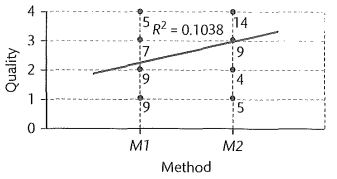


Fig 4.15 Observed qualities of fingerprint for two different lifting methods

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

Table 4.2 Measures for the strength of an association

Variable pairs defined by type	Symmetric measures	Directional measures
Nominal / nominal	Phi, ϕ (2 × 2 tables) Cramer's V Kappa, κ	Lambda, λ
Ordinal / ordinal	Gamma, Γ Kendall's tau-b, τ Spearman's rho*, ρ Coefficient of concordance	Somers' d
Interval / interval	Pearson's coefficient*, r	Linear regression**
Nominal / interval		Eta, η

*These measures are considered more fully in Section 4.1.
**Linear regression, developed in Section 2.1, is included here as a reminder that the standard regression calculation assumes zero uncertainty in x and calculates the regression of y *on* x, with y being the dependent variable.

(independent) variable can be used to predict the variation of the other (dependent) variable. In a *symmetric* association, there is no sense of direction and we measure only the extent to which the two variables vary in similar ways.

When selecting a possible analysis, it is useful to remember that, due to the one-way hierarchy between variable types, it is possible to analyse an interval variable as an ordinal variable and an ordinal variable as a nominal variable, but not in the reverse order. For example, Cramer's V could be used to measure the association between categories defined by ordinal variables (although the ranking of the variables will be lost), but it would not be possible to use Kendall's τ to analyse nominal data.

4.3.3 Cramer's *V* and Phi

The chi-squared statistic χ^2 works well as a test to identify the *significance* of a possible association, but it is not so useful in measuring the *strength* of the association, because its value depends on sample size. In the *hypothesis test*, the sample size is automatically taken into account by including the degrees of freedom in the calculation of critical values or p-values.

Cramer's V and Phi, φ, are *symmetric measures of association* (φ applies to 2 × 2 tables) based on the chi-squared statistic χ^2 between nominal variables, but *corrected* for sample size, N:

$$V = \sqrt{\frac{\chi^2}{N(k-1)}} \qquad \phi = \sqrt{\frac{\chi^2}{N}} \tag{4.8}$$

For larger than 2 × 2 tables For 2 × 2 tables only

where N is the total of recorded trials and k is the smaller of the number of rows or columns.

An approximate qualitative evaluation of these values is given in Table 4.3.

Table 4.3 Strength of association for Cramer's V and Phi

Value	Strength of association
0.0 > 0.10	Weak
0.10 > 0.30	Moderate
> 0.30	Strong

4.3.4 Goodman and Kruskal's Lambda

Lambda is an *asymmetric* (or directional) measure between nominal variables, in which one factor is measured as being *dependent* on the other, *independent*, factor.

The statistic calculates the 'proportional *reduction* in error' in predicting the *dependent* category of the next randomly chosen answer, based on whether it is either **before**, B, or **after**, A, taking into account the information of category numbers in the *independent* factor. The value of λ is given in Eqn 4.9 by the reduction in the probability of error, $pE_B - pE_A$,

$$\lambda = \frac{pE_B - pE_A}{pE_B} \tag{4.9}$$

where pE_B and pE_A are the probabilities of error 'before' and 'after' *knowing* the values of the independent factor.

It is common practice to put the independent variable as the column variable and the dependent variable in the rows, but, for reasons of consistency with other techniques in this book, we start with an example, in Fig 4.18, where the independent variable is the row variable, X.

	A	B	C	D
1		*Y1*	*Y2*	Total
2	*X1*	28	12	40
3	*X2*	18	22	40
4	Total	46	34	80

Fig 4.18 Calculating lambda, λ

If we take Y as the dependent factor and X the independent factor, we can calculate the value of λ by trying to predict into which Y category the next randomly chosen value will fall. We need to calculate the probabilities that our predictions will be *wrong*, based on the *observed* cell numbers.

Step 1 (Before)

We do not know whether the *next value* will be in the *Y1* or *Y2* category.

Without any knowledge of the X-value, we can only use the *total* numbers for the Y categories as predictors,

$$Y1(\text{total}) = 46 \text{ and } Y2(\text{total}) = 34$$

We would expect that a randomly chosen item would fall into the most probable, *Y1*, category, but the probability that it might fall into the other, *Y2*, category is given by

(80 – 46) / 80. This is the probability of error *before* knowing the value of X, and can be written in general as:

$$pE_B = \frac{N - (\text{Largest category total})}{N} \quad (4.10)$$

In this example, $pE_B = (80 - 46) / 80 = 34 / 80 = 0.425$

The equation might seem over complicated when there are only two categories for the dependent variable, because 80 – 46 just equals the value, 34, already given in the other cell. However the equation also covers situations where there are *more than* just two categories.

Step 2 (After)

We now know the value of X and there are two elements in the calculation with X equal to $X1$ or $X2$.

With X equal to $X1$, the probability of error would be (40 – 28) / 80 or,

with X equal to $X2$, the probability of error would be (40 – 22) / 80

Thus the total probability of error, given that we know the value of X, would be the sum of these two probabilities:

$$pE_A = \sum_{All\ rows} \left\{ \frac{\text{Row total} - \text{Largest frequency in row}}{N} \right\} \quad (4.11)$$

In this example, $pE_A = (40 - 28)/80 + (40 - 22)/80 = 0.375$

Step 3

The calculation of λ for Y dependent on X, using Eqn 4.9 gives

$$\lambda_{Y/X} = \frac{0.425 - 0.375}{0.425} = 0.118,$$

This can be interpreted by saying that a knowledge of the X values increases the predictability of Y by 11.8%.

We can also use the same data to calculate the value of lambda for X being dependent on Y:

$$pE_B = (80 - 40) / 80 = 40 / 80 = 0.5 \text{ and}$$
$$pE_A = (46 - 28) / 80 + (34 - 22) / 80 = 18 / 80 + 12 / 80 = 0.375$$
$$\lambda_{X/Y} = \frac{0.500 - 0.375}{0.500} = 0.250$$

Lambda values can be used to compare the strengths of bivariate relationships, e.g. compare different pairs of answers in a questionnaire.

Note that the lambda calculation does not work when the differences in both categories of the independent variable are in the same direction, and the calculation returns a zero value even when there is an association. For example, if X is considered to be the *dependent* variable in Fig 4.19(b), Y becomes the independent variable but with differences in the same direction 24 > 7 and 19 > 16, giving:

$$pE_B = (66 - 43) / 66 = 23 / 66 \text{ and}$$
$$pE_A = (31 - 24) / 66 + (35 - 19) / 66 = 7 / 66 + 16 / 66 = 23 / 66$$
$$\lambda_{X/Y} = \frac{23/66 - 23/66}{23/66} = 0$$

However, the calculation of Y dependent on X gives a non-zero value: $\lambda_{Y/X} = 0.161$.

4.3.5 Concordance of data pairs

A number of measures of association for *ordinal* factors are based on counting the numbers of concordant pairs, C, discordant pairs, D, and ties, T, within a whole data set.

X\Y	1	2	Total
1	*a*	*b*	*a* + *b*
2	*c*	*d*	*c* + *d*
Total	*a* + *c*	*b* + *d*	*N*

(a) Cell numbers

X\Y	1	2	Total
1	24	19	43
2	7	16	23
Total	31	35	66

(b) Example values

Fig 4.19 Concordance within a contingency table

We consider the 2 × 2 frequency table in Fig 4.19(a) with factors X and Y, in which we have the results of $N = a + b + c + d$ trials, where

a trials have resulted in values for X,Y of (1,1)

b trials have resulted in values for X,Y of (1,2)

c trials have resulted in values for X,Y of (2,1)

d trials have resulted in values for X,Y of (2,2)

We review all possible **pairs** of trials and classify them as concordant, C, or discordant, D, depending on the direction of differences in their values, using the 'rules' that:

(r,s) and (p,q) are **con**cordant if EITHER $r > p$ **and** $s > q$ OR $r < p$ and $s < q$

(r,s) and (p,q) are discordant if EITHER $r > p$ **and** $s < q$ OR $r < p$ and $s > q$

otherwise the trial 'pairs' are classified as ties, T.

Calculating the values for Fig 4.19(b):

$C = a \times d$ pairs of trials will be concordant : (0,0) is concordant with (1,1) $= 24 \times 16 = 384$

$D = b \times c$ pairs of trials will be discordant : (0,1) is discordant with (1,0) $= 19 \times 7 = 133$

$T_X = a \times b + c \times d$ pairs will be tied with just the *same* x-values $= 24 \times 19 + 7 \times 16 = 568$

$T_Y = a \times c + b \times d$ pairs will be tied with just the *same* y-values $= 24 \times 7 + 19 \times 16 = 472$

Goodman and Kruskal's gamma

Gamma is a measure of increase of the extent to which one variable increases in step with the other:

$$G = \frac{C-D}{C+D}. \tag{4.12}$$

Using the values from Fig 4.19(b):

$$G = \frac{384-133}{384+133} = 0.485.$$

However, this measure does not include ties in the calculation.

Kendall's tau-b

Kendall's tau was introduced in 4.1.2 with Eqn 4.5, which had the same form as Gamma, above, and did not allow for tied pairs. However, Kendall's tau-b does allow for ties, and provides a *symmetric* measure of association:

$$\tau_b = \frac{C-D}{\sqrt{C+D+T_x} \times \sqrt{C+D+T_y}}. \tag{4.13}$$

Using the values from Fig 4.19(b):

$$\tau_b = \frac{384-133}{\sqrt{384+133+568} \times \sqrt{384+133+472}} = 0.242.$$

Somer's D

Somer's D is a *asymmetric* (directional) measure of association, which uses a similar equation to Kendall's tau-b, but only includes one set of ties depending on the measured direction. For example, Somer's D for Y dependent on X is given by:

$$D_{Y/X} = \frac{C-D}{C+D+T_y}. \tag{4.14}$$

Using the values from Fig 4.19(b):

$$D_{Y/X} = \frac{384-133}{384+133+472} = 0.254 \text{ and } D_{X/Y} = \frac{384-133}{384+133+568} = 0.231.$$

4.3.6 Nominal by interval association, Eta

Eta, η, is a *directional* measure of the *strength* of association between the values of an *interval* variable, v, and a *nominal* variable or factor, F. Eta has possible values ranging from 0 for no association to 1 for strong association. We can demonstrate its use with the data set A in Fig 4.20 which shows six values of interval data, v, in column B, two at each level, 1, 2, and 3, of the factor F. The same data is also presented in the contingency table format in cells E2:G7, with a '1' to indicate the data entry at each of the relevant v values and F levels. In addition, the contingency table in J2:L7 shows a *second* data set, B, which has each of the values in set A but *duplicated* to give a set with a total of 12 values.

The factor levels, F, are given *numeric* values because this is a requirement of the SPSS analysis, but they are treated as *nominal* values with no sense of progression.The Eta value does not depend on the order in which the levels of the nominal variable are presented, as with the chi-squared analysis.

	A	B	C	D	E	F	G	H	I	J	K	L
1	*F*	*v*		*v* \ *F*	1	2	3		*v* \ *F*	1	2	3
2	1	6.9		4.4			1		4.4			2
3	1	5.5		5.5	1				5.5	2		
4	2	6.8		5.6			1		5.6			2
5	2	7.6		6.8		1			6.8		2	
6	3	5.6		6.9	1				6.9	2		
7	3	4.4		7.6		1			7.6		2	
8		Set A				Set A					Set B	

Fig 4.20 Nominal by interval association

Using SPSS we calculate the value of Eta by selecting '☑**-Eta**', under the 'Statistics' options (8.2.4), and obtain the value 0.840.

We can also perform a one-way ANOVA or Kruskal–Wallis test using the interval data in column B to *test* whether there is any significant difference in the mean or median values of v between the nominal levels of F, and we return the values shown in Table 4.4 for the two data sets.

Table 4.4 Difference between measures for the *existence of* and *strength of* association

	ANOVA p-value	Kruskal–Wallis p-value	Eta, η, v dependent
Set A (6 values)	0.159	0.276	0.840
Set B (12 values)	0.004	0.059	0.840

The results in Table 4.4 show that, for set A with six values, there are no significant differences between the values of v for the three levels of F, but 'taking more results' with the 12 values in set B, the tests are able to detect a significant difference in mean values and almost a difference in median values. Increasing the number of data values has increased the *power* of a test for the *existence* of an association. However, the *distribution* of values has not changed (Fig 4.20) between set A and set B, and it is this characteristic that describes the *strength* of the association. This is measured by Eta, and we see in Table 4.4, that this value is the same for the two data sets.

4.4 Agreement between variables

A test for correlation (e.g. with a *p*-value) *tests* whether a relationship *exists* between two variables or factors. However, in this section we measure the *agreement* between the variables, which is the extent to which the related pairs of data have the *same values*. We approach this concept of 'agreement' through a number of different contexts.

It is important to remember that hypothesis tests for agreement are actually testing for *disagreement*, and that a null hypothesis result does not imply total agreement, only that there is no evidence of disagreement.

4.4.1 R^2 goodness of fit

The most familiar example of fitting experimental data to a theoretical model is through linear regression and the derivation of a best-fit straight line. We see in 2.1.1 and 5.4.6 that it is the residuals that are the underlying measure of agreement between the values predicted by the analysis and the actual experimental values. The *overall* measure of agreement is the *coefficient of determination*, r^2, which can be calculated using Eqn 2.12:

$$r^2 = 1 - \frac{SS_{RESID}}{SS_{TOT}}$$

where SS_{RESID} is the sum of squares of the residuals and SS_{TOT} is the total sum of squares in the data.

The calculated value of r^2 (or R^2) is often reported in many analytical results (e.g. ANOVAs), giving a measure of the quality of agreement between the data and the best-fit parameters of a theoretical model. However, some care has to be taken when increasing the *number* of factors to describe the experimental data, because it becomes easier to achieve a better fit purely through random chance, and it is quite possible for the value of R^2 to *increase*, suggesting a better (but spurious) agreement. An 'adjusted' value of r^2, typically written as R^2(adj), takes into account the number of variables or factors that are used to achieve the fit, and, with more factors, the *adjusted* value, R^2(adj), may begin to *decrease*, indicating that there is no real advantage in increasing the model complexity.

In addition, the graphical display of residual values is also very useful in identifying where, *within* a data set, the mathematical model deviates from the experimental data, e.g. Fig 2.6. Residuals are also very useful for assessing normality and homoscedasticity (equality of variance) in an analysis (5.4.6).

4.4.2 Agreement between two related variables

Two samples with *related* variables have unique links between *pairs* of data. For example, in Fig 4.2, measurements are made at the same concentration by the two assays. We have seen that the change in these values can be described by correlation and regression calculations, but in this section we consider the level of *agreement* between the values in each pair.

Exact agreement. Each pair of measurements has the *same value*. Plotted on a scatterplot between the variables, this gives a straight line with slope = 1.0. The two variables must be measuring the same scientific *quantity* and with the same *units*.

Linear agreement. The change in the value of one variable is directly *proportional* to the change in the other. Plotted on a scatterplot this would give a straight line, but not necessarily with a slope equal to 1.0, or with intercept = 0.0. The two variables could be measuring different quantities.

Nonlinear agreement. The relationship between the variables follows a defined, but not linear, relationship between the values.

Rank agreement. Having ranked the variable values, the rank of each variable changes consistently with the rank of the other variable, either increasing or decreasing.

The strength of agreement for both *exact* and *linear* relationships are measured using Pearson's linear correlation coefficient, *r*, which is independent of the slope of the relationship (4.1.1), and the strength of *rank* agreement is measured using Spearman's correlation coefficient, ρ (rho) or Kendall's tau-b. For a curvilinear relationship it would be necessary to model the data using a nonlinear mathematical equation and calculate the goodness of fit, r^2, from the residuals. We use the following case study to develop relevant techniques.

Case study: Toxicity assays / 3. Agreement

—continued from 7.1.1 and 4.1.1, leading to 4.4.3

Fig 4.21 records measurements of the mortality (percentage deaths) of bacteria for increasing drug concentrations, *C*, (recorded as log(C)) using two assay methods, *A* and *B*, with each method repeated once. We wish to assess the *agreement between* the two methods and the *reproducibility within* each method.

The data in columns I, J, L, and M are used for the Bland–Altman plots in Fig 4.22(c) and Fig 4.23(c).

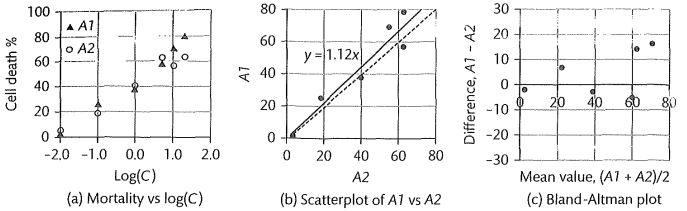
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Log(C)		A1	A2		B1	B2		(A1+A2)/2	A1-A2		(A1+B1)/2	A1-B1
2	-2.000		2.0	3.5		2.0	4.0		2.8	-1.5		2.0	0.0
3	-1.000		25.5	18.5		15.0	16.0		22.0	7.0		20.3	10.5
4	0.000		37.5	40.0		36.0	50.5		38.8	-2.5		36.8	1.5
5	0.699		57.0	62.0		33.0	54.5		59.5	-5.0		45.0	24.0
6	1.000		69.5	55.0		35.0	65.0		62.3	14.5		52.3	34.5
7	1.301		78.7	62.5		57.5	75.5		70.6	16.2		68.1	21.2

Fig 4.21 Replicate measurements of bacteria mortality using assays *A* and *B*

In this analysis, we compare *pairs* of variables separately, but in the following section (4.4.3) we develop the method of analysing multiple variables together. We first consider the *reproducibility* of assay *A*, by comparing the results of *A1* and *A2* in Fig 4.22.

Fig 4.22(a) plots both values of *A1* and *A2* against the *logarithm* of concentration, *C*. The use of the log axis presents concentrations related by multiplicative factors (e.g. dilutions) as evenly spaced points along the *x*-axis and avoids bunching of values at one end of the graph.

The initial options for comparing two variables include testing for a *linear* or *rank* agreement. We can then use linear regression for a *proportional* agreement and a measurement of the slope to identify *exact* agreement with slope = 1.0. Having confirmed correlation,



(a) Mortality vs log(C) (b) Scatterplot of *A1* vs *A2* (c) Bland-Altman plot

Fig 4.22 Reproducibility within *A* by comparing *A1* and *A2*

we could use a Bland–Altman plot to display *disagreements* between the variables and then a paired Wilcoxon test to test whether there is a significant *net* difference between the values.

Fig 4.22(b) shows the values of *A1* plotted against the *related* values of *A2*, together with the trendline and equation. For an *exact* agreement, the values should lie along the dashed line which has a slope $m = 1.0$ and intercept = 0. We can calculate the confidence interval for the best-fit slope passing through the origin (Eqn 2.16) as:

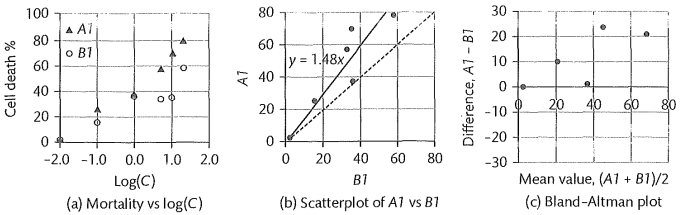
$$m = 1.12 \pm 0.20.$$

As this includes the possible value of $m = 1.00$, this result does not identify any significant difference between that the two sets of measurements.

The **Bland–Altman** plot in Fig 4.22(c) provides a more *sensitive* display of the agreement between measurements of the *same variable* than the simple *x–y* scatterplot. For each *A1A2* pair, the *difference* or *disagreement* between the values, $A1_i - A2_i$, (calculated in column J of Fig 4.21) is plotted on the vertical axis against their average (mean) value, $(A1_i + A2_i)/2$ (calculated in column I). If the values are in perfect agreement then they will all lie along the '0' horizontal axis, but any disagreements will appear as vertical deviations above and below the axis. The plot is good at highlighting any drift in agreement through the range of values.

The Bland–Altman plot displays the magnitude of the differences, $x_i - y_i$, between data pairs that ideally should be giving the same value. If we believed that the *uncertainties* were normally distributed and the same across the range, then we could use a *paired t*-test to test for a *net* difference in mean values. However, given that we are often measuring over a wide range of values, this condition is unlikely to be satisfied and we can use a paired Wilcoxon test for a net difference in median values. A significant *p*-value ($p < 0.05$) would suggest that there is *disagreement* between the values, although a non-significant *p*-value would not prove that there was total *agreement*. It is also important to note that the paired test only tests for a net difference over the *whole range*, and that a Bland–Altman plot that showed a clear progression from a negative difference at one end to a positive difference at the other end may give a non-significant *net* difference for the paired test.

Fig 4.23 shows similar plots for the comparison between *A1* and *B1*. We can see the greater disagreement as the data in (b) is significantly away from the line of unit slope and the points in the Bland–Altman plot in (c) all show a positive difference between *A1* and *B1*.



(a) Mortality vs log(C) (b) Scatterplot of *A1* vs *B1* (c) Bland-Altman plot

Fig 4.23 Agreement between *A* and *B* by comparing *A1* and *B1*

We now compare each pair of variables from Fig 4.21 in Table 4.5 giving values for Spearman's correlation coefficient and the associated *p*-value, together with the result of a paired Wilcoxon test for net differences between the values and, finally, the confidence interval for the best-fit slopes of the linear relationships.

Table 4.5 Pairwise comparisons of *A1*, *A2*, *B1*, and *B2*

Variable pair	Spearman's rho	Spearman's *p*-value	Paired Wilcoxon *p*-value	Linear slope *CI*
A1 / *A2*	0.943	0.005	0.345	1.12 ± 0.20
A1 / *B1*	0.829	0.042	0.043*	1.48 ± 0.35**
A1 / *B2*	1.000	0.000	0.463	1.01 ± 0.16
A2 / *B1*	0.771	0.072'	0.028*	1.30 ± 0.35
A2 / *B2*	0.943	0.005	0.249	0.89 ± 0.15
B1 / *B2*	0.829	0.042	0.028*	0.67 ± 0.12**

Note: For agreement between variables, we would expect to see $p < 0.05$ for Spearman's correlation test, with $p > 0.05$ for differences using a paired Wilcoxon test, and the confidence interval, *CI*, for the relative slope should include 1.0.
*shows significant disagreement between variables using Wilcoxon test
**shows significant disagreement because the slope is not equal to 1.00
'shows a lack of correlation

Considering the reproducibility between *A1* and *A2*, we see a significant correlation ($p = 0.005$), with no significant difference between the values (Wilcoxon $p = 0.345$). In addition, the linear relationship between *A1* and *A2* has a slope with a calculated confidence interval of 0.92 to 1.32 which includes the possible value of 1.00. These are all consistent with reproducible measurements.

Considering the agreement between *A1* and *B1*, the correlation is significant, but the paired Wilcoxon tests indicates a significant net difference between the values ($p = 0.043$) and the confidence interval of the slope is 1.13 to 1.83 which does not include 1.00 and suggests that assay *A* tends to record higher values than assay *B*.

Comparing *A2* and *B1*, the poor correlation ($p = 0.072$) gives a large *uncertainty* in the linear slope, which results in the inability of the Wilcoxon test to detect the significant disagreement.

4.4.3 Agreement between several variables

We now introduce two tests that can be used to measure the *strength* of the agreement between more than two variables. Kendall's coefficient of concordance performs an analysis equivalent to *correlation* between multiple variables, and if there is good *correlation* between variables we would expect a significant result with $p < 0.05$. The Friedman test analyses differences between *linked* values across multiple variables in the same way as the paired Wilcoxon test does for paired variables, and if there is good *agreement* between the variables, we would expect the Friedman test to give a 'no significant difference' result with $p > 0.05$. The two analyses become equivalent when the data is transposed, swapping rows for columns. See 6.4.6 for performing Friedman's test in Minitab and SPSS.

Kendall's coefficient of concordance, *KCC*, is a measure of the *overall* correlation between k related samples of data, and is calculated as:

$$KCC: \quad W = \frac{(k-1)\bar{r}+1}{k} \tag{4.15}$$

where $\bar{r}$ is the mean of the Spearman correlations between all sample pairs, provided that all the groups have the *same direction of correlation*. For many samples (large k), W tends to equal $\bar{r}$. Compared to the *kappa* statistic (4.4.4), in which results either agree or disagree, *KCC* has the advantage of *weighting* the amount of disagreement by using the difference in ranked values.

An equivalent chi-squared value can be derived for k samples of size n:

$$\chi^2 = k(n-1)W \tag{4.16}$$

with degrees of freedom, $df = n - 1$.

Case study: Toxicity assays / 4. Multiple comparisons

–continued from 7.1.1 and 4.4.2

Fig 4.21 records measurements of the mortality (percentage deaths) of bacteria for increasing drug concentrations, C, (recorded as log(C)) using two assay methods, *A* and *B*, with each method repeated once. We now wish to assess the level of overall agreement between *more than two* variables.

Taking the values from Table 4.5, we can calculate the mean value of the six Spearman correlation coefficients between pairs of the four samples *A1*, *A2*, *B,1* and *B2* giving:

$$\bar{r} = (0.943 + 0.829 + 1.000 + 0.771 + 0.943 + 0.829)/6 = 0.886$$

which, using Eqn 4.15 with $k = 4$ gives

KCC, $W = 0.914$

and then with $n = 6$, Eqn 4.16 gives

$$\chi^2 = 4*(6-1)*0.914 = 18.3.$$

The p-value can be calculated from the χ^2 and df, using the Excel function

$$p = \text{CHISQ.DIST.RT}(\chi^2, df) = 0.0026.$$

If there is agreement between variables we would expect a significant result with $p < 0.05$.

The same result can be obtained in Minitab or SPSS.

Minitab > Stat > Quality Tools > Attribute Agreement Analysis...
⊙-***Multiple columns*: Enter data columns,** e.g. *A1 A2 B1 B2*
Number of appraisers: 2–each assay is an 'appraiser'
Number of trials: 2–each assay has two trials
Known standard/attribute: *log(C)*
☑-**Categories of the attribute data are ordered**
→ Output: See Table 4.6

Table 4.6 Results from Kendall's coefficient of concordance calculations in Minitab (see 4.4.4 for Kappa)

		KCC value *W*	*p*-value for *KCC*	Kappa κ (using rank values)
Within assays	*A*	0.971	0.0837	0.6
	B	0.914	0.1035	0.4
Each assay vs standard	*A*	0.933	0.0003	0.8
	B	0.867	0.0009	0.7
Between assays		0.914	0.0026	0.6
All assays vs standard		0.900	<0.00005	0.75

'Within assay' measures the values separately between the *pairs* of results for each assay. The *KCC* values confirm that assay *A* is more *reproducible* than assay *B*. However, without the information of the standard value being measured, the reproducibility of each does not have the significance shown below.

'Each assay vs standard' measures the agreement between each assay pair *and* the known standard. The fact that we compare with log(*C*) and not *C* makes no difference because these analyses are nonparametric, and only take rank order into account. We now see that there is highly significant agreement between *A1* and *A2* measuring *C* with $p = 0.0003$ and also between *B1* and *B2* measuring *C* with $p = 0.0009$.

'Between assays' measures an overall agreement between *A1*, *A2*, *B,1* and *B2*.The values, $W = 0.914$ and $p = 0.0026$, agree with the values derived above from the pairwise correlation coefficients.

'All assays vs standard' demonstrates the overall agreement between the two assays and the measured concentration.

To perform the same test in SPSS, it is necessary to *transpose* the data so that each of six columns in SPSS corresponds to one value of the concentration, and the separate variables are on different rows, as in Fig 4.24.

SPSS

SPSS > Analyze > Nonparametric tests > Related Samples
Fields: *C1 C2 C3 C4 C5 C6*
Settings: ◉ **Customize tests**
☑ **Kendall's coefficient of concordance (*k* samples)**

	C1	C2	C3	C4	C5	C6
1	2.00	25.50	37.50	57.00	69.50	78.70
2	3.50	18.50	40.00	62.00	55.00	62.50
3	2.00	15.00	36.00	33.00	35.00	57.50
4	4.00	16.00	50.50	54.50	65.00	75.50

Fig 4.24 Data entry in SPSS for Kendall's coefficient of concordance

SPSS reports the same 'between assays' results with *KCC*, $W = 0.914$, and $p = 0.0026$ as above.

The **Friedman test** tests for *differences* in the median values of the multiple assays. In Minitab, the Friedman test requires the data to be presented as *univariate* data with the factor being tested identified as the *Treatment* and the other factor the *Block*, and the calculation in SPSS requires the assay data to be in separate columns, as in Fig 4.21. See 6.4.6 for implementation in Minitab and SPSS, which both give $p = 0.019$ giving a significant difference between the assays *A1*, *A2*, *B,1* and *B2*.

4.4.4 Agreement within a contingency table

For *ordinal* or *nominal* data it is useful to represent the agreement, or otherwise, between two variables by recording the *frequency* of their observations in a contingency table. As an example, we can consider two variables as being the results of two tests *A* and *B*, which could be, for example, two

- assessors judging the quality of (the same) cakes in a village fete
- methods of assessing the quality of (the same) fingerprints.

Each test is performed on a number of different *subjects* (cakes, fingerprints).

Fig 4.25 shows a number, $n = 40$, of subjects being tested, or assessed, in two ways A and B, with each test recording a value on a scale of 1 to 3. In each case we can say that there are two *assessors* or *tests* each with a single *trial* of 40 *subjects*. There are no replicates in this analysis. The raw results for tests *A* and *B* are entered as related samples in columns B and C. The result of each trial pair is then *counted* into one of the nine possible combination cells

	A	B	C	D	E	F	G	H
1	Subject	Test A	Test B		A \ B	1	2	3
2	1	1	2		1	11	2	3
3	2	3	3		2	4	7	1
4	3	2	3		3	1	3	7
5	4	3	3					
41	40	3	2					

Fig 4.25 Ordinal agreement between tests *A* and *B* (Subjects 5 to 39 are in hidden rows)

shaded in F2:H4. For example, the pair of values for subject 1, '1' for *A* in B2 and '2' for *B* in C2, contribute one to the total count of two in cell G2, etc.

Agreements between *A* and *B* contribute to frequencies along the main diagonal of the table from F2 to H4. Any *disagreements* contribute to the off-diagonal frequencies. If a subject gives test *B* a larger value than test *A*, then this will appear as an entry in the top right of the table, and vice versa if the result of test *B* is less than that of *A*. Kappa, κ, is a statistic that is used to measure the amount of agreement within a contingency table.

Cohen's kappa, κ, can be understood as a symmetrical measure of the agreement between *two* assessors. It is often referred to as a measure of 'inter-rater agreement' where two assessors each rate a number of items (e.g. quality of wines), and κ is a measure of the extent to which they give the same ratings.

A similar statistic, **Fleiss's kappa**, can be used when assessing agreement between *more than two* assessors. For recording agreement between three (or more) assessors it is necessary to use a three(or more)-dimensional table of values, which is possible mathematically, but not so easy to present on a two-dimensional sheet of paper.

The κ statistic does not lead to a hypothesis test, and there is no agreement on its value in terms of an absolute qualitative scale, although Landis and Koch(Landis J R and Koch G G 'The Measurement of Observer Agreement for Categorical Data'. *Biometric* 33, pp. 159–174, 1977.) have proposed the scale in Table 4.7.

Table 4.7 Strength of inter-rater agreement

Values of κ	Amount of agreement
< 0.0	None
0.00 – 0.20	Slight
0.20 – 0.40	Fair
0.40 – 0.60	Moderate
0.60 – 0.80	Substantial
0.80 – 1.00	Almost perfect

A significant problem with *kappa* is a dependence on sample size, in that the same *percentage* of agreement will, for different *total numbers*, give different values of κ. It is nevertheless useful as a statistic to identify differences between *similar sized* sample sets.

The statistic is calculated as

$$\kappa = \frac{Pr(O) - Pr(E)}{1 - Pr(E)} \tag{4.17}$$

where $Pr(O)$ is the proportion of 'observed' agreements and $Pr(E)$ is the proportion of 'expected' agreements calculated from the observed data.

We will use a simple example in Fig 4.26 which represents the results of two assessors, *A* and *B*, giving binary responses, *Y* or *N*, for a total of *T* items.

	A	B	C	D
1	*A \ B*	*Y*	*N*	Row totals
2	*Y*	*a*	*b*	*Ay*
3	*N*	*c*	*d*	*An*
4	Column totals	*By*	*Bn*	*T*

Fig 4.26 Calculation of Kappa, κ

The total number of pairs of assessment, $T = a + b + c + d$.
Ay, *An*, *By*, and *Bn* are the row and column totals for *A* giving *Y* and *N* and for *B* giving *Y* and *N* respectively.
The total number of *agreements observed* in the data are:

a items were given *Y* by *both* assessors and

d items were given *N* by *both* assessors.

Thus, the *overall* probability of a randomly selected trial resulting in an agreement is given by:

$$Pr(O) = \frac{a+d}{a+b+c+d} = \frac{a+d}{T} \tag{4.18}$$

Using the data from Fig 4.19(b): $a = 24$, $b = 19$, $c = 7$, $d = 16$, $T = 66$

$Pr(O) = 40 / 66 = 0.6061$

We also need to calculate the *expected* probability of agreement, $Pr(E)$, *given* the observed performances of *each* assessor:

Overall probability that assessor A records $Y = \frac{(a+b)}{(a+b+c+d)} = \frac{Ay}{T}$

Overall probability that assessor B records $Y = \frac{(a+c)}{(a+b+c+d)} = \frac{By}{T}$

The *random* probability that they *both* record $Y = \frac{Ay}{T} \times \frac{By}{T} = \frac{Ay \times By}{T^2}$

Overall probability that assessor A records $N = \frac{(c+d)}{(a+b+c+d)} = \frac{An}{T}$

Overall probability that assessor B records $N = \frac{(b+d)}{(a+b+c+d)} = \frac{Bn}{T}$

The *random* probability that they *both* record $N = \frac{An}{T} \times \frac{Bn}{T} = \frac{An \times Bn}{T^2}$

The random probability that A and B are in agreement is equal to the probability that they both record *Y or N*, which is the *sum* of the separate probabilities:

$$Pr(E) = \frac{\{Ay \times By + An \times Bn\}}{T^2} \tag{4.19}$$

Using the data from Fig 4.19(b) again: $a = 24$, $b = 19$, $c = 7$, $d = 16$, $T = 66$

$Pr(E) = (43 \times 31 + 23 \times 35) / 66^2 = 0.4908$

Then using Eqn 4.17: $\kappa = \frac{0.6061 - 0.4908}{1 - 0.4908} = 0.226$, which only rates as 'fair' agreement for this set of observed values.

A particular limitation of the kappa statistic for ordinal data with *several* levels is that kappa only assesses whether two assessors agree *exactly*, and does not assess the closeness of *disagreement*. For example, if two assessors differ by only one in a ranked answer, then this is rated the same as a much larger disagreement with a difference of five. This problem is addressed by Kendall's coefficient of concordance (4.4.3) which uses ranked values to record the magnitude of the disagreement.

4.4.5 **Binary agreement**

It is possible to test for agreement between *related binary* values using:

- McNemar's test for two samples
- Cochran's Q for *k* samples, where *k* may be more than two.

Case study: **Forensic questionnaire / 3. McNemar's test and Cochran's Q**

–continued from 9.2.1 and 8.2.1, leading to 6.2.1

Fig 4.27 reproduces the binary data responses given in Fig 8.10. We want to test for agreement between the responses.

McNemar's test

To understand McNemar's test, we consider the 2 × 2 table, H2:I3, in Fig 4.27 between the binary variables, *Q1* and *Q2*, in which

24 subjects give N to both *Q1* and *Q2*

16 subjects give Y to both *Q1* and *Q2*

	A	B	C	D	E	F	G	H	I
1	*Subject*	*Q1*	*Q2*	*Q3*	*Q4*		Q1\Q2	N	Y
2	1	N	N	N	Y		N	**24**	**19**
3	2	Y	Y	Y	Y		Y	**7**	**16**
4	3	N	N	N	N				
5	4	Y	N	Y	Y		McNemar's test:		
6	5	Y	N	N	N			χ^2 =	4.65
7	6	N	N	N	N			p =	0.031
8	7	N	Y	Y	N				
67	66	Y	N	Y	Y				

Fig 4.27 Nine questionnaire responses from 66 subjects (rows 9 to 66 are 'hidden' in the worksheet)

19 subjects give N for *Q1* and Y for *Q2*, and

7 subjects give Y for *Q1* and N for *Q2*.

Although there is a good measure of agreement between the two questions, 40 out of 66 subjects, the essential difference between the questions lies in the way in which the 26 disagreements are distributed, and for this we can use the McNemar's test.

McNemar's test is a hypothesis test for *square* tables using the chi-squared statistic, but it specifically tests for a specific difference between the 'off-diagonal' elements of the table. The McNemar test applies to 2 × 2 tables whereas the McNemar–Bowker version is used for larger square tables.

It is a useful measure which detects a difference in the *changes* between samples. For example, with 'before and after' questions, it can test whether there is a difference between the numbers of people who change their answer from 'no' to 'yes' and those who change it from 'yes' to 'no'. If McNemar's test detects a difference, then the *direction* of the difference can be seen from the numbers in the table.

McNemar's test can be illustrated by using the 2×2 table in Fig 4.26, with the null hypothesis:

H_0: The probability of an item randomly appearing in '*b*' is the same as the probability for '*c*': $p_b = p_c$.

The analysis tests for a significant difference between the off-diagonal elements of the table using the statistic (with $df = 1$):

$$\chi^2 = \frac{(b-c)^2}{b+c} \quad \text{or with a continuity correction similar to Yates} \quad \chi^2 = \frac{(|b-c|-1)^2}{b+c}. \tag{4.20}$$

It can be seen that the statistic is dependent only on the off-diagonal elements, *b* and *c*. Using the values of $b = 19$ and $c = 7$ in Fig 4.27, we calculate $\chi^2 = 4.65$ in I6 using the continuity correction, and then calculate the *p*-value in I7 (3.7.4) using degree of freedom, $df = 1$:

p = CHISQ.DIST.RT(I6,1) = 0.031.

