	0.041	0.088	0.065	0.055	0.071
12					*t*-statistic =	1.20	-0.38	2.45	1.39	1.02
13					Comparison =	0	0	1	0	0
14	Mann-Whitney test:									
15					Ranks A	6	2	1	3	6
16					A	5	7	2	2	5
17	*W*-statistic =	25			A	1	3	3	1	1
18					A	3	8	5	7	2
19	Proportion =	0.0588			B	7	5	4	8	3
20	StDev =	0.0024			B	4	6	8	4	8
21					B	2	4	7	5	4
22	*p*-range =	0.054	to	0.063	B	8	1	6	6	7
23										
24					W_A =	15	20	11	13	14
25					W_B =	21	16	25	23	22
26					Comparison =	0	0	1	0	0

Fig 3.52 Case study: River pH / 7. Monte Carlo analysis (columns I to NTS are hidden)

Steps 3, 4: *t*-test

For the *t*-test, we first use Eqn 3.6 in row 11 to calculate the pooled standard deviation for each sample pair, e.g.

[F11] = SQRT((3 * VAR.S(F2:F5) + 3 * VAR.S(F6:F9)) / 6)

and then Eqn 3.5 in row 12 to calculate the *t*-statistic, e.g.

[F12] = (AVERAGE(F2:F5) – AVERAGE(F6:F9)) / (F11 * SQRT(1/4 + 1/4))

For each of the generated 10,000 samples, we wish to test whether the *t*-value is *greater* (i.e. more extreme) than is observed for the experimental data. We do this by comparing the positive value (using the ABS() function) with the experimental value in B4, using

[F13] = IF(ABS(F12) > $B4,1,0)

which returns a '1' only if the positive value of the generated *t*-statistic (in F12) is *greater* than the experimental value (in B4). The formulae in F11, F12, and F14 are copied to all 10,000 data sets.

We then calculate in B6 the *proportion* of data sets that record a '1' in row 13. This is the proportion of random samplings where the null hypothesis generates a test statistic greater than the experimental value. This is the *p*-value:

p-value: [B6] = SUM(F13:NTU13) / 10000 = 0.0527

The standard deviation uncertainty in this proportion is calculated in B7, using Eqn 3.24:

[B7] = SQRT(B6 * (1 – B6) / 10000) = 0.0022

The confidence interval limits for the calculated *p*-value are then given in

[B9] = B6 – 1.96 * B7 and [D9] = B6 + 1.96 * B7

For the *t*-test in this particular example of randomly chosen data we get a *p*-value in the range 0.048 to 0.057, and this is consistent with the directly calculated *p*-value of 0.052 in 3.1.3.

Note that, every time the 'Enter' or 'F9 function' key is pressed, 10,000 new calculations will be performed giving new calculated confidence interval ranges for the *p*-value. We should find that, if we repeat the analysis many times, 95% of the confidence intervals should include the value 0.052.

Steps 3, 4: Mann–Whitney test

To perform the Mann–Whitney test on the two samples in F2 to F9, we first calculate the rank of each data value. For example the rank of the number in F2 (within the range of numbers in F2:F9) is calculated and recorded in F15 using the equation:

[F15] = RANK.AVG(F2,F$2: F$9)

This formula is then copied down to all cells F15:F22 to generate the ranks for the values in both data samples.

In F24 and F25 we calculate the values of W_A and W_B by simply calculating the total ranks for each of the samples, *A* and *B*. The formulae in F15 to F25 are copied to all columns up to NTU, giving 10,000 resamples of the Mann–Whitney calculation.

A pair of samples *A* and *B* will only give a value equal to, or more extreme than, the observed samples, if either W_A or W_B has a value equal to, or greater than, the experimental upper rank total, W_U, which is in B17. We can identify the data sets that fall into this category by using the comparison condition in row 26, for example:

[F26] = IF(OR(F24 >= $B17, F25 >= $B17), 1, 0)

which returns a '1' only if the generated sets show a more extreme variation from the null hypothesis than the experimental data.

As with the *t*-test, we then calculate in B19 the *proportion* of data sets that record a '1'. This is the proportion of random samplings where the null hypothesis will record a test statistic greater than the experimental value. This is the *p*-value:

p-value: [B19] = SUM(F26:NTU26) / 10000 = 0.0588

The standard deviation uncertainty in this proportion is calculated in B20, using Eqn 3.24:

[B20] = SQRT(B19*(1–B19)/10000) = 0.0024

The confidence interval limits for the calculated p-value are then given in

[B22] = B19 – 1.96*B20 and [D22] = B19 + 1.96*B20

For the Mann–Whitney test in this example calculation we get a p-value in the range 0.054 to 0.063, and this is consistent with the analysis in 3.5.1 where we did not reject the null hypothesis.

3.9.3 Chi-squared probabilities

The use of the Monte Carlo method has a distinct advantage over the standard chi-squared test when dealing with *low expected frequencies* in one or more categories (3.7.7). We develop the chi-squared case study to illustrate this application.

Resampling chi-squared: Excel analysis for Fig 3.53. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: Chi-squared / 3. Monte Carlo analysis

–continued from 3.7.2

The chi-squared test was used in 3.7.2 to assess whether the distribution of four experimental frequencies was significantly different from the expected ratios 9:3:3:1 predicted by Mendel's theory.

In this previous problem there was an expected frequency of at least five in each category. We now consider the same problem, but with a new set of experimental frequencies of 7, 8, 3, and 2 respectively in the four categories, which gives three categories with expected frequencies of less than five.

With the low data numbers, the standard chi-squared calculation is no longer reliable, and we use the Monte Carlo method as outlined in 3.9.1.

Step 1

In Fig 3.53, the experimentally observed numbers are entered in D3:D6, giving a total frequency of [D7] = 20.

From the expected ratios, 9:3:3:1 in B3:B6 we can calculate these ratios as probabilities in C3:C6 giving 0.563, 0.188, 0.188, 0.063 (to 3 dp), e.g.

[C3] = B3 / SUM(B$3:B$6)

We then calculate the expected values in E3:E6, e.g.

[E3] = D$7*C3

The chi-squared statistic, χ^2, for the experimental data is calculated in D8 using Eqn 3.14.