Using McNemar's test identifies a significant difference in agreement which did not appear by just comparing the *proportions* of the unrelated samples. The value of McNemar's test is that it compares only the off diagonal disagreement values, whereas these differences can be masked by a larger number of diagonal agreements when using the proportions test.

In SPSS, it is possible to perform McNemar's test via contingency table statistics (8.2.4) or by using the analysis of nonparametric related samples as below.

Cochran's Q

Cochran's Q is the equivalent of the McNemar's test that can be applied to more than two samples. It is not possible to define answers to three or more variables using a 2-D contingency table, and consequently the calculation of Cochran's Q in SPSS is performed using the analysis of related nonparametric variables:

SPSS > Analyze > Nonparametric Tests > Related Samples...
Objective: ◉-Customize analysis
Test Fields: *Q1 Q2 Q3 Q4*
Settings: ◉-Customize tests
☑-Cochran's Q (*k* samples)
→ Output: Fig 4.28

Hypothesis test summary

	Null hypothesis	Test	Sig.	Decision
1	The distributions of Q1, Q2, Q3 and Q4 are the same.	Related-samples Cochran's Q test	.039	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Fig 4.28 Cochran's Q for *Q1 Q2 Q3 Q4* (SPSS)

The output in Fig 4.28 reports that there is a significant difference ($p = 0.039 < 0.05$) between the four tests, but we do not immediately know where the difference might lie. We can then use McNemar's test between pairs of variables to locate possible differences, giving the results:

Q1 and *Q2*: $p = 0.031$ *Q1* and *Q3*: $p = 1.000$ *Q1* and *Q4*: $p = 0.629$
Q2 and *Q3*: $p = 0.052$ *Q2* and *Q4*: $p = 0.164$ *Q3* and *Q4*: $p = 0.648$

If we had used *only* the multiple McNemar's tests, then we would need to impose a Bonferroni correction (1.6.4) for the *significance level* by dividing it by the number of tests, giving the new significance level of 0.05/6 = 0.009. In this case, *none* of the paired tests would show significant differences. However, the use of the single Cochran's Q test does confirm that a significant difference does exist, and we can then use the McNemar paired tests as post hoc tests to identify the principal source of the difference, i.e. between *Q1* and *Q2*.

Part II

Analysing experimental data

Part II approaches an understanding of data analysis from the 'top down' *scientific* perspective of the need to answer specific questions. It starts with the assumption that the student has a set of experimental results and wishes to know what analyses would be applicable to that data and which would address the scientific questions being asked. The book therefore provides targeted support for the student at the time when he/she is well motivated to investigate and use the techniques developed, but it also provides support for a formal course in which students are given example data to analyse. The content develops the different analytical techniques through their implementation using Excel, Minitab and SPSS, but with extensive reference made to the underlying statistics developed in Part I.

Chapter 5. Project data analysis outlines the issues that confront students, normally during their final year, when faced with their own research project or dissertation. This does *not* approach the topic from the 'research design' perspective that would be required for a 'real' research project, but reflects the exploratory nature of most student projects. It concentrates on a 'what do I do now?' approach for the student who is faced with experimental data, either within a personal project, or in an extended exercise in a taught (e.g. MSc) course.

The remaining chapters then address issues that are defined by the structure of the experimental data and the scientific questions to be answered. Each section starts with example data sets, together with possible analytical options and methods of describing and visualizing the experimental results. They then continue with the use of SPSS, Minitab and Excel to implement relevant analytical techniques.

Chapter 6. Single response variable assumes that the student wishes to test for the effect of one or more factors that might affect a single measured variable. Typical analyses include the familiar *t*-tests, ANOVAs and their nonparametric equivalents, together with general and generalized linear models.

Chapter 7. Related variables considers two or more related variables which introduces a wide range of analyses based on specific linear and nonlinear relationships as well as more general *x*-*y* systems. Typical analyses include linear and nonlinear regression, correlation and agreement, but extend to include convolution techniques and component spectral analysis.

Chapter 8. Frequency data relates to the counting of experimental results that fall into specific categories, either defined by the categorical nature of the measurement

itself or by the use of probability density histograms in describing the distribution of interval data. This includes chi-squared calculations and associated contingency table statistics. It also addresses the specific issues raised by analysing and modelling binary data.

Chapter 9. Multiple variables provides an introduction to some of the techniques for handling data sets with many variables, including cluster and principal component analysis and multiple regression. It concludes with an example data set with multiple variables (similar to questionnaire data) and considers the different analytical 'questions' that can be asked, with reference to the techniques introduced earlier throughout the book.

Project data analysis

Introduction

In this chapter we are assuming that a student is conducting a 'research' project as part of a final year's study as an undergraduate, and has been asked to 'investigate' scientific behaviour within a specific topic. Having just finished collecting experimental results, he/she would, *in an ideal world*, already know what data analysis techniques to use because the experiments would have been conducted within a designed framework that fully anticipated the relevant statistical analysis. However, in common with many other students, not much thought may have been given to data analysis, or possibly the investigation has been of an exploratory nature and it was not possible to anticipate what analysis would be required.

Faced with experimental data without well-defined analytical options, the danger is that the student might grab any statistical test that will produce a p-value or even to rush off for help from a friendly statistician. However, the best approach is to sit back and carefully review the

- scientific objectives–what questions should the statistical analysis aim to answer?
- experimental results–how was the data collected, what factors were involved, replicate values, paired data, etc.?

No one can provide help until the student can explain clearly what it is he/she wants to achieve, the experimental results, and how they were collected. In fact, this actual process of reflection will often lead to a better insight of what analytical techniques can be used, and this section aims to help develop that overview.

Section 5.1 considers the initial steps in reviewing data and objectives, understanding the source of data uncertainties, and transforming the data into a format suitable for software analysis.

Section 5.2 presents examples of methods to identify experimental characteristics that are suitable for analysis.

Section 5.3 reviews the techniques and issues involved in transforming the data into a format required to match the analytical technique.

Section 5.4 discusses the relevance of normality and equality of variance for many analytical techniques and develops the approach, tests, and transformations that may be involved.

5.1 Preparing data for analysis

A student has just completed experimental work and recorded the results, but before thinking about any analysis, it is important to preserve the original data and any associated notes. It is essential to be able to check the original data, for example it may be necessary to check if a handwritten 7 has been misread as a 2. For electronic records, the original data should be saved in a secure memory store before copying the data into a new file, and all subsequent analysis should only be carried out on the duplicate data set.

5.1.1 Case studies

In this section we will meet the following case studies:

Football fantasy

Highlights the difference between *statistical* and *scientific* significances.
5.1.4 / 1. Significance: Can a distant football fan's actions affect the team's performance?

Fingerprint quality

An investigation into methods of lifting fingerprints and the resultant quality.
5.1.5 / 2. Organizing data entry: Considers the transfer of data from lab book to software analysis.

Ink analysis

Analyses the spectral responses of different black inks to identify a method of forensically differentiating between them.
5.1.6 / 1. Exploratory phase: Develops an overview of the experimental investigation.

5.1.2 Identifying the variables/factors

The key to understanding data is to identify and define all the variables and factors included in the investigation. The changing values associated with the system are the measured *variables* of the experiment, and the variable that describes the condition of an experiment (e.g. the temperature of reaction) will typically be called a *factor*. The variables/factors can be categorized under the headings of Action, Type, Levels, and Variability, and this process of categorizing for a given data set can be very helpful in clarifying the *understanding* of the data, and may help in deciding on a suitable analytical technique.

Under **Action** we first identify whether we are measuring a variable that is an 'input' to the system or whether it is an 'output' variable whose value is determined by the system. However, in interrelated (e.g. correlation) measurements we can measure two outputs without an obvious input (e.g. recording height and weight of an animal without knowing its age).

Under **Type** we distinguish whether a measurement is an interval, ordinal, or nominal value (1.2.2), or a frequency (count). In some cases, a frequency can be treated as an interval variable (6.1.1).

Under **Levels** we record the range of values that the variable could take. An interval variable may be *continuous* with values within a given range, an ordinal variable may have a number of *progressing* values, and a nominal variable may have a number of specific discrete *categories*. Frequencies have *integer* values.

Under **Variability** we identify whether the value is known exactly, or how replicate values could be expected to change. They may follow a known distribution, e.g. normal, Poisson, binomial. If it is an 'input' we decide whether it is a variable/factor whose value is *fixed* in the design or operation of the experiment or whether it is a variable/factor that has been *randomly* selected.

For example, in the calibration of a spectrophotometer, the absorbance relating to the concentration of a solution would be described by the data structure in Table 5.1(a). Both variables are continuous positive interval values. We assume that the experimental variations in absorbance will follow a normal distribution and that the values of concentration are determined by the specific standard solutions prepared for the experiment. The data structure in Table 5.1(b) relates to the contingency table in Fig 8.9 which counts the numbers of people, grouped as male and female, who show none, some, good, or excellent improvement following bacteriophage treatment. The distribution of observed frequencies based on an underlying probability is expected to show a Poisson distribution.

Table 5.1 Example descriptions of experimental variables/factors

(a) Absorbance vs concentration calibration data

Variable	Action	Type	Levels	Variability
Absorbance, A	Output	Interval	Continuous 0→	Normal
Concentration, c	Input	Interval	Continuous 0→	Fixed

(b) 2 × 4 contingency table data

Variable	Action	Type	Levels	Variability
Frequency	Output	Frequency	Integer	Poisson
Improvement	Input	Ordinal	Category 4 levels	Fixed
Sex	Input	Nominal	Category 2 levels	Fixed

5.1.3 Understanding the uncertainty in the data

The statistical analysis of experimental data frequently calculates *significance* by comparing a possible difference in the data with the random variations in that data. For this reason, it is important to be aware of

- the sources of uncertainties in the data
- the way in which the different types of uncertainty can be differentiated, and
- the propagation of uncertainties/errors.

Section 1.4 identifies the different types of measurement, subject, and probability uncertainties and develops the techniques for their quantification.

The importance of designing the experiment to collect the relevant uncertainty data is often overlooked. For example, the identification of an *interaction* term in a multifactorial ANOVA requires *replicate* measurements at the same factor levels (3.3.2) such that the analysis can distinguish between an interaction and experimental uncertainty.

A prior knowledge of the uncertainty in a measurement process can also change the approach to analysis. For example, the *t*-test relies on the *sample* data for calculating experimental uncertainty, but prior knowledge of this uncertainty allows the use of the more precise *z*-test (3.1.5). A similar benefit occurs if the experimental uncertainty is previously known when using straight line intercept calculations (2.2.3).

5.1.4 Scientific significance

Through most of a project the student may have been immersed in the science of the experiment, but it is easy to forget this context as soon as one enters the alien world of statistics to analyse the results. For example, *statistical* significance is a measure of the statistical probability with which an observed set of results could have occurred by chance, but, before we can conclude a *scientific* significance, we must be aware of the wider scientific context of the problem, as illustrated by the following case study.

Case study: Football fantasy / Significance

A football fan regularly watches his team play on television. He typically leaves the room for about 15 minutes during each match to collect refreshments, and, over several matches, gets the impression that his team is more likely to concede a goal if he is *out* of the room. Will his team do better if he stays in the room? He decides to conduct a hypothesis test which has the null hypothesis:

H_0: The probability of his team conceding a goal is not affected by whether he is in, or out, of the living room in his own house.

During each of the next six matches, he leaves the room for a randomly selected period of 15 minutes out of the 90 minutes of play. He would then expect,assuming that the null hypothesis is correct, that the average *proportion* of goals conceded during his absence would equal 15/90 = 0.167.

In fact, his team concede a total of 12 goals, five of which were while he was out of the room, which is greater than the randomly expected average of 12 × 0.167 = 2 goals, confirming his original impression. Should he now publish his findings?

The statistical approach to the results uses Fisher's exact test (3.8.2) to test whether the measured proportion of 5/12 is greater than 0.167 for a sample size of 12, and returns a statistically significant *p*-value = 0.037. Based on this statistical analysis he rejects the null hypothesis and concludes that leaving his living room significantly increases the probability of his football team conceding a goal!

His conclusion is clearly wrong—there is no *scientific* mechanism by which his presence in his own living room can affect how his team play on the football pitch many miles away. A *statistically* significant result does not necessarily mean that there must be a significant *scientific* effect.

5.1.5 Data entry into software

Initially, experimental data is likely to be organized in a 'collection' format, defined by the order in which the experiments were carried out, and is likely to consist of a number of sets of tabulated data recorded in different conditions and times.

Case study: Fingerprint quality / 2.Organizing data entry

–continued from 6.4.1

As a simple example, the columns A to D in Fig 5.1 record the assessed quality of fingerprints as *Quality* values (on a 0 to 5 ordinal scale), obtained using three different lifting methods, *Method* (*A*, *B*, *C*) at three different temperatures, *Temp* (10, 20, 30). It is also possible that some 'initial' analyses have been performed, as represented by the calculations of mean and standard deviations (SD) for replicate data values, but we now need to organize the data for entry into analytical software.

	A	B	C	D	E	F	G	H	I	J	K
1	**Collection format:**							**Analysis format:**			
2											
3	**Method A:**	Quality measurements			**Mean**	**SD**		*Rec*	*Temp*	*Quality*	*Method*
4	**Temp = 10**	2	**1**	**2**	**1.67**	**0.58**		**1**	**10**	2	A
5	**Temp = 20**	3	**3**	**2**	**2.67**	**0.58**		**2**	**20**	3	A
6	**Temp = 30**	2	**1**	**1**	**1.33**	**0.58**		**3**	30	2	A
7								**4**	10	2	B
8	**Method B:**	Quality measurements			Mean	SD		5	20	3	B
9	Temp = 10	2	3	2	2.33	0.58		6	30	3	B
10	**Temp = 20**	**3**	4	**2**	3.00	1.00		**7**	**10**	3	C
11	**Temp = 30**	**3**	**4**	**4**	3.67	0.58		**8**	**20**	4	C
12								**9**	**30**	**4**	C
13	**Method C:**	**Quality measurements**			Mean	SD		**10**	**10**	**1**	A
14	**Temp = 10**	**3**	**2**	3	2.67	0.58		**11**	**20**	**3**	A
15	**Temp = 20**	**4**	**3**	5	4.00	**1.00**		**12**	**30**	1	A
16	**Temp = 30**	**4**	3	3	3.33	0.58		**13**	**10**	3	B
17								**14**	**20**	4	B

Fig 5.1 Data in both 'collection' and 'analysis' formats (data in columns H to K extend to other rows)

Excel is very accommodating in the possible layouts for data analysis, generally accepting data for analysis in either rows or columns within the two-dimensional spreadsheet. This can be very useful for presenting data and laying out complex calculations, and is the ideal medium for collecting and organizing experimental results. It is also possible to carry out a range of basic statistical analyses using Excel, e.g. initial calculations for the means and standard deviations as in Fig 5.1. However, for more advanced analysis it is probably necessary to use other dedicated software packages. These include a number of possible Excel 'add-ins' that can expand Excel's capability, but in this book we will mainly consider the separate packages: Minitab and SPSS.

Both Minitab and SPSS accept data in a strictly column format, and are *not* two-dimensional spreadsheets. This has the advantage of establishing discipline in organizing the data and requires careful thought about how the data is structured. Hence, the next task may be

transcribing data from a 'collecting' format in Excel to an 'analysing' format for Minitab or SPSS. The columns I to K in Fig 5.1 record the same data as in columns A to D, but reorganized into the required column (stacked) format with each column identifying a separate variable or factor. When moving data from *rows* to *columns* in Excel, it is often useful to use the 'paste special' method and check the 'transpose' option which will automatically switch the copied data from a row to column format (or vice versa).

It is important to remember that most software analyses expect to use the *individual raw data values* and *not* calculated means and standard deviations. The software calculations use the variation between *individual* values as the means of estimating the inherent experimental uncertainty for comparison with any differences that may be due to factor effects.

Each row in the column format will record a 'unit' of measurement, which is often described as a 'record' or as a 'subject' (from the analysis of questionnaires). Each *row* will have either:

- **Univariate response variable.** The same *single* variable (e.g. *pH*, fish weight, response on a Likert scale) is recorded in a single column (column J in Fig 5.1), and the conditions under which it was recorded for each subject are identified by values in the other columns.
- **Related response variables.** Related measurements have two, or more, response variables associated with the same record or subject being measured. These may be either:
  - **bivariate or multivariate data** in which *different* variables measure aspects of the same experimental system, e.g. *Quality, Temp, Method* recorded as columns C1, C2, and C3-T in Fig 5.2(a),

    or
  - **repeated measures** in which the *same* variable is measured under different conditions for the *same* subject, e.g. *%T* for different inks in Fig 3.40(a) at the same wavelength. If just two values are measured, e.g. the measurements of bacterial contamination of the same surface *before* and *after* cleaning, then this may lead to the familiar *paired t*-test.

Data in **Minitab** is entered into a worksheet (Fig 5.2(a)) in which each *numeric* column has a label, C1, C2 etc. and a space to add a column name. If *text* is entered into a column it adds a '–T' to the column number, but it is possible to change between *text* and *numeric* columns using:

Minitab > Data > Change Data Type

↓	C1	C2	C3-T	C4
	Entry Direction	Quality	Method	
1	10	2	A	
2	20	3	A	
3	30	2	A	
4	10	2	B	
5	20	3	B	
6	30	3	B	

(a) Minitab worksheet

	Temp	Quality	Method	MethodN
1	10.00	2.00	A	1.00
2	20.00	3.00	A	1.00
3	30.00	2.00	A	1.00
4	10.00	2.00	B	2.00
5	20.00	3.00	B	2.00
6	30.00	3.00	B	2.00

(b) SPSS data editor: Data view

Fig 5.2 Extracts from the data files for Minitab and SPSS for data from Fig 5.1

The printed outputs in Minitab appear in the session window and graphs appear in separate pop-up windows. A *project* file with the '.mpj' extension consists of a worksheet(s) to hold the data and a session window to record the results, although it is possible to save the worksheets separately as '.mtw' files.

SPSS holds data in data editor files with the extension '.sav' and separately saves results in viewer files with the extension '.spv'. The SPSS data editor has two 'views': Data view as in Fig 5.2(b) for the data values, and variable view as in Fig 5.3 for defining the qualities for each variable. In Fig 5.2(b) we have added a variable *MethodN* which is the same as *Method*, but coded 1 to 3 so that it could be used as a scale variable instead of a nominal variable.

	Name	Type	Width	Decimals	Label	Values	Missing	Columns	Align	Measure	Role
1	Temp	Numeric	8	2		None	None	6	Center	Scale	Input
2	Quality	Numeric	8	2		None	None	5	Center	Scale	Input
3	Method	String	1	0		None	None	5	Center	Nominal	Input
4	MethodN	Numeric	8	2		None	None	6	Center	Scale	Input

Fig 5.3 SPSS data editor: Variable view

The data values appear in the data view, and their characteristics are defined in the variable view, where the key values to define are:

Name which cannot include spaces or punctuation marks (except for a full stop in the body of the name)

Type which describes the data entry, e.g. numeric, string, date, etc.

Measure which can be scale, ordinal, or nominal. In general it is useful to define an ordinal *variable* as having a scale *measure*, but this can depend on the analysis being carried out.

Label provides an additional and more flexible description of the variable.

The SPSS output, including printouts and graphs, appear in the output viewer (.spv) file.

5.1.6 Reviewing data and objectives

When completing a final year project, a student's motives for using statistical analysis can easily become confused. The student may feel (probably correctly) that the final grade will depend on demonstrating familiarity with statistical analysis. In which case, he/she may start looking for *any* analysis that might accept the data rather than an analysis that matches the *objectives* of the research. It is important to first carefully review the experimental results and then consider possible statistical analyses in the *context* of the original scientific objectives.

Describing data

A useful first step that is often overlooked is to *describe* raw data values. Not only will it be necessary to describe the experimental results for the project report, but the process of visualizing the data will help immensely in seeing what is required for any further analysis. We use the following case study as an example, but we also put 'describing the data' as a first step in identifying analytical methods in most sections of Chapters 6, 7, and 8.

Case study: **Ink analysis / 1. Exploratory phase (overview)**

—leading to 5.2.3, 2.2.2, 3.6.2, 3.3.3, and 6.4.8

As part of a forensic investigation to characterize and distinguish between four black inks, the percentage transmission, *%T*, of light (Fig 5.4) is measured using a visual spectral comparator over wavelengths from 500 nm to 800 nm. For the purposes of forensic classification, we need to identify a common characteristic that will generate a simple measureable parameter that *differentiates* between the inks. We first develop an overview of the experimental investigation.

5.2.3 / 2. Analytical characteristics: Identifies test statistics from raw data.

2.2.2 / 3. Exact *y*-intercept: Calculates the confidence intervals for intercepts at 50%T.

3.6.2 / 4. Repeated measures: Tests for a difference in %T at 'linked' wavelengths.

3.3.3 / 5. ANCOVA analysis 1: Differentiates between inks with wavelength as a covariate using Excel and Minitab.

6.4.8 / 6. ANCOVA analysis 2: Differentiates between inks with wavelength as a covariate using SPSS.

By plotting their spectral responses on the graph in Fig 5.4 we see that all inks show a near zero transmittance over the main visual range up to about 650 nm—hence they all appear 'black'. However, the plot does allow us to identify a possible method of differentiation, by recording the long wavelength 'cut-off' points at which their spectra cross the 50%T level. In the next step in this case study example (5.2.3), we focus on different *statistical* methods that can be used to test for these possible differences.

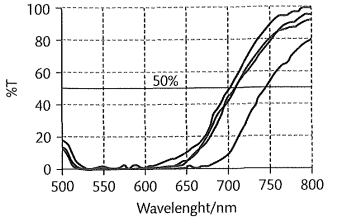


Fig 5.4 Transmission spectra for black inks

Reviewing objectives

With a good understanding of what the experimental data looks like, it is useful to sit back and review the ultimate aims of the research and see how they may help to identify any specific analysis. Table 5.2. uses the 'Ink analysis' case study to establish a hierarchy of objectives, starting with the title of the project.

Table 5.2 Hierarchy of objectives in the 'Ink analysis' case study

Overall aim (title)	Ink analysis. An investigation into the forensic analysis of black ink
Objectives	Identify methods of differentiating between black inks
Experimental data	Univariate values of *%T* as a function of wavelength provide a spectral analysis of the different inks, Fig 5.4
Analytical characteristics	Select long wavelength cut-off values at 50%T in the spectrum
Types of analysis	Test for significant differences at 50%T
Specific analyses	- Confidence intervals for 50%T intercepts (2.2.2) - Repeated measures in %T with linked wavelengths (3.6.2) - ANCOVA for differences in %T with wavelength a covariate (3.3.3)

Having reviewed both the experimental data and objectives,it may be necessary to identify a specific data characteristic for testing (Section 5.2), transform the data (Section 5.3), or consider the normality and variance conditions for analysis (Section 5.4). Otherwise, the various analytical options in Chapters 6, 7, 8, and 9 should be investigated, according to the data type and objectives for analysis.

5.2 Deriving test characteristics

If the research was somewhat exploratory (typical for a student project), it may have raised questions and possible analyses that were not expected. It would be fortunate if it were possible to take the raw results from the exploratory data and enter them directly into a statistical test, but in many cases it will be necessary to manipulate the original data in some way. However, the possibilities for data rationalization are endless, and the best we can do here is to provide some of the major techniques together with some examples which may provide some basis of experience for future problems.

For example, it may be necessary to

- select a sub-set of the data relevant to the focus of the investigation (5.2.3).
- combine data to arrive at new variables that represent relevant specific scientific values (5.2.4).
- transform the response variable according to a theoretical relationship (5.2.5).

Even after identifying analytical data it may still be necessary to

- transform data to provide a near-normal distribution of experimental data (5.4.7).
- use linearization to transform one, or both, of two interrelated variables such that their theoretical relationship can be described by a straight line (Section 2.3).

5.2.1 Case studies

In this section we meet the following case studies:

Bacterial growth

Investigates the effect of different antibacterial cleaning agents on the growth of bacteria.

5.2.2 / 1. Exploratory phase: Identifies characteristics capable of analysis.

Ink analysis

Analyses the spectral responses of different black inks to identify a method of forensically differentiating between them.

5.2.3 / 2. Analytical characteristics: Identifies test statistics from raw data.

Chemotaxis index

The chemotaxis experiment initially treats nematodes with different concentrations of a therapeutic drug, and then measures their capacity for migration towards a food supply in a segmented agar plate.

5.2.4 / 2. Deriving analytical statistic: Starting with *raw data values*, it calculates the *chemotaxis index* that is then used to analyse the data.

Mean kinetic temperature

Models the effect of raised temperature on the deterioration of stored pharmaceutical products.

5.2.5 / Modelling the analytical variable: Uses a theoretical model to *weight* the output data.

5.2.2 Beyond the exploratory phase

The following case study illustrates how an exploratory study into the growth of bacteria can be used to identify aspects for further research. In principle, the *same* data should not be used to *both* identify possible test characteristics *and* then to carry out the analysis, because the data for the analysis should be *independently* obtained. However, in the student project there is usually no time to collect new data, but it is acceptable to use the existing data to *illustrate* the statistical techniques that could be used, provided that reservations are stated about using the same data twice for both identification and analysis.

Case study: Bacterial growth / 1. Exploratory phase (overview)

–linking to: 3.1.1, 3.4.3, 7.3.5

The general aim of this exploratory project was to assess the effect of using different antibacterial cleaning agents on the growth of bacteria. The investigation records the effect on the quantity of genetically modified bioluminescent E-coli as a function of time. The graphs in Fig 5.5 plot luminesce, *L*, for three increasing concentrations, *C1*, *C2*, and *C3*, of the cleaning agent. In this exploratory phase we first identify possible test statistics from the raw experimental data.

3.1.1 / 2. Difference in slopes using *t*-test: Demonstrates the essential statistics of a *t*-test.

3.4.3 / 3. Difference in slopes as an interaction: Demonstrate interaction in an ANOVA.

7.3.5 / 4. Using smoothing convolutes: Develops a best-fit curve to raw data as drawn in Fig 5.5, and calculates maximum and minimum values.

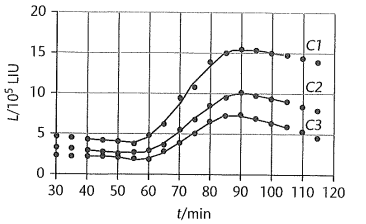


Fig 5.5 Growth of bacterial populations in presence of antibacterial agents. The process of drawing these best-fit *curved* lines is developed in 7.3.5

Following the exploratory phase, represented by the curves in Fig 5.5, it may become evident that there is some effect of particular interest that could be tested more carefully, e.g. defining the difference between the curves.

There is an initial lag before the bacteria enter a growth phase, and the student wishes to compare the different curves just within this growth phase plus find characteristics that define the *scientific* performance and which can be tested *statistically*.

The two most obvious statistical choices are:

The slope of the line between 60 min and 85 min, which would be the rate of bacterial growth. Statistical tests for difference in slopes are developed in 3.1.1 and 3.4.3.

The difference between the minimum and maximum values, either as a difference or a ratio. This calculation is considered in 7.3.5 by using smoothing convolutes to calculate the best estimates for difference between maximum and minimum.

The actual choice will depend on the science of the problem.

5.2.3 Selecting analyses

Exploratory data typically covers a wide range of values hoping to identify regions of analytical interest. We need then to pick out a reduced range of values suitable for specific analysis. In 2.1.5 we see how a 'linear' calibration graph may have a high end curvature, but, by selecting a useful working range, the calibration may still be linear, and in 5.2.2 we see the identification of the growth phase of bacteria for analysis.

In the following case study, the full exploratory data from the spectrophotometric analysis of three inks gives an overall description, but if we wish to differentiate between the spectra, we need to concentrate on a specific range of values.

Case study: **Ink analysis / 2. Analytical characteristics**

–continued from 5.1.6, leading to 2.2.2, 3.6.2, 3.3.3, and 6.4.8

We saw in Fig 5.4 the spectral responses for four black inks, but we now need to identify simple measureable parameters that differentiate between the inks.

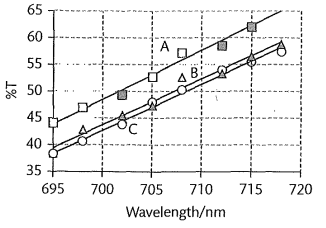


Fig 5.6 Black ink transmission spectra

The only visual difference between the ink spectra given in Fig 5.4 is that they have different cut-off points at the long wavelength end of their spectra. We can define this mathematically by recording the wavelength, λ_{50}, where the transmittance passes the 50 %T point. One ink is clearly different, with a cut-off wavelength, λ_{50}, at about 745 nm. We can then compare the other three inks, *A*, *B*, and *C*, by analysing the specific section of the graph in Fig 5.6, between 40 %T and 60%T.

The most direct method is to calculate the wavelengths with which they each cross the 50%T line and test for any significant *horizontal* difference (2.2.2).

An alternative method is to test for a *vertical* difference between the lines, but the problem here is that the change in %T with wavelength (a covariate) is an additional vertical variation which confounds a simple analysis such as a *t*-test. The first method uses the fact that measurements for each line are made at the *same* wavelengths, and this is a unique factor that links the corresponding measurement in each sample. This allows us to use a *paired* *t*-test between each pair of lines or a *repeated measures* test between all three lines (3.6.2). For the second method, we consider a situation where the wavelength measurements for each line are not at the same values, and we *demonstrate* the use of an ANCOVA analysis (3.3.3) by testing for a vertical difference while taking into account the wavelength covariate.

5.2.4 Combining data

Depending on the science of the system, it may be necessary to take more than one response variable and mathematically combine them with a defined formula to obtain a *derived* analytical characteristic.

Case study: **Chemotaxis / 2. Deriving analytical characteristics**

–continued from 6.3.1, leading to 5.4.6

The aim of the investigation of is to see whether the pre-treatment with a drug affects the mobility of nematodes. The experimental procedure of chemotaxis uses an agar plate which is divided into three zones with an attractive food supply (NaCl) in section A and a control in section B. The nematodes are introduced into section C and might then be expected to migrate preferentially to the food in section A. Sections A and B also contain spots of sodium azide to paralyse the nematodes once they arrive in either of the sections.

The numbers of worms in each section A, B, and C are recorded in Fig 5.7, where each row represents one measurement. The experiment is repeated at three levels of pre-treatment, *Treat*, using three concentrations of a chemotherapeutic drug, with eight replicates at treatment level 0, seven at level 1 and nine at level 2. A number of rows have been hidden in the worksheet (shown by the horizontal lines) to save space.

	A	B	C	D	E	F	G	H	I	J
1	RecNo	Treat	A	B	C	A-B	A+B+C	CI	Mean	St. Dev
2	1	0	35	6	54	29	95	0.31		
3	2	0	40	8	62	32	110	0.29		
8	7	0	37	14	66	23	117	0.20		
9	8	0	34	8	61	26	103	0.25	0.27	0.08
10										
11	9	1	14	20	52	-6	86	-0.07		
12	10	1	30	10	57	20	97	0.21		
16	14	1	40	19	65	21	124	0.17		
17	15	1	29	17	48	12	94	0.13	0.15	0.11
18										
19	16	2	19	32	55	-13	106	-0.12		
20	17	2	27	11	50	16	88	0.18		
26	23	2	38	29	41	9	108	0.08		
27	24	2	23	26	59	-3	108	-0.03	0.01	0.09

Fig 5.7 Results data for chemotaxis measurements

Note that some rows have been hidden at the horizontal lines to save space

The chemotaxis index is calculated as the ratio

$$CI = (A-B)/(A+B+C)$$

where *A*, *B*, and *C* are the numbers of nematodes in the various sections after one hour.

The aim is to test whether different pre-treatment affects the value of the index. There are then two *apparent* options for calculating values for *CI*:

1. (Incorrect option) For each of the three *treatment levels*, calculate total values of *A*, *B*, and *C* separately, and then calculate *one* value of *CI*.
2. For each *measurement* (row) calculate the value of *CI* for that row as in column H, and then calculate the mean and standard deviations of the *CI* values for each treatment as in columns I and J.

This is similar to the problem in the 'DIY dice' case study (1.4.6) in that case option 1 loses any information about the *experimental variation* in replicate measurements of *CI*. It is only in option 2 that we can calculate the standard deviation variation. The software analysis must calculate the experimental uncertainty to be able to test whether any observed differences could have occurred by random chance.

The calculated mean and standard deviation, *CI*, values suggest that there is an apparent difference between the treatments, but we now want to perform a hypothesis test to test whether this difference is statistically significant.The next step in the analysis is to copy the *individual* calculated values of *CI* for each measurement into a data column in Minitab or SPSS, together with a second column which identifies the value of *Treat* related to that measurement, and then use an ANOVA analysis (5.4.6 and 6.3.1).

5.2.5 **Modelling response variables**

Perhaps the most common use of a theoretical transformation is in the transformation of variables to obtain a straight line relationship (Section 2.3). For example, in the decay of a bacterial colony the population will fall according to an exponential relationship with time, but by taking the logarithm of the population, *N*, it is possible to see a linear relationship of ln(*N*) against time, *t*. However, we now consider the use of a theoretical transformation to develop a *weighting* factor for the experimental data.

Case study: **Mean kinetic temperature / Modelling the analytical variable**

In this case study, a pharmaceutical product is intended to be kept refrigerated at a temperature which will fluctuate around 5°C. However, due possibly to refrigerator failure or delayed transport between refrigerators, the product is exposed to a relatively high temperature surge for a short period, as shown in Fig 5.8.

The rate of decay, *v*, of the product increases with temperature according to the Arrhenius's equation:

$$v = A \times \exp\left\{-\frac{\Delta H}{R \times T_K}\right\}$$

where T_K is the temperature in degrees Kelvin, ΔH is the activation energy, R is the gas constant = 8.31 J mol^{-1}K^{-1}, and A is a constant which will cancel out in our calculations.

We wish to calculate the mean kinetic temperature (*MKT*) which is the *constant* storage temperature that would result in the same overall decay as the varying temperature profile shown in the graph. We estimate that ΔH = 85 kJ mol^{-1} for this product.

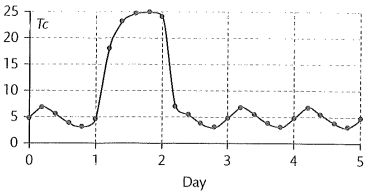


Fig 5.8 Temperature fluctuation with time

The exponential relationship means that the rate of decay is not linear with temperature, and a high temperature will have a proportionally greater effect than a lower temperature. This means that simply taking the *arithmetic* average of all temperatures will underestimate the amount of decay. We will need to provide the higher temperatures with an increased 'weighting' of importance.

The exponential term in the equation becomes the 'weighting' factor for each data point. Hence to calculate an average for the rate of decay for all *n* data points we need to calculate the average of the 'weights' from each point, *i*, which will be given by:

$$\bar{v} = \frac{A \times \sum_i \exp\left\{-\frac{\Delta H}{R \times T_K}\right\}}{n}$$

Defining the MKT_K (in degrees K) as that constant temperature that would produce the same overall ageing as observed, the average rate will then be given by

$$\bar{v} = A \times \exp\left\{-\frac{\Delta H}{R \times (MKT)_K}\right\}$$

By combining the above two equations, and with some rearrangement, we get

$$(MKT)_K = \frac{-\left(\Delta H / R\right)}{\ln\left\{\frac{\sum_i \exp\left\{-\frac{\Delta H}{R \times T_K}\right\}}{n}\right\}}$$

We can implement this calculation easily in Excel.

MKT analysis: Excel analysis for Fig 5.9. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell

	A	B	C	D	E	F	G
1	Time	Data			Weighting		
2		T /C			exp(-(ΔH/R)/(T+273.15))		
3							
4	0	4.9	Average T_C =	8.31	1.08E-16	MKT_C =	12.58
5	0.2	6.8			1.38E-16		
6	0.4	5.5			1.17E-16		
7	0.6	3.82			9.33E-17		
8	0.8	3.1			8.47E-17		
9	1	4.7			1.05E-16	ΔH =	85000
10	1.2	18.1	Temp surge		5.70E-16	R =	8.31
11	1.4	23.2			1.04E-15	ΔH/R =	10223.23
12	1.6	24.6			1.23E-15		
13	1.8	24.9			1.27E-15	Sum / n =	2.89E-16
14	2	24			1.14E-15	ln(Sum/n) =	-35.78
15	2.2	7.1			1.44E-16	MKT_K =	285.73
16	2.4	5.5			1.17E-16		
28	4.8	3.1			8.47E-17		
29	5	4.8			1.06E-16		

Fig 5.9 MKT calculation (rows 17 to 27 are hidden)

The values for ΔH and R are entered in cells G9 and G10 and the ratio calculated in [G11] = G9/G10.

The calculation of weighted values is performed in column E, with, for example,

[E4] = EXP(–G$11/(B4+273.15))

with the addition of 273.15 to convert °C to °K.

The above equation for MKT_K is then evaluated, starting with the exponential term:

$$\sum_i \exp\left\{-\frac{\Delta H}{R \times T_K}\right\}: \qquad [G13] = \text{SUM(E4: E29)/COUNT(E4: E29)} = 2.89\times10^{-16}$$

(we could have just used) [G13] =AVERAGE(E4:E29)

We then take the natural log: [G14] = LN(G13) = –35.78

MKT in °K is then calculated: [G15] = G11/G14 = 285.73

Finally, the MKT in °C is calculated in G4 by subtracting 273.15 from G15:

MKT_C = 12.58

For comparison, we also calculate in D4 the simple *arithmetic* mean of all the temperatures

Average temperature = 8.31.

We see how the weighted average gives a much higher effective 'mean' temperature.

5.3 Transforming and weighting data

There are a number of reasons why the data might not be suitable for use directly in analysis, and it may be necessary to apply a *mathematical transformation* to all the values or to *weight the importance* of individual values.

The most common reasons to *transform* data include:

improving graphical presentation

linearization for regression analysis

meeting normality and equality of variance conditions.

Data transformation also occurs as a *link function* within the GsdLM analysis (3.4.7).

Weighting is used to accommodate the fact that different data, perhaps measured with greater accuracy, should be given greater effect, or leverage, in the calculations (5.2.5, 5.3.4).

We start in 5.3.2 with the practicalities of data transformation in Excel, SPSS, and Minitab, and then 5.3.3 reviews common transformations and refers to other sections of the book where these transformations are also addressed. Finally 5.3.4 considers the situation in which the uncertainty varies for different measured values of y, and introduces the method of *weighting* to give a measure of importance to each data value in the analysis that matches the known uncertainty in the value.

5.3.1 Case studies

In this section we will meet the following case studies:

Species abundance

The investigation aims to identify whether different management has affected the species abundance on different sites.

5.3.2 / 2. Transforming data: Demonstrates the transformation processes in Minitab and SPSS.

Experimental uncertainties

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

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

5.3.2 Software transformation

In transforming data, we use a mathematical function (e.g. log) to generate a new value, y', for each original data value, y: $y' = f(y)$.

In x–y analyses, we may wish to transform either or both variables, for example:

A *loglog* transformation would take the logs of both variables:
$y' = \ln(y)$ and $x' = \ln(x)$.

A *loglinear* transformation would only take the logs of the y-variable:
$y' = \ln(y)$ and $x' = x$.

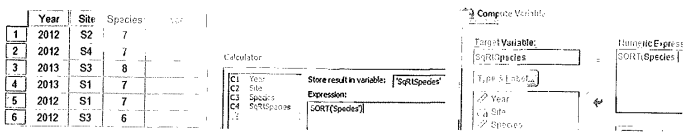
Transforming data can be performed easily within Excel, SPSS, and Minitab. Starting with the y-data in one column, the process creates a new column of the transformed y'-values.

Transforming data: Minitab and SPSS transformations in Fig 5.10. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell

Case study: **Species abundance / 2. Transforming data**

–continued from 5.4.7, returning to 5.4.7

The data values in Fig 5.10 (a) show the first six random rows in Minitab of the number, *Species*, of plant species observed per quadrat in ten quadrats in four sites, *S1*, *S2*, *S3*, and *S4*, taken at the same time of year in each of two years. We use this as example data for demonstrating the mathematical transformations processes in Minitab and SPSS, and we look at the effect of transformations on the normality of the data in 5.4.7.



(a) Original data (b) Minitab using 'calculator' (c) SPSS using 'compute variable'

Fig 5.10 Transforming a variable in Minitab and SPSS

In Excel we can use the relevant *functions*, e.g. SQRT(), LN(), but in Minitab and SPSS we use the processes illustrated in Fig 5.10 (b) and (c). We label a new data column, e.g. *SqRtSpecies*, and then direct the software to generate the transformed values within that column. The relevant expressions can be typed in directly or they can be selected from the options list within dialogue boxes:

Minitab > Calc > Calculator...
Store result in variable: *SqRtSpecies*
Expression: SQRT(*Species*)

SPSS > Transform > Compute Variable...
Target variable: *SqRtSpecies*
Numeric Expression: SQRT(*Species*)

The *arcsine* transformation (5.4.7) requires the entries ASIN(SQRT()) in Excel and Minitab, and ARSIN(SQRT()) in SPSS.

5.3.3 Common transformations

Graphical presentation

Data transformation can be used to improve the *visualization* of data. If, when the data is plotted on an x–y graph, it is too spread out or too tightly packed towards one end of a scale, then it may be useful to transform the *scale* variable. The most common remedy is to use a log transformation for one or both variables. The logarithm to base 10 can be convenient for *display* because the unit divisions on the scale now represent *multiplicative factors* of ten, giving a very clear visual interpretation of scale. However, if already using natural logarithms in the data analysis, it may be better to continue to use these logs to avoid confusions.

Linearization

The process of linearization can be used if it is expected that the x–y data follows a specific nonlinear mathematical relationship (e.g. exponential decay or power equation). After linearizing the data, it is then possible to use the powerful technique of linear regression to produce and analyse a best-fit straight line.

These techniques include:

changing the variable, 2.3.1.

linearizing an exponential relationship, 2.3.4.

using logarithms for power relationships, 2.3.5.

Normality and equality of variance transformations

In Section 5.4, we consider the situations in which the data distribution and the variances of samples do not meet the requirements for an analytical procedure (e.g. ANOVA), but can be transformed into an acceptable form.

5.3.4 Weighting data

The technique of 'weighting' data is frequently used, both in pure statistics and in a scientific context, to represent the varying importance, or effect, of different values in the data.

In the 'Mean kinetic temperature' case study (5.2.5), we used a theoretical equation to derive weightings that reflected the increased importance of higher temperatures in the decay of a pharmaceutical product.

The following case study demonstrates how the results from samples of different sizes and uncertainties can be combined to give one overall result.

Case study: **Experimental uncertainties / 5. Weighting values**

—continued from 1.4.6

We can demonstrate the use of weighting by considering a situation where five students are asked to measure blood alcohol level, in units of mg of alcohol per 100 ml of blood, using a method which has a known standard deviation uncertainty of 2.0. Without receiving specific instructions, they all used different numbers of replicate measurements (5, 1, 8, 10, and 2) to calculate their best estimate values as shown in column B of Fig 5.11. Each student has also calculated, in column E, the uncertainty, u, in their best estimate using the standard error from Eqn 1.21 based on the known standard deviation of 2.0 and their individual sample size.

The problem is 'how to combine their results into one best estimate value?'

	A	B	C	D	E	F	G
1	Student	Mean	No. of	Approx.	Measurement	Weighting	Weighted
2		value, v	replicates, n	sum = $v \times n$	uncertainty, u	$w = 1/u^2$	value = $v \times w$
3	1	65.2	5	326	0.89	1.25	81.5
4	2	64.3	1	64.3	2.00	0.25	16.075
5	3	62.9	8	503.2	0.71	2	125.8
6	4	63.2	10	632	0.63	2.5	158
7	5	64.8	2	129.6	1.41	0.5	32.4
8							
9	Totals	320.4	26	1655.1		6.5	413.775
10	Mean	64.08		63.66			63.66

Fig 5.11 Weighting results according to their uncertainties

The simple mean value of their results is calculated by taking the total of values in B9 and dividing by five to get the simple average in B10 of 64.08. However, this calculation has given the same *importance* to every value, even though the separate values clearly have different associated uncertainties, and it is likely to be unduly biased by the less accurate values.

We can correct this bias because we are given the numbers of replicates for each measurement, and we can work out, in column D, the sum of the values recorded by each student. For example, for student 1, the mean value of 65.2 from five measurements tells us that the sum of his/her five measurements was $65.2 \times 5 = 326.0$. In this way, we can calculate backwards to the overall sum of all 26 measurements made by all students, obtaining the value of 1655.1 in D9. We can then calculate the true mean value of *all* measurements in D10 as 1655.1 / 26 = 63.66.

We now consider the situation where *we do not know* the number of replicates and ignore the data in columns C and D, so that the only information we have is the uncertainties in column E quoted by each student. We choose to work *backwards* from these uncertainties, u, to infer relative sample sizes, n. We start with Eqn 1.21 which gives the uncertainty, u, as being inversely proportional to the square root of the sample size, and then rearrange this equation such that the number of replicates, n, is *inversely proportional to the square of uncertainty*:

$$u \propto \frac{1}{\sqrt{n}} \quad \text{giving} \quad n \propto \frac{1}{u^2} \quad \text{and hence} \quad w = \frac{1}{u^2} \tag{5.1}$$

We can therefore calculate a *weighting factor*, w, proportional to the sample size, n, which is the reciprocal of the square of the uncertainty. The individual weighting factor values are calculated in column F, e.g.

[F3] = 1/E3^2 = 1.25

For each datum, the value is multiplied by its associated weighting factor to derive the weighted value in column G, e.g.

[G3] = B3*F3 = 81.5

The mean value in G10 is then calculated by adding all the weighted values in G9 and dividing by the sum of the weighting factors in F9, giving 413.775 / 6.5 = 63.66, the same result as previously calculated above using replicate numbers.

In this example we see how a weighting factor which is the *inverse* of the *square* of the uncertainty provides the correct importance weighting for data values with different uncertainties. When used in software packages, it is only necessary to give the weighting factors as *relative* values as we do in this example (i.e. they do not add up to 1.00), because the calculation automatically normalizes the proportion by dividing by the sum of the factors (in F9).

5.4 Normality and homoscedasticity

Many analytical techniques make the assumptions that:

- sample data is derived from populations with *normal distributions.*
- different sample groups are homoscedastic, i.e. have the *same variance.*

In this section we review the methods for *testing* these assumptions, and consider data *transformations* that convert a data set which does not meet these requirements into one which does (or nearly does).

However it is important to emphasize that the robust *t*-tests and GLM/ANOVAs remain viable analytical techniques unless the assumptions are *grossly* violated, and, for *exploratory* investigations, these analyses continue to give useful output information, provided that reservations about the validity of any *p*-values are made explicit by a discussion of the statistical limitations. In particular, in a *student project*, a full discussion of possible tests and their limitations is an appropriate way to demonstrate a sound understanding of all the issues involved. The exploratory results can then be used to develop new investigations which can be designed to satisfy the statistical criteria.

5.4.1 **Case studies**

In this section we meet the following case studies:

Chemotaxis index

The chemotaxis experiment initially treats nematodes with different concentrations of a therapeutic drug, and then measures their capacity for migration towards a food supply in a segmented agar plate.

5.4.6 / 3. Normality and homoscedasticity: Demonstrates the use of *residuals* to assess the normality and equal variance conditions necessary for the ANOVA.

Species abundance

The investigation aims to identify whether different management techniques have affected the species abundance at different sites.

5.4.7 / 1. Normality transformation: Uses transformations to improve data normality.

5.4.2 Analytical approach

The first step in assessing the normality and homoscedasticity of experimental data is to consider the *science* involved (5.4.3), and only use *statistical* analysis as a subsequent check of the assumptions. The first question is then: 'Is there any evidence that the data will *not* have a normal distribution?'

If there is *no prior information* (5.4.3) suggesting that the data is *not* normal, proceed with the analysis and then perform the necessary tests to confirm the *validity* of the analysis. There are then two approaches, depending on the data structure and sample sizes:

- For simple tests, such as a two sample *t*-test, there may be enough data values in *each* sample to make reasonable assessments of the normality (5.4.5) of each sample separately and test for a difference in variance (5.4.4). It is then possible to decide, beforehand, whether to use the parametric or nonparametric alternative.
- For more complex analyses with multiple levels of a number of factors, there may be only a few (or even just one) values in each combination of conditions, and the most direct method of assessing normality and homoscedasticity is to analyse the *residuals* (5.4.6) that remain *after* performing the parametric analysis (e.g. ANOVA). This is effectively a 'post hoc' method of checking the validity of the assumptions for using the parametric test in the first place

If the data is *probably* not normal, due to some well-defined reasons for non-normality, then there are established transformations that can be used (5.4.7). In other cases it may be appropriate to try a series of different transformations or go directly to nonparametric tests.

5.4.3 Anticipating normality

Fortunately, we can treat very many routine experimental measurements as normal. For relatively small random uncertainties, with no specific bias in either direction, the variations will usually follow a normal distribution. In addition, the central limit theorem confirms that when we take the mean values of several measurements, the distribution of the mean values will tend to be normal, even if the distribution of individual measurements is not normal.

The best approach to assessing the normality of experimental data is therefore to consider the reasons that it might *not* be normal. There are situations in which data is clearly *not* normal, including:

- binary and ordinal data in which only specific values can occur.
- systems where there are additional factors occurring *within* the 'sample' measurements, e.g. the exam results of a student cohort might show a bimodal (two peaks) distribution. corresponding to two sub groups entering the course with different previous knowledge.

- frequency data, in which the observed value is dependent on an underlying *low value* of *probability*, will follow a Poisson distribution (1.3.3), e.g. radioactive decay.

There are also situations, for replicate interval data, in which the normality condition *might* be violated because it is *skewed* in one direction or the other:

- Experimental values that are close to a *limiting value*, e.g. recording values of 4.2 with an 'uncertainty' of 0.5 when it is known that the maximum possible value is 5.0. The distribution is likely to be *skewed* away from the limiting value.
- Proportions, particularly with values 0 to 0.3 or 0.7 to 1.0, or percentages with values 0 to 30% or 70% to 100%. The distribution can be skewed away from either 0 (0%) or 1.0 (100%). However, proportions in the middle range of 0.3 to 0.7 are likely to be symmetrical and thus more likely to be normal.
- Experimental variations that are greater than about 5% to 10% of the measured value (assuming 0 is a limiting value).

Finally, it is useful to ask if there is any *previous knowledge* of the distribution of similar types of measurements. The approach reported by others (e.g. from published papers) can also be a useful starting point, but it is important to check whether it also works for one's own data.

5.4.4 Differences in variance

Differences in variance are not often a problem when using a *t*-test for small differences in mean values between two *similar* data samples. However, the variance, σ^2, of a measurement often increases with the mean value, μ, being measured, so that ANOVA analyses involving a wide range of measured values may also find significant differences in variance.

For data whose uncertainty is determined by the Poisson distribution (e.g. types of frequency data), the variance equals the mean value, $\sigma^2 = \mu$. This specific relationship can then lead to the successful use of a square root transformation (5.4.7) to counteract the problem. The GsdLM also has the Poisson distribution as a standard distribution option (3.4.7).

However, the relationship between variance and measured value is often not well defined, and may be dependent on characteristics of the different samples, e.g. any characteristic that differentiates between well and ill patients is likely to have a greater range of possible values for the 'ill' condition than for the 'well' condition. In such cases it may be possible to try different transformation options.

The various tests to compare variances are given in 6.2.4 and 6.3.4, and their analysis through residuals is introduced in 5.4.6.

5.4.5 Testing normality

The key parameters that indicate deviations from normality are:

- **skewness** in which the data which does not have a symmetrical probability, with one 'tail' extending further than the other.
- **kurtosis** in which, compared to the ideal bell-shaped curve, the data may be either flatter or more sharply peaked at the central value.

Although the most frequently used analyses(e.g. *t*-tests, ANOVAs) are known to be robust for deviations from normality, they can become unreliable when the distribution is asymmetric with a long tailed, i.e. with significant, *skewness*.

The main hypothesis tests for normality are

Anderson–Darling (Minitab)

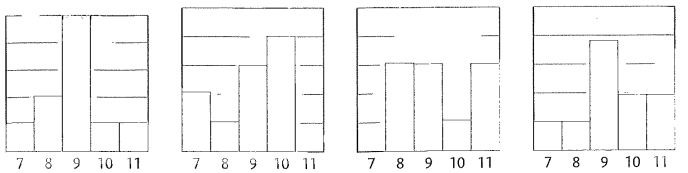
Shapiro–Wilk (SPSS)

which have the null hypothesis

H_0: Data source population has a normal distribution.

Consequently, if $p > 0.05$, then there is no significant evidence that the distribution is *not* normal and, unless we have other reasons to suspect non-normality, we would usually treat the data as normal. The use of Minitab and SPSS to test for normality in a single data set is given in 6.1.6 and 8.1.6.

The normality criterion requires that the sample values have been randomly chosen from a source *population* which has the normal probability distribution, but, unless we have a very *large* sample of replicate measurements, it is unlikely that our *sample* will also appear with the neat bell shape. This is illustrated in Fig 5.12 which shows the frequency distributions of four samples, each of ten data values, all selected at random from the same source population which has a normal distribution with a mean of 9.5 with a standard deviation of 1.5. Each sample is shown with the results of the Shapiro–Wilk (SPSS) and the Anderson–Darling (Minitab) normality tests.



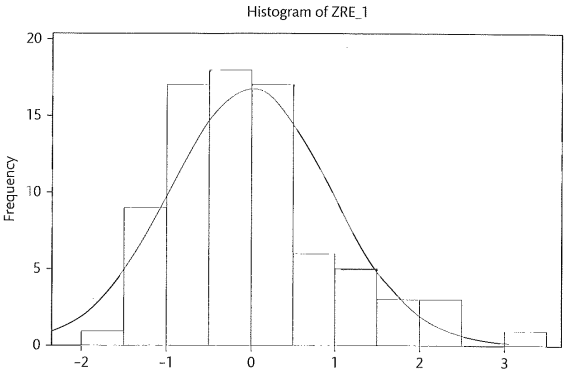
(a) Sample A (b) Sample B (c) Sample C (d) Sample D

Shapiro–Wilk, p =	0.807	0.019	0.444	0.558
Anderson–Darling, p =	0.656	0.016	0.487	0.602

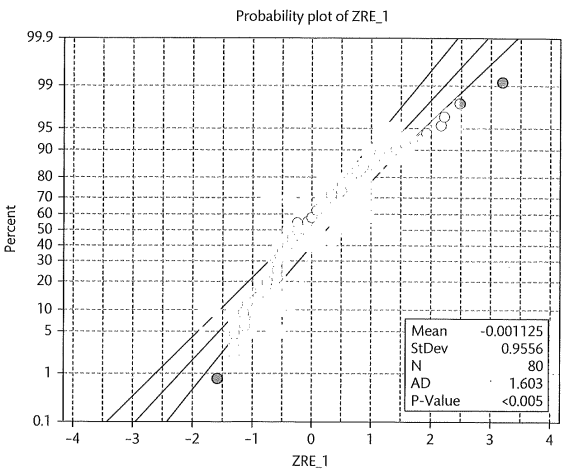
Fig 5.12 Random samples from a normally distributed population

Just by *looking* at the sample distributions it would be impossible to say whether they were drawn from a normal distribution or not, but the *p*-values suggest (with $p > 0.05$) that samples, A, C, and D, can be accepted as normal data. However, for sample B the normality tests with $p < 0.05$ suggest, *incorrectly*, that the data is not normally distributed. These examples illustrate the difficulties in assessing normality based on small samples of data. In fact, for experimental data, the first approach should be a consideration (5.4.3) of the *scientific* system from which the data was collected.

In addition to the hypothesis tests, it is useful to use normality plots that provide a useful graphical presentation.



(a) Histogram with normal plot using: **Minitab >Graph > Histogram...**



(b) P–P normality plot using: **Minitab > Graph > Probability Plot...**

Fig 5.13 Graphical assessment of normality using Minitab

Fig 5.13(a) shows the histogram for the residuals, ZRE_1, from Fig 5.18(b), together with a best-fit normal curve. The data is skewed to the right with skewness = 1.064 (from Table 5.3).

Fig 5.13(b) shows the same data plotted on a P–P normality curve which plots the individual data values (on the *x*-axis) against the expected proportional values of a normal data set. A data set with a near normal distribution would have values that all lie along the central diagonal line and within the curved confidence intervals on either side.

If the data is *skewed* then the observed values will extend further towards one end of the curve. Fig 5.13(b) shows the *positively* skewed data with values spread out to the right.

If it has significant *kurtosis*, then it will be curved away from the line symmetrically in both directions, e.g. Fig 5.13(b) shows positive kurtosi.

The output also gives the *p*-value (<0.005) for the Anderson–Darling normality test, confirming the deviation from normality.

The Q–Q plot (Fig 5.19(a)) provides a similar presentation to the P–P plot, but using *quartile* values instead of proportions.

Analysing residuals (Minitab): Analysis for Fig 5.14 data. See also 6.4.5. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell

Analysing residuals (SPSS): Analysis for Fig 5.14 data. See also 6.4.5. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell

5.4.6 Using residuals

Residuals are the differences between the experimentally measured values and the values predicted by the analytical model (2.1.1). The normality and homoscedasticity requirements for the data *also* apply to residuals in the analysis, and we can use the distribution of residual values as a 'post hoc' test for the overall quality of fit of the analysis (4.4.1).

If our experimental design is such that we have a large number of *replicates* in each sample then it would be possible to assess the normality of *each sample* separately, but we usually only have a few replicate measurements at each of the possible conditions of the experiment. For example, in linear regression there is often only one measurement at each *x*-value, or in a factorial investigation there may be only a very few replicates at each possible combination of factor levels. By using the residuals we can group all values into just one data set for analysis.

The residual value from each data point is calculated, and then 'standardized' by dividing by the standard deviation of all the residuals. The relevance of standardization is that we have a reasonable idea of how we expect normally distributed data to be spread out. For example, the probability of observing a value more than two standard deviations from the mean is about 1 in 20.

Case study: Chemotaxis index / 3. Normality and homoscedasticity

—continued from 6.3.1 and 5.2.4, returning to 6.3.1

Using the data derived from Fig 5.7, we have values for the chemotaxis index, *CIndex*, in Fig 5.14 with 24 values distributed over three treatment levels. We wish to test whether the data meets the normality and homoscedasticity (equality of variance) conditions for a parametric ANOVA analysis.

	A	B	C	D	E	F
1	*Treat*	*CIndex*	*Treat*	*CIndex*	*Treat*	*CIndex*
2	0	0.31	1	-0.07	2	-0.12
3	0	0.29	1	0.21	2	0.13
4	0	0.17	1	0.13	2	0.09
5	0	0.26	1	0.30	2	0.01
6	0	0.43	1	0.17	2	0.06
7	0	0.26	1	0.17	2	0.02
8	0	0.20	1	0.13	2	-0.06
9	0	0.25			2	0.08
10					2	-0.03

Fig 5.14 Chemotaxis index values (shown unstacked in separate treatment groups)

The one-way GLM/ANOVA analysis of this data is described more extensively in Section 6.3, but we will concentrate here on testing that the data meets the necessary parametric criteria. The data must be first stacked with all the *CIndex* values in one column.

Minitab

In Minitab we can produce a direct analysis of residuals and/or choose to save the residual values as *SRES1* in an empty column for later analysis.

Minitab > Stat > ANOVA > General Linear Model > Fit General Linear Model ...
Reponses: *CIndex* *Factors: Treat*
> Graphs ... It is useful to select ▼ ***Standardized*** for **Residuals for plots**
Select ◉ ***Individual plots***, e.g.
☑ ***Normal plot of residuals*** to show normality
☑ ***Residuals versus fits*** show equality of variance
or
Select ◉ ***Four in one***
> Storage ... Use ◉ ***Standardized residuals*** to save residuals into the next empty column.
→ Output in Fig 5.15

The top two plots in Fig 5.15 are similar to those using SPSS and are discussed below. In addition, this 'four in one' plot also gives a histogram of residual values which is not inconsistent with a normal distribution. It also shows the residuals for each data value stepping through the data, which does not show any significant non-random patterns of behaviour, with only one residual more than two standard deviations from the mean.

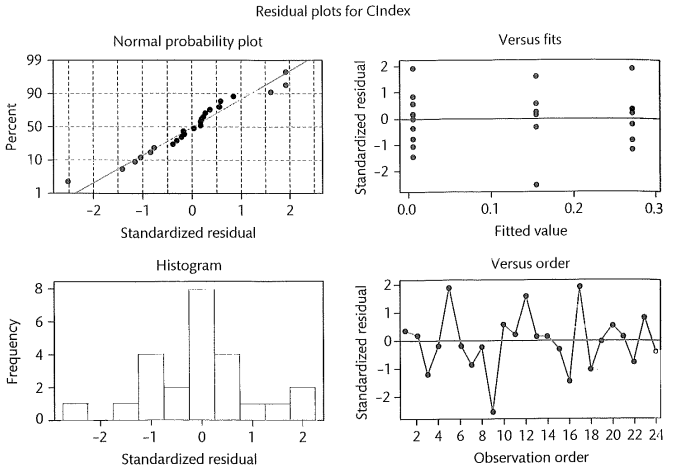


Fig 5.15 'Four in one' residual plots (Minitab)

The saved residuals, *SRES1*, can be analysed as a single data sample (8.1), using

Minitab >Stat > Basic Statistics > Graphical Summary ...

which will perform the Anderson–Darling normality tests and give values for skewness and kurtosis.

SPSS

In SPSS, it is easier to run the GLM/ANOVA analysis first, requesting that the residuals for each data value are saved into a new column in the data editor:

SPSS > Analyze > General Linear Model > Univariate ...
Dependent variable: *CIndex* **Fixed factor(s):** *Treat*
> Save: ☑ **Standardized residuals.** which saves the residuals in a new column with the name *ZRE_1* and label ***Standardized Residual***
> Options: Display ☑ ***Homogeneity tests***
→ The homogeneity test reports Levene's test (Fig 5.16 (a)), which, in this case, shows no significant differences ($p = 0.845$) in variances between the levels of *Treat*.

Levene's Test of Equality of Error Variances[a]

Dependent Variable: CIndex

F	df1	df2	Sig.
.170	2	21	.845

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Treat

(a) Test for homoscedasticity

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Standardized Residual for CIndex	.109	24	.200	.965	24	.541

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

(b) Tests for normality

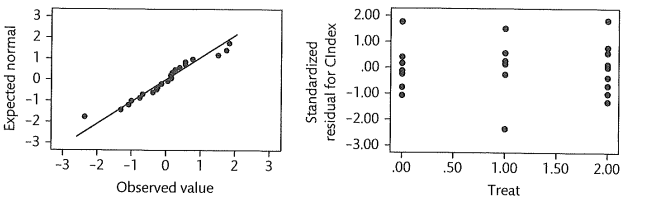
Fig 5.16 Tests for homoscedasticity and normality (SPSS)

The saved residuals can then be analysed using:

SPSS > Analyze > Descriptive Statistics > Explore...
Dependent List: ***Standardized residual***
> Plots: ☑ ***Normality plot with test.***
→ The output will include the standard statistics with skewness and kurtosis, tests for normality, Fig 5.16 (b), and the Q–Q normality plot in Fig 5.17 (a).

It is also possible to plot the variation of residuals for the different levels of *Treat*:

SPSS > Graphs > Legacy Dialogs > Scatter/Dot...
Simple scatter: Define
Y axis: ***Standardized residual***
X axis: *Treat*
→ Output Fig 5.17(b)



(a) Q–Q Normality plot

(b) Plot of variation against factor level

Fig 5.17 Normality and homoscedasticity plots (SPSS)

The normality plots in both SPSS and Minitab show that the data follows a reasonably normal distribution. The standardized residual plots show a similar spread of values at each of the three treatment levels, hence we can also assume that we have a homogeneity of variance.

5.4.7 Data transformations

Any data transformation will affect both normality and the homogeneity of variance, but generally both conditions react in a similar way and a suitable transformation for one often also addresses problems with the other. In practice, it is probably best to focus on developing

the equality of variance, as deviations from normality are well tolerated by the main analytical techniques.

If there is no guidance from previous knowledge in choosing a suitable transformation (e.g. arcsine for proportions), then try the simplest transformations first: square root, logarithmic, before more complex options.

We now consider the most common types of transformation for general data values.

Square root transformation

takes the square root of all the data values:

$$y' = \sqrt{(y)} \text{ or } y' = \sqrt{(y+0.5)} \tag{5.2}$$

(the '+0.5' is often included for data with low values of y).
This equalizes variance for samples where $\sigma^2 = \mu$ and reduces *positive* skewness.

The square root transformation is a mild transformation that is particular useful for positively skewed data and when the sample variances increase in proportion to their mean values. The Poisson distribution (1.3.3) is a particular example which occurs if there is a relatively low probability of observing a specific event, e.g. in counting appearances of rare plants. This distribution is positively skewed, and has the specific characteristic that the expected variance in the data will be equal to its mean value. See the 'Species abundance' case study later in this section.

Logarithmic transformation

takes the logarithm of all the data values:

$$y' = \log(y) \text{ or } y' = \log(y+1) \tag{5.3}$$

(the '+1' is often included for data including 0 values, because log (0) becomes 'minus infinity'). This also reduces *positive* skewness, and reduces variances for samples with larger mean values.

The log to base 10 transformation has the advantage that it produces a data axis that is conveniently defined by 'powers of ten'. See the 'Species abundance' case study later in this section.

Box–Cox transformations

The Box–Cox approach uses a family of transformations which are defined by a parameter, λ, whose value can be adjusted to suit the experimental distribution. The transformation can only be applied to non-zero data values.

$$y' = y^{\lambda} \text{ if } \lambda \neq 1 \quad \text{or} \quad y' = \ln(y) \text{ if } \lambda = 1 \tag{5.4}$$

If $\lambda = 1$, the transformation becomes a simple logarithmic transformation, and if $\lambda = 0.5$, the transformation becomes a square root transformation. When selecting a Box–Cox transformation, the software analyses the data before recommending the optimum value of λ for the transformation. See the general regression example in 7.2.5.

Arcsine transformation

This is used for the transformation of proportions, P, or percentages, $P\%$.

$$P' = \arcsin(\sqrt{P}) \quad \text{or} \quad P' = \arcsin(\sqrt{P\%/100}) \tag{5.5}$$

A measured proportion has values that are limited at both ends, 0.0 and 1.0, of its possible range. The data tends to show a *positive* skewness for proportions between 0 and 0.3 which is corrected by the *square root* factor, and *negative* skewness between 0.7 and 1.0 which is corrected by the *arcsine* function. For proportions within the central region, 0.3 to 0.7, skewness is less likely to be a problem and transformation may not be necessary.

Case study: **Species abundance/ 1. Normality transformation (overview)**

–leading to 5.3.2

The data values in Fig 5.18(a) show the first six random rows in SPSS of the number, *Species*, of plant species observed per quadrat in ten quadrats in four sites, *S1*, *S2*, *S3*, and *S4* taken at the same time of year in each of two years. Two of the sites received different management compared to the other two, and the aim of the experiment is to test whether the different management has had any effect on the numbers of observed species. We start by trying different transformations to improve data normality.

5.3.2 / 2. Transforming variables: Demonstrates the transformation process in Minitab and SPSS

Transforming for normality: SPSS analysis leading to Fig 5.18 and Table 5.3. See also 5.3.2. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell

	Year	Site	Species
1	2012	S2	7
2	2012	S4	7
3	2013	S3	8
4	2013	S1	7
5	2012	S1	7
6	2012	S3	6

(a) Initial data entry

	Year	Site	Species	ZRE_1	SqRtSpecies	ZRE_2	LnSpecies	ZRE_3
1	2012	S2	7	.70	2.65	.78	1.95	.85
2	2012	S1	7	-.60	2.65	-.52	1.95	-.44
3	2013	S3	8	.32	2.83	.37	2.08	.40
4	2013	S1	7	.76	2.65	.66	1.95	.95
5	2012	S1	7	.32	2.65	.41	1.95	.49
6	2012	S3	6	-1.08	2.45	-1.05	1.79	-.93

(b) Transformed data and saved residuals

Fig 5.18 Numbers of species at four sites (Six rows shown randomly out of a total of 80 rows)

Table 5.3 shows the results of performing the ANOVA/GLM analysis (as in 6.4.4), testing for the main effects of the *Year* and *Site* and also the interaction *Year*Site*. A significant result for *Year*Site* would indicate that the *different* management of *some* sites resulted in a difference in the numbers of species, but $p = 0.312$ in Table 5.3 suggests that this interaction is not significant. However our main interest in this data is in the *normality* and *homoscedasticity* of the values.

Within the operation of the ANOVA/GLM, the option of saving the standardized residuals as ZRE_1 in an available column allows an analysis of these residuals using *Explore*, giving the values in Table 5.3. The results for *Species* show that, although there is no significant difference in variance between samples ($p = 0.888$), the data is *not* normally distributed, with $p = 0.000$ for the Shapiro–Wilk test, and both skewness and kurtosis are significant:

Skewness = 1.064, which is more than twice the standard error of 0.269

Kurtosis = 1.163, which is more than twice the standard error of 0.532.

The resultant P–P normality plot using Minitab is given in Fig 5.13(b) and Q–Q plot in Fig 5.19(a) using SPSS, in which the positive skewness is shown by the points extending further to the top right of the plot, and the positive kurtosis is shown by the curvature of the data.

Table 5.3 Results of GLM/ANOVA analysis of data in Fig 5.18(b)

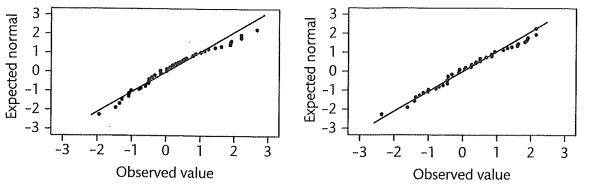
ANOVA results:	Species	SqRtSpecies	LnSpecies
Year	$p = 0.057$	$p = 0.047$	$p = 0.041$
Site	$p = 0.004$	$p = 0.003$	$p = 0.002$
Year*Site	$p = 0.312$	$p = 0.311$	$p = 0.321$
Levene's test for homogeneity of variance	$p = 0.888$	$p = 0.874$	$p = 0.669$
Residuals analysis:	*ZRE_1*	*ZRE_2*	*ZRE_3*
Skewness (St error = 0.269)	1.064	0.651	0.257
Kurtosis (St error = 0.532)	1.163	0.215	−0.114
Normality test, Shapiro-Wilk	$p = 0.000$	$p = 0.022$	$p = 0.241$
Normality plots	Fig 5.13 (a)/(b) Fig 5.19 (a)		Fig 5.19 (b)

Two transformations of the data are made using

SPSS > Transform > Compute Variable...

producing respectively the square root of the values, *SqRtSpecies*, and the log of the values, *LnSpecies*. The ANOVA/GLM analysis is repeated for each new data set, producing new sets of residuals ZRE_2 and ZRE_3 and giving the results in Table 5.3, and the final SPSS data set in Fig 5.18(b).

Although the gentle square root transformation reduces both skewness and kurtosis, the data is still significantly non-normal ($p = 0.022$), but using the log transformation the data can now be considered to be sufficiently near normal ($p = 0.241$). The transformation has reduced skewness and kurtosis to acceptable levels, with the result that the Q–Q normality plot in Fig 5.19(b) now has the data values lying close to the 'normal' line.



(a) Q–Q Normality plot for *Species* (b) Q–Q Normality plot for *LnSpecies*

Fig 5.19 Normality plots for initial and transformed data sets (SPSS)

Single response variable

Introduction

This chapter presents the techniques used to analyse the effect that one or more factors may have on a single response variable. The data can be described as *univariate* and may be either *interval* or *ordinal* data. Some *frequency* values can be treated as interval values, provided that the values are sufficiently large (6.1.1), but, for the analysis of frequencies or probabilities, also refer to Chapter 8.

Section 6.1 presents analyses relevant to *single samples* of replicate data (e.g. data descriptions, one sample *t*-test, Wilcoxon test).

Section 6.2 assumes *two data samples*, which is essentially a special case of a one factor analysis with just two levels (e.g. independent samples *t*-test, Mann–Whitney test).

Section 6.3 relates to investigations that test, or measure, the effect of just *one factor*, but with more than two levels (e.g. one-way ANOVA, Kruskal–Wallis test, repeated measures).

Section 6.4 develops investigations of more than one factor together with possible interactions. The GLM is used as a flexible approach for conducting ANOVA calculations, and the effect of a covariate term is addressed through the ANCOVA.

6.1 One sample

The simplest set of experimental results is a set of replicate measurements of a *single* value, for which we will normally be aiming to

derive the best-estimate values (1.4.1) for the mean, median, standard deviation, etc. of the population from which these experimental results have been drawn, or to

test for a difference between the experimental data and a specified test value.

If the data leads to a 'counting of values' or *frequency* values (e.g. data set *D9* in Fig 6.1), then you should also refer to Section 8.1, although for larger frequency values, you can treat the values as *continuous* data (e.g. data set *D10* in Fig 6.1).

6.1.1 Example data

Single data samples can be of different data *types* (5.1.2) and expressed using different *level* formats, as illustrated in Fig 6.1.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Data set:	D1		D2	D3	D4		D5	D6		D7	D8		D9	D10
2	Type:	Interval		Ordinal	Ordinal	Ordinal		Binary	Binary		Nominal	Nominal		Frequency	Frequency
3	Levels:	continuous		text	integer	rank		text	integer		text	coded		integer	'continuous'
4		7.57		SA	2	1		F	1		AB	1		6	52
5		7.15		A	1	2		F	1		AB	1		2	55
6		7.33		N	0	3		M	0		Ab	2		9	49
7		6.43		D	-1	4		F	1		ab	4		4	46
8		6.68		SD	-2	5		F	1		AB	1		3	57
9		6.97		D	-1	4		M	0		aB	3		6	53
10		7.39		N	0	3		M	0		aB	3		4	49
11		7.75		A	1	2		F	1		AB	1		7	53
12		6.75		N	0	3		F	1		AB	1		3	39
13		7.18		N	0	3		F	1		AB	1		3	52
14		7.18		SA	2	1		F	1		aB	3		8	52
15		6.22		A	1	2		M	0		ab	4		8	41

Fig 6.1 The first 12 values from each of 10 data samples (50 values in each)

Each data sample in Fig 6.1 represents a set of 50 *replicate* measurements made under the *same experimental conditions*, and with the important assumption that every value is assumed to be *independent* of any other value, i.e. the recorded value of one measurement is not affected by any other measurement in the sample.

- *D1* shows 12 values of a set of 50 continuous *interval* values randomly drawn from a normal population with mean, $\mu = 7.00$ and standard deviation, $\sigma = 0.5$.
- *D2*, *D3*, and *D4* all relate to the same set of *ordinal* values of Likert questionnaire responses, but expressed using different level values. The text form (*D2*) gives the abbreviations *SA*– strongly agree, *N*– neutral, *D*–disagree, etc., and these are coded (*D3*) by an integer value from +2 to −2. The same data is also given a ranked value (*D4*).
- *D5* and *D6* record *binary* data in text and numeric forms respectively. Binary data can be considered as either ordinal or nominal data with only two possible states. This data records the observations of 50 frogs as being either male, *M*, or female, *F*, leading to a measurement of proportions (6.1.7).
- *D7* and *D8* record *nominal* values in text and *coded* forms respectively. See Section 8.1 for the analysis of this type of data using categorical frequencies.
- *D9* and *D10* record observed *frequency* values which must be integer. Frequency data is considered in Chapter 8. However, if the frequency values are sufficiently high, as for data set *D10*, it is possible to treat the data as *continuous interval* data.