[D8] = (D3 – $E3)^2 / $E3 + (D4 – $E4)^2 / $E4 + (D5 – $E5)^2 / $E5 + (D6 – $E6)^2 / $E6

giving $\chi^2 = 7.022$

Three of the expected frequencies in C3:C6 are less than five, which prevents us from comparing the chi-squared value with critical values from tables or using the standard calculation of p-value, but we can continue with the Monte Carlo method.

	A	B	C	D	E	F	G	H	I	J	NTU	NTV
1	**Category**	**Expected**	**Expected**	**Observed**	**Expected**		1	2	3	4	9999	10000
2		**ratios**	**proportions**	**numbers**	**numbers**							
3	AB	9	0.5625	7	11.25		11	9	13	9	7	12
4	Ab	3	0.1875	8	3.75		3	7	3	6	3	6
5	aB	3	0.1875	3	3.75		2	3	3	5	7	1
6	ab	1	0.0625	2	1.25		4	1	1	0	3	1
7			**Total =**	20								
8		**Chi-squared Statistic =**		7.022	**Re-sampled =**		7.022	3.467	0.622	3.467	7.022	3.467
9			***p* =**	0.081	**Comparison =**		1	0	0	0	1	0
10			**StDev =**	0.0027								
11	***p*-range (99% *CI*) =**		0.074	to	0.088							

Fig 3.53 Case study: Chi-squared / 3. Monte Carlo analysis (columns K to NTT are hidden)

Step 2

We now generate the first random sample of values in G3:G6, based on the null hypothesis that a total of D7 = 20 individual events are randomly allocated to the four categories with the probabilities in C3:C7. The first value in G3 generated by using the binomial distribution to randomly allocate a number out of a possible 20 based on the probability in C3:

[G3] = BINOM.INV($D7, $C3, RAND()) = 11

We now have 20 – G3 events left to allocate to the remaining three categories. Hence we allocate a number to G4, out of a possible D7-G3, using the binomial distribution but with a new probability calculated by dividing C4 by the sum of the remaining probabilities C4:C6:

[G4] = BINOM.INV(D7 – G3,$C4 / SUM($C4 : $C6),RAND()) = 3

We perform a similar allocation for G5 based on the numbers not allocated in G3 or G4:

[G5] = BINOM.INV(D7 – G3 – G4,$C5 / SUM($C5 : $C6),RAND()) = 2

Then the remaining numbers from the original 20 in D7 are entered into G6

[G6] = $D7 – G3 – G4 – G5 = 4

The formulae in G3:G6 are then copied to column NTV to give 10,000 randomly generated samples which are all based on the null hypothesis that the observed frequencies occurred randomly according to the probability ratios of 9:3:3:1.

Step 3, 4

For every resampled data set we now calculate the chi-squared statistic in row 8 by copying the calculation in D8 to all cells G8:NTV8.

The next step is to test in row 9 whether the chi-squared value for each sample in G8:NTV8 is greater than, or equal to, the experimentally observed value in D8, using for example:

[G9] = IF(G8 >= $D8,1, 0)

The IF() function returns a '1' if the resampled data gives a chi-squared value that is equal to, or more extreme, than the observed.

The *proportion* of '1s' in row 9 equals the probability that the observed (or greater) chi-squared value could have occurred by chance from the null hypothesis, and is therefore equal to the *p*-value for the test. This proportion is calculated in D9:

p-value: [D9] = SUM(G9:NTV9) / 10000 = 0.081

The standard deviation uncertainty in this proportion is calculated in D10, using Eqn 3.24:

[D10] = SQRT(D9 * (1−D9) / 10000) = 0.0027

In this example, we calculate the 99% confidence interval limits for the calculated *p*-value (to compare with SPSS below), for which the relevant *z*-value is 2.58:

[C11] = D9 − 2.58 * D10 and [E11] = D9 + 2.58 * D10

In this *particular* calculation, the 99% confidence interval range for the *p*-value is from 0.074 to 0.088, and, since these values are all greater than 0.05, we conclude that there is not enough evidence for a significant difference from the specified frequency ratios.

We can use SPSS to perform the same Monte Carlo analysis.

SPSS

Starting with the data as in Fig 3.54, it is necessary to first *weight* the cases (8.1.3, 8.2.4) to give the correct frequency weighting to each category:

SPSS > Data > Weight Cases....
◉-Weight cases by:
Frequency variable: *Freq*

and we then use the chi-squared analysis:

SPSS > Analyze > Nonparametric tests > Legacy Dialogs > Chi-Square...
Test variable list: *GTypeN* (categories must be defined by numeric values)
> Exact... ◉-Monte Carlo
Expected values: Either accept all categories as equal ***or***
◉-Values: Add expected frequency values in ascending order of the numeric categories, e.g. enter 9 **Add** 3 **Add** 3 **Add** 1 **Add** if the values of *GTypeN*, 1, 2, 3, 4 describe the categories *AB*, *Ab*, *aB*, *ab*.

The 99% confidence interval in Fig 3.54(b) is 0.070 to 0.084 which is consistent with the results given by the Excel model above, allowing for the fact that each new set of random

	GType	GTypeN	Freq
1	AB	1.00	7.00
2	Ab	2.00	8.00
3	aB	3.00	3.00
4	ab	4.00	2.00

(a) Frequency data entry

Test Statistics

			GTypeN
Chi-Square			7.022[a]
df			3
Asymp. Sig.			.071
Monte Carlo Sig.	Sig.		.077[b]
	99% Confidence Interval	Lower Bound	.070
		Upper Bound	.084

a. 3 cells (75.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.3.

b. Based on 10000 sampled tables with starting seed 2000000.

(a) Chi-squared results

Fig 3.54 Monte Carlo analysis for a 'goodness of fit 'test in SPSS

numbers will generate a slightly different result. SPSS also reports the *starting seed* used to generate the particular set of random numbers used.

Comparing data

Introduction

Many scientific investigations arrive at the need to *compare* sets of experimental data, either testing for the *existence* of some relationship or measuring the *strength* of that relationship. Chapter 3 introduced methods for hypothesis testing, and now this chapter brings together a range of different techniques which provide measures of association and agreement between theoretical models and experimental data, and also between different experimental measurements of the same quantities.

Section 4.1 further develops the statistics of *parametric correlation* from Section 2.1, and also introduces methods of *nonparametric correlation*.

Section 4.2 further develops the statistics for testing *association* from Section 3.7, and also introduces *Fisher's exact test* and the ability to test for *progression* in factors.