6.1.2 Analytical options

We list below *some* of the most common analyses used for one sample data, together with links for further information. The issues associated with choosing whether to use *parametric* or *nonparametric* tests are developed in Section 5.4.

Describing data (6.1.3)

- Graphical plots: Boxplot, bar chart, histogram, stem and leaf plot

  Numerical statistics: Mean, median, standard deviation, confidence interval, etc.

Tests / Measurements:

- Is the **mean** value different from a *specified value*? One sample *t*-test for normal data (6.1.4, 3.1.2)
- Is the **median** value different from a *specified value*? Wilcoxon test (nonparametric) (6.1.5, 3.5.2)

  Is the data distribution **normal**? Anderson–Darling, Kolmogorov–Smirnov, Ryan–Joiner (similar to Shapiro–Wilk) tests (6.1.6, 8.1.6)

  Is the **distribution** different from a *specified distribution*? Kolmogorov–Smirnov test (6.1.6, 8.1.6)

  Do the values occur **randomly** above and below a *specified value*? Runs test (nonparametric) (6.1.6)

  Use a **goodness of fit** test to compare with a *specified distribution* of frequencies: Chi-squared goodness of fit test (8.1.5, 3.7.2)

  Is the **proportion** of numerical values above and below a defined value different from a *specified proportion*? Binomial test (6.1.6)

  Is the **proportion** of binary values different from a *specified proportion*? Proportion tests (6.1.7)

6.1.3 Describing the data

It is useful to summarize the data values using numerical and/or categorical statistics and graphical plots.

Numerical statistics (Section 1.5) can be calculated for any data with *numerical* values, e.g. integer, ordinal, ranked, or coded data. The sample statistics (e.g. mean, standard deviation) are best estimates that describe the population from which the sample was drawn. The *relevance* of particular statistics will depend on the data type (for example a measure of skewness has no meaning for ordinal data), and the *reliability* of the distribution statistics (e.g. skewness, kurtosis, Fig 6.2) will be low for small data sets. An important derived statistic is the *confidence interval* (1.5.2) which gives the best estimate range for the true *mean* of the data.

Categorical statistics (Section 8.1) can be applied to all data types and counts the frequencies with which specific values occur in the data set. The distribution of values can be represented using a stem and leaf plot (Fig 6.3), bar graphs, or histograms (Figs 6.4, 6.5).

The simplest **graphical plot** that gives an immediate overview of a data set is the **boxplot** introduced in 1.1.2. In addition, various software options, **Excel** (> **Insert** > **Charts**), **Minitab** (> **Graph**) and **SPSS** (> **Graphs** > **Legacy Dialogs**), have the facilities for drawing a wide range of individual graphs and data plots—see also 8.1.3. The following options for Excel, SPSS, and Minitab give examples of methods for summarizing and presenting experimental data.

Describing sample data (Excel): Descriptives and graphs. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Describing sample data (Minitab): Descriptives and graphs. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Describing sample data (SPSS): Descriptives and graphs. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Excel

Excel, with its two-dimensional worksheet, is a very useful format for tabulating, presenting, and calculating results. It also provides a range of graphical outputs, which can be easily edited for printing.

Excel uses a variety of *dynamic* functions, *fx*, to calculate different statistics individually, e.g. AVERAGE(), STDEV.S(), etc., and a set of summary statistics is also available through the Data Analysis Add-In:

Excel > Data > Data analysis > Descriptive statistics
– calculates a range of statistics.

SPSS

Descriptive statistics can be accessed through three main menu choices as below, with 'Descriptives' giving the main numerical statistics, 'Frequencies' adding data plots and 'Explore' providing greater flexibility with the ability to split the data on the basis of factors in other columns.

SPSS > Analyze > Descriptive statistics >Descriptives...
Variable(s): e.g. *D1*
> Options: e.g. mean, standard deviation, standard error of the mean, skewness, kurtosis
→ Fig 6.2

An example of the output in Fig 6.2 gives statistic values and their standard errors. When using these statistics, we can make a rough estimate of the *confidence deviation* (Eqn 1.22) as being *twice* the standard error. For example, the skewness in Fig 6.2, would have a *confidence interval* of $0.130 \pm 2 \times 0.337$ giving a range from −0.54 to 0.80, which, because it includes 0.0, suggests that the distribution could be normal.

Descriptive Statistics

	N	Mean		Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Std. Error	Statistic	Statistic	Std. Error	Statistic	Std. Error
D1	50	7.0262	.06995	.49465	.130	.337	-.287	.662
Valid N (listwise)	50							

Fig 6.2 SPSS: Example of output from the 'Descriptives' option

SPSS > Analyze > Descriptive statistics > Frequency...
Variable(s): e.g. *D2 D9*
> Statistics: mean, mode, median, standard deviation, skewness, kurtosis, quartiles, etc.
> Charts: Bar charts (Fig 6.4 for *D2*), histograms (similar to Fig 6.6 for *D9*) or pie charts (similar to Fig 6.7 for *D7*)

SPSS > Analyze > Descriptive statistics > Explore...
Dependent List: e.g. *D1*
> Statistics: Option to select numerical parameters
> Plots: Boxplot and outliers, histogram, stem and leaf plot (Fig 6.3), normality plot and test (Fig 5.19)


```
D1 Stem-and-Leaf Plot
 Frequency     Stem &  Leaf
     1.00        5 .  9
     7.00        6 .  2224444
    17.00        6 .  55566778888899999
    16.00        7 .  0001111333333334
     8.00        7 .  56667778
     1.00        8 .  3
 Stem width:     1.00
 Each leaf:      1 case(s)
```

Fig 6.3 SPSS stem and leaf plot for *D1*

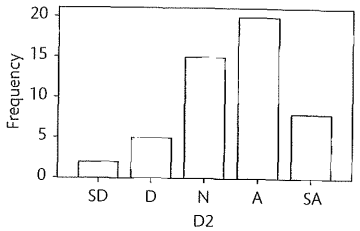


Fig 6.4 SPSS bar chart for *D2*

It is often necessary to edit the graph produced in the SPSS output window. For example, in Fig 6.4, it was necessary to change the horizontal axis labels from a default *alphabetical* order into the *ranked* order. This was achieved by double-clicking on the chart to open the chart editor, then using a right click on the data bars to open the relevant properties window and changing the order under 'Categories'.

Descriptive Statistics: D9

Variable	Mean	SE Mean	StDev	Q1	Median	Q3	Skewness	Kurtosis
D9	6.120	0.347	2.455	4.000	6.000	8.000	0.56	-0.12

Fig 6.5 Minitab: Example of descriptive statistics for data set D9

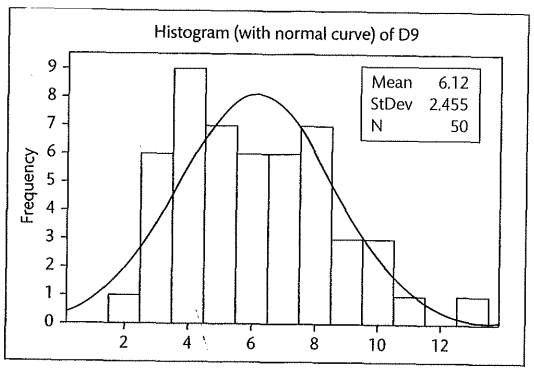


Fig 6.6 Minitab histogram with normal curve for *D9*

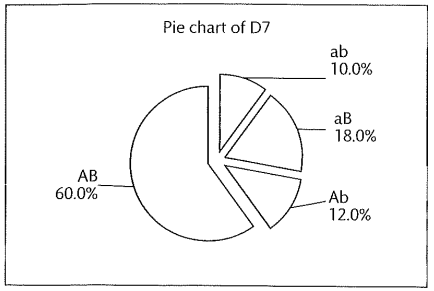


Fig 6.7 Minitab pie chart of *D7*

Minitab

In addition to the individual graph options under > **Graph** (e.g. pie chart in Fig 6.7), Minitab has a range of options for displaying data:

Minitab > Stat > Basic Statistics > Display Descriptive Statistics...
Variable(s): e.g. *D9*
By variable(s): Used to identify subgroups within the column of data.
> Statistics: mean, mode, median, standard deviation, skewness, kurtosis, quartiles, etc.
> Graphs: ☑-Histogram of data, with normal curve
→ Output: Fig 6.5 displays the sample statistics and Fig 6.6 gives the histogram

Minitab > Stat > Basic Statistics > Graphical Summary...
Variable(s): e.g. *D9*
By variable(s): Used to identify subgroups within the column of data.
→ Output: Produces a graphical output which includes the histogram in Fig 6.6, the Anderson–Darling test for normality, the sample statistics in Fig 6.5, and confidence intervals for the mean, median, and standard deviation.

Minitab > Stat > Tables > Tally Individual Values...
→ Output: Counts the number of occurrences of each value in the data set.

One sample tests (Minitab): Analysis for *t*-test and Wilcoxon test. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

One sample tests (SPSS): Analysis for *t*-test and Wilcoxon test. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

6.1.4 One sample *t*-test

The **one sample *t*-test** (3.1.2) tests for a significant difference between the sample *mean* of *normally* distributed data and a *specific value*. For illustrative purposes, we will test whether the mean of the data set, *D1*, is significantly different from 7.2, and, as the true population mean is actually 7.0, we might expect to detect a difference.

SPSS

SPSS > Analyze > Compare Means > One Sample T-test
Test variable: Select data, e.g. *D1*
Test value: Enter test mean value, e.g. 7.2
→ Output: Fig 6.8

One-Sample Test

	Test Value = 7.2					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
D1	-2.484	49	.016	-.17380	-.3144	-.0332

Fig 6.8 SPSS output for *t*-test

With $p = 0.016$ it is possible to state that there is a significant difference between the mean and the test value of 7.2.

Minitab

Minitab > Stat > Basic Statistics > 1-Sample t ...
▼ One or more samples, each in a column
Enter sample: e.g. *D1*
☑-Perform hypothesis test
Hypothesized mean: e.g. 7.2

Minitab gives the same results in Fig 6.9 as SPSS, except that the confidence interval is expressed differently, e.g. the upper *CI limit* of 7.1668 in Minitab is equal to the *difference*, −0.0332, in SPSS from the test mean of 7.2 with 7.2 − 0.0332 = 7.1668.

Variable	N	Mean	StDev	SE Mean	95% CI	T	P
D1	50	7.0262	0.4946	0.0700	(6.8856, 7.1668)	-2.48	0.016

Fig 6.9 Minitab output for *t*-test

6.1.5 Wilcoxon test

The Wilcoxon test is the *nonparametric* equivalent of the *t*-test, testing for a significant difference between the sample *median* and a specific value.

SPSS

The Wilcoxon test is performed in SPSS through '**Analyze > Nonparametric Tests > One Sample...**' and is given in 6.1.6.

Minitab

Minitab > Stat > Nonparametrics > 1-Sample Wilcoxon...
Variables: e.g. *D1*
⊙-Test median: e.g. 7.2

This gives results in Fig 6.10, which show a significant difference ($p = 0.020$) from the specified value of 7.2.

```
Test of median = 7.200 versus median not = 7.200

          N for   Wilcoxon          Estimated
    N     Test  Statistic      P     Median
D1  50      50      396.0  0.020      7.020
```

Fig 6.10 Minitab output for Wilcoxon test

Nonparametric tests (SPSS): Nonparametric tests. See also 6.4.6. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

6.1.6 SPSS nonparametric tests

The 'nonparametric tests' option in SPSS provides a range of tests (including the above Wilcoxon test) that do not assume a normal distribution, including the option of testing for normality itself:

SPSS > Analyze > Nonparametric Tests > One Sample...
Fields: Select data, e.g. *D1*
Settings: ◉-Customize tests
☑-**Compare observed binary probability to hypothesized (binomial test)** (3.8.2)
Options: For continuous field, enter *cutpoint*, e.g. 7.2
☑-**Test observed distribution against hypothesized (Kolmogorov-Smirnov** test) (8.1.6)
Options: Select a distribution(s) for comparison: e.g. normal
☑-**Compare median to hypothesized (Wilcoxon** signed-rank test) (3.5.2)
Hypothesized median: Enter defined median value, e.g. 7.2
☑-**Runs test**
Options: For continuous field, leave the *cutpoint* as the default sample median

The output for the example data, *D1*, is given in Fig 6.11. For reference, the values in the data set *D1* were actually derived randomly from a normal population with a mean (and median) value of 7.00 and a standard deviation of 0.50, giving a sample mean of 7.03 and a sample median of 7.00.

1. The runs test records no significant evidence ($p = 0.568$) for any *non-random ordering* of the data values above and below the sample median. This supports the expectation that the data values have been obtained *independently* of each other.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The sequence of values defined by D1<=7.00 and >7.00 is random.	One-Sample Runs Test	.568	Retain the null hypothesis.
2	The categories defined by D1 <=7.20 and >7.20 occur with probabilities 0.5 and 0.5.	One-Sample Binomial Test	.066	Retain the null hypothesis.
3	The median of D1 equals 7.20.	One-Sample Wilcoxon Signed Rank Test	.020	Reject the null hypothesis.
4	The distribution of D1 is normal with mean 7.03 and standard deviation 0.49.	One-Sample Kolmogorov-Smirnov Test	.967	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Fig 6.11 SPSS: Nonparametric analyses of randomly selected normal data with mean of 7.0

2. Given that the value of 7.20 is not equal to the true median value, we would expect that the proportions of randomly selected values would *not* be the same above and below this value. However the binomial test is not quite *powerful* (1.6.3) enough ($p = 0.066$) to find evidence for a difference for this particular data set.
3. The Wilcoxon signed rank test identifies a significant difference ($p = 0.020$) between the sample median of 7.00 and the test value of 7.20, agreeing with the Minitab result in Fig 6.10.
4. Test for normality. The Kolmogorov–Smirnov test accepts ($p = 0.967$) that the distribution could be normal with calculated values of mean and standard deviation close to the true values.

6.1.7 Proportions

One proportion (SPSS): Analysis for Fig 6.1 data. See also 3.8.2. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: Frogs / 1. Introduction (overview)

– leading to 3.8.2 and 3.8.3

Randomly selecting a sample of 50 frogs in column H in Fig 6.1 from a large lake, it was found that 37 were female. We wish to test whether the observed proportion of 37/50 is significantly *greater than* an expected test proportion of 0.6, or significantly *different from* an observed proportion of 30 females out of 50 randomly selected from a *second* lake.

3.8.2 /2. One proportion: Develops the binomial and other statistics for the exact one sample test.

3.8.3 /3. Two proportions: Develops the statistics for the two sample test.

The 'Frogs' case study sets two problems:

One proportion test, observing 37 female frogs in a sample of 50, has the null hypothesis:

H_0: The probability that each randomly selected frog will be female is 0.6.

Two proportion test, observing 37 female frogs in a sample of 50 from one lake and 30 out of 50 from a second lake, has the null hypothesis:

H_0: The probability that a randomly selected frog will be female is the same in both lakes.

The statistics of possible analytical techniques are developed in 3.8.2 and 3.8.3 together with the use of **Minitab** to perform tests based on the normal approximation, exact binomial tests, and a chi-squared test for the two proportion problem.

If the data is presented as in Fig 6.1 column H, with *individual* observations, then we use SPSS to perform the binomial test as in 6.1.6 using:

SPSS > Analyze > Nonparametric Tests > One Sample...

with

☑-**Compare observed binary probability to hypothesized (binomial test)**
> Options...: Hypothesized proportion: 0.6

which reports a one-sided p-value = 0.030 for a significant difference.

However, if we have just the two frequency *totals* then we can still perform the binomial test as above by first weighing the *F* and *M* categories with the frequencies 37 and 13 respectively as in Fig 8.4.

6.2 Two samples

This section considers two samples of measurements of the *same property*, but measured under different conditions. It uses the example of *pH* measurements from two rivers, in which case, the specific rivers can be considered to be two *levels* of one river *factor*. The analysis here also differentiates between *unrelated* and *related* measurements of the same property, but for *interrelated* data between two samples measuring *different* properties see Chapter 7.

6.2.1 Example data

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	D1				D2				D3			D4							
2	*pH*	*River*		*Meter*	*A*	*B*	*A-B*		*Grp*	*Q5*		*Q1*	*Q2*	*Q3*	*Q4*		Q1\Q2	N	Y
3	**6.56**	A		M1	**6.56**	**6.47**	**0.09**		G2	1		N	N	Y	Y		N	**24**	**19**
4	**6.52**	A		M2	6.52	**6.4**	**0.12**		G1	0		Y	Y	Y	Y		Y	**7**	**16**
5	6.7	A		M3	6.7	6.49	**0.21**		G2	-2		N	N	N	N				
6	6.61	A		M4	6.61	6.55	**0.06**		G2	0		Y	N	Y	Y		McNemar's test:		
7	**6.47**	B							G2	0		Y	N	N	N			χ^2 =	**4.65**
8	**6.4**	B							G1	0		N	N	N	N			*p* =	**0.031**
9	**6.49**	B							G1	2		N	Y	Y	N				
10	**6.55**	B							G1	0		N	Y	N	N				
11									G1	0		N	N	Y	N				
68									G2	-1		Y	N	Y	Y				
69																			
70												Sum of "Y"s =		**23**	**35**	**23**	**26**		
71												Proportion of "Y"s =		**23/66**	**35/66**	**23/66**	**26/66**		

Fig 6.12 Example data from the River *pH* and Forensic Questionnaire case studies (rows 12 to 67 are 'hidden' in the worksheet)

Case study: River *pH* / 1. Overview

In Fig 6.12, data set *D1* in column A shows four *replicate pH* measurements recorded in *each of two* rivers, *A* and *B*. The data source is identified in column B by the *text* variable, *River*, either *A* or *B*. In this format, there is no link or relationship between a specific value measured in one river and a specific value in the other river, and consequently the data samples are described as *independent* or *unrelated*.

Data set, *D2*, in columns E and F, shows the same *pH* measurements, but in this case, four different *pH* meters, *M1*, *M2*, *M3*, and *M4* were used, each making one of the measurements from each river. There are now specific links between pairs of measurements from each river. For example, the value 6.56 in *A* is *uniquely* linked to 6.47 in *B* because they were both measured using *M1* and no other value was measured using *M1*. The two *related* samples *A* and *B* are also described as *paired*. In this section we test for differences as both *unrelated* and *related* samples.

3.1.3 / 2. Two sample *t*-test: Develops the statistics of the *independent samples t-test*.

3.2.2 / 3. ANOVA calculations: Develops the statistics underpinning the *analysis of variance* calculations.

3.4.2/ 4. GLM, ANOVA, and the *t*-test: Demonstrates the *equivalence* of the ANOVA and *t*-test, leading to the GLM.

3.5.1 / 5. Mann–Whitney test: Develops the statistics of the Mann–Whitney test as an example of *nonparametric* testing.

3.6.1 / 6. Paired *t*-test: Introduces the use of four separate *pH* meters as *unique links* between pairs of data to develop the statistics of the paired *t*-test.

3.9.2 / 7. Resampling technique: Demonstrates the use of resampling to *estimate* the same results as the direct tests.

Case study: Forensic questionnaire / 4. Ordinal and binary responses

–continued from 9.2.1, 8.2.1, and 4.4.5

Data sets *D3* in columns I and J and *D4* in columns L to O in Fig 6.12 are extracts of the responses to a forensic questionnaire on the interpretation of evidence. The *same* questions are answered by two groups of people, *G1* and *G2* (possibly different age groups or sexes), with the answers *Q5* on an ordinal scale from –2 to + 2 and *Q1* to *Q4* as binary responses.
In 6.2.6 we use nonparametric tests for differences in the median values and distributions of Q5 responses between the different groups and in 6.2.9 we test for differences in the proportions of binary answers.

The variables in Fig 6.12 are summarized in Table 6.1.

Table 6.1 Variable characteristics (5.1.2)

Variable	Action	Type	Levels	Variability
Sets: D1 and D2				
pH	Output	Interval	Continuous	Normal ?
River	Input	Nominal	*A* / *B*	Fixed
Meter	Input	Nominal	*M1*/*M2* etc.	Fixed
Set: D3				
Grp	Input	Nominal	*G1* / *G2*	Fixed
Q5	Output	Ordinal	– 2 to + 2	
Set: D4				
Q1 to *Q4*	Output	Binary	*Y* / *N*	

In Table 6.1, the data marked 'normal ?' is expected to have a normal distribution, but this can be checked using *residuals* within the analysis (5.4.6). The nominal variables, *River*, *Meter*, and *Grp*, are identified as 'Fixed' because the subjects have not been selected randomly as representative of *all* rivers, meters, and groups.

6.2.2 Analytical options

When dealing with two samples, it is essential to distinguish between *independent* (*unrelated*) samples and *paired* (*related*) samples. Related data with three or more samples is called 'repeated measures' (Section 3.6). *Independent* sample data is normally entered in a *single* column, as data set *D1* in Fig 6.12, and it would make no difference if we changed the order of the values in sample *A* (A3:A6) without changing the order of values in sample *B* (A7:A10). *Related* data is normally entered in *separate* columns and, as for the data set *D2*, there are specific links between paired values in the same rows, and it would not be possible to change the order in E2:E6 without changing the order in F2:F6.

The issues associated with choosing whether to use *parametric* or *nonparametric* tests are developed in Section 5.4.

Describing data (6.2.3)

- Graphical plots: Combined boxplots, differences vs value for paired data

Tests / measurements:

- Are the sample distributions **normal**?: Section 5.4
- Is there a difference in **variance** (and standard deviation)?: *F*-test (normal distribution), Levine's test (any continuous distribution) (6.2.4, 3.2.1)

Unrelated samples:

- Is there a difference between **mean** values (for normally distributed data)?: Independent samples *t*-test for equal variances, Welch's modified test for unequal variances (6.2.5, 3.1.3)
- Is there a difference between **median** values (nonparametric test)?: Mann–Whitney test (6.2.6, 3.5.1)
- Comparing **distributions** of values: Kolmogorov–Smirnov test (not suitable for small samples) (6.2.6), Chi-squared test for association (suitable for a few categories of nominal or ordinal data) (3.7.4)

Related samples:

- Is there a difference between **mean** values (for normally distributed data)?: Paired samples *t*-test (6.2.7, 3.6.1)
- Is there a difference between **median** values (nonparametric test)?: Paired Wilcoxon test (6.2.8)
- Comparing **distributions** of nominal or ordinal values: Cross-tabulation and chi-squared contingency table (8.2.4)

6.2.3 Describing the data

The characteristics of *individual* data samples can be described using the methods in 6.1.3. For *unrelated* samples, the 'Explore' analysis in SPSS provides both numerical and graphical data descriptions, e.g.

SPSS > Analyze > Descriptive statistics > Explore...
Dependent List: e.g. *Q5* **Factor List:** *Grp*
> Statistics: Option to select numerical parameters
> Plots: Boxplots and outliers (Fig 6.13), histogram, stem and leaf plot, normality plot and test.

Specific graphs can also be produced under the graph menu in both Minitab and SPSS:

Minitab
Minitab > Graph > Boxplots...
Select *One Y* and *With Groups*
Graph variable: *Q5*
Categorical variables: e.g. *Grp*
→ Output: Similar to Fig 6.13

SPSS
SPSS > Graphs > Legacy dialogs > Boxplot...
Select *Simple* - **Define**
Variable: *Q5*
Category Axis: *Grp*
→ Output: Fig 6.13

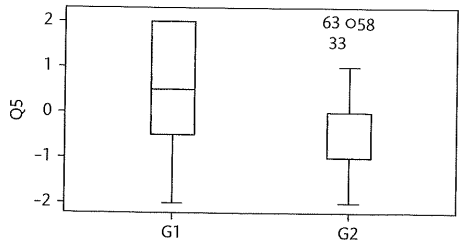


Fig 6.13 SPSS boxplots for *Q5* responses for groups *G1* and *G2*

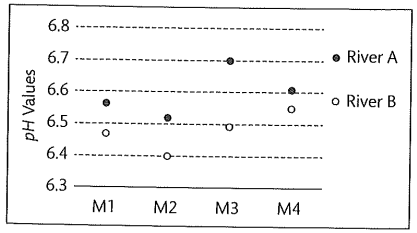


Fig 6.14 Related *pH* data values plotted against meter

The *Q5* boxplots for *G1* and *G2* in Fig 6.13 both record values over the whole range −2 to + 2, but with most *G1* values being '+ 2' and most *G2* values being '0' with just three '+ 2' values identified as outliers (record numbers 33, 58, and 63). It is possible to view the numbers in each category by using separate stem and leaf plots (Fig 6.3) for each variable.

For the *related pH* samples in Fig 6.14 it can be useful to plot both sets of data on a 'line' graph in Excel with each row (meter) being the categories on the *x*-axis. This can show whether there is any pattern in the differences between the pair values, and, for example, we can see that *M2* appears to be biased, giving the lowest readings for both rivers.

6.2.4 Comparing variances

We can use the parametric ***F*-test** and nonparametric **Levene's test** for a difference in **variance**:

Excel > Function > F.TEST (*array1, array2*)
For Fig 6.12 data, enter A3:A6, A7:A10 for *array1, array2*
→ Returns the two-tail *p*-value.

Minitab > Stat > Basic Statistics > 2 Variances...
→ Returns *p*-values for *F*-test and Levene's test

SPSS > Analyze > Compare Means > Independent Samples T test
Levene's test is performed *within* the *t*-test analysis, as in 6.2.5 below

For the *D1 pH* data in Fig 6.12, the two-tailed *F*-test gives $p = 0.718$ and Levene's test $p = 0.618$. There is therefore no evidence of a difference in variance, and we then assume 'equal variance' for further analyses.

Two sample tests (Minitab): Analysis of variance, means, and medians. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

6.2.5 Two sample *t*-test

See 3.1.3 for the development of the two sample *t*-test for unrelated data, which assumes that the two samples are normally distributed and have equal variance. If the variances are not equal, it is possible to use the Welch's modified *t*-test, but, as the standard *t*-test is a robust test and the variance test can be unreliable for small samples, it is not necessary to routinely test for a difference in variance unless there is reason to believe that the variances are likely to be different. The tests can be performed using:

Two sample tests (SPSS): Analysis of variance, means, and medians. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Excel

Excel > Function > T.TEST (*array1,array2, tails, type*)
For Fig 6.12 data, enterA3:A6,A7:A10 for *array1,array2*. Enter 1 or 2 for the number of *tails*, and, for *type* enter 1 for related/paired data, 2 for equal variance, or 3 for unequal variance.
→ Output: Equal variance gives, *p* (2-tailed) = 0.0520 and for unequal variance, *p* (2-tailed) = 0.0540

SPSS

SPSS > Analyze > Compare Means > Independent samples T-test...
Test variable(s): *pH*
Grouping variable: *River*
Define groups...: Enter *A* and *B* to identify the two samples
→ Output: Fig 6.15

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
pH	Equal variances assumed	.297	.606	2.418	6	.052	.12000	.04962	-.00142	.24142
	Equal variances not assumed			2.418	5.715	.054	.12000	.04962	-.00291	.24291

Fig 6.15 SPSS output from two sample *t*-test

In Fig 6.15, Levene's test gives $p = 0.606$, indicating that there is no evidence for a difference in variance, and hence it would be safe to use the 'not significant' result, $p = 0.052$, of the standard *t*-test, with 'equal variances assumed'.

Minitab

Minitab > Stat > Basic Statistics > 2-Sample t...
▼ ***Both samples are in one column***
Samples: e.g. *pH*
Sample IDs: e.g. *River* (in Minitab 16 these are called *subscripts*)
> Options...
Define expected difference between means and select either 1- or 2-tailed test
☑**-Assume equal variances** - choose equal or unequal variances
→ Output: Fig 6.16

```
Difference = mu (A) - mu (B)
Estimate for difference:  0.1200
95% CI for difference:  (-0.0014, 0.2414)
T-Test of difference = 0 (vs not =): T-Value = 2.42  P-Value = 0.052  DF = 6
Both use Pooled StDev = 0.0702
```

Fig 6.16 Minitab output from two sample *t*-test assuming equal variance

When calculating with *equal* variance, Minitab reports the value of the pooled standard deviation (0.0702) calculated using Eqn. 3.6. For calculations with *unequal* variance, Minitab would give *p* (2-tailed) = 0.054, and with no 'pooled standard deviation'. Both results fail to find any significant difference between the two rivers.

It is useful to compare the data in the above results for SPSS and Minitab with those obtained in Fig 3.3.

6.2.6 Nonparametric tests

The Mann–Whitney test is the nonparametric equivalent to the two sample *t*-test for unrelated data, testing for a difference in *median* values.

The Kolmogorov–Smirnov test tests for a difference in the rank *distribution* of values in the two samples.

Using SPSS for the ***Q5* data** in respect of the different groups, *G1* and *G2*:

SPSS > Analyze > Nonparametric Tests... > Independent Samples...
Objective: ◉ Customize analysis
Test Fields: *Q5* **Groups:** *Grp*
(this option will not accept the groups in *scale* format)
Settings: ◉ Customize tests
☑-Mann-Whitney U test (2 samples)
☑-Kolmogorov-Smirnov test (2 samples)
→ Output: Fig 6.17

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Q5 is the same across categories of Grp.	Independent-Samples Mann-Whitney U Test	.031	Reject the null hypothesis.
2	The distribution of Q5 is the same across categories of Grp.	Independent-Samples Kolmogorov-Smirnov Test	.195	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Fig 6.17 SPSS output from independent samples testing

The differences between the two results in Fig 6.17 is that the Mann–Whitney U test identifies a difference ($p = 0.031$) just between the *median* values of the two samples, whereas the Kolmogorov–Smirnov test is unable to identify a significant difference ($p = 0.195$) in the *relative shapes of the distribution* of values within each sample.
It is also possible to perform the Mann–Whitney test in SPSS using the 'Legacy' option:

SPSS > Analyze > Nonparametric Tests... > Legacy dialogs > 2 Independent Samples

Using **Minitab** for the ***pH* data** for a nonparametric test between rivers *A* and *B*:

Minitab > Stat > Nonparametrics > Mann-Whitney
(The two samples must be in different columns)
First Sample: *A*
Second Sample: *B*
→ Output: Fig 6.18

```
Point estimate for ETA1-ETA2 is 0.1200
97.0 Percent CI for ETA1-ETA2 is (-0.0300,0.3000)
W = 25.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0606
```

Fig 6.18 Mann–Whitney test using Minitab

The nonparametric Mann–Whitney test for a difference between the two rivers returns a 'no significance' result ($p = 0.061$) in agreement with that of the independent samples *t*-test above.

6.2.7 Paired *t*-test

For related samples, the parametric paired *t*-test can be performed for normally distributed data, using:

Excel

Excel > Function > T.TEST (*array1,array2, tails, type*)
For Fig 6.12 data, enterE3:E6,F3:F6 for *array1,array2*. Enter 1 or 2 for the number of *tails*, and, for *type*, enter 1 for related data.
→ Output gives the two-tailed *p*-value: $p = 0.0342$

SPSS

SPSS > Analyze > Compare Means > Paired-Samples T-test
Paired variables: Enter *A* and *B* as the two variables for **Pair 1**
→ Output: Fig 6.19

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 A - B	.12000	.06481	.03240	.01688	.22312	3.703	3	.034

Fig 6.19 SPSS output for paired samples *t*-test

Minitab

Minitab > Stat > Basic Statistics > Paired t...
▼ *Each sample is in a column*
Sample 1: *A*
Sample 2: *B*
(Minitab calculates the first sample minus the second sample values)
→ Output: Fig 6.20

```
95% CI for mean difference: (0.0169, 0.2231)
T-Test of mean difference = 0 (vs not = 0): T-Value = 3.70  P-Value = 0.034
```

Fig 6.20 Minitab output for paired samples *t*-test

We can now see that, by taking into account the variations between the meters, the paired *t*-tests are able to detect a significant difference ($p = 0.034 < 0.05$) between the mean *pH* values of the two rivers.

6.2.8 Paired Wilcoxon test

The paired Wilcoxon test is the nonparametric equivalent to the paired *t*-test. This is not performed in the standard version of Excel.

Paired tests (Minitab): Analysis for paired *t*-test and Wilcoxon test. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Paired tests (SPSS): Analysis for paired *t*-test and Wilcoxon test. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

SPSS

SPSS > Analyze > Nonparametric Tests > Related Samples...
Objective: ◉-Customize analysis
Test Fields: *A B*
Settings: ◉-Customize tests
☑-Wilcoxon matched-pair signed-rank (2 samples)
→ Output: Fig 6.21 giving a 'no significance' result with $p = 0.068$

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between A and B equals 0.	Related-Samples Wilcoxon Signed Rank Test	.068	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Fig 6.21 SPSS output for paired Wilcoxon signed rank test

Minitab

To perform the Wilcoxon paired test in Minitab, we first calculate the differences between the values of *A* and *B* for each data pair, and record the values in column G in Fig 6.12. We then carry out a one sample Wilcoxon test to test whether the *A*–*B* data has a median value that is significantly different from 0.

Minitab > Stat > Nonparametrics > 1-Sample Wilcoxon...
Variables: *A–B*
◉-Test median: 0
→ Output: $p = 0.100$, which does not show a significant difference.

The nonparametric test is not as *powerful* as its parametric equivalent, failing to find a difference in median values between the two rivers.

6.2.9 Unrelated binary data

Two proportions (SPSS): Analysis for Fig 6.22. See also 3.8.3. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Considering the Forensic Questionnaire binary data in Fig 6.12, we *could* choose to treat the four data samples, *Q1*, *Q2*, *Q3* and *Q4* as *unrelated*. In this case the best that we could do is to calculate the proportions of 'Y' outcomes in each sample as shown in row 71, and test for any significant differences.

To test whether there is a significant difference between the two extreme *proportion* values 23/66 for *Q1* and 35/66 for *Q2* we use the two-proportion test in Minitab (see 3.8.3), and find a non-significant $p = 0.053$ for Fisher's exact test, which suggests that there is insufficient evidence to find a difference between the samples. For SPSS we need to weight (8.1.3) the frequency values as in Fig 6.22(a) and then use crosstabs and contingency table statistics (8.2.4) to give the results in Fig 6.22(b). The two proportion test produces a 2 × 2 contingency table which means that we should use either the Yates continuity correction

or the Fisher's exact test, which give $p = 0.054$ and 0.053 respectively, agreeing with the Minitab result.

	Answer	Question	Freq
1	Y	Q1	23
2	N	Q1	43
3	Y	Q2	35
4	N	Q2	31

(a) Data entry for 'weighting'

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.429[a]	1	.035		
Continuity Correction[b]	3.721	1	.054		
Likelihood Ratio	4.455	1	.035		
Fisher's Exact Test				.053	.027
N of Valid Cases	132				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 29.00.

(b) Calculated chi-squared values

Fig 6.22 Crosstabs and 2 × 2 contingency table analysis (SPSS)

However, we can use the full information available by treating the data as *related*, and test for *agreement* between samples (4.4.5) by either:

- comparing two *variables* at a time, using *cross-tabulation* to derive a 2 × 2 contingency table (8.2.4) whose analysis can include McNemar's test for agreement between the values or
- comparing more than two variables together, using Cochran's Q, which is equivalent to the McNemar test for multiple samples.

6.3 One factor

In Section 6.2 we analysed differences between two data samples, which could be considered as *two levels* of one factor. In this section we extend the analysis to *more than two levels* of the one factor.

6.3.1 Example data

	A	B	C	D	E	F	G	H	I	J
1	Chemotaxis data:							Forensic questionnaire data:		
2	*Treat*	*CIndex*	*Treat*	*CIndex*	*Treat*	*CIndex*		*Year*	*T1*	*T2*
3	0	0.31	1	-0.07	2	-0.12		3	88	87
4	0	0.29	1	0.21	2	0.18		3	57	56
5	0	0.17	1	0.18	2	-0.09		3	68	67
6	0	0.26	1	0.30	2	0.01		2	36	33
7	0	0.44	1	0.17	2	0.06		3	81	78
8	0	0.26	1	0.17	2	0.02		2	42	31
9	0	0.20	1	0.13	2	-0.06		3	60	63
10	0	0.25			2	0.08		3	71	77
11					2	-0.03		2	41	34
12								2	53	51
68								2	43	48

Fig 6.23 Data from the Chemotaxis Index (unstacked) and Forensic Questionnaire case studies (rows 13 to 67 are 'hidden' in the worksheet)

Case study: **Chemotaxis index / 1. One factor analysis (overview)**

The chemotaxis experiment initially treats nematodes with different concentrations of a therapeutic drug, and then measures their capacity for migration towards a food supply in a segmented agar plate. The effect of the pre-treatment factor, *Treat*, on the derived chemotaxis index, *CIndex*, is tested using a one-way ANOVA.

The data in Fig 6.23, columns A to F, gives 24 calculated (5.2.4) chemotaxis index values, *CIndex*, for three levels of the pre-treatment factor, *Treat*. Note that it is *not* necessary that the samples are all the same size. For convenience of display, the data in Fig 6.23 has been shown 'unstacked', in that the *unrelated* replicate samples for the different levels are given in separate columns. For entry into software, we must put all response values, *CIndex*, into the *same* column (stacked) with the levels of *Treat* for each entry given in a separate column.
In this section we demonstrate the one-way ANOVA analysis (parametric and nonparametric):

6.3.3 Clustered boxplots used for *describing* data

6.3.4 Test for normality and homoscedasticity

6.3.5 One-way ANOVA

6.3.6 post hoc tests

6.3.7 Kruskal–Wallis test (nonparametric one-way ANOVA)

5.2.4 / 2. Deriving the analytical statistics: Starting with *raw data values* in a lab notebook, we consider the calculation of the *chemotaxis index* that is then used to analyse the data.
5.4.6 / 3. Normality and homoscedasticity: We use *residuals* to assess the normality and equal variance conditions necessary for the ANOVA analysis.

Case study: **Forensic questionnaire / 5. Repeated measures**

The data in columns H to J in Fig 6.23 is an extract from the full forensic questionnaire data set in Fig 9.12, and gives the results *T1* and *T2* of two tests performed by 66 subjects who are identified as falling into two *year* groups. The tests were conducted *before* and *after* some additional tuition classes, and in 6.3.8 we are interested in comparing the effect of the classes on the performances of the two groups. We use this data as an example of *repeated measures* (Section 3.6) in which the same measurement (i.e. the test) is performed more than once on the same subject.

The variables in Fig 6.23 are summarized in Table 6.2. Note that it is not known initially whether *CIndex* is normally distributed within each *treat* group, although there is no prior information that it is *not* normal. We see in Fig 9.13 that the distribution of *T1* (and probably *T2*) is actually bimodal due to the two *year* groups, but we will initially treat the distribution *within* each *year* as being normal.

Table 6.2 Description of variables in Fig 6.23

Variable	Action	Type	Levels	Variability
Chemotaxis				
CIndex	Output	Interval	Continuous	Normal ?
Treat	Input	Ordinal	0, 1, and 2	Fixed
Forensic				
T1 and *T2*	Output	Interval	Continuous	N[illegible] ?
Year	Input	Ordinal	2 and 3	Fixed

6.3.2 **Analytical options**

The issues association with choosing whether to use *parametric* or *nonparametric* tests are developed in Section 5.4.

Describing data (6.3.3)

- Graphical plots: Boxplots, factor plots

Tests / Measurements:

- Test for **normality** and **homoscedasticity** (homogeneity of variance): 6.3.4 and 5.4.6
- Is there a difference between the **mean/median** values for different levels of the factor?: GLM/ ANOVA (normal data) (6.3.5), Kruskal–Wallis test (nonparametric) (6.3.7), GsdLM (6.4.7)
- Use **post hoc** tests to locate significant differences: 6.3.6, 3.2.4

Repeated measures data:

- Is there a difference between **repeated** values (within-subject)?: 3.6.2, 6.3.8
- Is there a difference between the **mean** values for different levels of the factor (between-subject)?: 6.3.8

6.3.3 **Describing the data**

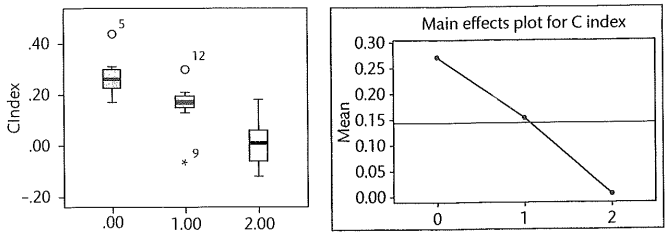
The methods for describing data here are similar to those used for unrelated samples in 6.2.3 and for individual data in 6.1.3. In particular, the 'Explore' option in SPSS provides a comprehensive range of numerical and graphical outputs for data that can be separated by factor levels.

One of the most effective plots for the raw data is the comparative boxplot in Fig 6.24(a) which can be produced through the graph menu in both Minitab and SPSS:

Minitab > Graph > Boxplot... > One Y, With Groups
Graph variables: *CIndex* **Categorical variables:** *Treat*

SPSS > Graphs > Legacy dialogs> Boxplot...>Simple
> Define: Variable *CIndex* **Category axis:** *Treat*

The fairly symmetrical boxplots suggest that the data for each treatment will be normally distributed (5.4.6). There are just three outliers identified, with one, marked by a star, considered to be an extreme outlier.



(a) Boxplots (SPSS) for three treatments

(b) Factor plot (Minitab)

Fig 6.24 Displaying one factor variations

Factor plots, as in Fig 6.24(b) also show the variation of the *mean* values for the different samples. They can be requested from within the ANOVA analyses (6.3.5).

6.3.4 Normality and equality of variance (homoscedasticity)

The assessment for both the normality and homoscedasticity is developed in Section 5.4 by analysing the distribution of the *residuals* after fitting a GLM to the data. It is often convenient to perform these checks *within the analysis itself* as a *confirmation* that the analysis is valid. The ANOVA analysis of residuals for the Chemotaxis data is performed in detail using **Minitab and SPSS** in 5.4.6.

Minitab also has a separate test for the equality of variance.

Minitab >Stat > ANOVA > Tests for Equal Variances...
▼ ***Response data are in a column for all factor levels***
Reponse: *CIndex* **Factors:** *Treat*
> Options...: Opting to base the test on the normal distribution will use Bartlett's test, otherwise the analysis uses Levene's test
→ Output ☞Fig 6.25


```
Bartlett's Test (Normal Distribution)
Test statistic = 0.55, p-value = 0.758

Levene's Test (Any Continuous Distribution)
Test statistic = 0.16, p-value = 0.853
```

Fig 6.25 Minitab tests for differences in variance between samples

SPSS performs Levene's test for variance within the ANOVA calculation (6.3.5), with the results given in Fig 6.26.

Levene's Test of Equality of Error Variances[a]

Dependent Variable: CIndex

F	df1	df2	Sig.
.170	2	21	.845

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Treat

Fig 6.26 Levene's test for homoscedasticity from within GLM/ANOVA (SPSS)

The results in Figs 6.25 and 6.26 indicate that there are no significant differences in the variances of the samples for the three *Treat* levels.

If the data does not meet the normality and homoscedasticity criteria then we cannot rely on the significance of the *p*-values from the analysis, and we would need to use some other analysis or transform the data (5.4.7) to meet these conditions.

6.3.5 GLM / ANOVA

An analysis of variance (ANOVA) calculation tests for a significant difference between the mean values of three or more samples. The basic calculation and interpretation of ANOVAs are developed in Section 3.2, but, in practice, it is often more convenient to use the ANOVA analysis within the GLM analysis (3.4.2).

The criteria for an ANOVA is that the random distribution of values has a normal distribution and the variances of all samples are equal (homoscedasticity). However, the GLM/ANOVA techniques are very robust and still produce reliable results for limited deviations from these conditions.

GLM/ANOVA (Minitab): Analysis leading to Figs 6.27(a), 6.28, and 6.30. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

GLM/ANOVA (SPSS): Analysis leading to Figs 6.27(b), 6.29, and 6.31. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Minitab

Minitab > Stat > ANOVA > General Linear Model > Fit General Linear Model...
Reponses: *CIndex*
Factors: *Treat*
→ Output: Fig 6.27(a)

and then for the *post hoc* tests and data plots:

Minitab > Stat > ANOVA > General Linear Model > Comparisons...
Response: *CIndex* **Type of comparison: ▼ *Pairwise***
Select a *post hoc* test (3.2.4), e.g. ☑-*Tukey*
Choose terms for comparison: Double click on *Treat* to see: C Treat
> Graphs... ☑**-Interval plot for difference in means**
> Results...
☑**-Grouping information**
☑**-Tests and confidence intervals**
→ Output: Same information as in Fig 6.28

and factor plots can be obtained through:

Minitab > Stat > ANOVA > General Linear Model > Factorial plots...

```
Analysis of Variance for CIndex, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS   Adj MS      F      P
Treat    2  0.30098  0.30098  0.15049  16.13  0.000
Error   21  0.19593  0.19593  0.00933
Total   23  0.49691

S = 0.0965919   R-Sq = 60.57%   R-Sq(adj) = 56.82%
```

(a) Minitab

Tests of Between-Subjects Effects

Dependent Variable: CIndex

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.305[a]	2	.152	16.638	.000
Intercept	.496	1	.496	54.207	.000
Treat	.305	2	.152	16.638	.000
Error	.192	21	.009		
Total	.956	24			
Corrected Total	.497	23			

a. R Squared = .613 (Adjusted R Squared = .576)

(b) SPSS

Fig 6.27 ANOVA outputs from Minitab and SPSS

SPSS

SPSS > Analyze > General Linear Model > Univariate
Dependent variable: *CIndex* **Fixed factor(s):** *Treat*
Random factor(s): Identify any random factors
Covariate(s): Include any covariates
> Model... Allows choice of factors to be included in the test–not relevant for one-way ANOVA
> Post Hoc...Post HocTests for: *Treat* and select tests e.g. ☑**-Bonferroni,** ☑**-Sidak**
> Options... Allows additional analyses, e.g.
☑**-Tests for homogeneity of variance** and
☑**-Residual plots**
→ Output : Fig 6.27(b), Fig 6.26, and Fig 6.29

Fig 6.27 shows the presentation of the standard ANOVA table of results (3.2.3) from both Minitab and SPSS, with $p = 0.000$ indicating difference(s) between the levels with a highly significant p-value that is less than 0.0005. However, we need to perform post hoc tests (3.2.4) to decide which *pairs* of samples are significantly different.

6.3.6 Post hoc comparison tests

The post hoc comparison tests are requested within GLM/ANOVA for both Minitab and SPSS (6.3.5). The interpretation and the variety of possible tests is discussed in 3.2.4.

The results given in Fig 6.28 are obtained for the 'Chemotaxis' case study.

```
Grouping Information Using Tukey Method and 95.0% Confidence
Treat  N      Mean  Grouping
0      8  0.271241  A
1      7  0.154530  A
2      9  0.005903     B
Means that do not share a letter are significantly different.

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable CIndex
All Pairwise Comparisons among Levels of Treat
Treat = 0  subtracted from:
Treat    Lower   Center    Upper   --+---------+---------+---------+----
1      -0.2426  -0.1167   0.0091              (---------*---------)
2      -0.3835  -0.2653  -0.1472   (---------*---------)
                                   --+---------+---------+---------+----
                                  -0.36     -0.24     -0.12      0.00

Treat = 1  subtracted from:
Treat    Lower   Center    Upper   --+---------+---------+---------+----
2      -0.2712  -0.1486  -0.02609            (----------*---------)
                                   --+---------+---------+---------+----
                                  -0.36     -0.24     -0.12      0.00
```

Fig 6.28 Minitab 16 output for Tukey post hoc test
(Minitab 17 provides the same information in a rearranged format)

In Fig 6.28, the *grouping information* shows that Treatments 0 and 1 both contain the letter 'A' and Treatment 2 only contains the letter 'B'. The interpretation is that there is a significant difference between Treatments 2 and both 0 and 1, and that there is no significant difference between 0 and 1. This is confirmed by reference to the confidence intervals shown as bracketed ranges, because the difference between 0 and 1 has a confidence interval from −0.24 to + 0.009, which includes *zero* and therefore gives the possibility that there is *no* difference. The confidence intervals between 2 and both 0 and 1 do not overlap zero showing that their differences are significant.

Multiple Comparisons

Dependent Variable: CIndex
Bonferroni

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	1.00	.1168	.04953	.084	-.0121	.2456
	2.00	.2669*	.04650	.000	.1460	.3879
1.00	.00	-.1168	.04953	.084	-.2456	.0121
	2.00	.1502*	.04823	.016	.0247	.2756
2.00	.00	-.2669*	.04650	.000	-.3879	-.1460
	1.00	-.1502*	.04823	.016	-.2756	-.0247

Based on observed means.
The error term is Mean Square(Error) = .009.
*. The mean difference is significant at the .05 level.

Fig 6.29 Bonferroni post hoc test with SPSS

The Bonferroni post hoc tests in Fig 6.29 report both confidence intervals of differences and p-values. For example, the difference between Treatments 0 and 1 has a confidence interval (reversed in signs) between −0.01 to + 0.25, similar to the Tukey results in Fig 6.28. The p-values confirm the significant differences between 2 and 0 ($p < 0.0005$) and 1 ($p = 0.016$), in addition to the 'no significant' difference between 0 and 1 ($p = 0.084$).

6.3.7 **Kruskal-Wallis test**

The Kruskal–Wallis test is the nonparametric equivalent of a one-way ANOVA, and is based on the distribution probabilities of the data when expressed as ranked values (3.5.2). It is necessary to use this test for ordinal or ranked data, or for interval data that does not satisfy the normality and homoscedasticity conditions required by the ANOVA. However, for the purposes of illustration we use the Chemotaxis data here as a comparative example.

Minitab

Stat > Nonparametrics > Kruskal-Wallis...
Response: *CIndex* **Factor:** *Treat*
→ Output: Fig 6.30

```
Kruskal-Wallis Test on CIndex
Treat     N    Median  Ave Rank      Z
0         8  0.256819      19.0   3.18
1         7  0.170732      13.0   0.22
2         9  0.008065       6.3  -3.31
Overall  24                12.5
H = 13.64  DF = 2  P = 0.001
```

Fig 6.30 Kruskal-Wallis test output for Minitab

SPSS

Analyze > Non Parametric Tests > Independent Samples...
Test Fields: *CIndex*
Groups: *Treat* (cannot be a scale variable—if necessary change description to ordinal)
Settings: ◉-Customize tests
☑-Kruskal-Wallis 1-way ANOVA (*k* samples)
→ Output : Fig 6.31

Using the Legacy Dialogs in SPSS, it is possible to enter the factor (group) as a *scale* variable, and it is possible (and necessary) to define the range of factor levels to be tested.

Analyze > Nonparametric Tests > Legacy Dialogs >*K* Independent Samples
Test variable: *CIndex*
Grouping variable: *Treat* (0,2) (define the ends of the chosen range of values)
☑-Kruskal-Wallis H
→ Output : Same as Fig 6.31

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of CIndex is the same across categories of Treat.	Independent-Samples Kruskal-Wallis Test	.001	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Fig 6.31 Kruskal-Wallis test output for SPSS

The results of $p = 0.001$ for both Minitab and SPSS shows a highly significant difference between at least one pair of the three samples, consistent with the ANOVA results.

6.3.8 **Repeated measures**

Repeated measures 2: SPSS analysis leading to 6.32. See also 3.6.2. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

We have met the power of related measurements in the paired t-test and Wilcoxon test in 6.2.7 and 6.2.8, and, extending this to more than two variables, we have already used a *repeated measures* analysis in 3.6.2 to identify a significant difference between three variables.

In this analysis, the data in columns H to J in Fig 6.23 has repeated measures between the tests *T1* and *T2* taken *before* and *after* additional classes. Applying the terminology, the 'within-subject' variation is the *horizontal* difference between *T1* and *T2* for each subject row, and the 'between-subject' variation describes how the *T1-T2* difference might vary *vertically* between the two year groups.

Using SPSS

SPSS > Analyze > General Linear Model >Repeated Measures...
The first step is to describe the 'within subjects' variation by giving it a name, and, if you wish, a name for the 'between subjects' measure.
Within Subject Factor Name: e.g. *TestDiff*
and entering the number of related variables
Number of Levels: 2 **> Add**
Measure Name: e.g. *YearGroup* **> Add**
> Define
Within Subjects Variables: Click across *T1* and *T2* to give T1(1.Year Group) and T2(2.Year Group)
Between Subjects Factors: Click across *Year*
For more than two factor groups it is also possible to include post hoc tests
→ Output : Fig 6.32

Tests of Between-Subjects Effects
Measure: YearGroup
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	368453.986	1	368453.986	2532.365	.000
Year	34168.168	1	34168.168	234.200	.000
Error	9337.142	64	145.893		

(a) Between subjects

Tests of Within-Subjects Contrasts
Measure: YearGroup

Source	TestDiff	Type III Sum of Squares	df	Mean Square	F	Sig.
TestDiff	Linear	.073	1	.073	.008	.927
TestDiff * Year	Linear	53.103	1	53.103	6.172	.016
Error(TestDiff)	Linear	550.692	64	8.605		

(b) Within subjects

Fig 6.32 Repeated measures output (SPSS)

The primary result in Fig 6.32(a) is for the test for a possible difference in overall mean values *between* the two *year* groups, and concludes ($p = 0.000$) that there is a significant difference in mean values between the two groups. This is confirmed by the clearly bimodal distribution of scores for *T1* in Fig 9.13.

The result of particular interest here is in Fig 6.32(b), which concentrates on the differences *within* individual subjects and concludes that the average difference between *T1* and *T2* is not significantly different ($p = 0.927$) from 0, i.e. the additional classes do not appear to have made any *overall* difference. However, the interaction (3.3.2) term, *TestDiff* Year*, between the *TestDiff* and the *Year* group, is significant with $p = 0.016$, which suggests that the effect of the additional classes is different between the two *Year* groups. This can be demonstrated by analysing the differences, *T2 – T1*, separately for the two *Year* group samples. Using Explore in SPSS, we get:

Mean difference for Year 3 = +1.35

Mean difference for Year 2 = –1.25

It appears that the extra classes have helped Year 3 giving them an increased score but have confused Year 2 and actually reduced their score.

Refer to 3.6.2 for the interpretation of Mauchly's test for *sphericity* which tests for the equality of variance when there are three or more repeated measures.

6.4 Multiple factors and interactions

This section analyses a single interval or ordinal response variable which may be dependent on a *number* of input factors or variables. It extends the use of the ANOVA for multiple levels of one factor to analyse multiple *factors* and possible *interactions* between those factors, and in 6.4.8 we also introduce the analysis of covariance (ANCOVA) which includes the effect of a continuous input *variable* that is correlated with the response variable.

6.4.1 Example data

We use two main case studies to introduce various aspects of multiple factor analysis, one with *interval* response data and one with *ordinal* data. We also include, in 6.4.8, data from the 'Ink analysis' case study to demonstrate the use of an ANCOVA analysis.

Case study: Boxing performance / 1. Multifactorial analysis (overview)

In an experiment to test for the effect of dehydration on boxing performance, six amateur boxers (a,b,c,d,e,f) each carried out two simulated boxing bouts (of three rounds each) in each of two states of hydration: euhydration (*E*) (normal state) and dehydration (*D*). The measured performance variable is the number of punches in each round, and a random extract from the results is given in Fig 6.33.
In this section:

6.4.3 Clustered boxplots describe the data and interaction plots identify key behaviour patterns.

6.4.4 The main analysis using GLM/ANOVA identifies significant factors and interaction.

6.4.5 The tests for normality and homoscedasticity support the validity of the analysis.

9.1.6 / 2. Multiple regression: Uses stepwise regression and general regression to identify the significant factors in a best-fit model.

	A	B	C	D	E	F	G	H	I
1	RecNo	Punches	Subject	Hydrat	HydratN	Round	Bout	H*R	H*B
2	11	131	e	E	1	2	1	2	1
3	3	129	c	E	1	1	1	1	1
4	62	117	b	D	2	5	2	10	4
5	42	127	f	D	2	1	1	2	2
6	24	135	f	E	1	4	2	4	2
7	16	150	d	E	1	3	1	3	1
8	55	147	a	D	2	4	2	8	4
9	51	132	c	D	2	3	1	6	2
10	40	132	d	D	2	1	1	2	2
11	59	122	e	D	2	4	2	8	4
12	31	159	a	E	1	6	2	6	2

Fig 6.33 Extract of data from the 'Boxing' case study

When entering data into Excel, it is useful to give all the data values a unique record number, as in column A of Fig 6.33. This allows the data to be sorted under various headings when editing the data, but then to use the record number as a key to re-sort the data back into its original order.

The *Punches* data is an integer frequency, but, given the high values, we can treat it as an *interval* variable. The level of hydration has been recorded as *E* or *D* under *Hydrat*, but we have also added a numeric code under *HydratN*, because SPSS will only recognize the numeric value for certain analyses. There are six possible rounds, with one to three occurring within *Bout* = 1 and four to six occurring within *Bout* = 2. The values of the variables *H*R* and *H*B* are calculated by multiplying *HydratN* by *Round* and *Bout* respectively, and their use is introduced for multiple regression in 9.1.6. The aim is to investigate whether the level of hydration affects performance over different rounds and bouts.

The data structure (5.1.2) is summarized in Table 6.3.

Table 6.3 Data structure for the variables in Fig 6.33

Variable/factor	Action	Type	Levels	Variability
Punches	Output	Frequency / interval	Continuous	Normal
Hydrat	Input	Nominal	*E* and *D*	Fixed
HydratN	Input	Coded	1 and 2	Fixed
Round	Input	Ordinal	1 to 6	Fixed
Bout	Input	Ordinal	1 and 2	Fixed
*H*R*	Input	Nominal	Derived, 1 to 12	Fixed
*H*B*	Input	Nominal	Derived, 1 to 4	Fixed

	A	B	C	D	E	F	G	H	I
1	*Rec*	*Temp*	*Quality*	*Method*		*Temp\Method*	A	B	C
2	1	10	2	A		10	2, 1, 2	2, 3 ,2	3, 2, 3
3	2	20	3	A		20	3, 3 ,2	3, 4, 2	4, 3, 5
4	3	30	2	A		30	2, 1, 1	3, 4, 4	4, 3, 3
5	4	10	2	B					
6	5	20	3	B		*Temp*	A	B	C
7	6	30	3	B		10	2	2	3
8	7	10	3	C		10	1	3	2
9	8	20	4	C		10	2	2	3
10	9	30	4	C		20	3	3	4
11	10	10	1	A		20	3	4	3
12	11	20	3	A		20	2	2	5
13	12	30	1	A		30	2	3	4
14	13	10	3	B		30	1	4	3
15	14	20	4	B		30	1	4	3
16	15	30	4	B					

Fig 6.34 Three formats for presenting *quality* as function of two factors *temp* and *method* (data in columns A, B, C, and D extend to further rows)

Case study: **Fingerprint quality / 1. Multifactorial analysis (overview)**

The data in Fig 6.34 records the assessed quality of fingerprints as *Quality* (on a 0 to 5 ordinal scale), obtained using three different lifting methods, *Method* (A, B, C) at three different temperatures, *Temp* (10, 20, 30). There are three replicate measurements at each of the nine possible combinations of factor levels, and the data is presented in three ways:

- *stacked* as in columns A, B, C, and D
- *unstacked* in cells F6:I15 (used for Friedman two-way ANOVA)
- replicate measurements *tabulated* in cells F1:I4.

The case study investigates different methods and conditions for collecting fingerprints for forensic analysis.
In this section:

6.4.3 Identify key behaviour patterns using clustered boxplots and interaction plots.

6.4.6 The main analysis uses nonparametric Friedman test and Kendall's coefficient of concordance to identify factor significance.

6.4.7 Uses the generalized linear model with a logit transformation for ordinal data.

5.1.5 / 2. Organizing data entry: We consider the transfer of data from lab book to software analysis.

The data structure (5.1.2) is summarized in Table 6.4.

Table 6.4 Data structure for the variables in Fig 6.34

Variable/factor	Action	Type	Levels	Variability
Quality	Output	Ordinal	0 to 5	Ordinal
Temp	Input	Interval	10, 20, 30	Fixed
Method	Input	Nominal	*A, B, C*	Fixed

The data would normally be entered into software analysis using the column format with the individual response values, *quality*, in column C, and *identified* by the relevant levels of the two factors, *temp* and *method*, in columns B and D respectively. Only the first 15 records are illustrated in the figure.

It is also possible to enter the data in an 'unstacked' format in F7:I15 with three samples representing the three *method* levels, *blocked* by the different temperatures. This is used for the Friedman nonparametric two-way ANOVA in 6.4.6 for a difference between methods, but it is also possible to test for a *temp* effect by re-entering and 'rotating' the data, to give between three columns for the three *temp* levels blocked by *method*.

6.4.2 **Analytical options**

The issues association with choosing whether to use *parametric* or *nonparametric* tests are developed in Section 5.4.

Describing data

Graphical plots: Clustered boxplots, Factor and interaction plots: 6.4.3

Tests / Measurements:

- Testing for **normality** and **homoscedasticity** (homogeneity of variance): 5.4 and 6.4.5

Is there a difference between the **mean/median** values due to factors?: GLM/ANOVA (normal data) (6.4.4), Friedman test (nonparametric) (6.4.6), GsdLM (6.4.7)

Is there an **interaction** between factors?:GLM/ANOVA (6.4.4 and 3.3.2)

Use **post hoc** tests to locate significant differences: 6.3.6, 3.2.4

Including **covariant** factors: 6.4.8

Repeated measures data:

Is there a difference between **repeated** values (within-subject)?: 3.6.2, 6.3.8

Is there a difference between the **mean** values for different levels of the factor (between-subject)?: 6.3.8

6.4.3 **Describing the data**

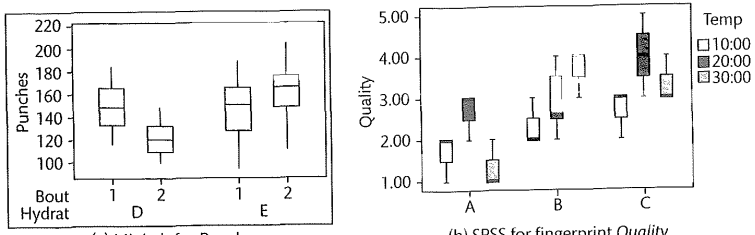
Graphical plots are particularly useful for multifactorial data as they can begin to show some of the structure that is not immediately obvious in tables of numbers. The boxplots in Fig 6.35 are quick and effective ways of displaying the *raw data* in your results, and are produced through the graph menu in both Minitab and SPSS, using

Minitab > Graph > Boxplot... > One Y, With Groups
Graph variables: *Punches* **Categorical variables:** *Hydrat Bout*
(entering the factors in the order in which the boxplots are to be grouped)

Factor plots (Minitab): Analysis leading to Figs 6.35(a), 6.36(b). Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Factor plots (SPSS): Analysis leading to Figs 6.35(b), 6.36(a). Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

SPSS > Graphs > Legacy dialogs ... > Boxplot... > Clustered
> Define: Variable: *Quality* **Category Axis:** *Method* **Define Clusters by:** *Temp*



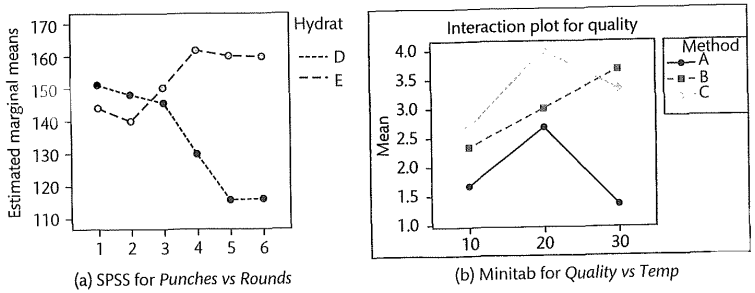
(a) Minitab for Punches

(b) SPSS for fingerprint *Quality*

Fig 6.35 Clustered boxplots in Minitab and SPSS

The clustered (grouped) boxplots in Fig 6.35(a) relate to the 'Boxing' case study in which the numbers of *Punches* are recorded for two factors, *Bout* (1 or 2), and *Hydrat* (E or D). The *Bout* boxplots can be grouped hierarchically within *Hydrat* as shown, or vice versa. The boxplots show no major differences between the different samples, except that it is useful to note that going from *Bout* 1 to 2 for dehydration, *D*, the number of punches falls, but going from *Bout* 1 to 2 for euhydration, *E*, the number rises. We will see that this is the effect of a significant *interaction* between bout and hydration.

The boxplots in Fig 6.35(b) relate to the 'Fingerprints' case study with *Quality* recorded for *Temp* and *Method*. The only clear variation is a general increase in quality from method *A* to *C*.



(a) SPSS for *Punches vs Rounds*

(b) Minitab for *Quality vs Temp*

Fig 6.36 Interaction plots in Minitab and SPSS

Interaction and factor plots, as in Fig 6.36, calculate the *mean values* of any replicate data values and plot these against the different combinations of factor levels. The plots use the GLM/ANOVA analyses to calculate the sample mean values, and are a useful *visualization* of the data variations *even for data that fails to meet the normality and homoscedasticity requirements* for the full ANOVA analysis.

In Minitab, the plots are available under the ANOVA submenu:

Minitab > Stat > ANOVA > Main Effects Plot... or Interactions Plot...

In SPSS, the plots are available under the Plot option within the GLM analysis

SPSS > Analyze > General Linear Model > Univariate ...> Plots...

Fig 6.36(a) is from the 'Boxing' case study and shows how the numbers of *Punches* varies for six rounds for different levels of *Hydrat*, D and E. Again we have evidence of an *interaction* in that the number of punches drops in the later rounds, but *only* for those subjects who are dehydrated.

Fig 6.36(b) relates to the Fingerprint case study and shows how the mean value of *Quality* changes with temperature for the three methods, *A*, *B*, and *C*. The main consistent characteristic is that, overall, method *C* has a greater average quality than *B* or *A*.

6.4.4 GLM/ANOVA

The ANOVA calculation (3.2, 3.3) can test simultaneously for the significance of multiple factors and their interactions, and is easily performed using the GLM (Section 3.4). The key data requirements for performing an ANOVA are normality and homoscedasticity (equal variance at all factor levels), and checks to confirm that the data meets these requirements are performed *within* the ANOVA group of analyses (5.4.5 and 6.4.5). These results can be reported as the 'quality of the model', but if the data fails to meet the requirements, it is necessary to move to nonparametric analyses.

With two or more factors, we may choose to test for the significance of *individual* factors (e.g. *Hydrat* and *Bout*) and also for a possible *interaction* between factors (*Hydrat*Bout*). The identification of interactions is developed in Section 3.3.2. However, we can only test for an interaction if there are *replicate* measurements, i.e. if there are at least two measurements made at each combination of factor levels.

Comparison (post hoc) tests (3.2.4 and 6.3.6) are requested within the analysis dialogue windows, and it is also possible to include the effect of *covariates* (6.4.8) on the analysis.

The data for Minitab and SPSS must be in the form of *unrelated* samples entered as a single column of univariate data (B in Fig 6.33 and C in Fig 6.34), but with the factor levels defined by the values in separate columns.

Multifactorial GLM/ANOVA (Minitab): Analysis leading to Fig 6.37. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Multifactorial GLM/ANOVA (SPSS): Analysis leading to Fig 6.37. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

SPSS

SPSS > Analyze > General Linear Model > Univariate...
Dependent variable: *Punches*
Fixed factor(s): *Subject Hydrat Bout*
Random factors(s): Include any random factors (3.4.4)
Covariate(s): Include any covariates (6.3.8)
> Model... *Subject Hydrat Bout Hydrat*Bout* (we identify just one of the possible interactions)
> Plots... Select any factor plots

> **Post Hoc...** Select any post hoc tests (3.2.4 and 6.3.6)
> **Options...** e.g. Homogeneity test and spread vs level plot

The results in Fig 6.37 show that there is a significant difference between subjects ($p < 0.0005$) as would be expected, with hydration a significant factor with *Hydrat* $p < 0.0005$ and the interaction between hydration and the bout also significant with *Hydrat*Bout* $p < 0.0005$. Although the p-value for the bout is greater than 0.05, we would still consider it to be a significant factor because of its involvement in the interaction term. The use of post hoc tests to identify the location of the differences between factor levels is introduced in 3.2.4 and demonstrated in 6.3.6.

Tests of Between-Subjects Effects

Dependent Variable: Punches

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	28228.944[a]	8	3528.618	11.421	.000
Intercept	1485226.125	1	1485226.125	4807.315	.000
Subject	12991.458	5	2598.292	8.410	.000
Hydrat	6068.347	1	6068.347	19.642	.000
Bout	654.014	1	654.014	2.117	.151
Hydrat * Bout	8515.125	1	8515.125	27.561	.000
Error	19463.931	63	308.951		
Total	1532919.000	72			
Corrected Total	47692.875	71			

a. R Squared = .592 (Adjusted R Squared = .540)

Fig 6.37 Two-way ANOVAs with interaction for the 'Boxing' case study data (SPSS)

The analysis in **Minitab** follows a very similar structure, with the interaction term explicitly defined as a model factor:

Minitab > Stat > ANOVA > General Linear Model > Fit General Linear Model...
Reponses: *Punches* **Factors:** *Subject, Hydrat, Bout*
> Model: Highlight *Hydrat* and *Bout* in **Terms in the model**
Cross predictors and terms in the model -Add
The terms in the model should now include *Subject, Hydrat, Bout, Hydrat*Bout*
→ Output: Standard ANOVA table with results as in Fig 6.37

The inclusion of the product *Hydrat*Bout* under Model requests that the ANOVA tests for an interaction between these factors, and the output gives the standard ANOVA table of results with the same values as in Fig 6.37. Post hoc test and factorial plots can be obtained by now using:

Minitab > Stat > ANOVA > General Linear Model > Comparisons...
Minitab > Stat > ANOVA > General Linear Model > Factorial plots...

Excel provides a two factor ANOVA test through the add-in 'Data Analysis'. The data for Excel must be held in a table of values with the two factors defining the rows and columns (as in F6 to I15 in Fig 6.34), *together* with the row and column labels.

Excel provides separate tests depending on replication:

Excel > Data > Data analysis > ANOVA: Two-Factor *with* Replication
(replicate values must be in successive *rows* as in G7:I15)
Input range: *F6:I15* (this range must include row and column labels)
Rows per sample: *3*
Output range: Select a new worksheet or a cell for the top left hand cell of data output area.
(Excel automatically tests for an interaction between the factors.)

Note that, if there are no replicates, it is not possible to test for an interaction:

Excel > Data > Data analysis > ANOVA: Two-Factor *without* Replication
Input range: Define the data range from top left cell to bottom right cell.
☑**-Labels:** - Check if the defined range includes the row and column labels.
Output range: Select top left hand cell of data output or new worksheet.

Excel provides the ANOVA output table in the standard format.

6.4.5 Checking for normality and homoscedasticity

The ANOVA calculation assumes that the random data variations are described by a normal distribution and with an equal variance for all measurement conditions. The consideration of these criteria is developed in more detail in Section 5.4, in which we see that the first step is to consider whether there is any evidence in the raw data to suggest that it does *not* comply with the requirements.

In respect of the 'Boxing' case study, the relevant variable is *Punches*, which is actually an *integer* variable, but, because the variation is considerably greater than just one unit and does not approach any limiting value (e.g. '0'), we can treat it as a *continuous* variable. We can also see from the boxplots in Fig 6.35(a) that the random variations due to different subjects are small compared to the median values, and we can conclude that the variations are likely to be *symmetrical* about mean values. In addition, the *spreads* of the boxplots are similar for all the experiment conditions so that we do not expect to see any significant differences in variance.

In respect of the 'Fingerprint' case study, we know immediately that the response data, *Quality*, is an *ordinal* variable, and, with just six possible levels limited by 0 and 5, we would not expect a parametric ANOVA calculation to give reliable estimations of significance (p-values).

The main approach to *testing* these criteria is from within the ANOVA analysis itself, by analysing the *residuals* between the best-fit model and the experimental data (5.4.5), either directly and/or by saving the individual residual values and testing them afterwards.
Within the **Minitab** dialogue we can include:

> Graphs ... It is useful to select ▼ *Standardized* for **Residuals for plots**
Useful plots are:
☑**-Normal plot of residuals** to show normality
☑**-Residuals versus fits** show equality of variance or
select ⦿**-Four in one** (Fig 5.15)
> Storage ... Use ☑**-Standardized residuals** to save residuals an empty column as *SRES1*

Normality and homoscedasticity (Minitab): Analysis of the 'Boxing' case study. See also 5.4.6. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Normality and homoscedasticity (SPSS): Analysis of the 'Boxing' case study. See also 5.4.6 and 6.3.4. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

The saved residuals can then be tested for normality:

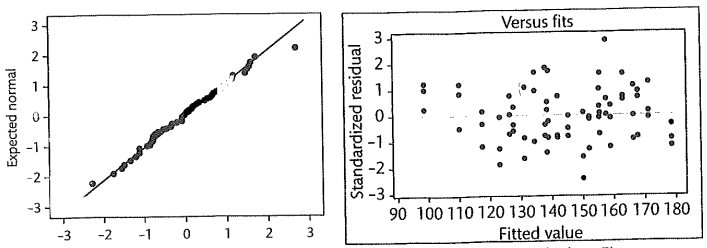
Minitab>Stat>Basic Statistics>Normality Test...
Variables: *SRES1*
Select a specific test, e.g. ◉**-Anderson-Darling**

Within the SPSS dialogue we can include:

> Save: ☑***-Standardized residuals,*** which saves the residuals in a new column with the Name *ZRE_1* and Label *Standardized Residual*
> Options: Display ☑**-Homogeneity tests**

The saved residuals can then be analysed using:

SPSS > Analyze > Descriptive Statistics > Explore...
Dependent List: *Standardized residual*
> Plots: ☑**-Normality plot with test**



(a) SPSS: Normality plot of saved residuals (b) Minitab: Residuals vs Fits

Fig 6.38 Testing for normality and homoscedasticity in the 'Boxing' case study

Examples of the results for the 'Boxing' case study are given in Fig 6.38. The Q–Q plot (5.4.5) of residuals in Fig 6.38(a) shows the *residuals* fitting closely to the diagonal normality line, and the normality tests give Shapiro–Wilk, $p = 0.941$ (SPSS) and Anderson–Darling, $p = 0.214$ (Minitab), both confirming that these is no significant deviation from normality.

The plot of residuals 'Versus Fits' in Fig 6.38(b) shows the spread of experimental data about the calculated values (Fits), and there does not appear to be any significant change in the spread (variance) for the different results along the x-axis.

Minitab also provides a separate test for the homogeneity of variance requirement:

Minitab > Stat > ANOVA > Test for Equal Variances...
▼ ***Response data are in a column for all factor levels***
Response: *Punches* **Factors:** *Subject Hydrat Bout*

and gives the following results:

Levene's test (for any continuous distribution), $p = 0.999$

or by choosing the normal distribution under **Options...**

Bartlett's test (assuming a normal distribution), $p = 0.974$

both of which confirm that we can assume homoscedasticity.

6.4.6 Nonparametric ANOVAs

The Friedman test is the *nonparametric* equivalent of the two-way ANOVA, testing for a difference in the median values of one factor while *blocking* the other factor. For the data in Fig 6.34 in cells G7:I15, it tests for a significant difference between the ranked values of methods *A*, *B*, and *C*, by treating each row as measurements made under the *same* conditions of the factor, *Temp*. The test provides no information about any significance in the row factor, and, if we wish to test for the significance of *Temp*, we would need to *transpose* the rows and columns such that there was a column of data for each value of *Temp*.