Section 4.3 considers the *strength* of the association between factors, and reviews a range of possible methods of measurement.

Section 4.4 reviews the concept of *agreement* in various contexts, including the 'goodness of fit' of analytical models, and agreements between variables and within contingency tables.

4.1 Correlation

The parametric statistics of correlation were introduced through the analysis of the straight line in 2.1.3, and these are now reviewed in relation to testing for the *existence* of, and measuring the *strength* of, *linear* correlation. We now also introduce nonparametric methods for correlation that are appropriate for ordinal data and which do not specifically assume that the relationship between the variables is that of a straight line.

4.1.1 Linear correlation

Linear correlation is a measure of the extent to which one variable increases in the same *ratio* as the increase in a second variable. The **correlation coefficient, *r***, between two variables x and y can be defined as *the proportion of the variation in y that is predicted by the variation in x.*

The variation in x can be represented by its standard deviation, s_x. If the slope of the line of regression of y against x is m, then the variation in x translates into a *predicted* variation in y given by $m \times s_x$. The *actual* variation in y is given by s_y, and thus the correlation coefficient, r, is given by the ratio:

$$r = \frac{\text{Variation in } y \text{ predicted by } x}{\text{Actual variation in } y} = \frac{m \times s_x}{s_y} \qquad (4.1)$$

Pearson's correlation coefficient, r, (or product moment coefficient) is the standard parametric statistic for linear correlation (2.1.3), and the square of its value is the **coefficient of determination, r^2**, which assesses the 'goodness of fit' (4.4.1) of the data to a straight line.

The related hypothesis test calculates whether the best-fit slope is significantly different from zero, with the null hypothesis:

H_0: The best-fit straight line for the data has a *zero* slope.

The value of the correlation coefficient can range from +1 when all the data values fall exactly on a straight line with a *positive* slope to −1 (perfect negative correlation) where they all fall on a straight line with a *negative* slope.

The *value* of the correlation coefficient, r, is NOT dependent on the slope, m, of the best-fit straight line except that:

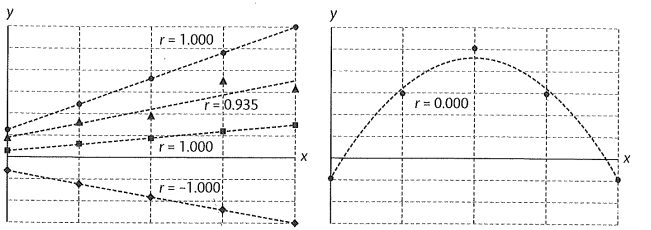
if the slope of the best-fit straight line is zero, $m = 0$, then $r = 0$.

if m is positive then r is also positive, and if m is negative then r is also negative.

Fig 4.1(a) shows examples of two data sets (circles and squares) with perfect *positive* correlation, $r = 1.000$, but with *different slopes*. A third data set (diamonds) has perfect *negative* correlation, $r = -1.000$. In all these cases the data points all lie exactly on the best-fit line. A fourth data set (triangles) has a best-fit line with a positive slope and with a correlation coefficient, $r = 0.935$.

Fig 4.1(b) shows a data set with an obvious correlation between x and y, but the correlation is not *linear*, and the calculated linear correlation coefficient actually has a zero value, $r = 0.000$. It is important to remember that r refers specifically to the correlation of data along a *straight* line.

The calculation of the correlation coefficient is *symmetrical* between x and y, and there is no suggestion that one variable is *dependent* and the other *independent* (2.1.1). Correlation between variables does not imply *causation*, and, in fact, we will see (4.1.4) that a bivariate



(a) Linear correlation (b) Nonlinear correlation

Fig 4.1 Examples of correlation in x–y scatterplots

correlation between two variables can appear because they are both dependent on the variation of third variable.

The *significance* of a calculated value of r is a measure of whether the apparent correlation could have occurred by chance. It depends on the number of data pairs, n, and can be assessed by comparing r with published sets of critical values. It is also possible to calculate an equivalent p-value in Excel, via a t-value, using the equation:

$$p = \text{T.DIST.2T}(X, n-2) \tag{4.2}$$

where the value of X is calculated as

$$X = r \times \sqrt{\frac{n-2}{1-r^2}} \tag{4.3}$$

We will use the following case study to demonstrate the calculation of the parametric correlation coefficient using Eqns 4.1, 4.2, and 4.3, and use the same data to demonstrate the nonparametric correlation coefficients introduced in 4.1.2.

Case study: **Toxicity assays / 2. Correlation**

–continued from 7.1.1, leading to 4.4.2

Parametric and non-parametric correlation: Excel analysis for Fig 4.2. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Two types of assay, *B1* and *B2*, have been used to measure the percentage of cell deaths due to exposure to different drug concentrations, C mM, with results given in Fig 4.2. The *ranked* values for *C*, *B1*, and *B2* are also given respectively as *CR*, *BR1*, and *BR2*.

	A	B	C	D	E	F	G	H	I	J	K
1	C/Mm	LogC	B1 (x)	B2 (y)					CR	B1R	B1R
2	0.01	-2.00	2.0	4.0					1	1	1
3	0.1	-1.00	15.0	16.0		m =	1.407		2	2	2
4	1	0.00	36.0	50.5		s_x =	19.157		3	5	3
5	5	0.70	33.0	54.5		s_y =	28.176		4	3	4
6	10	1.00	35.0	65.0		r =	0.957		5	4	5
7	20	1.30	57.5	75.5		p =	0.003		6	6	6
8											

Fig 4.2 Cell death and drug concentration. The ranked values are analysed in 4.1.2.

We wish to compare the level of agreement between the two assay methods, *B1* and *B2*, in Fig 4.2 by calculating the *correlation* between the numbers of deaths using assay *B1* with those recorded using assay *B2*. Fig 4.3(a) plots *B2* as y against *B1* as x on a scatterplot using the *interval* values directly. If the two assays were in perfect *agreement* we would expect equal values at each concentration giving a straight line with a slope, $m = 1.0$, intercept, $c = 0$ and with a correlation coefficient, $r = 1.00$, but the actual trendline, with $m = 1.41$, suggests that *B2* gives *proportionately* higher results than *B1*. However, in this analysis we are just testing for *correlation* and the extent to which *B1* and *B2* values fall close to a straight trendline, and we use Pearson's r to measure the *strength* of this linear correction. The p-value tests whether there is *any* true correlation, i.e. whether the slope is *significantly* different from 0.