The statistics of Kendall's coefficient of concordance are introduced in 4.4.3 where it is developed to test for agreement between samples. It provides a similar analysis to the Friedman test in that it tests for a difference between the column values while blocking the rows.

We also see in the next section that the GsdLM can be used to perform the same analysis using a logit transformation for the ordinal data.

Nonparametric ANOVA (Minitab): Analysis leading to Fig 6.39(b). Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Nonparametric ANOVA (SPSS): Analysis leading to Fig 6.39(a). See also 6.1.6. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

SPSS

SPSS > Analyze > Nonparametric Tests > Related Samples...
Fields: *ABC*
Settings: ◉**-Customize tests**
☑**-Kendall's coefficient of concordance (*k* samples)**
☑**-Friedman's 2-way ANOVA by ranks (*k* samples)**

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of A, B and C are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.014	Reject the null hypothesis.
2	The distributions of A, B and C are the same.	Related-Samples Kendall's Coefficient of Concordance	.014	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

(a) SPSS summary

```
Kendall's Coefficient of Concordance

For Method being blocked by Temp:
    Coef   Chi - Sq  DF       P
0.476703   8.58065   2  0.0137

For Temp being blocked by Method:
    Coef   Chi - Sq  DF       P
0.456790   8.22222   2  0.0164
```

(b) Minitab

Fig 6.39 Nonparametric two-way ANOVAs

In these analyses, each *row* of data is blocked separately, effectively giving nine levels to the factor *Temp*, and does not take into account the *replicates* at each level. Fig 6.39 (a) gives the

same p-value = 0.014 for both tests which identifies a difference between the distribution of values in *A*, *B*, and *C*, showing a significant effect for *Method*. To identify where this difference lies it is possible to view the plot in Fig 6.35(b), but it would also be possible to perform paired Wilcoxon tests separately between pairs of *A*, *B*, and *C*, using a Bonferroni correction for their significance (1.6.4).

In Minitab, the **Friedman** test requires that the data is presented as *univariate* data with the test factor identified as the Treatment and the other factor the Block. However, Minitab requires that there are *no replicate* Block (i.e. *Temp*) values, and cannot directly analyse the data in Fig 6.34. This can be overcome by replacing *Temp* with a *dummy* variable with values from 0 to 9 in column F, in which case the test gives the same p-value, $p = 0.014$.

Minitab

Minitab > Stat > Nonparametrics > Friedman...
Response: Enter column holding data
Treatment: Enter column with factor being tested
Blocks: Enter column with factor to be blocked

To perform the **Kendall's concordance** test, Minitab will accept the data either in univariate form or as related data in the same format as SPSS. Using the univariate input:

Minitab

Minitab > Stat > Quality Tools > Attribute Agreement Analysis...
Attribute: *Quality* (response variable)
Samples: *Method* (factor being *tested*)
Appraisers: *Temp* (factor being *blocked*)
Known standard/attribute: Include any 'correct answer' data if known
☑**-Categories...** ordered (check when response variable is *ordinal*)

The result in Fig 6.39(b) again gives $p = 0.014$ for the significance of a *Method* effect, and, by swapping round the dialogue entries for *Samples* and *Appraisers*, we see that the effect of Temp is also significant with $p = 0.016$.

6.4.7 Generalized linear model

We could not reliably use a GLM/ANOVA for the 'Fingerprint' case study because the response data was *ordinal*, and we used the nonparametric analyses to test the two factors separately. However, it is possible to use the GsdLM (3.4.7), which performs a single hypothesis test that analyses both factors together by transforming the *ordinal* data values using a cumulative logit function (8.3.3).

Generalized linear model (ordinal logistic): SPSS analysis leading to Fig 6.40. See also 3.4.7. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

SPSS

SPSS > Analyze > Generalized Linear Models> Generalized Linear Model...
> Type of model: ◉**-Ordinal logistic**
>Response: Dependent variable: *Quality*

> Predictors: Factors: *Temp Method*
> Model: *Temp Method*

The results in Fig 6.40 show that both factors are significant, which is consistent with the conclusions from Fig 6.39.

Tests of Model Effects

Source	Type III Wald Chi-Square	df	Sig.
Temp	7.136	2	.028
Method	11.202	2	.004

Dependent Variable: Quality
Model: (Threshold), Temp, Method

Fig 6.40 SPSS: Output from the GsdLM

6.4.8 Analysis of covariance, ANCOVA

ANCOVA 2: SPSS analysis leading to Figs 6.41 and 6.42. See also 3.3.3. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

The typical analysis in an ANOVA has factors which have a limited number of specific levels, e.g. *bout, method*, but we now consider the additional effect of a variable with a continuous range of possible values.

Case study: Ink analysis / 6. ANCOVA analysis 2

—continued from 3.3.3

The graph in Fig 5.6 gives the percentage transmissions, *%T*, for three different *inks*, *A*, *B*, and C, which could be considered as a one factor problem. However, the *%T* value is also affected linearly by the *wavelength* of the measurement, which is then called a *covariate*. The modification of the simple ANOVA to accommodate this covariate factor is called an ANCOVA.

The calculation using this data is carried out in 3.3.3 using Excel and Minitab, in which it is assumed that the *%T* value varies linearly with the *wavelength*, and the first step is to perform a linear regression to measure the relationship (i.e. slope) between the two variables, *%T* and *wavelength*. Once the best-fit relationship is known, a customized 'correction' can be applied to each value of *%T* to compensate for the wavelength effect, and then it is possible to treat the problem as a simple ANOVA analysis.
The analysis using SPSS is as follows:

SPSS > Analyze > General Linear Model > Univariate...
Dependent variable: *%T*
Fixed factor(s): *Ink*
Covariate(s): *Wavelength*
> Options...
Display Means for: *Ink*

☑-Compare main effects
Confidence interval adjustments: ▼ Bonferroni
☑-Parameter estimate

The results for the between-subjects effects give $p = 0.000$ for *wavelength*, confirming that it is indeed a significant covariate. The values for the coefficients, B, in the parameter estimates shown in Fig 6.41 give the linear relationship between them (with the inks coded with integer values) as:

$$\%T = 0.875 \times \text{Wavelength} - 569$$

which is consistent with the calculations in 3.3.3 using data for inks A and B.

Parameter Estimates

Dependent Variable: T

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	-569.422	16.126	-35.311	.000	-603.061	-535.784
Wavelength	.875	.023	38.328	.000	.827	.922
[Ink=A]	6.039	.426	14.189	.000	5.151	6.927
[Ink=B]	.894	.426	2.100	.049	.006	1.782
[Ink=C]	0[a]	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

Fig 6.41 Parameter estimates in ANCOVA results (SPSS)

The between-subjects effects also give $p = 0.000$ for *ink*, which detects a significant difference between at least two inks. The post hoc test for this difference is conducted by using the Bonferroni comparison of the main effect, giving the results in Fig 6.42, which detects a significant difference between *A* and both *B* and *C*, but no significant difference ($p = 0.146$) between *B* and *C*. This is consistent with the graphs as presented in Fig 5.6.

Pairwise Comparisons

Dependent Variable: T

(I) Ink	(J) Ink	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
A	B	5.145*	.426	.000	4.033	6.257
	C	6.039*	.426	.000	4.927	7.151
B	A	-5.145*	.426	.000	-6.257	-4.033
	C	.894	.426	.146	-.218	2.006
C	A	-6.039*	.426	.000	-7.151	-4.927
	B	-.894	.426	.146	-2.006	.218

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Fig 6.42 Bonferroni comparison of *inks* (SPSS)

Related variables

Introduction

This chapter considers the analysis of *related* data samples, in which there are unique links between pairs of values in each sample. The content builds on Section 4.4 to measure the *agreement* between two different assessments that are measuring the *same* quantity, and refers to Section 3.6 for successive measurements of the *same* variable, possibly 'before' and 'after' an intervention, leading to a *paired* analysis for two samples or *repeated measures* for multiple samples. However, a main focus of the chapter is to build on Sections 2.1 and 2.3 to develop the analysis of interrelated samples that measure *different* quantities, often described by an x–y graph, e.g. in an absorbance vs concentration calibration graph. It also develops convolution and spectral analysis techniques to address more complex exploratory x–y data and time series data.

Section 7.1 develops the basic parametric and nonparametric methods with which we can analyse the *regression*, *correlation*, and *agreement* between two variables.

Section 7.2 considers the methods that are available to analyse data that is expected to have a specific *nonlinear* relationship.

Section 7.3 introduces some less common techniques for handling *general x–y data* whose behaviour is not described by a simple mathematical function.

7.1 Regression, correlation, and agreement

A correlation analysis measures the extent to which a change in one variable is related to a change in the other. It can test whether the observed relationship could have occurred by chance, but it can also measure the *strength* of the relationship. Linear correlation measures the extent to which the change in one variable is *proportional* to a change in the other, but nonparametric correlation just measures the extent to which one variable increases (or decreases) *in step with* the other. For two variables that are correlated, a *regression* analysis calculates the values that *quantify* the relationship (e.g. slope and intercept) between a response variable and a predictor variable.

Chapter 2 developed the *statistics* associated with linear correlation and regression. The analyses can be performed when the variables are recording *different* scientific quantities, e.g. between the values of absorbance and concentration with different units of measurement. However, we can also look for *agreement* between variables when they have the *same units*, e.g. when comparing the proportions of cell deaths recorded by different assays. Chapter 4 introduced the techniques associated with the tests and measurements of nonparametric correlation, association, and agreement.

7.1.1 Example data

The most common form of *related* data is *x–y* data that is expected to produce a *straight line*. The theory and practice for this is introduced in Chapter 2, and you are referred to the case studies:

Best-fit straight line (2. Introduction)

which develops the statistics of linear regression and its use in analysing scientific data.

The assessment of agreement within *categorical* or *binary* data is introduced in 7.1.6 and analysed primarily using *crosstabulation* and *contingency tables* (8.2.4). Relevant case studies are:

Association (8.2.1)

Forensic questionnaire (8.2.1)

We also meet *related* data in *paired* and *repeated measures* analyses in the case studies:

River *pH* (3.6.1)

Ink analysis (3.6.2)

The following two case studies give examples in which the related variables are measuring *different* quantities (i.e. absorbance and concentration) and in which they are 'repeated' measurements of the *same* quantity (i.e. mortality).

Case study: **Spectrophotometer calibration / 1. Calibration (overview)**

In Fig 7.1(a) the data in columns A and B provide an example of interrelated *interval* data. A spectrophotometer typically analyses the concentration, *Conc*, of a chemical sample by recording the absorbance, *Abs*, of light passing through the sample. It is initially *calibrated* by measuring absorbance values for the known concentrations of prepared standards, as shown in Fig 7.1(b). In 7.1.3 and 7.1.4 we assess the linearity of the calibration line using residual plots and correlation measurements.

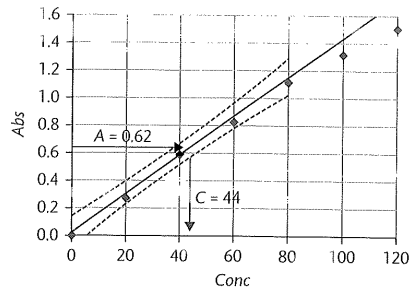
7.1.5 / 2. Measuring an unknown solution. The absorbance of an unknown solution is measured as 0.62, and, using the calibration line,we calculate the confidence interval, 44 ± 3, for the concentration of the unknown solution.

2.1.5 / 3. Linearity range. The linearity of calibration is analysed using residuals and correlation coefficients.

2.2.1 / 4. Calibration result. Calculation of the confidence interval of an unknown solution using Excel.

	A	B
1	*Conc, C*	*Abs, A*
2	mg/L	
3	0	0.00
4	20	0.27
5	40	0.58
6	60	0.82
7	80	1.12
8	100	1.31
9	120	1.50

(a) Calibration data



(b) *x-y* Scatterplot

Fig 7.1 Spectrophotometer calibration

Case study: **Toxicity assays / 1. Comparative assays (overview)**

Two types of assay, *A* and *B*, have been used to measure the percentage of cell deaths (mortality), Fig 7.2, due to exposure to different concentrations, C mM, (also entered as its logarithm, log(C)) of an antibacterial agent. Each assay was tested twice, and we test for a nonparametric *correlation* between pairs of *A1*, *B1*, and log(C), etc. and we can look for numerical *agreement* between *A* and *B*.

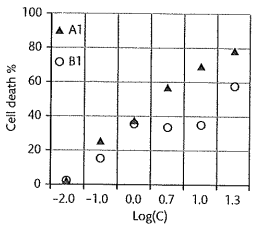
4.1.1 / 2. Correlation. Develops measures of *correlation* between pairs of variables.

4.4.2 / 3. Agreement. Develops measures of *agreement* between pairs of variables.

4.4.3 / 4. Multiple comparisons. Measures agreement and correlation between *more than two* variables.

	A	B	C	D	E	F
1	**Conc**	**Log(C)**	**A1**	**A2**	**B1**	**B2**
2	0	-2.0	2.0	3.5	2.0	4.0
3	0.1	-1.0	25.5	18.5	15.0	16.0
4	1	0.0	37.5	40.0	36.0	50.5
5	5	0.7	57.0	62.0	33.0	54.5
6	10	1.0	69.5	55.0	35.0	65.0
7	20	1.3	78.7	62.5	57.5	75.5

(a) Experimental data



(b) Line graph
(not for interval *x-y* data)

Fig 7.2 Comparison of assay results

7.1.2 Analytical options

We list below *some* of the most common analyses used for related data, together with links for further information. The statistics of linear regression and correlation are developed in depth in Chapter 2, and Chapter 4 develops the association and agreement between variables.

Describing data

- Graphical plots: x–y scatterplots (Note that the 'line' plot in Excel uses a *categorical* x-axis and is not suitable for x–y data), residuals, and Bland–Altman plots (7.1.3).
- Numerical statistics: Slope and intercept of straight line. Correlation coefficients.

Tests / measurements:

- **Nonparametric correlation** to test whether one variable increases *in step with* the other: Spearman's rho and Kendall's tau-b (4.1.2)
- **Linear correlation** to test whether there is a *linear* relationship between the variables: Pearson's correlation coefficient (2.1.3, 4.1.1).
- **Straight line analysis**: See Chapter 2 for linear regression analysis of x–y data and use of Excel, slope and intercept (2.1.1), errors and uncertainty (Section 2.2).
- **Calibration** calculations based on a straight line: Calculations of an unknown x-value (2.2.1), exact x/y intercepts (including standard additions) (2.2.2)
- **Nonlinear** regression: Linearization techniques (Section 2.3), nonlinear regression (2.4.3, Section 7.2).
- **Binary** regression: See 8.3.4.
- **Agreement** and **reproducibility**: Section 4.4 develops relevant methods including Bland–Altman plot for interval data, Kappa for ordinal data, t-test and Wilcoxon test, and for more than two variables, Kendall's coefficient of concordance is a nonparametric measure of correlation and Friedman's test measures rank differences.
- **Association** between categorical variables: Crosstabulation and chi-squared (8.2.4).

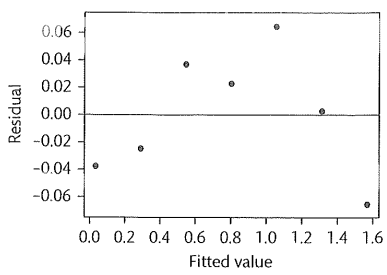
7.1.3 Describing the data

The most direct method of presenting the relationship between two numeric values is with an 'x–y scatterplot', together with a best-fit trendline and error bars if required. The example in Fig 7.1(b) uses the *Abs/Conc* data, but the trendline shown is based only on the first five points (as explained in 7.1.5 below).

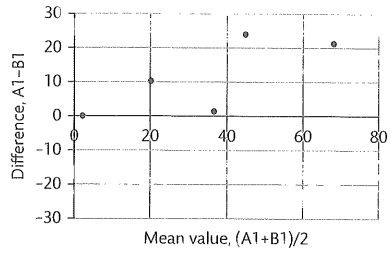
The 'line' graph in Excel is *not* generally appropriate for x–y data, because the x-axis is for *categorical* data. For example, Fig 7.2(b) uses a 'line' graph of $A1$, with each point 'labelled' by the value of log(C), but it is clear that the x-axis values are not *proportional* to log(C).

Very often, the important information can be hidden in a simple x–y plot, and Fig 7.3 illustrates two ways in which slight, but important, differences can be highlighted.

Fig 7.3(a) is a plot of *residuals* for the data in Fig 7.1(a), showing the differences between each data point and the best-fit straight line. This plot shows a deviation of the two upper points, away from the direction of the lower portion of the graph, and in the calibration calculations below we decide to use only the lower 'linear' five data points.



(a) Residual values for data in Fig 7.1(a)



(b) Bland–Altman plot for data in Fig 7.2(a)

Fig 7.3 Differentiated values

Fig 7.3(b) is a Bland–Altman plot that gives the *differences* between the values of $A1$ and $B1$ plotted as a function of their average value, in which it can be seen that $A1$ is generally scoring higher than $B1$.

7.1.4 Correlation

The parametric statistics of correlation between two interval variables (bivariate) are developed in 2.1.3 and 4.1.1, with the statistics of nonparametric correlation being developed in 4.1.2. The basic calculations can be accessed in SPSS and Minitab through:

SPSS > Analyze > Correlate > Bivariate...
Variables: y x
☑ Pearson ☑ Kendall's tau-b ☑ Spearman's

Minitab > Stat > Basic Statistics > Correlation...
Variables: y x
☑ Display p-values

SPSS provides the correlation coefficients and p-values for Pearson's linear correlation coefficient, r, and also for the nonparametric values of Kendall's tau, τ, and Spearman's rho, ρ. Minitab 16 gives the Pearson's r results directly, and Spearman's rho can be calculated using:

Minitab > Stat > Tables > Cross Tabulation and Chi-Square...
For rows: y **For columns:** x
> Other Stats...: ☑ Correlation coefficients for ordinal categories

In Excel it is possible to use the functions CORREL(), PEARSON(), or 'Correlation' in Data Analysis Tools to calculate the linear correlation coefficient. The derivation of the p-value in Excel is given in 2.1.3.

Table 7.1 gives results for some of the pairs of variables in Figs 7.1 and 7.2. The calculated correlation coefficients, r, measure the *strength* of the correlation, and the p-values give the hypothesis test significances for the *presence* (or not) of correlation.

Table 7.1 Selected correlation statistics for data from Figs 7.1 and 7.2

	Pearson's *r*		Spearman's ρ		Kendall's τ–b	
Data pair	*r*	*p*	ρ	*p*	τ	*p*
Abs/Conc	0.997	0.000	1.000	0.000	1.000	0.000
A1/A2	0.954	0.003	0.943	0.005	0.867	0.015
A1/B1	0.918	0.010	0.829	0.042	0.733	0.039

Note that the rounding of values implies that 1.000 is '≥ 0.9995' and 0.000 is '<0.0005'.

For the calibration data, *Abs/Conc*, the *p*-value is not relevant because it is already known that the variables are strongly correlated, but the *strength* of the linear correlation, $r = 0.997$, is an important measure for the quality of the *calibration* line (see 7.1.5). The values for *A1/A2* arise from analyses using the same method and provide a measure of the *reproducibility* of assay *A*, and those for *A1/B1* provide a measure of the *agreement* between the different assays *A* and *B*.

The correlation between two variables may be dependent on their joint correlation with a third variable, and it is possible to take this into account by calculating *partial* correlation coefficients. This analysis is developed in 4.1.4.

7.1.5 Linear regression and calibration

The following case study uses a straight line calibration to calculate the confidence interval of the concentration of an unknown sample.

Calibration uncertainty 2: Minitab and SPSS analysis. See also 2.2.1. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: Spectrophotometer calibration / 2. Measuring an unknown sample

—continued from 7.1.1, leading to 2.1.5

We use the calibration data that is given in columns A and B of Fig 7.1 to calculate the concentration of an unknown solution, based on the analysis developed in 2.2.1. Three replicate measurements of the unknown solution give an average absorbance of 0.62.

The basic regression calculations in Minitab and SPSS are accessed through:

Minitab > Stat > Regression > Regression > Fit Regression Model...
Responses: *Abs*
Continuous predictors: *Conc*
> Graphs... ☑ Residuals versus fits
→ Output: Fig 7.3(a) and Fig 7.4

SPSS > Analyze > Regression > Linear...
Dependent: *Abs*
Independent(s): *Conc*
→ Output: Gives the same values as in Fig 7.4


```
Abs = - 0.0006 + 0.0140 Conc

Predictor       Coef    SE Coef       T      P
Constant    -0.00060    0.01375   -0.04  0.968
Conc       0.0139850  0.0002808   49.81  0.000
S = 0.0177567   R-Sq = 99.9%    R-Sq(adj) = 99.8%

Analysis of Variance
Source          DF       SS       MS        F      P
Regression       1  0.78232  0.78232  2481.20  0.000
Residual Error   3  0.00095  0.00032
Total            4  0.78327
```

Fig 7.4 Linear regression output values in Minitab or SPSS

It is useful to look at the printout of the residuals, Fig 7.3(a), as this give a good indication of the *linearity* of the calibration line, and in this example we see a straight line portion up to a concentration of about 80 mg/L and then a distinct curvature. In addition, for a good calibration line we would expect values of linear correlation, *r*, greater than 0.999, but for *Abs/Conc* in Table 7.1 the correlation coefficient $r = 0.997$. However, if we only use the values up to *Conc* = 80, we now find good linear correlation with $r = 0.999$—see also the analysis in 2.1.5.

As it appears that the calibration data is curved above *Conc* = 80, we decide to use only the first five calibration values and, repeating the regression calculation, we obtain the results in Fig 7.4, which gives the slope, $m = 0.0140$, and intercept, $c = -0.0006$, of the best-fit straight line. If we were confident that the zero adjustment of the instrument was exactly set so that 0.0 absorbance corresponded to 0.0 concentration, then we could *force* the best-fit line through the origin giving zero intercept, *c*.

The confidence deviations, *Cd*, of slope and intercept can be calculated by multiplying the standard error (SE Coef in Fig 7.4) by the relevant *t*-value (3.18) for $n-2$ degrees of freedom (where $n = 5$ is the number of data pairs in this example). This then gives the confidence interval (Eqn 1.23) for the slope.

$$m = 0.0140 \pm 0.00028 \times 3.18 = 0.0140 \pm 0.0009$$

The relevance of the R^2 and R^2 (adj) values (99.9% and 99.8%) as measures of the 'quality of fit' are explained in 2.1.3 and 4.4.1.

The unknown solution has an absorbance, *Abs* = 0.62, which allows us to calculate the unknown concentration by rearranging Eqn 2.1:

$$Conc = (0.62 - (-0.0006)) / 0.0140 = 44.3$$

We can calculate the confidence interval for this result by using the Excel method developed in 2.2.1. However, the measured absorbance is close to the *middle* of the calibration range and it is possible to use the approximate method. The first step is to calculate the standard error of regression, SE_{REG} from the sum of squares of the residuals from Fig 7.4, using Eqn 2.14:

$$SE_{REG} = \sqrt{\frac{SS_{RESID}}{n-2}} = \sqrt{\frac{0.00095}{5-2}} = 0.0178$$

Then, using Eqn 2.19, we calculate the standard uncertainty, u_x, in the x-interpolated value (where k is the number of replicates in the measurement of the unknown value):

$$u_x \approx \frac{SE_{REG}}{m} \times \sqrt{\left\{\frac{1}{k}+\frac{1}{n}\right\}} = \frac{0.0178}{0.0140} \times \sqrt{\frac{1}{3}+\frac{1}{5}} = 0.928$$

The confidence deviation is then calculated using Eqn 2.21 with a t-value calculated for $n-2$ degrees of freedom:

$$Cd = 0.928 \times 3.18 = 2.95$$

This then gives the confidence interval for the unknown concentration (to 1 dp) as:

$$Conc = 44.3 \pm 3.0 \text{ (95\% CI)}$$

Note that if the unknown absorbance value was towards the ends of the calibration range, or beyond, then the uncertainty would increase and the full uncertainty calculation in Excel (2.2.1) would be required.

7.1.6 Agreement between results

It is not appropriate to talk about agreement between the values of absorbance and concentration in Fig 7.1, because they are two different *quantities*. However, it is relevant to consider the agreement between the assays' results of Fig 7.2 because they are aiming to record the *same values*.

The analysis of the data in Fig 7.2 is developed in detail in 4.4.1, 4.4.2, and 4.4.3, together with the underlying statistics. In overview, for the assessment of the agreement between two variables, we can use:

- tests for *correlation* which should show strong significance and a *low* p-value.
- linear regression and correlation between the variables to assess whether the change in one is *proportional to* or *equal to* the change in the other. A slope of $m = 1.00$ in regression indicates that the *changes* are *equal* to each other.
- a *paired* t-test or Wilcoxon test to test for a zero *overall* difference between values, which should give a *high* p-value.
- Bland–Altman plot to display the *differences* on a scatterplot as a function of the *averages*, which should give near zero differences across all values.

For the two assays *A1* and *B1* in Fig 7.2, Spearman's correlation gives $p = 0.042$ which shows that there is correlation between the assays, but the slope of linear regression, $m = 1.48$, (Table 4.5) shows a difference in the value response, which is confirmed by the Wilcoxon test with $p = 0.043$ indicating a significant overall difference between the assays. These results are also consistent with the Bland–Altman plot in Fig 7.3(b)which shows that the assays are *correlated*, but that they do not give the same *values*, with *A1* giving increasingly higher values than *B1*.

For comparison between *more than two* variables, we see the use of Kendall's Coefficient of Concordance (4.4.3) in combining the multiple correlations between variables to produce an overall statistical value. Friedman's repeated measures analysis also provides a complimentary analysis for the difference in values between multiple variables.

McNemar's test and Cochran's Q (4.4.5) can be used to test for the agreement between two or more samples of binary data.

7.2 Nonlinear relationships

In this section, we consider the analysis of x–y relationships that are expected to follow mathematical relationships other than that of the simple straight line (7.1). The following section (7.3) deals with more *general* experimental x–y data that cannot be modelled directly using these methods, including periodic data.

7.2.1 Example data

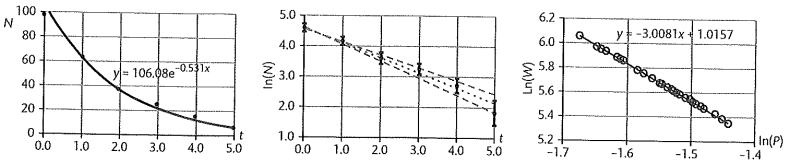
Different scientific mechanisms lead to data defined by a wide variety of mathematical models:

- Exponential relationships (2.3.3), e.g. radioactive decay and the growth and decay of bacterial populations.
- Power and reciprocal relationships (2.3.1, 2.3.2), e.g. thermal emission of radiation and the gas laws.
- Complex relationships (2.3.6), e.g. the Michaelis–Menten and Arrhenius equations.

Fig 7.5 illustrates different approaches to nonlinear data which we develop through the case studies:

Exponential decay (7.2.3) which uses radioactive decay as an example for the different methods of analysis that are available to describe any form of exponential growth or decay.

Rowing (7.2.4) which determines the (mathematical) power relationship between the power, W watts, and pace, P seconds per metre, of competitive rowers.



(a) Exponential decay with exponential trendline

(b) Linearized exponential decay with linear trendline

(c) ln(*Power*) vs ln(*Pace*) in 'Rowing' case study

Fig 7.5 Graphical examples of nonlinear relationships

7.2.2 Analytical options

Describing data

- Graphical plots: x–y scatterplots using nonlinear trendlines (e.g. Fig 7.5(a)). A 'line' graph can be used to plot *interval* y-data against *categorical* x-data (e.g. 7.2(b)). Convolutes can be used to produce localized 'best-fit' lines to nonlinear data (7.3.5 and 7.3.6).

Tests/Measurements:

- **Transform to a linear relationship**

  The basic techniques of linearization should be reviewed first by reference to Section 2.3, giving the main options for transforming one or both variables such that a linear relationship can be obtained. The mathematics of linear regression is then used to analyse the relationship.

- **Fit to a mathematical model**

  Section 2.4 develops an iterative approach to nonlinear regression using the Excel add-in 'Solver', which allows the use of different data distributions (e.g. Poisson) and optimization criteria (e.g. maximum likelihood estimation). The 'Exponential decay' case study in 7.2.3 demonstrates this analysis performed for nonlinear regression by Minitab and SPSS, and in 3.4.7 it demonstrates the use of the GsdLMmodel.

- **Derive the mathematical model**

  The experimental data may be used to derive the model itself, as a way of understanding the science of the relationship (7.2.4, 7.2.5).

An important point to check when using a nonlinear analysis is that the criteria for the analysis are still met, i.e. the standard process of linear regression assumes that the uncertainties in the data show a normal distribution and that the variances are the same throughout the data set. We use the 'Exponential decay' case study to explore these issues, with the use of *weighting* in 2.2.4 for linearized data, and the iterative process of Solver in 2.4.3 for handling different underlying statistical distributions.

Nonlinear regression (Minitab): Analysis for Fig 7.6(a). See also 2.4.3. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

7.2.3 Iterative nonlinear regression

The common approach to nonlinear data is to use one of the linearization techniques developed in Section 2.3. These transform the data into a linear relationship in which it is possible to use the statistics of linear regression to *calculate* the constants (slope and intercept) of the best-fit straight line. In this section, we aim to fit a *nonlinear* mathematical model to the data. However it is not always possible to calculate the coefficients of a complex model directly, and it is sometimes necessary to use a process of *iteration*, which works through repetitive cycles of 'trial and error'.

In 2.4.3 we use the Excel add-in 'Solver' to introduce the *iteration* process for an exponential decay, and in this section we demonstrate the use of Minitab and SPSS in using iteration to perform a nonlinear regression, which

1. assumes that the best-fit line can be described by a specific mathematical relationship, e.g. an exponential, $y = Ae^{Bx}$,
2. starts with a reasonable *guess* of the values of the constants, i.e. A and B, that describe that relationship, then
3. measures the*sum of squares of the residuals*, SS_{RES}, between the values predicted by the model and the experimental values, and then
4. adjusts the values of the constants, A and B, to minimize the value of SS_{RES}.

Nonlinear regression (SPSS): Analysis for Fig 7.6(b). See also 2.4.3. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

There are some limitations with iterative processes, particularly with more complex systems.

For example, when trying to find the *minimum* value in a test statistic, the iteration might get stuck with different parameter values that give a *local* minimum value. Sometimes this problem can be overcome by starting the iteration with different initial values.

The radioactive decay calculation below is used as an example representative of the many forms of exponential growth and decay.

Case study: Exponential decay / 6. Nonlinear regression using Minitab and SPSS

–continued from 2.3.4, 2.4.3, and 3.4.7

Nuclear radiation occurs when atomic nuclei decay from one state to another. Each nucleus has the same probability of decay at any time, with the result that the intensity of radiation decreases as the number of original nuclei falls. The rate of decay is defined by the time it takes for half of the atoms to decay–the half-life, $T_{1/2}$ (2.3.3).

Fig 7.5(a) plots the exponential decay of the radioactive count, N, as a function of time, t, using the data from Fig 2.20.

Using the data in Fig 2.20, it is necessary, in both Minitab and SPSS, to define the mathematical relationship, either by entering the equation directly or by using the menu options to select from example relationships.

Minitab > Stat > Regression > Nonlinear Regression...
Response: *N*
There are *three* options for entering the relationship to be fitted to the data:
> Use Catalog...: Enables the selection of one of a menu of standard relationships.
> Use Calculator...: Enables the use of functions to create the relationship.
or **Edit directly:** We can use this to enter: A*exp(B*t)
It is necessary to enter *starting* values for the constants.
> Parameters...: Enter guesses for starting values of A and B for the iteration,
e.g. A = 50, B= -0.5 (if you have no idea, try just zero values!)
→ Output: Fig 7.6(a)

The process for SPSS is very similar:

SPSS > Analyze > Regression > Nonlinear...
Dependent: *N*
Model Expression: A*EXP(B*t)–select functions and operators to help.
> Parameters...: It is necessary to enter *starting* values for the constants
e.g. A(50), B(−0.5)(as for Minitab)
→ Output: Fig 7.6(b)

```
Starting Values for Parameters
Parameter  Value
A             50
B           -0.5

Equation
N = 97.8601 * exp(-0.476281 * t)

Parameter Estimates
Parameter  Estimate  SE Estimate
A           97.8601      2.11541
B           -0.4763      0.01916

Summary
Iterations        6
Final SSE   20.6051
DFE               4
MSE         5.15127
```

(a) Output from Minitab

Iteration history[b]

Iteration number[a]	Residual sum of squares	Parameter	
		A	B
1.0	3849.550	50.000	-.500
1.1	34.589	97.808	-.451
2.0	34.589	97.808	-.451
2.1	20.609	97.884	-.476
3.0	20.609	97.884	-.476
3.1	20.605	97.861	-.476
4.0	20.605	97.861	-.476
4.1	20.605	97.860	-.476
5.0	20.605	97.860	-.476
5.1	20.605	97.860	-.476

Derivatives are calculated numerically.

a. Major iteration number is displayed to the left of the decimal, and minor iteration number is to the right of the decimal.

b. Run stopped after 10 model evaluations and 5 derivative evaluations because the relative reduction between successive residual sums of squares is at most SSCON = 1.000E-008.

(b) Output from SPSS

Fig 7.6 Nonlinear regression using Minitab and SPSS

The result from Minitab using six iterations and SPSS using ten model evaluations give the same derived equation:

$$N' = 97.86 \times e^{-0.476}$$

This agrees with the values calculated by Solver in 2.4.3. Fig 7.6(b) shows the iteration process for SPSS from the starting values of $No = 50$ and $k = -0.5$ to the final values of $No = 97.86$ (3 sf) and $k = -0.476$, when the iteration stops because successive iterations make no significant reduction on the residual sums of squares. There are only a few steps in this particular analysis because the starting value for k was chosen quite close to the true value, but the number of steps would obviously increase the further the iteration has to travel.

7.2.4 Deriving the mathematical model

The following case study now demonstrates the use of nonlinear regression to calculate the unknown *power* of a relationship between variables (2.3.5).

Deriving the model: Excel analysis for Fig 7.7. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Case study: Rowing performance/power and pace

Ten students, all with some years rowing experience, took part in an investigation into the effect of four different 'warm up' periods on their performance over a subsequent 2.0 km timed trial using rowing machines. The rowing machine for each student was calibrated using an initial trial to adjust the drag factor, in order to compensate for differences between students. Fig 7.7 gives a selection of results showing average power values, *W* watts, recorded as a function of the time, *t* seconds, taken to complete the 2.0 km course. The aim of this part of the analysis is to test the validity of the following relationship (assuming that it is independent of any warm up period):

$$W = 2.8 / P^3$$

where *P* is the pace measured in seconds per metre, and is given by $P = t/2000$.

We start by assuming that the relationship between power, *W*, and pace, *P*, is a general mathematical power equation given by:

$$W = A \times P^B$$

taking natural logs of both sides of the equation (2.3.2) gives:

$$\ln(W) = \ln(A) + B \times \ln(P)$$

and, if we plot $\ln(W)$ against $\ln(P)$, the slope will be the value of *B*.

The first step is to calculate in column E the values of *Pace*, using *Pace* = *Time*/2000, and then to transform both *Power* and *Pace* by taking the natural logs of columns C and E to produce columns of $\ln(W)$ and $\ln(P)$. We use Excel functions to perform the linear regression of $\ln(W)$ against $\ln(P)$, arriving at the values:

Slope, $m = -3.008$
Intercept, $c = 1.016$

The power, *B*, in the equation is given directly by the slope, *m*, giving $B = -3.0$

	A	B	C	D	E	F	G	H	I	J
1	*Student*	*WarmUp*	*Power, W* watts	*Time* seconds	*Pace, P* s/m	ln(*W*)	ln(*P*)			
2										
3	9	0	259	442	0.221	5.557	-1.510		Slope =	-3.008
4	2	0	272	435	0.2175	5.606	-1.526		Intercept =	1.016
5	8	2	291	425	0.2125	5.673	-1.549			
6	6	0.5	226	463	0.2315	5.421	-1.463		*B* =	-3.008
7	10	1.25	268	437	0.2185	5.591	-1.521		*A* =	2.761
8	9	1.25	274	434	0.217	5.613	-1.528			

Fig 7.7 Random selection from 40 results for power and pace in rowing

The constant, A, in the equation is given by $\ln(A)$ = intercept, c, which can be rearranged to give:

$$A = e^c = \exp(1.016) = 2.76$$

The resultant equation:

$$W = 2.76 \times P^{-3} = 2.76 / P^3$$

agrees very closely, within experimental error, with the accepted equation.

General regression (Minitab): Analysis for Fig 7.8. See also 3.4.3. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

7.2.5 General regression

We demonstrate the use of general regression analysis, including the Box–Cox transformation (5.4.7), by using the data in Fig 7.8 which shows the *numbers of occurrences* of two types of animal behaviours, *B1* and *B2*, observed *together* on different days under varying environmental conditions. We wish to investigate whether we can use regression to derive an approximate relationship between the number of *B1* behaviours and the number of *B2* behaviours.

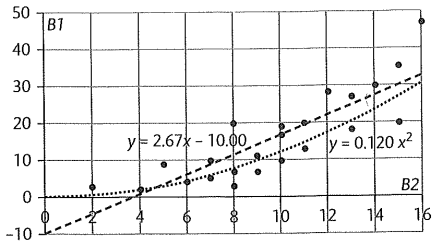


Fig 7.8 Frequency of observations of behaviours *B1* and *B2* occurring together

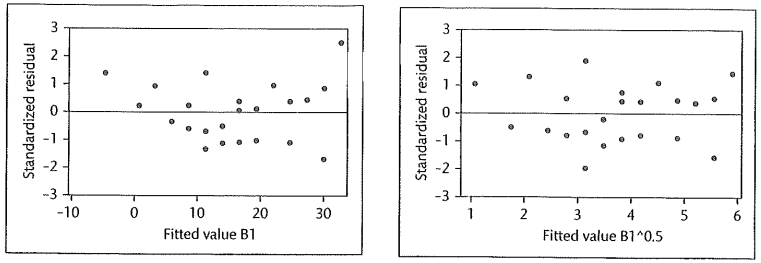
We start the analysis by performing a *simple linear regression* between the two variables, entering the *related* values of *B1* and *B2* into two columns in Minitab:

Minitab > Stat > Regression > Regression > Fit Regression Model...
Responses: *B1* **Continuous predictors:** *B2*
> Graphs: Residuals for plots: ☑ ***Standardized***
☑ ***Residuals versus fits*** to assess the equality of variance

The regression derives the best-fit *straight line* as shown by the straight, dashed trendline in Fig 7.8 with the equation:

$$B1 = -10.0 + 2.67 \times B2$$

The analysis also plots the residuals shown in Fig 7.9(a).



(a) Direct data values (b) With Box-Cox transformation

Fig 7.9 Residuals against the fitted value for data from Fig 7.8

This interpretation is unsatisfactory for two reasons:

- the straight line with its '−10' intercept on the *B1* axis is not a good representation of the science as the true frequency *B1* cannot be negative, and
- the residuals in Fig 7.9(a) show increasing variance with value.

We repeat the regression, but now request a Box–Cox transformation:

> Options... Box–Cox transformation
◉ *Optimal λ*

Minitab calculates the 95% confidence interval for the optimum value of lamda, λ, (5.4.7) as being between −0.054 and 0.695, and then chooses to use the rounded value of $\lambda = 0.5$. This gives a square root transformation for *B1*, resulting in a best-fit regression equation:

$$(B1)^{0.5} = 0.362 + 0.346 \times B2$$

However, the value of the constant, 0.362 with a standard error of 0.441, is not significantly different from *zero*, so we can approximate this equation to

$$\sqrt{(B1)} \approx 0.346 \times B2$$

which, by squaring both sides of the equation, gives:

$$B1 \approx 0.120 \times (B2)^2$$

The transformed regression gives a better fit to the data, with

- the square relationship plotted on Fig 7.8 as the dotted curve, and
- the residuals showing a more even variance in Fig 7.9(b) across the range of fitted values.

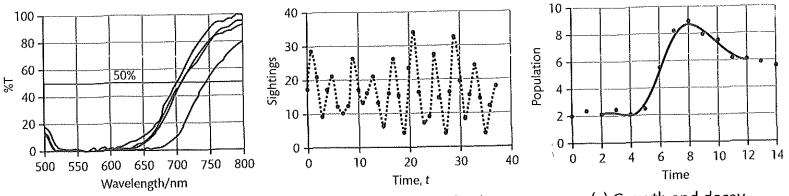
However, this analysis is only indicative of a *possible* mathematical relationship, and a more direct approach, as in 7.2.4, would be required to test whether a simple power relationship was relevant in this case.

7.3 General x–y data

Sections 7.1 and 7.2 developed the analyses of x–y curves that are expected to follow a single mathematical model. In this section, we consider x–y relationships that cannot be analysed by using an *overall* mathematical description, but do contain sections or patterns of behaviour that can be analysed by various techniques.

In 7.3.4 and 7.3.5 we consider methods of drawing best-fit curves through the experimental data points, and in 7.3.7 we illustrate the use of autocorrelation and spectral analysis to identify any periodic patterns hidden within the data.We also introduce the techniques of convolution in 7.3.5 and 7.3.6 as localized best-fit methods of analysing varying data.

7.3.1 Example data



(a) Spectral response (b) Time-series periodic data (c) Growth and decay

Fig 7.10 Examples of general x–y data

Fig 7.10(a) relates to the case study:

Ink analysis (5.1.6) giving the spectral responses of four black inks from different sources. The problem is to identify a forensic characteristic that can be used to differentiate the inks.

Fig 7.10(b) relates to the case study:

Porpoise sightings (7.3.7), an example of 'time-series' data in which there appears to be *periodically* varying components. The dashed line is not a best-fit line, but is a smoothed line 'joining the dots', which is included as a visual aid.

Fig 7.10(c) relates to the case study:

Bacterial growth (5.2.2, 7.3.5), an example of time-dependent data, recording sections of growth and decay. The localized line of best-fit was created using the smoothing convolute developed in 7.3.5.

7.3.2 Analytical options

Describing data

- Graphical plots: x–y scatterplots with a best-fit line. 7.3.4 identifies the problems of using polynomials for a best-fit line and 7.3.5 develops the use of smoothing convolutes.

Tests / Measurements:

- **Identify relevant characteristics:** The human brain is one of the best analytical tools for picking out patterns in graphs, giving another reason for displaying the data graphically.
- **Isolate specific characteristics:** The data recorded by the exploratory study may contain superfluous data outside the range required for analysis, and it is sometimes necessary to identify just the specific sections or characteristics that may address the scientific objectives of the investigation (7.3.3).
- **Transform to a linear relationship:** If the whole data set can be described by a specific mathematical relationship (e.g. exponential decay), then it may be possible to use linearization techniques (Section 2.3) or nonlinear analysis (7.2.3).
- **Identify periodic components:** In time series data we often see the influence of periodic factors. These can be analysed using autocorrelation techniques (7.3.7).

7.3.3 Identifying relevant analytical characteristics

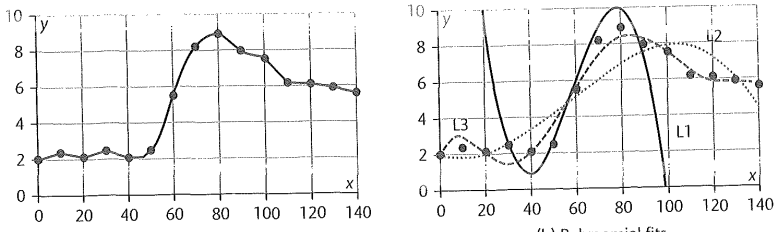
The data produced by an exploratory investigation often asks more questions than it answers. The first step is to produce a graphical presentation of the data that can highlight aspects worthy of more detailed analysis. In 7.3.4 we consider fitting an overall polynomial curve to smooth out the random variations in the data, but then, in 7.3.5, we use localized polynomial curves to calculate *best-fit values* for the data which can be used to draw the best-fit curve in 7.10(c). In 7.3.6 we use similar convolutes to *differentiate* the curve and plot its slope, highlighting such features as maxima and minima and points of maximum slope.

The graphs given in Fig 7.10 give examples of identifying different analytical features that are capable of statistical analysis:

(a) In distinguishing between the spectral curves of the four inks, there is very little difference in transmission over the main visible spectrum (they all appear black), but there are differences in the wavelengths at which the lines pass the 50% transmission mark at the high wavelength 'cut-off'. The case study (5.1.6) uses different methods to measure and test for these differences.

(b) The graph appears to show strong *periodic patterns*, which we can analyse using autocorrelation and spectral analysis (7.3.7).

(c) The portion of scientific interest is the bacterial growth period in the middle section of the curve. Initially in 5.2.2, the case study differentiates between different responses by comparing the *slopes* of this growth, and then in 7.3.5, we use the best-fit values from the smoothing convolute to estimate the confidence interval of the *difference between maximum and minimum* values.



(a) Joining the dots (b) Polynomial fits

Fig 7.11 Options for fitting a curved line to a set of data points

7.3.4 **Describing the data**

The data points in Fig 7.11(a) are based on the data in rows 1 and 2 of Fig 7.13, and it is natural to want to 'improve' the visual quality of the graph by adding a best-fit curve. As a starting point, we could just 'join the dots' by using the *smoothed* line in Excel, but this is not generally appropriate because it implies that each point is 100% accurate, when, in fact, we know that the 'dots' should appear randomly on *either side* of a best-fit curve.

We can try fitting a curve defined by a polynomial equation as in Eqn 7.1

$$y = b_0 + b_1x + b_2x^2 + b_3x^3 + \cdots + b_nx^n \qquad (7.1)$$

The maximum value of n in the polynomial in Eqn 7.1 is called the *degree* of the polynomial. A polynomial with degree $n = 2$ is called a quadratic equation, $n = 3$ gives a cubic, $n = 4$ a quartic, and $n = 5$ a quintic, etc. The number of maximum and minimum values that can appear in the graph is equal to $n-1$, with a quadratic equation, $n = 2$, having *one* turning point in the graph (either a maximum or a minimum), and a cubic equation, $n = 3$, having *two* turning points in the graphs (see lines L1 and L2 in Fig 7.11(b)).

A polynomial of degree n can make an *exact* fit through $n + 1$ points, i.e. it can pass through every point. For example, a straight line is a polynomial with degree 1, and will fit exactly through two points. Similarly, a quadratic equation can make a line fit *exactly* through $2 + 1 = 3$ data points. However, trying to fit a polynomial of degree n through *more* than $n + 1$ points will require a 'best-fit' curve, which does not necessarily pass through any point exactly.

The use of polynomials to fit experimental data is illustrated in Fig 7.11(b).

- **L1** (solid line) is a best fit for a *cubic* equation, but only for the *six points* $x = 40$ to 90. Although it produces a reasonable fit for this limited range of data, it clearly cannot fit the rest of the data.
- **L2** (dotted line) uses another best-fit *cubic* equation, but for *all* of the data points. The need to include all the data dramatically reduces the overall goodness of fit.
- **L3** (dashed line) uses a polynomial of degree $n = 6$, which gives a closer overall *statistical* fit, but the oscillation between $x = 0$ and 40 does not have any equivalence in the real data.

The main problems in using increasingly complicated polynomials to fit experimental data points are that

- different sections of the real experimental data may be generated by different scientific processes, which cannot be matched by a single *overall* mathematical relationship, and that
- an artificially complex *mathematical* relationship is unlikely to represent the underlying simple *scientific* processes that are generating the data points.

In 7.3.5 we see how we can use 'convolutes' to achieve a compromise by fitting a polynomial to short ranges of data, but still derive a best-fit curve to the whole data set.

7.3.5 **Smoothing convolutes**

Case study: **Bacterial growth / 4. Using smoothing convolutes**

—continued from 5.2.2

The data for this analysis is drawn from Fig 7.13, and is similar to that depicted in Fig 5.5. We wish to develop a localized best-fit curve to smooth experimental variations, from which we can also derive an 'averaged' best-fit value for each data point.

We then use the best-fit values to estimate the confidence interval between maximum and minimum values.

Using convolutes: Excel analysis for Figs 7.13 and 7.14. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Instead of aiming to fit one *single* polynomial equation simultaneously to all of the data points, the process of convolution derives best-fit curves that cover the data over short *local* ranges. A small set of values (convolutes) are combined with a section of data values to produce a single derived 'best-fit' value, and then, *by moving* the convolute throughout the whole data set, it generates a new modified data set. The relationship between the original data set and the derived data set depends on the choice of convolute values, and we will consider two types:

- Smoothing convolutes to provide a best-fit curve to complex data,
- Differentiating convolutes (7.3.6) to calculate slopes and identify maxima, minima, etc.

In Fig 7.13 the y-values in row 2 give luminescence measurements of bacterial populations as a function of time, x, in row 1. We wish to produce a best-fit curve for this data.

As an illustration, we first fit a cubic polynomial in Fig 7.12(a), using a least squares fit with Solver (Section 2.4), to *only the five* data points around $x = 70$. The y-value of this polynomial at $x = 70$ is calculated to be $y = 8.03$, and this is the *best-fit* y-value *at this point*.

This process is then repeated using the five data points around $x = 80$ (see Fig 7.12(b)), and we get a best-fit y-value at $x = 80$ of $y = 8.72$. In Fig 12(c) the process is repeated again around $x = 90$, giving the best-fit $y = 8.26$.

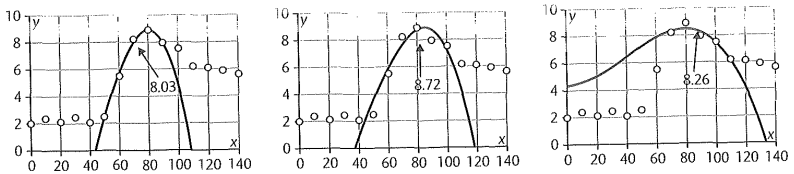


Fig 7.12 Fitting a cubic curve to five points at a time

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	x	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	
2	y	2.1	2.4	2.1	2.5	2.1	2.5	5.5	8.2	8.9	8.0	7.5	6.2	6.1	5.9	5.6	
3							↓	↓	↓	↓	↓						Sum
4	C1:						-3	12	17	12	-3						35
5																	
6	y'						2.34	2.23	2.08	2.90	5.40	8.02	8.76	8.27	7.23	6.48	5.99
7	y' - y	(residuals)					0.24	-0.27	-0.02	0.40	-0.10	-0.18	-0.14	0.27	-0.27	0.28	-0.11

Fig 7.13 Applying the convolute

The above procedure of calculating a best-fit polynomial for *every* point would be too time consuming for practical use, but we can obtain very similar results by using the process of convolution with convolute values published by Savitsky A and Golay M J E ('Smoothing and Differentiation of Data by Simplified Least Squares Procedures'. *Analytical Chemistry* 36(8): 1,627–1,639, 1964).

The derivation in Fig 7.13 uses a set of 'convoluting integers', {−3,12,17,12,−3}, held in G4:K4, together with the sum of their values, 35, in Q4. The calculation in I6 for the five data points around $x = 70$ multiplies each of the five data values by the corresponding convolute value in the same column, adding the five products, and dividing by the convolute sum:

$$[I6] = \frac{-3\times2.5+12\times5.5+17\times8.2+12\times8.9-3\times8.0}{35} = 8.02$$

This gives a best-estimate y-value at $x = 70$. We can then 'move' this convolute left and right to repeat the process to calculate the best-fit values for all values of x. In practice, we perform this operation in Excel by simply copying the equation along cells in row 6, provided that we have entered the appropriate '$' signs in the equation to lock the column values for the convolute values:

[I6] = ($G4 * G2 + $H4 * H2 + $I4 * I2 + $J4 * J2 + $K4 * K2)/$Q4

The convolute calculations give values similar to those calculated using the separate polynomial calculations. They represent the weighted average of the data at each point, taking into account the values of the five local points, and we use them to plot a best-fit curve as in Fig 7.10(c). The curve must stop two values from each end, otherwise the convolute would overlap the end of the raw data values.

We can also use the best-fit data for further calculations, but first we will need to estimate the *uncertainty* in the data by comparing the experimental values with the best-fit values. We do this by calculating the individual residual differences in row 7, and use Eqn 1.20 to estimate the experimental standard deviation as:

$$s = \sqrt{s^2} = \sqrt{\frac{\sum_i (x_i - x)^2}{(n-1)}} = 0.244$$

The normal calculation for the standard error is given by Eqn 1.21:

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

but, in this case, the $n = 5$ data points do not have an equal 'averaging' effect, and we must *weight* (2.2.4) their individual variances using their convolute values:

$$\left(\frac{-3}{35}\right)^2 + \left(\frac{12}{35}\right)^2 + \left(\frac{17}{35}\right)^2 + \left(\frac{12}{35}\right)^2 + \left(\frac{-3}{35}\right)^2 = 0.697$$

so that we can estimate the standard error:

$SE = 0.697 \times 0.244 = 0.17$

In calculating the difference between the maximum and minimum values, the best estimate maximum and minimum values in row 6 are 8.76 and 2.08 respectively, which give a *difference* between maximum and minimum of 6.68. Combining (1.4.4) the standard error in each measurement, gives a standard error for the *difference* of

$0.17 \times \sqrt{2} = 0.24$

The original experimental uncertainty calculation is based on 11 values, which gives a t-value of 2.23, which then gives a confidence deviation:

$Cd = 2.23 \times 0.24 = 0.54$

The confidence interval for the difference between maximum and minimum values can be rounded conservatively to give:

$CI = 6.7 \pm 0.6$

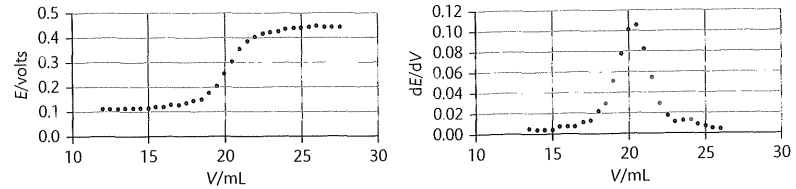
Other convolutes are possible. The simplest has a constant value convolute, {1,1,1,1,1}/5, which just adds up the five data points and divides by five. This is a simple average which is moved through the data–a *moving average*, also called *boxcar* averaging. You should note that the moving average *trendline* in Excel calculates the average of the data points at the *end* of the convolute range, and not, as we have done it, in the middle. This means that there is a 'delay' along the x-axis and the averaged trendline is displaced along the x-axis, for example by two units for a five element convolute.

Savitsky and Golay also published larger convolutes and higher order polynomials, e.g. for a convolute with seven values:

Cubic, $n = 3$:	−2	3	6	7	6	3	−2	Sum: 21
Quintic, $n = 5$:	5	−30	75	131	75	−30	5	Sum: 231

7.3.6 **Differentiating convolutes**

In addition to a smoothing function, convolutes can be used to derive the *slope* of the curve. For example, Fig 7.14(a) gives the results of a potentiometric titration, recording potential, E, versus the amount of added titrant, V, with the end point of the titration being the point of *steepest* slope.



(a) Curve of potential vs volume added

(b) Differentiated curve giving slope vs volume added

Fig 7.14 Potentiometric titration

The convolute {−22, +67, +58, 0, −58, −67, +22}/252 fits a cubic curve to the data and provides the slope of the data at the central point with the assumption that there is unit distance between each point along the x-axis. Fig 7.14(b) gives the result of this convolution, showing that the point of maximum slope is for an added volume of about $V = 20.3$ mL.

In the calculations for Fig 7.14(b) we have also divided each value by the separation between data points, 0.5 mL, so that the curve becomes the *first* differential, dE/dV, of the original E vs V curve. It is also possible to use the same convolution again to obtain the *second* differential which will then show the top of the peak in Fig 7.14(b) as a zero slope crossing point on the axis, precisely identifying the end point of the titration.

7.3.7 **Spectral analysis**

Spectral analysis: SPSS and Minitab analyses for Figs 7.16 and 7.18.
Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Variables that are recorded over a period of time often show some form of periodic behaviour (e.g. diurnal, annual), and statistical analysis can help in identifying specific periods hidden within the data.

We start with the concept of *autocorrelation*, and, in order to understand this technique, it is useful to use the simple function F(0) in Fig 7.15 which has a varying value with a well-defined repetition period of $T = 4$ time units.

The process of autocorrelation measures the correlation of a function with a *time-shifted* version of itself. If we take the original function, F(0), and shift it by two units, called the *lag* time, we obtain the function F(2), and similarly for F(3), F(4), and F(8). The autocorrelation function, ACF, is then a plot of the correlation values against the lag time.

For a lag time = 2, autocorrelation measures the *correlation* between F(0) and F(2), getting a value $r = -1.0$ because F(2) behaves in exactly the *opposite* way to F(0), i.e. the peaks in F(0) correspond to the troughs in F(2). Autocorrelation repeats this process for different lag times. Function F(3) has a lag time of three units, and the peaks of F(3) fall in between the peaks and troughs of F(0) resulting in zero correlation, $r = 0.0$, but function F(4) with a lag time of four units now falls exactly *in step* with F(0) resulting in perfect correlation, with $r = 1.0$. The function F(8) with a lag time of eight units is again in perfect correlation with $r = 1.0$.

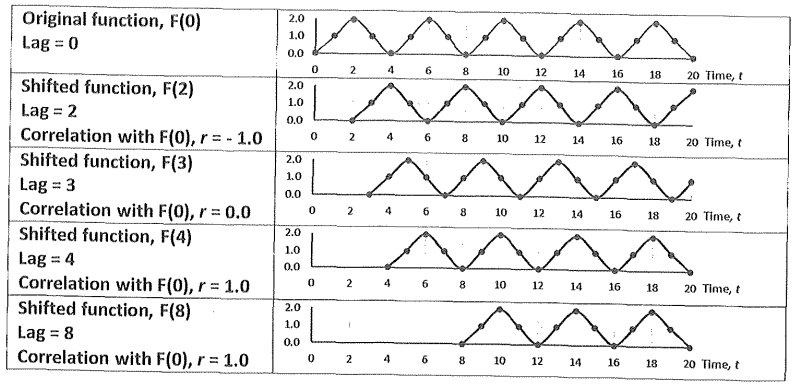
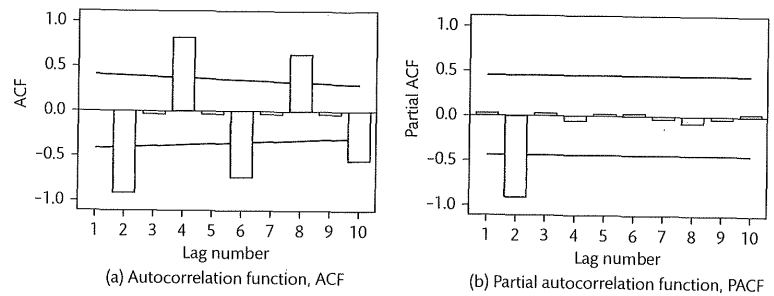


Fig 7.15 Autocorrelation

The autocorrelation function, ACF, for the function F(0), entered as *Data*, can be obtained in Minitab or SPSS via:

Minitab > Stat > Time Series >
Autocorrelation...
or
Partial Autocorrelation...
Series: *Data*
◉ **Number of lags:** e.g. 10
→ Output: Similar to Fig 7.16

SPSS > Analyse > Forecasting >
Autocorrelations...
Variables: *Data*
☑ ***Autocorrelations***
☑ ***Partial autocorrelations***
> Options
Maximum number of lags: e.g. 10
→ Output: Fig 7.16



(a) Autocorrelation function, ACF

(b) Partial autocorrelation function, PACF

Fig 7.16 ACF and PACF using SPSS

We can see in Fig 7.16(a) that the autocorrelation function, ACF, confirms the interpretation in Fig 7.15 with *positive* correlation for lag times of four and eight, and *negative* correlation for lag times of two, six, and ten. We would like to use the ACF as an analytical tool to identify hidden periodic components in the data. However, the basic ACF can lead to possible *ambiguity* in that, for example, the positive correlation at lag = 8 could be due to the periodic variation in the data of $T = 4$ as above, but it could also be due to a different component with exactly double the period of $T = 8$.

The *partial* autocorrelation function, PACF, can be used to avoid the ambiguity in the ACF, in that any further correlation between the delayed functions is 'cut-off' after the first correlation. Fig 7.16(b) shows the partial autocorrelation for the function F(0), and it can be seen that only the first negative correlation appears uniquely at *Lag* = 42, which means that if other larger period components were present, then these would appear *separately* at larger lag times.

The lines drawn in Fig 7.16 show the 95% confidence interval limits, confirming that a period of $T = 4$ is a significant component in the data.

We see how frequency components within time-dependent data can be identified with ACF or PACF. It is now useful to be able to display these periodic (or spectral frequency) components within a spectral analysis that plots out the components as a function of their periods.

Case study: **Porpoise sightings / Spectral analysis**

Fig 7.17 records the number of sightings of porpoises in equal periods, recorded in each of four seasons over 14 years. We wish to analyse the variations to identify any significant patterns of variation.

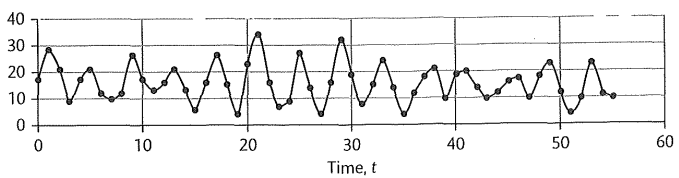
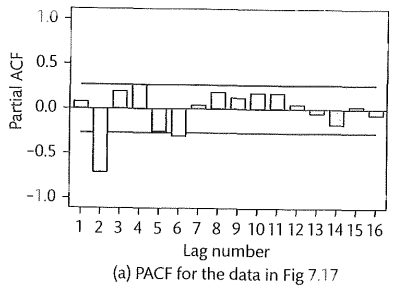


Fig 7.17 Frequency of porpoise sightings per quarter year

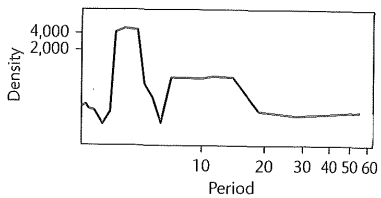
We can derive the partial autocorrelation function, PACF, given in Fig 7.18(a). The first negative correlation in the PACF suggests a significant frequency with a period of $2 \times 2 = 4$ time periods, which is equal to four seasons, i.e. an annual variation. However, there also appears to be another significant component hidden within the data with a period of about $2 \times 6 = 12$ seasons, or three years.

It is also possible to produce a 'spectral density' plot that displays the different frequency components as peaks against a horizontal period or frequency axis.

SPSS > Analyse > Forecasting > Spectral Analysis...
Variables: *Data*
☑ **Spectral density**
◉ **By period**
→ Output: Fig 7.18 (b)



(a) PACF for the data in Fig 7.17



(b) Spectral analysis of the data in Fig 7.17

Fig 7.18 Partial autocorrelation and spectral analysis

The spectral analysis plot in Fig 7.18(b) shows the major component with a period of about four quarters, and also shows a less defined component with an approximate period of about ten quarters or two and a half years. The existence of frequency components in the data might suggest further investigation into whether the additional variations could be linked to any other factor over the 14-year period.

Spectral analysis can be a useful exploratory tool for identifying hidden periodic patterns within the data, and we have only given a simplified introduction to its use. The general analysis of 'time-series' data rapidly becomes more complex, and seeking the help of specific statistical advice is recommended.

Frequency data

Introduction

This chapter develops techniques which analyse the frequencies and probabilities of observed events. It first introduces methods for describing and managing the data either as individual observations or as tabulated frequencies, and then builds on the contingency table statistics developed in Sections 3.7 and 4.2. Finally, the content addresses the modelling of binary systems and analysing the probabilities of a system being in a particular state.

Section 8.1 presents analyses relevant to *single samples* of categorical data, e.g. data descriptions, 'goodness of fit' test.

Section 8.2 develops the statistics of the *contingency table* and the use of *cross-tabulation* to generate the table from individual observations.

Section 8.3 develops the analysis of binary systems using *logistic regression* and the description of probabilities using *ROC* plots.

8.1 Single variable

In this section we consider single samples of data for which we can perform a *frequency* analysis. We may have simple *categorical* data in which the individual observations already fall into specific categories. Alternatively, the raw data may consist of a *distribution* of many *interval* values, and the first step is to group the values into specific categories (binning), and then count the numbers (frequencies) of records that fall into each category in a table (tabulation). Once this is done it is possible to plot the distribution of frequencies on a frequency bar or column graph (Fig 8.2) or histogram (Fig 8.5). The actual processes of binning and tabulation are described in 8.1.7 and 8.1.8. The chi-squared statistics for testing the distribution of observations across categories is developed in 3.7.2.

8.1.1 Example data

Case study: **Chi-squared / 1. Genotypes (overview)**

This case study introduces examples of the chi-squared family of analyses. In Fig 8.1, column B records 200 observations in which the response variable is recorded as one of four genotypes, defined by the *text* categories, *GTypeT*: *AB*, *Ab*, *aB*, or *ab*. Column C gives the same values, but the categories are *numerically* coded, *GTypeN*: 1, 2, 3, and 4 respectively. It is sometimes necessary to recode text labels

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		Type:	Nominal	Nominal	Category	Freq	Ratio		Interval			Bin		Freq
2		Variable:	*GTypeT*	*GTypeN*	*GTypeT*		(expected)		*BAlc*		Cutpoints	Range	BinNo	*BAlc*
3			AB	1	ab	8	1		78.8		75	< 75	1	1
4			AB	1	aB	39	3		82.7		76	75 - 76	2	3
5			Ab	2	Ab	28	3		79.8		77	76 - 77	3	6
6			ab	4	AB	125	9		78.9		78	77 - 78	4	22
7			AB	1	Total	200	16		77.4		79	78 - 79	5	30
8			aB	3					76.6		80	79 - 80	6	26
9			aB	3					78.8		81	80 - 81	7	27
10			AB	1					77.3		82	81 - 82	8	22
11			AB	1					86.3		83	82 - 83	9	13
12			AB	1					81		84	83 - 84	10	7
13			aB	3					79.4		85	84 - 85	11	1
14			ab	4					78.5		86	85 - 86	12	1
15			AB	1					76.6			>86	13	1
16			AB	1					80.8				Total	160
17			aB	3					81					
162			AB	1					77.2					
163			AB	1										
202			Ab	2										

Fig 8.1 Typical data sets using a *frequency* analysis (Rows 18 to 161 and 164 to 201 are 'hidden' in the worksheet)

as numbers, or vice versa, to suit the input requirements of different software analyses. Column F gives the *tabulated* frequencies for the same data, in which the occurrences of each category are counted and recorded alongside the category label in column E. In this section, we use this data to demonstrate the use of frequency bar (or column) graphs (8.1.3), the one-way 'goodness of fit' chi-squared test (8.1.5), and tabulation (8.1.7).

3.7.2 / 2. One-way 'goodness of fit' test: Develops the underlying statistics of the one-way analysis.

3.9.3 / 3. Monte Carlo analysis: Uses a re-sampling technique to perform a 'goodness of fit' test when the expected frequencies are too low for the standard chi-squared analysis.

Case study: **Blood alcohol / 9. One sample analysis**

—introduced in 1. Introduction

In Fig 8.1, column I records 160 replicate measurements of blood alcohol level, *BAlc*, (mg of alcohol per 100ml of blood) from a source population with a mean of 80 and a standard deviation of 2.0. Column L defines possible value ranges (*categories* or *bins*), and column N records the tabulated numbers (or frequencies) of measurements that fall into each range.

The main difference between the genotype and blood alcohol data in Fig 8.1 is that the categories of *GTypeT* are defined by the *science involved*, and are few in number, but the categories (bins) used for *BAlc* are defined purely as a way of *describing* the statistical distribution of values. Data similar to *GTypeT* or *GTypeN* would be either *nominal* (in this example) or *ordinal* (e.g. assessment levels) but the data similar to *BAlc* would typically be *interval*.

Another form of 'frequency data' occurs where the measured response variable is *itself* a frequency. For example in radioactive decay (where the recorded activity can be measured as the 'number of counts per second'), or in the growth of a bacterial population (measured as the number of 'colony forming units', *cfu*). We see in 6.1.1 that we can treat higher frequency values as continuous *interval* data, and, in particular, we analyse radioactive decay as interval data in 2.3.4.

8.1.2 Analytical options

We list below *some* of the most common 'frequency' analyses, together with links for further information.

Describing data

- Graphical plots: Boxplot (1.1.2), bar chart, histogram (8.1.3), stem and leaf plot (Fig 6.3).
- Numerical statistics: Mean, median, standard deviation, confidence interval, etc. (6.1.3).

Frequency data (Minitab): Descriptives and graphs. Scan here to watch the video or find it via www.oxford textbooks. co.uk/orc/ currell/

Tests / measurements:

- Chi-squared 'goodness of fit test' tests whether the **distribution** between defined categories is different from a *test distribution* of frequencies (8.1.5, 3.7.2).
- Tabulation and binning of data are the processes of **counting** and **grouping** frequencies (8.1.7/8).
- The **normality** of a distribution of data values can be tested (8.1.6) using Anderson–Darling, Kolmogorov–Smirnov, and Ryan–Joiner (similar to Shapiro–Wilk) tests.
- The Kolmogorov–Smirnov test can be used to test whether a **distribution** of values is different from a *standard distribution* (normal, Poisson, binomial, or uniform) (8.1.6).

  Testing the **proportion** of data in specific categories (6.1.7 and Section 3.8).

Frequency data (SPSS): Descriptives and graphs. Scan here to watch the video or find it via www.oxford textbooks. co.uk/orc/ currell/

8.1.3 Describing categorical data

For categorical data, given as a list of *individual* categories, e.g. *GtypeT* in column A, we can use Minitab or SPSS to plot bar graphs directly and print out the tabulated frequencies for each category.

Minitab

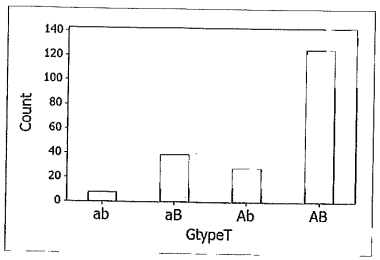
Minitab > Graph > Bar Chart...
Bars represent: For a column of values select: ***Counts of unique values***
For a single sample of values select: ***Simple***
Categorical variable: *GtypeT*
→ Output Fig 8.2(a)

The tabulated counts can be read from the bar chart or printed using:

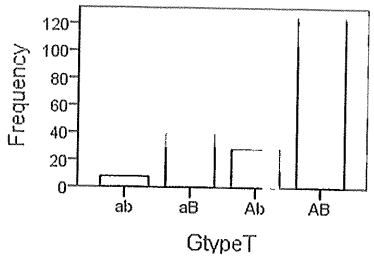
Minitab > Stat > Tables > Tally Individual Variables... or **Descriptive Statistics...**
→ Output: Plot as in Fig 8.3(a)

SPSS

SPSS > Analyze > Descriptive statistics > Frequencies...
Variable(s): Identify categorical data, e.g. *GtypeT*
> Charts: ◉-Bar charts (for categorical *GtypeT* data)
→ Output: Bar chart as in Fig 8.2(b) and tabulated frequencies in Fig 8.3(b).



(a) Minitab



(b) SPSS

Fig 8.2 Frequency bar charts for data from Fig 8.1

Tally for Discrete Variables: GtypeT

GtypeT	Count
ab	8
aB	39
Ab	28
AB	125
N=	200

(a) Tally chart (Minitab)

GtypeT

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	ab	8	4.0	4.0	4.0
	aB	39	19.5	19.5	23.5
	Ab	28	14.0	14.0	37.5
	AB	125	62.5	62.5	100.0
	Total	200	100.0	100.0	

(b) Frequencies and percentages (SPSS)

Fig 8.3 Tabulated frequencies

The tabulated results in Fig 8.3 are useful when the data is presented as individual records (as in column B). If the data has already been tabulated as *frequencies* (as in column E), the same bar graphs can be obtained using:

Minitab

Minitab > Graph > Bar Chart...
Bars represent: For a column of values select: ***Values from a table***
For a single sample of values select: ***Simple***
Graph variables: *Freq*
Categorical variable: *GtypeT*
→ Output Fig 8.2(a)

SPSS

In SPSS it is necessary to *weight* categories, *ab*, *aB*, *Ab*, and *AB*, with the respective frequencies, by entering the values as in Fig 8.4 and using:

SPSS > Data > Weight Cases...
◉-Weight cases by:
Frequency variable: *Freq*

	GtypeT	Freq
1	ab	8.00
2	aB	39.00
3	Ab	28.00
4	AB	125.00

Fig 8.4 Data entry for 'weighting' data in SPSS

After performing the weighting operation, each row in the data will be treated as being repeated the number of times given by the *Freq* value in that row. For example, when the variable, *GtypeT*, is used it will be treated as 8 values of *ab*, 39 of *aB*, 28 of *Ab*, and 125 of *AB*, allowing the same set of instructions for a bar chart as above.

8.1.4 Editing histograms

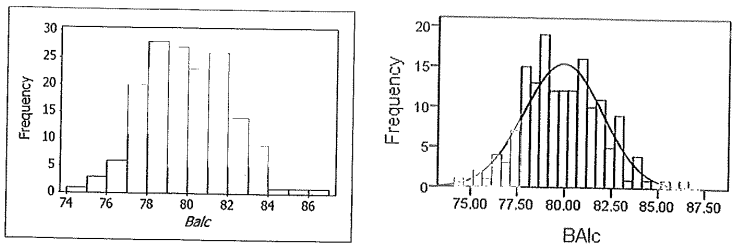
The process of obtaining the histogram (1.3.1) of a sample of *interval* data (as in column I) involves *binning* and *tabulation*. Binning is a process of splitting up the data range into categories or *bins* (column L), and then tabulation is the process of counting how many data values fall into each bin. The *cutpoints* (column K) are the divisions that separate each of the bins. Binning and tabulation are considered as separate processes in 8.1.7 and 8.1.8 but we now see that they also become an integral part of editing a histogram when describing interval data.

Minitab

Minitab > Graph > Histogram...
For a single sample of values select: ***Simple***
Graph variables: *BAlc*
→ Output: Produces a graph that can be edited, Fig 8.5(a)
To change the 'binning':
> Right click on the *x*-axis, and select **Edit X Scale...**
> Select **Binning** tab:
Interval type: Check ◉-Cutpoint
Interval definition: Check ◉-Midpoint/Cutpoint positions and
Enter values with spaces: 74 75 76 77 78 79 80 81 82 83 84 85 86

SPSS

SPSS > Analyze > Descriptive Statistics > Frequencies...
Variable(s): Identify data, e.g. *BAlc*
> Charts: ◉-Histograms (for scale *BAlc* data)
☑-Show normal curve on histogram
→ Output: Produces a graph that can then be edited, Fig 8.5(b)
To change the 'binning':
> Double click on the graph to open the editing window
> Right click on the histogram, and **Select ▸ This Histogram Bar**
> Select **Binning** tab:
x-axis: Check **◉-Custom, ◉-Interval width**: Enter 1.0
For **☑-Custom value for anchor** Enter 80 or 80.5 to define position of cutpoints



(a) Minitab (b) SPSS

Fig 8.5 Histograms of the *same* data

The reason for the difference in the histogram shapes in Fig 8.5 (a) and (b) is a difference in the cutpoints and bin widths chosen to define the bins, but these can be edited as described in the derivations above.