Calculating the slope, m, between *B1* and *B2* using the Excel function:

m: [G3] = SLOPE(D2:D7, C2:C7) = 1.407

and the sample standard deviations, s_x and s_y, for the two samples,

s_x: [G4] = STDEV.S(C2:C7) = 19.157

s_y: [G5] = STDEV.S(D2:D7) = 28.176

Using Eqn 4.1, Pearson's product moment correlation, r, is

r: [G6] = (G3 * G4)/G5) = 1.407 * 19.157 / 28.176 = 0.957.

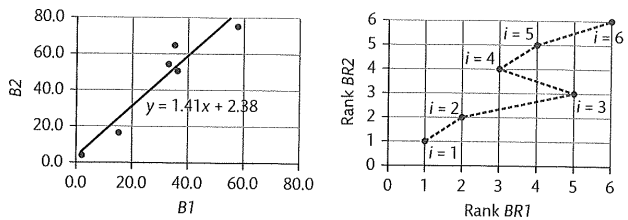
It is also possible to get this result directly in Excel by simply using the function CORREL() or PEARSON().

The p-value can be calculated using Eqns 4.2 and 4.3:

p-value: [G7] = T.DIST.2T(G6 * SQRT((6-2) / (1– G6^2)),4) = 0.003.

Since the p-value is less than the default significance level of 0.05, we accept that there is significant evidence of linear correlation between *B1* and *B2*.

Section 2.1.3 develops an alternative method of calculating Pearson's correlation coefficient, together with the use of Minitab and SPSS.



(a) Interval data (parametric) (b) Ranked data (nonparametric)

Fig 4.3 *B1* and *B2* data plotted from Fig 4.2

4.1.2 **Nonparametric correlation**

The two main nonparametric correlation statistics are Spearman's rho, ρ or r_S, and Kendal's tau-b, τ, in which it is only the relative *ranking* (1.1.2) between two data values that is important, and the magnitude of the *difference* between them is irrelevant.

Fig 4.3(b) plots the *ranked* values, *BR1* and *BR2*, of the data from Fig 4.2 as the x and y coordinates, with the points joined by a dotted line to indicate the *order* of increasing concentration. Two data points would be *positively* correlated if, when moving from one point to the other, the ranking changes in the *same way* for both values in the data pair, giving a *positive slope* in a scatterplot. They would be negatively correlated if their ranks changed in opposite directions giving a negative slope in the scatterplot. For example, in Fig 4.3(b) the point labelled $i = 4$ is negatively correlated with $i = 3$, but it is positively correlated with all other data points.

Measures of correlation for the data record the extent to which *all* the data pairs show the *same direction* of change. Perfect correlation occurs if the change in one variable is always in the same direction (either positive or negative) with respect to a change in the other variable.

Spearman's rank correlation coefficient is calculated by first identifying the rank of the values in each sample, calculating the difference, d_i, in these ranks for each data pair, and then using the equation

$$r_s = 1 - \frac{6\sum_i d_i^2}{n(n^2-1)} \tag{4.4}$$

where n is the number of data pairs.

As with the Pearson's correlation coefficient, the values of the Spearman's correlation coefficient range between −1 and +1, and their significance can be tested by comparing the positive value with a table of critical values.

Kendall's tau, τ, is an alternative nonparametric correlation coefficient which uses the same ranked data as in Spearman's calculation. It calculates the numbers of concordant, C, and discordant, D, pairs of values within samples (4.3.5). A concordant pair of values has both ranks changing in the same direction, giving a positive slope in Fig 4.3(b), and a discordant pair change in opposite directions, giving a negative slope. For example, points 3 and 6 would be a concordant pair but 3 and 4 would be discordant.

$$\tau = \frac{C-D}{0.5n(n-1)} = \frac{C-D}{C+D} \tag{4.5}$$

The expression for τ in Eqn 4.5 assumes that there are no *tied* ranks with equal pair values, which can result in some pairs of values being neither concordant nor discordant. This situation is addressed using a modification of the simple formula to calculate Kendal's tau-b, which is introduced in 4.3.5, but we use the simpler formula here to understand the basic principles.

Using the data from Fig 4.2, the ranked values for *BR1* and *BR2* have been transposed into rows 2 and 3 in Fig 4.4. Each of the six data pairs has been identified by the value of the label, i, in row 1.

	A	B	C	D	E	F	G	H	I
1	i	1	2	3	4	5	6		
2	x_i (*B1R*)	1	2	5	3	4	6	n =	6
3	y_i (*B2R*)	1	2	3	4	5	6		
4	d_i	0	0	2	-1	-1	0		
5	d_i^2	0	0	4	1	1	0	Σd_i^2 =	6
6								r_S =	0.829
7	a	b =	2	3	4	5	6		
8	1		C	C	C	C	C	n_C =	13
9	2			C	C	C	C	n_D =	2
10	3				D	D	C		
11	4					C	C	τ =	0.733
12	5						C		

Fig 4.4 Calculation of Spearman's and Kendal's tau correlation coefficients

For **Spearman's correlation coefficient**, we first calculate, in row 4, the differences d_i in rank for each data pair, i, and then, in row 5, the square of these differences d_i^2.

The sum of the d_i^2 values is calculated in I5, $\Sigma d_i^2 = 6$, and then the correlation coefficient is calculated in I6 using Eqn 4.4.

p or r_S: [I6] = 1−6*I5/(I2*(I2^2−1)) = 0.829

For **Kendal's tau** we consider every possible pair of values identified by the different i values, and decide whether they are concordant, with ranks changing in the *same* direction, or discordant with ranks changing in *opposite* directions.

For example, if we take the values $i = 3$ and 4 in columns D and E:

$x_3 = 5$ and $x_4 = 3$, giving $x_3 > x_4$, but

$y_3 = 3$ and $y_4 = 4$, giving $y_3 < y_4$

In this case, the x-rank does not change in the same direction as the y-rank, and this is then a *discordant* pair. In the 'results' section between A7 and G12 we then enter a D in E10 to record that the pair defined by $a = 3$ and $b = 4$ is discordant. By comparison, for the values $i = 3$ and 6, the x-rank and y-rank both change in the *same* direction, and thus form a *concordant* pair, recorded as C in G10, defined by $a = 3$ and $b = 6$.

We simply record the total numbers of concordant, $n_C = 13$, and discordant pairs, $n_D = 2$, in I8 and I9 respectively. Kendal's tau is then calculated using Eqn 4.5

τ: [I11] = (I8−I9)/(0.5*I2*(I2−1)) = 0.733

SPSS performs the calculations for nonparametric correlation coefficients, as above, and their associated p-values directly:

SPSS > Analyze > Correlate > Bivariate.... Variables: *BR1 BR2*
☑-Kendall's tau-b ☑-Spearman's
→ Output: Fig 4.5

Correlations

			BR1	BR2
Kendall's tau_b	BR1	Correlation Coefficient	1.000	.733*
		Sig. (2-tailed)	.	.039
		N	6	6
	BR2	Correlation Coefficient	.733*	1.000
		Sig. (2-tailed)	.039	.
		N	6	6
Spearman's rho	BR1	Correlation Coefficient	1.000	.829*
		Sig. (2-tailed)	.	.042
		N	6	6
	BR2	Correlation Coefficient	.829*	1.000
		Sig. (2-tailed)	.042	.
		N	6	6

*. Correlation is significant at the 0.05 level (2-tailed).

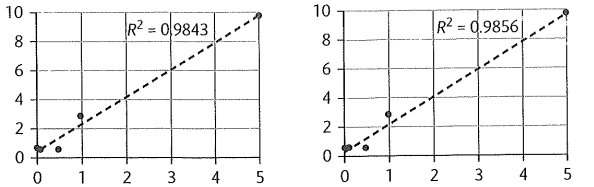
Fig 4.5 SPSS Nonparametric correlation

Fig 4.5 gives the correlation coefficients of 0.829 and 0.733 as calculated above, and also gives the equivalent two-tailed p-values recorded as 'Sig'.

Spearman's correlation coefficient in Minitab can be obtained by using the contingency table statistics via crosstabs (8.2.4):

Minitab > Stat > Tables > Cross Tabulation and Chi-Square...
Rows: *BR1* **Columns:** *BR2*
> Other Stats...: ☑-Correlation coefficients for ordinal categories

4.1.3 Scientific context of correlation



Linear correlation: p = 0.001
Nonparametric correlation: p = 0.285
(a) y-values: 0.52, 0.50, 0.49, 2.80, 9.68

Linear correlation: p = 0.001
Nonparametric correlation: p = 0.037
(b) y-values: 0.52, 0.50, 0.55, 2.80, 9.68

Fig 4.6 Effect of bunched measurements at one end of a correlation

It is useful to compare the conclusions from Pearson's and Spearman's correlation tests using some example data shown in Fig 4.6. It is not uncommon to see student data with multiple values bunched towards one end of the graph, and we use this to calculate the p-values for the two forms of correlation. The only difference between graphs (a) and (b) is that the y- value of the middle data point has a very small change from 0.49 to 0.55.We see that, for the *parametric* analysis, the R^2 value is almost unchanged between the graphs and the p-value remains a highly significant 0.001. However, the small change in the actual y-value of the middle point changes the *nonparametric* p-value from a non-significant 0.285 to a significant 0.037. The small experimental difference makes very little difference to the parametric calculation, but, because the data values are so close together in the bunch the difference changes the *rank* order between the second and third point (from 0.50 > 0.49 to 0.50 < 0.55) giving a very different nonparametric result. It is important to be aware of how experimental variations may affect the statistical interpretation of your data.

4.1.4 Bivariate and partial correlation

In situations where there are more than two related samples, it is possible to measure the correlations, pairwise, between each possible pair of samples. A *bivariate* correlation relates to the simple correlation calculated solely between each pair, without taking into account the variations of any other sample. However, it is possible that an *apparent* correlation exists between two samples because they are both also correlated with a third sample. A *partial* correlation between two samples takes into account (or controls for) the third sample.

Case study: Correlated variables / 1. Bivariate and partial (overview)

–leading to 3.4.5

Fig 4.7(a) gives a response variable, *R*, and three related input variables, *v1*, *v2*, and *v3*. We start here by investigating the correlations between *R*, *v1*, and *v2*, and in Section 3.4 we compare these results with the data produced by a general regression analysis.

3.4.5 / 2. Sums of squares: Demonstrates the difference between sequential and adjusted sums of squares, leading to 3.4.6 and the difference between 'pure error' and 'lack of fit' in a regression model.

	A	B	C	D
1	*R*	*v1*	*v2*	*v3*
2	10.00	2.24	0.94	0.94
3	12.30	2.39	1.11	1.12
4	9.60	1.71	0.76	0.76
5	8.00	1.85	0.89	0.91
6	9.80	1.65	0.85	0.85
7	9.60	2.18	1.12	1.12
8	8.20	2.08	0.81	0.81
9	9.50	2.30	0.91	0.91
10	7.40	1.69	0.72	0.72
11	11.90	2.50	1.25	1.25

(a) Data

Correlations

		R	v1	v2
R	Pearson Correlation	1	.678*	.784**
	Sig. (2-tailed)		.031	.007
	N	10	10	10
v1	Pearson Correlation	.678*	1	.818**
	Sig. (2-tailed)	.031		.004
	N	10	10	10
v2	Pearson Correlation	.784**	.818**	1
	Sig. (2-tailed)	.007	.004	
	N	10	10	10

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

(b) SPSS output

Fig 4.7 Bivariate correlations between *R*, *v1*, and *v2*

Bivariate and partial correlation: Analysis for Fig 4.7. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

SPSS calculates the direct bivariate correlations in Fig 4.7(b) using:

SPSS > Analyze >Correlate>Bivariate...
Variables: *R v1 v2*
☑-Pearson

The bivariate correlation suggests that there is a significant correlation between *v2* and *R* with $p = 0.007$ and between *v2* and *v1* with $p = 0.004$. As *v2* shows significant correlation with both *R* and *v1*, it is not surprising that there is also correlation between *v1* and *R* with $p = 0.031$.

Correlations

Control Variables			R	v1
v2	R	Correlation	1.000	.102
		Significance (2-tailed)	.	.794
		df	0	7
	v1	Correlation	.102	1.000
		Significance (2-tailed)	.794	.
		df	7	0

(a) Partial correlation between *R* and *v1*, controlling for *v2*

Correlations

Control Variables			R	v2
v1	R	Correlation	1.000	.542
		Significance (2-tailed)	.	.131
		df	0	7
	v2	Correlation	.542	1.000
		Significance (2-tailed)	.131	.
		df	7	0

(b) Partial correlation between *R* and *v2*, controlling for *v1*

Fig 4.8 Partial correlations between *R*, *v1*, and *v2*

SPSS performs the partial correlation between R and $v1$, while controlling for $v2$:

SPSS > Analyze >Correlate > Partial...
Variables: *R v1*
Controlling for: *v2*

The *partial* correlation between R and $v1$ in Fig 4.8(a)measures the variability in R related to $v1$ *after* the effect of $v2$ has been taken into account. We see that this now gives $p = 0.794$ which suggests that most of the variation in R can be predicted by $v2$. Similarly R also showed significant correlation with $v1$, so that the partial correlation between R and $v2$, controlling for $v1$, leaves less variation to be described by $v2$ giving $p = 0.131$. These results do not specifically decide whether there is a *scientific* cause and effect link between R and $v1$ or between R and $v2$, although the relative magnitudes of the correlation coefficients do indicate the strongest direct relationships.

We can compare these results with those in 3.4.5 where we perform a general regression analysis to model R on $v1$ and $v2$, and find the same partial p-values in Fig 3.31.

4.2 Tests for association

The concept of association between two factors in a contingency table of frequencies was introduced in 3.7.4, and the concept of an ANOVA interaction between factors in 3.3.2. In this section we:

- investigate the similarities and differences between the analytical techniques that test for association and interaction.
- develop further methods of analysis for contingency tables: Fisher's exact test, Mantel–Haenszel linear by linear association chi-squared test, and Eta for nominal-interval association.

The practical procedures for using Minitab and SPSS for association within a contingency table are demonstrated in Section 8.2.

4.2.1 Association and interaction

We start by reviewing the difference between the terms *association* and *interaction* and their underlying analytical processes.

	A	B	C	D	E	F	G	H
1		B1	B2	Totals			B1	B2
2	A1	31	**19**	50		A1	**35.7, 25.7**	**16.8, 20.6**
3	A2	**20**	**30**	**50**		A2	**16.3, 22.7**	**32.2, 26.4**
4	Totals	51	49	100				
		Data set (a)					Data set (b)	
5		Association					Interaction	

Fig 4.9 Comparing association and interaction

Fig 4.9 shows two sets of data in shaded cells. Set (a) in B2:C3 gives *frequency* values, and set (b) gives two *replicate interval* values in each cell in G2:H3. Both sets of data are obtained under conditions defined by factors A and B, each with two levels.

For set (a), 100 observations/trials have been made, each one of which *falls* into one of the cells defined by the *observed values* for A and B, giving a total count or frequency for each cell. For set (b), a total of eight experiments/observations have been conducted under conditions *defined* by A and B, and the *observed result* recorded in the relevant cell.

The data is presented in Fig 4.10 (a) and (b) respectively, in which the response values are plotted against the values for A, with the values for B differentiated by circular and triangular markers. The two lines, one for $B1$ and one for $B2$, join the *values themselves* in set (a) and the *mean* of each data pair in set (b). The lines provide an indication of the change in response due to the change in level for A.

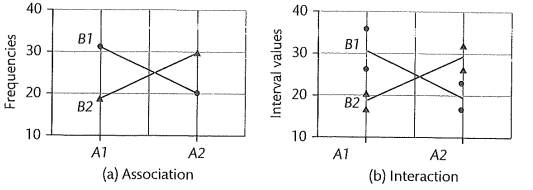


Fig 4.10 Factor plots for association and interaction

It certainly appears that the *response to a change in the value (level) of A depends on the value (level) of B*. The fact that the slopes of the two lines are not parallel suggests that there is an *association* in (a), and an *interaction* in (b), between the factors A and B.

The relevant tests for an *association* in (a) give results:

Corrected (Yates, 3.7.5) chi-squared = 4.002 giving $p = 0.045$

Fisher's exact test (4.2.3) gives $p = 0.045$.

The relevant GLM/ANOVA test (3.3.2) for an *interaction* in (b) gives $p = 0.034$.

Both analyses show a significant effect ($p < 0.05$), but it is useful to reflect on the underlying analytical processes. We see in 3.3.2 that the identification of an interaction in the GLM/ANOVA calculation requires the replicate measurements under identical conditions, such that it is possible to derive an estimate of the true experimental uncertainty in the measurement, assuming a normal uncertainty distribution. Without the replicate values in set (b) it would be impossible to distinguish between any interaction and experimental error. However, set (a) does not have replicates, but, with frequency values, the analysis uses the probabilities of the binomial distribution to estimate the uncertainty directly from the values themselves. Using Eqn 1.21, the standard deviation uncertainty in a frequency, N, is equal to $\sqrt{N}$, giving a standard deviation uncertainty in each of the values in (a) of approximately $\sqrt{25} \sim 5.0$.

The relatively large data uncertainties in (a) and variations in (b) explain why the association and interaction, which appear very obvious from the lines in Fig 4.10, are only just statistically significant, with p-values just less than 0.05.

A major difference between the two analytical methods is in the assumptions of the underlying statistical distribution. Analysis of (a) assumes a binomial/Poisson distribution, whereas the GLM for (b) assumes a normal distribution. The aim of the unifying *GsdLM* (3.4.7) is to incorporate the option of different distributions.

4.2.2 Tests for association

Table 4.1 lists possible hypothesis tests for the *existence* of an association, and Table 4.2 lists analyses that measure the *strengths* of association. The most common tests, e.g. Pearson's chi-squared analysis, treat the levels of the factors as nominal categories, with no sense of progression from one category to the next. However, many situations involve ordinal data, e.g. giving an opinion on a Likert scale, and the linear-by-linear test can be useful in that it takes into account this sense of progression between the levels of the two factors.

A common problem encountered, particularly in student experiments, is insufficient time to collect enough data to avoid having low expected frequencies in some cells of the table. We consider possible solutions in 8.2.7, either combining factor levels to reduce the number of cells or by using the Monte Carlo resampling approach.

Table 4.1 Tests for association in a contingency table

Statistic	Comment	Link
Pearson's chi-square	Standard statistic, but the test conclusions can be unreliable for low category numbers	3.7.4
Yates continuity correction	Correction when degrees of freedom = 1	3.7.5
Likelihood ratio	Alternative to Pearson's chi-squared value, usually giving larger values for smaller sample sizes	3.7.6
Fisher's exact test	Exact calculation based on binomial distribution. Tests for a difference in proportions in 2 × 2 tables	4.2.3
Linear-by-linear test	Test for an association between ordinal/linear factors, using a correlation analysis to identify a progression between factor levels.	4.2.4
McNemar's test	Tests for a difference in the off-diagonal elements of a 2 × 2 table. McNemar–Bowker test is similar to McNemar's test, but for larger tables.	4.4.5
Monte Carlo analysis	The Monte Carlo resampling techniques is useful for situations with low expected frequencies.	3.9.3

4.2.3 Fisher's exact test

R A Fisher addressed the contingency table problem by considering the probabilities with which different combinations of frequencies could occur while keeping the *same* row totals and column totals. In this way, he was able to develop an 'exact' test that calculated the cumulative probability that the observed distribution of values, or any more extreme distribution, could have occurred by chance.

Computer software can handle large contingency tables, but we can demonstrate the principle by analysing the simple 2 × 2 table between factors X and Y in Fig 4.11, with individual cell frequencies, a, b, c, and d, giving a total of N observed events. The null hypothesis is that the *distribution* of frequency values in each row is the same, from which it follows that the *distribution* of frequency values in each column is also the same.

	A	B	C	D
1		Y1	Y2	Total
2	X1	a	b	a+b
3	X2	c	d	c+d
4	Total	a+c	b+d	N = a+b+c+d

Fig 4.11 2 × 2 contingency table

Step 1

We use the **binomial coefficient** to calculate the *number of ways* that N randomly allocated items would result in the individual numbers of items, a, b, c, and d, for the particular set of the observed *column totals*.
In the *first* row:

Number of ways of selecting exactly a items in column 1 from a total of $a + b = {}_{a+b}C_a$

and in the *second* row:

Number of ways of selecting exactly c items in column 1 from a total of $c + d = {}_{c+d}C_c$

The total *number of ways* of obtaining the specific arrangement overall $= {}_{a+b}C_a \times {}_{c+d}C_c$

Step 2

We calculate the *probability* of obtaining the distribution a, b, c, and d, by dividing the number of ways from Step 1 by the total number of ways that the particular set of *column totals* could have occurred by chance.

Number of ways of selecting exactly $a + c$ items in column 1 from a total of $N = {}_{N}C_{a+c}$

The probability, P, that N randomly allocated items, with the defined row and column totals, would give the specific distribution of values, a, b, c, and d is:

$$P = \frac{{}_{a+b}C_a \times {}_{c+d}C_c}{{}_{N}C_{a+c}} \tag{4.6}$$

The calculations in Steps 1 and 2 are performed using Excel in Fig 4.12 for $a = 24$, $b = 19$, $c = 7$, and $d = 16$. We use the COMBIN() function to derive the binomial coefficients in F2, F3, and H2, and then Eqn 4.6 for P in H4.

Fisher's exact test: Excel analysis for Fig 4.12. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

	A	B	C	D	E	F	G	H
1		Data		Totals				
2		24	19	43	$_{a+b}C_a$ =	8.005E+11	$_{N}C_{a+c}$ =	6.406E+18
3		7	16	23	$_{c+d}C_c$ =	2.452E+05		
4	Totals	31	35	66			P =	0.0306

Fig 4.12 Calculation of the probability of a given distribution

Step 3

The final step in calculating the ***p*-value** is to calculate, and sum, the probabilities that the null hypothesis could also randomly produce more *extreme* distributions, but all with the *same row and column totals*. Fig 4.13 gives the most probable of the extreme distributions by decreasing the '7' by one each time and adjusting all other values to keep row and column *totals* constant. In each case the probability, *P*, for that distribution is calculated:

25	18	26	17	27	16	28	15	etc.
6	17	5	18	4	19	3	20	
gives P = 0.0096		gives P = 0.0022		gives P = 0.0004		gives P < 0.0001		

Fig 4.13 Calculation including more 'extreme' distributions

The total probability of the null hypothesis producing the observed distribution, or one of the more extreme distributions, will be given by the sum of all probabilities, *P*:

$$p = 0.0306 + 0.0096 + 0.0022 + 0.0004 + \ldots = 0.0428 \text{ (4 sf)}.$$

This gives a *p*-value that has been *calculated* exactly. However, the scientific *interpretation* of this *p*-value is not so exact as it tends to give slightly more Type II errors than it should, due to the fact that moving from one distribution to the next (as in Fig 4.13) shows discrete jumps and is not a smooth distribution.

It is also important to note that we have calculated a one-tailed *p*-value, by only including extreme distributions in the same sense as the observed distribution–i.e. with increasing values along the diagonal from top left to bottom right. For a two-tailed test (as for the chi-squared test), we would need to include extreme distributions with increasing values along the bottom left to top right. Unfortunately, the situation is not symmetrical and we cannot just double the one-tailed value (Eqn 1.27), but if we perform the additional calculations we would find $p = 0.070$. For example, if we use the two-proportion test in Minitab (3.8.3) to compare 24/43 with 7/23 we find that $p = 0.043$ for the 'greater than' option and $p = 0.070$ for the 'not equal' option.

The use of the binomial test in SPSS is demonstrated in 6.1.7 and 6.2.9.

4.2.4 Linear by linear association

The standard contingency table is defined by *nominal* variables, and the *order* in which rows and columns are placed makes no difference to the chi-squared calculation—see

for example the nominal categories, *Science*, *English*, and *History* in Fig 3.47. We now introduce the Mantel–Haenszel *linear by linear association* chi-squared test, which can be used for a contingency table when a row or column category is defined by an *ordinal* or *interval* variable.

The data in Fig 4.14 (a) and (b) both result from using two different methods, *M1* and *M2*, to lift fingerprints from difficult surfaces. The different columns represent the quality of the resultant prints recorded on an ordinal scale from 1 (poor) to 4 (excellent). The numbers of prints with the different qualities were recorded in the separate cells, giving a total of 30 prints using *M1* and 32 using *M2*.

The *linear by linear association chi-squared* is calculated, with degrees of freedom, $df = 1$, using:

$$Q_{MH} = (N-1) \times r^2 \qquad (4.7)$$

where *r* is the Pearson correlation coefficient.

The values for Pearson's and the linear by linear association chi-squared are calculated for each of the contingency tables in Fig 4.14 (a) and (b). The results in Fig 4.14 (a) show that, although there is not enough evidence for an association just based on nominal values ($p = 0.057$), the additional information of a *progressive* change between ordinal quality levels gives $Q_{MH} = 6.33$ with a significance of association of $p = 0.012$.

Quality:	1	2	3	4
M1	9	9	7	5
M2	5	4	9	14

Pearson's chi-squared = 7.522 giving $p = 0.057$
Q_{HM} = 6.33 giving $p = 0.012$
(a) Initial column order

Quality:	1	2	3	4
M1	9	5	7	9
M2	5	14	9	4

Pearson's chi-squared = 7.522 giving $p = 0.057$
Q_{HM} = 0.34 giving $p = 0.559$
(b) Interchanging columns 2 and 4

Fig 4.14 Effect of changing the order of categories

Set (b) actually has the same values as (a), but the difference is that the results for quality '2' have been swapped with quality '4'. The Pearson's chi-squared analysis tests whether there is a significant difference in the *distribution* of values between *M1* and *M2*, but *without* any sense of progression, and because the order of the columns makes no difference, it results in the same, non-significant, *p*-value for both sets, $p = 0.057$. However, the change in the linear by linear chi-squared value from the significant $p = 0.012$ in (a) to the non-significant $p = 0.559$ demonstrates that the data for (a) has an increasing number of prints *in proportion to* the quality of the print, whereas this is not the case for (b).

It is important to note that the test here is specifically for a *linear* association, and consideration must be made of the underlying scientific processes being tested. For example, it is possible that method *M2* may have the enhanced ability to provide *medium* and *good* quality prints, but has a technical limitation in not being able to produce *excellent* quality, in which case the expected relationship with quality would not be truly linear.

Fig 4.15 shows the quality of each separate fingerprint plotted against the method (*M1* or *M2*) used. The numbers by each data point give the numbers of prints giving the same value (the same frequencies as in the contingency table).

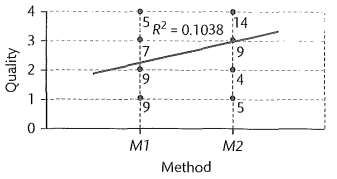


Fig 4.15 Observed qualities of fingerprint for two different lifting methods

The best-fit trendline is shown, together with the coefficient of determination, $r^2 = 0.1038$.

We can see from the slope of the best-fit line in Fig 4.15 that there is an apparent increase in the mean quality value between *M1* and *M2*. We can also test for a difference in median value by using a Mann–Whitney test for the two samples, *M1* and *M2*, from which we get $p = 0.011$ which is consistent with the Q_{HM} result of $p = 0.012$. Tests for correlation also give $p = 0.011$ and 0.010 for Pearson's and Spearman's correlation respectively.

Using the Pearson's value for $r = 0.322$ ($= \sqrt{r^2}$), for $N = 62$, we can calculate Q_{HM} using Eqn 4.7.

$$Q_{HM} = 61 \times 0.322^2 = 6.32$$

which is consistent with the value derived in SPSS.

4.3 Strength of association

In Section 3.7 we used the chi-squared statistic to perform a hypothesis test for a contingency table to test for a possible difference in the distribution of row values between different columns or vice versa. We were testing for the *existence* of an association between the factors that define the rows and columns. We now want to be able to measure the *strength* of that association.

For clarity of calculations we will use a simple 2 × 2 contingency table, but the concepts can be applied to larger tables.

4.3.1 Association and agreement

In describing the strength of association and agreement within a contingency table, we can consider the two variables as *X* and *Y*, each with categories described as 1 and 2.

	A	B	C	D
1		*Y1*	*Y2*	**Total**
2	***X1***	*a*	*b*	***a+b***
3	***X2***	*c*	*d*	***c+d***
4	Total	*a+c*	*b+d*	***N = a+b+c+d***

Fig 4.16 2 × 2 contingency table

The categories *X1*, *X2*, *Y1*, and *Y2* could be nominal values, but if they are ordinal or interval values we assume that the sense of progression would be from levels 1 to 2 in both variables.

The association between the variables could be either *symmetric*, with no specific dependence on either variable, or *directional* (asymmetric), in which the difference due to one variable *depends* on the other. A *directional* association could be in either direction (*X* dependent on *Y* or *Y* dependent on *X*), but, for consistency in our calculations, we will normally make the row variable, *X*, the *independent* variable, and the column variable, *Y*, the *dependent* variable. In this way our table is similar to an *x*–*y* scatterplot that has been rotated clockwise by 90°.

In mathematics, a table of values as in Fig 4.16 would be described as a matrix. The values *a* and *d* are said to be on the 'diagonal' of the matrix (top left to bottom right) and *c* and *b* are 'off-diagonal' elements.

Values *a* in B2 show that both *X* and *Y* record level '1', i.e. they are in *agreement*. Similarly values *d* in C3 also show agreement, both recording level '2'. However, values *b* and *c* in C2 and B3 both show *disagreement* (or negative agreement) with different values for *X* and *Y*.

An *association* (3.7.4) is demonstrated by a difference in the *distribution* of values for different rows and for different columns, and is only treated as a positive quantity.

20	18	38	3	3	38
19	22	2	37	37	2
(a)		(b)		(c)	

Fig 4.17 Examples of association and agreement

We can differentiate between association and agreement using the example tables in Fig 4.17:

(a) has an approximately even distribution of values and shows no overall association or agreement.

(b) has a strong association, e.g. with different proportions for the two rows, and shows strong positive *agreement* for ordinal data.

(c) also has strong association, but shows strong *disagreement* (or negative agreement) for ordinal data.

4.3.2 Measures of association

Tests for the *existence* of an association were given in Table 4.1, and now Table 4.2 identifies possible measures for the *strength* of an association between pairs of variables, grouped according to variable types: nominal, ordinal, and interval.

We can also divide the measured 'association' into two types: symmetrical and directional. In a *directional* (or asymmetric) association we measure how a knowledge of one