8.1.5 Chi-squared 'goodness of fit' test

The chi-squared 'goodness of fit' test, which compares the observed and expected frequencies within a single set of categories, is developed in 3.7.2. We now use Minitab and SPSS to analyse the data in columns E and F, which is the same as in Fig 3.46

Minitab

Minitab > Stat > Tables > Chi-Squared Goodness-of-Fit Test (One Variable)...
Enter the data either as one of:
◉-Observed counts: Column containing 8, 39, 28, 125 ***or***
◉-Categorical data: *GtypeT*
Under **Test** enter the frequency values for comparison as one of: **◉-*Equal proportions*** or
◉-Specific proportions: 0.0625, 0.1875, 0.1875, 0.5625

Chi-squared goodness of fit (Minitab): Analysis for Fig 8.6. See also 3.7.2. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Chi-squared goodness of fit (SPSS): Analysis for Fig 8.7. See also 3.7.2. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

(the proportions can be calculated: 1/(1+3+3+9) = 0.0625, etc. or

◉-**Proportions defined by historical counts:** 1, 3, 3, 9

For either of the last two above options, select either: ***Input Column*** if the comparison values are in a column or ***Input Constants*** to enter values directly as shown in this example.

→ Output in Fig 8.6

Chi-Square Goodness-of-Fit Test for Categorical Variable: GtypeT

Category	Observed	Historical Counts	Test Proportion	Expected	Contribution to Chi-Sq
ab	8	1	0.0625	12.5	1.62000
aB	39	3	0.1875	37.5	0.06000
Ab	28	3	0.1875	37.5	2.40667
AB	125	9	0.5625	112.5	1.38889

N	N*	DF	Chi-Sq	P-Value
200	0	3	5.47556	0.140

Fig 8.6 Output from chi-squared 'goodness of fit' test (Minitab)

SPSS

SPSS > Analyze > Nonparametric Tests > One Sample...
Objective: ◉-**Customize analysis**
Fields: Select data, e.g. *GtypeT*
Settings: ◉-**Customize tests**
☑-**Compare observed probabilities to hypothesized (chi-squared)**
Options: Select either
◉-All categories have equal proportions ***or***
◉-Customize expected probability. Enter relative frequencies as in Fig 8.7(a).
→ Output in Fig 8.7(b)

Expected probabilities:

Category	Relative Frequency
ab	1
aB	3
Ab	3
AB	9

(a) Entering relative frequencies

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The categories of GtypeT occur with the specified probabilities.	One-Sample Chi-Square Test	.140	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

(b) Summary output

Fig 8.7 Chi-squared 'goodness of fit' test (SPSS)

Alternatively in SPSS it is possible to use an earlier version of the test retained within the 'Legacy Dialogs' as described in 3.9.3.

The standard outputs from chi-squared tests typically present the observed frequencies, together with the calculated expected frequencies, the calculated chi-squared value, and *p*-value. The output from SPSS in Fig 8.7(b) is a summary with just the *p*-value, but the full information can be obtained by double-clicking on the summary output in the Output window. The reported statistics in Figs 8.6 and 8.7 agree with those calculated using Excel in Fig 3.46, and since $p = 0.140$ is more than the default significance of 0.05 we conclude that the observed distribution is not significantly different from the expected ratios and may have occurred by random chance.

8.1.6 Testing distributions

The use of Minitab and SPSS to test specifically for the *normality* of a set of experimental data is developed in Section 5.4. In addition, we can use the Kolmogorov–Smirnov test in SPSS for differences from a range of *standard* distributions:

Testing distributions (Minitab): Analysis for distributions and Fig 8.8(b). Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Testing distributions (SPSS): Analysis for distributions and Fig 8.8(a). Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

SPSS

SPSS > Analyze > Nonparametric Tests > One Sample...
Objective: ◉-**Customize analysis**
Test Fields: Select data (only scale values), e.g. *BAlc*
Settings: ◉-**Customize tests**
☑-**Kolmogorov–Smirnov**
> Options: Select Normal, Uniform, Poisson, Exponential
→ Output in Fig 8.8(a)

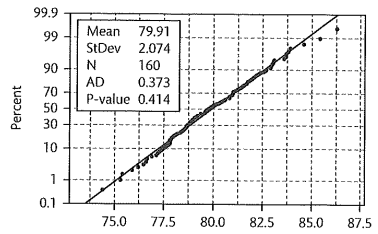
It is possible to *define* a specific test distribution for comparison by entering values for the mean and standard deviation of the test distribution. However, it is common to use the default which allows the software to calculate the distribution that is *closest* to the data, and then perform the test for a significant difference from that calculated distribution.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of BAlc is normal with mean 79.91 and standard deviation 2.07.	One-Sample Kolmogorov-Smirnov Test	.646	Retain the null hypothesis.
2	The distribution of BAlc is uniform with minimum 74.40 and maximum 86.30.	One-Sample Kolmogorov-Smirnov Test	.000	Reject the null hypothesis.
3	The distribution of BAlc is Poisson with mean 0.00.	One-Sample Kolmogorov-Smirnov Test	.	Unable to compute.
4	The distribution of BAlc is exponential with mean 79.91.	One-Sample Kolmogorov-Smirnov Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

(a) SPSS distribution tests



(b) Minitab probability plot and test for normality

Fig 8.8 Distribution tests for *BAlc*

The output in Fig 8.8(a) shows that the data does *not* show a significant difference from a normal distribution, but that it is significantly different from a uniform or exponential distribution. It is unable to compare the *continuous* values of the *BAlc* data with a Poisson distribution because a Poisson distribution must have *integer* values.

It is also possible to select the Kolmogorov–Smirnov test directly from:

SPSS > Analyze > Nonparametric Tests > Legacy Dialogs...

SPSS also provides normality plots (5.4.5) and Kolmogorov–Smirnov and Shapiro–Wilk tests using:

SPSS > Analyze > Descriptive statistics > Explore...
Dependent List: *BAlc*
> Plots: ☑-Normality plots with tests
→ Output similar to Fig 8.8(b)

Minitab provides a specific test for normality:

Minitab > Stat > Basic Statistics > Normality Test
Variable: *BAlc*
Select a specific normality test, e.g. **◉-Anderson–Darling**
→ Output in Fig 8.8(b)

Fig 8.8(b) shows a normality plot (5.4.5) in which the data is closely aligned with the neutral diagonal line which indicates that the data is close to a normal distribution. This is confirmed by the $p = 0.414$ result of the Anderson–Darling (AD) test in the data box, from which we conclude that there is no evidence that the distribution is *not* normal.

Minitab also provides a specific test for a Poisson distribution:

Minitab > Stat > Basic Statistics > Goodness-of-Fit Test for Poisson...

which uses a one-way chi-squared 'goodness of fit' test.

8.1.7 Tabulation of data

If the data has a limited number of discrete categories (e.g. *GTypeT* in Fig 8.1), we can *count* the occurrences within each category, and list these frequencies in a table. This is the process of *tabulation*. One of the oldest ways of recording a count is to use a *tally chart* where occurrences are recorded as strikes which build up in blocks of five, for example:

𝍸 // = 7.

Minitab uses this terminology when counting the occurrences of specific values in data.

Minitab

Minitab > Stat > Tables > Tally Individual Variables...
Variables: *GTypeT*
☑-Select required statistics and/or storage of output values in data table
→ Output: Returns the frequency values given in column F, and the count values can also be stored in new columns under Tally1 and Tally 2

SPSS can also produce tabulated results using

SPSS > Analyze > Descriptive statistics > Frequencies...

8.1.8 Binning

Binning is a process in which numeric values are identified as falling into specific *value ranges*, called *bins*. The *total* range of possible values is divided into a limited number of individual bins, defined by 'cut-points' which are the values between adjacent bins—see columns K and L in Fig 8.1.

Binning can be done interactively during the editing of a histogram (8.1.4) for both Minitab and SPSS.
SPSS also has a specific binning option:

SPSS > Transform > Visual binning...
Variables to bin: *BAlc*
Binned variable: Enter a name for a new variable to hold bin values, e.g. *BinNo*
> Make Cutpoints: Define the bin locations and sizes, e.g.
First Cutpoint Location: 75.0 ***Number of cutpoints:*** 12 ***Width:*** 1.0
→ Output: Saves a new variable, *BinNo*, in the next available column such that each entry records the bin number (1 to 13 in this example) for each value in *BAlc*.

Having identified the bin number for each datum, it is possible to tabulate the values, and find the frequency within each bin, using the methods in 8.1.3.

Excel

We can bin frequency data in Excel, in Fig 8.1, by first using the functions, MAX() and MIN(), to find the maximum and minimum values, 74.4 and 86.3, in the *BAlc* data. We can then define (for example) 12 cutpoints, 75 to 86, in cells K3:K14, which will create 13 bins. The range for each bin is shown by text in cells L3:L15.

The binning process uses an 'array' function and the process must be carefully followed:

- Highlight the target cells N3:N15 to hold the output frequency values.
- Using the function FREQUENCY(), identify the *data range* I3:I162 and the *bin range* K3:K14.
- Holding down the Shift and Ctrl key *together*, press Enter.

Excel can also produce a histogram with the above data using data analysis tools.

8.2 Contingency tables

Section 8.1 developed the frequency statistics for categories defined by the *levels* of just one factor or variable, e.g. genotypes, blood alcohol levels. In this section, the categories are

defined by the levels of *two* factors, which provide the rows and columns to create the cells of a 2-D contingency table. The statistics of the contingency table have been developed in 3.7.4 and then Sections 4.2, 4.3, and 4.4 developed tests/measures for *association* and *agreement* between these two factors.

8.2.1 Example data

	A	B	C	D	E	F	G	H	I	J	K	L
1	Improvement:	None	Some	Good	Excellent			*Sex*	*SexN*	*Improv*	*ImprovN*	*Freq*
2		*N / 1*	*S / 2*	*G / 3*	*E / 3*	Totals:		F	1	N	1	13
3	Female, F	**13**	**18**	**159**	**72**	262		F	1	S	2	18
4	Male, M	**26**	**28**	**166**	**68**	288		F	1	G	3	159
5	Totals:	39	46	325	140	550		F	1	E	4	72
6								M	2	N	1	26
7	Improvement:	*N+S*	*G+E*	Totals:				M	2	S	2	28
8	Female, F	31	**231**	262				M	2	G	3	166
9	Male, M	54	**234**	288				M	2	E	4	68
10	Totals:	85	465	550								

Fig 8.9 Case study: Association / 1. Bacteriophage treatment

Case study: **Association / 1. Bacteriophage treatment (overview)**

The contingency table, B3:E4, in Fig 8.9 shows the numbers of male and female patients who have received bacteriophage treatment and shown no, *N*, some, *S*, good, *G*, or excellent, *E*, improvement. The data in columns H to L contain the same information as in the table, with the sex of the patient identified both as a nominal (text) variable *Sex* and a numeric variable *SexN*. The improvement is given both by the nominal variable *Improv* and by the numeric variable *ImprovN*. We have included a numeric code for each variable because some analyses require the data presented in specific formats. In this section we use Minitab and SPSS to test for an association between the level of improvement and the sex of the patient.

3.7.4 / 2. Contingency table: Develops the underlying test statistics by testing for an association between the sex of children and their preferred study subjects.

	A	B	C	D	E	F	G	H	I	J	K	L
1	*Grp*	*Q1*	*Q2*	*Q3*	*Q4*		G1+G2:	Q1\Q2	N	Y		*Q6*
2	**G2**	**N**	**N**	**Y**	Y			N	24	19		**0**
3	G1	Y	**Y**	**Y**	Y			Y	7	16		**-2**
4	G2	N	**N**	**N**	**N**							**-1**
5	**G2**	**Y**	N	**Y**	**Y**		**G1:**	**Q1\Q2**	**N**	Y		**1**
6	**G2**	**Y**	**N**	**N**	**N**			**N**	11	16		**-2**
7	**G1**	**N**	**N**	**N**	**N**			**Y**	1	8		**2**
8	G1	N	Y	Y	N							**2**
9	**G1**	**N**	Y	N	**N**		**G2:**	**Q1\Q2**	**N**	Y		**2**
10	G1	N	N	Y	N			N	13	3		**1**
11	**G1**	**N**	Y	**N**	**N**			**Y**	6	8		**1**
12	G1	**N**	**Y**	**N**	**Y**							**3**
67	**G2**	**Y**	**N**	**Y**	**Y**							**0**

Fig 8.10 Case study: Forensic questionnaire / 2. Crosstabs and contingency table (Rows 13 to 66 are 'hidden' in the worksheet)

Case study: **Forensic questionnaire / 2. Crosstabs and contingency table**

—continued from 9.2.1, leading to 4.4.5 and 6.2.1

Fig 8.10 is derived from forensic questionnaire results in Fig 9.12, and gives the *binary* responses to four questions, *Q1*, *Q2*, *Q3*, and *Q4*, in a questionnaire on the interpretation of forensic evidence for two groups of people defined by *G1* and *G2*. The contingency table in I2 : J3 records the number of specific *Q1 / Q2* pairs in the whole data set, but the tables in I6 : J7 and I10 : J11 are *layered* to include only groups *G1* and *G2* respectively. *Q6* gives a response on a −3 to +3 Likert scale, which is analysed in 8.2.7.

8.2.2 Analytical options

Describing data

- **Graphical plots**: Clustered and 3-D bar charts (8.2.3).
- **Numerical statistics**: Present the frequencies using the contingency table values.
- **Layering tables**: Differentiates between members of different groups (8.2.8).

Tests / Measurements:

- **Cross-tabulation** (8.2.4) is the process of creating a *contingency table* from lists of paired observations.
- **Contingency table** statistics (3.7.4, 8.2.4) provide tests for the *existence* of an association between factors, in addition to measures for *strength* of this association and *agreement* between values.
- Identify a **progressive** association between ordinal factors (8.2.5).
- **Data consolidation** (8.2.6) can be used to vary the detailed objective of the analysis.
- Due to **low count values** it may be necessary to combine factor levels together or to use a Monte Carlo analysis (8.2.7).
- **2 × 2 binary** tables are effectively two *proportions* and are considered in 6.1.7 and 6.2.9.

The basic tests for the *existence* of an association in contingency tables are developed in 3.7.4 and Section 4.2:

- Pearson's chi-square, χ^2, and the likelihood ratio, *G*, test for an association between *nominal* factors, but they do not test for any sense of progression from one category to the next.
- Yates continuity correction is used when the degrees of freedom in the calculation is equal to 1.0.
- Fisher's exact test (4.2.3) provides the exact binomial test for association in a 2×2 table, which can also be viewed as a test for a difference in *proportion* between rows or columns.
- The linear by linear (Mantel–Haenszel) association (4.2.4) tests for a progressive *linear* association between the values of two *interval* variables, measured using Pearson's correlation coefficient.

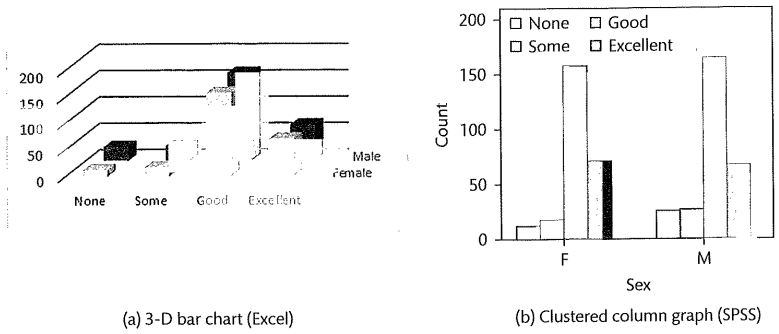
Measures for the *strength* of association between nominal factors include:

- Phi and Cramer's V (4.3.3) take into account the number of data values involved.
- Goodman and Kruskal's Lambda (4.3.4), Gamma and Somer's D (4.3.5), Kendall's tau-b (4.3.5), Spearman's, ρ, and Pearson's r measure the strength of association of ordinal and interval variables.
- Eta, nominal-by-interval association, (4.3.6) measures the strength of association between nominal and interval factors.

Measures for *agreement* between nominal factors include:

- Kendall's coefficient of concordance (4.4.3) and Cohen's and Fleiss's kappa (4.4.4) measure the amount of *agreement* between related variables.
- McNemar's and Cochran's Q tests (4.4.5) measure the agreement between *binary* data values.

8.2.3 Describing the data



(a) 3-D bar chart (Excel)

(b) Clustered column graph (SPSS)

Fig 8.11 Plotting categorical data

The 3-D bar chart in Excel is a very convenient way of visualizing the frequency values in each category.

Excel > Insert > Column chart ▼ Select from the 3-D options
> Select data source:
Chart data range: B3:E4 (from Fig 8.9)
Edit each data series by entering series names: Male/Female
Edit horizontal labels by entering B1:E1 as the axis label range
→ Output: Fig 8.11(a)

Clustered column graphs also show the grouping of data within the categories:

Minitab

Minitab > Graph > Bar Chart...
Bars represent ▼ Values from a table
One column of values: *Cluster*
Graph variables: *Freq*
Categorical variables for grouping: *Sex Improv*
→ Output: Similar to Fig 8.11(b)

For SPSS it is necessary to ***weight*** each row by the *Freq* values, using the method given below in 8.2.4, and then:

SPSS > Graphs > Legacy Dialogs > Bar...
Clustered and then
> Define
Category Axis: *Sex*
Define Clusters by: *Improv*
→ Output: Fig 8.11(b)

8.2.4 Contingency tables and cross-tabulation

We may already have the data in terms of frequency values within a contingency table. If so, it is possible to use Minitab for a basic chi-squared analysis by entering a table of *frequency values*, as in B3:E4 in Fig 8.9, *directly* into the worksheet, and using the selection 'Summarized data in a two-way table' in either of menu selections:

Minitab > Stat > Tables > Chi-Square Test for Association... or
Minitab > Stat > Tables > Cross Tabulation and Chi-Square...
and then: **Columns containing table**: Enter the columns for the table

The full range of analyses in SPSS is only available using *crosstabs* which require every observation to be listed separately. For example, for the data in Fig 8.9 with columns for *Sex*, *SexN*, *Improv*, *ImprovN*, it would require:

13 rows with values 'F, 1, N, 1',
18 rows with 'F, 1, S, 2',
159 rows with 'F, 1, G, 3', etc.

The need for individual observations can be avoided in SPSS by using the process of 'weighting' to enter each combination only once, but identify the frequency with which each row is to be 'duplicated'. Each different combination of values, corresponding to one cell in the table, is entered in a separate row as in columns H to K and the frequencies for each particular combination cell are entered as *Freq* values in a separate column, L.

Crosstabs and contingency tables (Minitab): Analyses for Figs 8.12/13/14/15. See also 3.7.4. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Crosstabs and contingency tables (SPSS): Analyses for Figs 8.12/13/14/15/16. See also 3.7.4. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

After running the instruction to weight cases:

SPSS > Data > Weight Cases...
◉ Weight cases by
Frequency variable: *Freq*

each row would be treated as though it was repeated the number of times given by the *Freq* value.

We now use cross-tabulation, or *crosstabs*, which is the process of counting the numbers of observation *pairs* that fall into each cell of a contingency table, and allows related data values, such as *Q1* and *Q2*, to be analysed using contingency table statistics.
In SPSS:

SPSS > Analyze > Descriptive statistics > Crosstabs ...

and Minitab

Minitab > Stat > Tables > Cross Tabulation and Chi-Square...

and then both:

Rows: Select the factor to define rows of the table. e.g. *Sex* or *SexN* or *Q1*

Columns: Select the factor to define columns of the table. e.g. *Improv* or *ImprovN* or *Q2*

Layer: If required it is possible to group the data into 'layers' using a further factor. e.g. *Grp*

The reason for entering a *numeric* value is to enable the factor to be treated as an ordinal value with a sense of *progression* from one category to the next (8.2.5). Having 'set up' the contingency table, the menu options then allow the choice of a variety of analysis options summarized above in 8.2.2.

SPSS:

> **Statistics:** Select specific analyses as required:
☑-**Chi-square** ☑-**Correlation** ☑-**Phi and Cramer's V**
☑-**Lambda** ☑-**Gamma** ☑-**Somer's d**
☑-**Kendall's tau-b** ☑-**Kappa** ☑-**McNemar**
> **Cells:** Check for required output, e.g.
☑-**Observed counts** ☑ **Expected counts**

Minitab:

> **Chi-Square...** Check for required output, e.g.
☑-**Chi-square analysis** ☑-**Expected cell counts**
> **Other Stats...** Select specific analyses as required:
☑-**Fisher's exact test for 2×2 tables** ☑-**Cramer's V-square statistic**
☑-**Goodman and Kruskal lambda and tau** ☑-**Measures of concordance for ordinal categories**

The results from these analyses are given in Figs 8.12/13/14/15.

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

     None, N  Some, S  Good, G  Excellent, E  Total
1         13       18      159            72    262
       18.58    21.91   154.82         66.69
       1.675    0.699   0.113          0.423

2         26       28      166            68    288
       20.42    24.09   170.18         73.31
       1.524    0.636   0.103          0.384

Total     39       46      325           140    550

Chi-Sq = 5.556, DF = 3, P-Value = 0.135
```

(a) 'Contingency table' case study (Minitab)

Q1 * Q2 Crosstabulation

			Q2		
			N	Y	Total
Q1	N	Count	24	19	43
		Expected Count	20.2	22.8	43.0
	Y	Count	7	16	23
		Expected Count	10.8	12.2	23.0
Total		Count	31	35	66
		Expected Count	31.0	35.0	66.0

(b) 'Forensic' case study (SPSS)

Fig 8.12 Contingency table calculations

The contingency table output from Minitab in Fig 8.12(a) prints the observed value, the expected value, and contribution to the chi-squared value for each cell (e.g. 13, 18.58, and 1.675 respectively in the top left cell). The sum of the individual contributions give a total chi-squared value, $\chi^2 = 5.556$ (Eqn 3.19) which, for degrees of freedom, $df = 3$ (Eqn 3.20), gives a p-value = 0.135.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3.875[a]	1	.049		
Continuity Correction[b]	2.923	1	.087		
Likelihood Ratio	3.958	1	.047		
Fisher's Exact Test				.070	.043
McNemar Test				.029[c]	
N of Valid Cases	66				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 10.80.
b. Computed only for a 2×2 table
c. Binomial distribution used.

Fig 8.13 Hypothesis tests for significant association between *Q1* and *Q2* including McNemar's test (SPSS)

For the 'Forensic questionnaire' case study, SPSS gives the contingency table for the 'Forensic' case study as part of its output in Fig 8.12(b), and, in Fig 8.13, the calculations for the basic 'chi-squared' tests from Section 3.7, using the observed and expected counts:

Pearson's chi-square, Eqn 3.19:

$$\chi^2 = \frac{(24-20.2)^2}{20.2} + \frac{(19-22.8)^2}{22.8} + \frac{(7-10.8)^2}{10.8} + \frac{(16-12.2)^2}{12.2} = 3.9$$

Yates continuity correction, Eqn 3.21:

$$\chi^2 = \frac{(3.8-0.5)^2}{20.2} + \frac{(3.8-0.5)^2}{22.8} + \frac{(3.8-0.5)^2}{10.8} + \frac{(3.8-0.5)^2}{12.2} = 2.9$$

Likelihood ratio, Eqn 3.22:

$$\chi^2 = 2\times\left\{24\ln\left(\frac{24}{20.2}\right) + 19\ln\left(\frac{19}{22.8}\right) + 7\ln\left(\frac{7}{10.8}\right) + 16\ln\left(\frac{16}{12.2}\right)\right\} = 4.0$$

The p-value for Fisher's exact test is derived in 4.2.3 and a different (not binomial) calculation for McNemar's test in 4.4.5 gives a similar value of $p = 0.031$.

In terms of deciding on the significance of an association, the results in Fig 8.13 appear to be contradictory. The basic chi-squared test and the likelihood ratio both suggest significance, however their reliability is suspect for 2×2 tables with just one degree of freedom. The Yates continuity correction and the Fisher's exact test both give more cautious results, suggesting that there is not enough real evidence for a significant association.

The interesting result is for the McNemar test which is actually testing for a difference in the way the respondents change their minds between questions (4.4.5). The result concludes that significantly more people change their mind, *Q1* to *Q2*, from N to Y (19) than from Y to N (7).

Figs 8.14 and 8.15 give the additional symmetric and directional measures of association calculated in Sections 4.2 and 4.3. Minitab produces the same values as SPSS except that it gives Cramer's V *squared* = $0.2423^2 = 0.587$.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Nominal by Nominal	Phi	.242			.049
	Cramer's V	.242			.049
Ordinal by Ordinal	Kendall's tau-b	.242	.117	2.049	.040
Measure of Agreement	Kappa	.226	.111	1.968	.049
N of Valid Cases		66			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Fig 8.14 Symmetrical measures of association between *Q1* and *Q2* (SPSS)

Directional Measures

			Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Nominal by Nominal	Lambda	Symmetric	.093	.116	.766	.444
		Q1 Dependent	.000	.000	.[c]	.[c]
		Q2 Dependent	.161	.194	.766	.444
	Goodman and Kruskal tau	Q1 Dependent	.059	.057		.051[d]
		Q2 Dependent	.059	.056		.051[d]
Ordinal by Ordinal	Somers' d	Symmetric	.242	.117	2.049	.040
		Q1 Dependent	.231	.113	2.049	.040
		Q2 Dependent	.254	.122	2.049	.040

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Cannot be computed because the asymptotic standard error equals zero.

d. Based on chi-square approximation

Fig 8.15 Directional measures of association between *Q1* and *Q2* (SPSS)

8.2.5 Progression within the table

	A	B	C	D	E	F	G	H	I	J	K	L
1		1	2	3	4	Totals:		1	2	3	4	Totals:
2	1	13	18	159	72	262		13	159	18	72	262
3	2	26	28	166	68	288		26	166	28	68	288
4	Totals:	39	46	325	140	550		39	325	46	140	550

Fig 8.16 Contingency tables with categories swapped

The table B2:E3 in Fig 8.16 reproduces the data from Fig 8.9 with *ordinal* values describing the *progression* between levels of improvement, *ImprovN*. In Fig 8.12(a) the result, $p = 0.135$, suggests that there is no significant improvement factor, but we now take into account the fact that there is a sense of progression in *ImprovN*, and carry out an analysis using SPSS as in 8.2.4, requesting the statistics of:

☑-Chi-square ☑-Correlation ☑-Kendall's tau-b ☑-Eta

and find that:

Pearson's chi-square		$p = 0.135$
Linear by linear association, Eta		$p = 0.027$
Interval by interval	Pearson's correlation	$p = 0.027$
Ordinal by ordinal	Spearman's correlation	$p = 0.049$
	Kendall's tau-b	$p = 0.047$

The analysis gives the same p-value for the chi-square value (for an association between *nominal* levels), but the linear by linear association test gives $p = 0.027$, which suggests that there is indeed a progressive association between the *ordinal* levels. The same p-value occurs for the Pearson's measurement of linear correlation, and the nonparametric measurements of correlation also record significant associations. The direction of correlation can be assessed by the correlation coefficients, which are negative, indicating that the improvement obtained by females is significantly greater than that by males.

As a confirmation of the effect of the ordinal measures, the second table H2:K3 in Fig 8.16 has the same values as in B2:E3 but the columns 2 and 3 have been reversed. Applying the same analysis to this table we obtain the *same* significance value for chi-square, $p = 0.135$, in respect of a possible difference between rows in the *distribution* of values across columns. However, the tests for a *progression* in values across the columns now show that there is no longer a significant relationship between *ImprovN* and *SexN*, with the linear by linear association, $p = 0.270$, and Spearman's rho and Kendall's tau-b, $p = 0.279$.

This analysis shows that even when the standard chi-squared test fails to find a significant difference, it is possible to test specifically for a progressive (or correlation) relationship between the two variables.

8.2.6 Data consolidation

It is important here to be quite clear about the *scientific* conclusion that can be drawn from the *statistical* results of the contingency table in Fig 8.12(a). In this case, the *non-significant* result of $p = 0.135$, actually answers the question 'Is there a difference in the *distribution* of responses by male and female patients over the *four* categories?' and reports that the observed distribution could have been observed by chance.

However, if our research is *exploratory*, we would be entitled to ask whether different *questions* might have given different conclusions. For example, we can ask the question 'Is there a difference between male and females in whether the improvement can be rated good or not?', in which case, *N* and *S* would combine to a 'No' category and *G* and *E* would combine

to a 'Yes' category and giving the 2×2 table B8:C9 in Fig 8.9. Fisher's exact test for two proportions (3.8.3) then gives a significant result of $p = 0.025$ for a difference between males and females who show *good* improvement.

The process of *combining* factor levels increases the frequencies in the remaining cells of the table thereby increasing the *power* of the analysis, but at the same time reduces the range of questions that can be asked of that data. However, it is clearly not appropriate to claim a significant effect just because you have sliced up the data in different ways until you get p less than 0.05. This is acceptable as an exploratory tool, but if you believe that you have found a possible significance, the next step would be to redesign and rerun the data collection focussing on the new hypothesis for confirmation.

Data consolidation is also used below in dealing with the problem of too *few* observations in too *many* factor categories.

8.2.7 Low expected frequencies

	A	B	C	D	E	F	G	H
1	Score:	-3	-2	-1	0	+1	+2	+3
2	G1	1	1	2	7	10	10	5
3	G2	3	3	5	9	8	1	1
4								
5	Score:			-3/2/1	0	+1	+2/3	
6	G1			4	7	10	15	
7	G2			11	9	8	2	

Fig 8.17 Questionnaire scores on a Likert scale of –3 to +3 for question *Q6*

If we take crosstabs between the group, *Grp*, and the response, *Q6*, from Fig 8.10, we obtain the contingency table A1:H3 in Fig 8.17, in which rows 2 and 3 give the number of responses from two groups *G1* and *G2* to question *Q6* on a Likert scale of –3 to +3. If we perform a chi-squared analysis on the table to test whether the distribution of values in row 2 is different from the distribution in row 3, we get $p = 0.038$, which suggests that there is a difference. However we also get a warning that there are eight cells with expected counts of less than five, which means that we cannot be confident that the p-value is reliable.

As the Likert scale is for an *ordinal* value, we could use data consolidation (8.2.6) by combining the –3, –2, and –1 results and the +2 and +3 results as shown in rows 6 and 7. The chi-squared analysis then gives $p = 0.004$, confirming a significant difference between the groups. Although this gives a significant result for the new range of answer categories, it is necessary to be cautious and avoid 're-grouping the answers' until the p-value becomes less than 0.05! However, it is a useful result for exploratory data as it can help design more effective and focussed questions in any future questionnaire.

The situation is different if the categories are *nominal* values (e.g. observations of different animal species), in that we may not be able to combine them into meaningful groups. In this case we can use the Monte Carlo analysis (3.9.3) which employs a resampling technique to estimate a p-value, even for low expected frequencies. Using the Monte Carlo option in SPSS:

SPSS > Analyze > Descriptive statistics > Crosstabs ...
Row(s): *Grp* **Columns(s):** *Q6*
> Exact: ☑-Monte Carlo
> Statistics: ☑-Chi-squared
→ Output: Fig 8.18

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Monte Carlo Sig. (2-sided)		
				Sig.	99% Confidence Interval	
					Lower Bound	Upper Bound
Pearson Chi-Square	13.353[a]	6	.038	.030[b]	.026	.035
Likelihood Ratio	14.807	6	.022	.042[b]	.037	.047
Fisher's Exact Test	13.191			.028[b]	.024	.032
N of Valid Cases	66					

a. 8 cells (57.1%) have expected count less than 5. The minimum expected count is 1.82.
b. Based on 10000 sampled tables with starting seed 508741944.

Fig 8.18 Monte Carlo analysis for chi-squared statistics

Fig 8.18 gives the calculated value for Pearson's chi-squared, $p = 0.038$, but it also gives a 99% confidence interval range for the p-value calculated by the Monte Carlo method, between 0.026 and 0.035, which confirms the significant association.

8.2.8 Layered contingency tables

It is possible that the data used to generate a contingency table can be divided into subgroups according to another variable in the data. For example, in Fig 8.10 the people answering questions *Q1* and *Q2* can be identified as belonging to either group, *G1* or *G2*, and we may wish to investigate whether there is a difference in the association of *Q1* and *Q2* between the two groups. We can do this by producing contingency tables for each sub-group by entering *Grp* into the **Layer** option in the analysis.

The results of layering by *Grp* for *Q1*/*Q2* is given in Table 8.1.

Table 8.1 Results from layered contingency tables

Group	Pearson's chi-squared	Fisher's exact test	McNemar's test
Combined G1 and G2	$p = 0.049$	$p = 0.070$	$p = 0.029$
G1	$p = 0.102$	$p = 0.219$	$p = 0.000$
G2	$p = 0.029$	$p = 0.057$	$p = 0.508$

Using Fisher's test for the 2×2 contingency table there is no p-value less than 0.05, although it is possible to see a difference in the results given for the two groups. However,

the interesting statistics are the results of McNemar's test which identifies differences in the numbers of respondents *changing* their answers in different directions. It is significant ($p < 0.0005$) that the majority of changes in group *G1* are 16 from *N* (for *Q1*) to Y (for *Q2*) with only one in the opposite direction, whereas there is no significant difference ($p = 0.508$) for *G2*, which suggests that the intervention occurring between *Q1* and *Q2* is having an effect on how those in *G1* answer the question, but not on those in *G2*.

8.3 Binary output data

Binary data has just two possible categories that could be defined in a variety of ways: True/False, Heads/Tails, Yes/No, 0/1, etc. Many important scientific outcomes depend on the *probability* of finding an individual in one of the two possible states. For example, this could be in the diagnosis of whether a single patient is suffering from a particular disease or not, and then, for a whole population of individuals, this translates into an expected *proportion* of people with this disease. This section develops statistical models that predict the underlying binary probabilities.

8.3.1 Example data

The analysis of binary *proportions* using Minitab and SPSS is introduced in 6.1.7 and 6.2.9, with reference to the underlying statistics developed in Section 3.8, and the use of 2×2 contingency tables for binary data developed in Section 8.2.

Two examples of data with *binary* output values dependent on *interval* input variables are given in Figs 8.19 and 8.20.

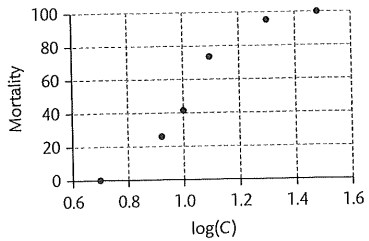


Fig 8.19 Percentage *mortality* of nematodes as a function of the log of a drug concentration

Worksheet 1 ***

→	C1	C2-T	C3	C4	C5	C6	C7	C8
	RecNo	Group	v1	v2	v5	v6	v9	EPRO1
1	1	A	74.7	41.4	56.1	68.2	20.8	0.785
2	2	B	89.5	30.1	39.7	51.1	23.8	0.139
3	3	A	61.7	22.9	48.5	67.9	49.2	0.974
4	4	A	77.2	35.7	57.3	64.4	50.2	0.931
5	5	B	70.5	14.8	41.8	46.6	9.3	0.069
6	6	A	72.6	47.0	64.5	70.5	48.9	0.965

Fig 8.20 Variables, *v1*, *v2*, *v5*, *v6*, *v9*, and *EPRO1* (in Minitab 16 or FITS1 in Minitab 17) as possible *predictors* of group *A* or *B*.

In Fig 8.19 the binary state, alive or dead, of *individual* nematodes determines the percentage of dead nematodes (mortality) in an overall *population*. The 'LC50' case study (8.3.3) develops nonlinear regression techniques to analyse this data.

Fig 8.20 shows the first 6 of 100 subjects from the 'Screening test' case study (8.3.4) which develops binary regression and ROC curves to model the prediction of the group *A* or *B* of an individual subject as a function of the interval variables, *v1*, *v2*, *v5*, *v6*, and *v9*.

8.3.2 Analytical options

For binary variables, we analyse the *probability* of individuals being in a particular state. If we are considering a sample of *n* individuals, or items, each of which may be in one of two states, then this probability determines the overall *proportion* of individuals/items that are in a particular state.

Describing data

- Techniques for describing categorical data are given in 8.1.3 and 8.2.3. We introduce here deviance curves (Fig 8.25) and ROC curves (Fig 8.27).

Tests / Measurements:

- Fisher's exact binomial tests to compare **proportions** with a target value or with another proportion (Section 3.8, 4.2.3, 6.1.7, 6.2.9).
- **Probit and logit** transformations of a proportion (or percentage) to a linear function (8.3.3).
- **Binary regression** to develop a model to predict binary probability (8.3.4).
- Use of **ROC curves** for the relationship between sensitivity and specificity (8.3.5).

8.3.3 Logit and probit linearization

The following case study considers the *probabilities* of individuals being in a specific state, and the consequential *proportion* of a population in that state.

Case study: LC50 / Logit and probit

In preparation for a chemotaxis measurement (6.3.1), it is necessary to find the LC50 value for the concentration of a chemotherapeutic drug which kills 50% of nematodes within a specified time. The percentage dying (mortality) for a range of relative concentrations, C, of a standard solution is recorded in Fig 8.19, and we wish to use a process of 'regression' to obtain a best-fit relationship that allows us to estimate the concentration at which 50% of the nematodes die.

Logit and probit: Excel analysis for Fig 8.22.
Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

Mortality is the overall *proportion*, *P*, (or percentage, *P%*) resulting from the binary condition that each nematode in the population is either alive or dead. The graph in Fig 8.19 records mortality plotted against the *log* of the concentration because it is often convenient

to use log to base 10 for the x-axis as the *unit* values then give the concentration axis displayed in 'powers of 10'.

A typical calculation derives the LC50 value which is the concentration at which 50% ($P = 0.5$) of the population has died, and to do this we need to derive a best-fit line through the data points and calculate the concentration at which the proportion equals 50%. The problem is that we do not have an easy way of fitting a best-fit equation to such a curve, as the relationship between proportion, P, and the log of the concentration, log(C), is clearly nonlinear. In fact the relationship cannot be linear because log(C) can have theoretical values from $-\infty$ to $+\infty$ whilst the values of P are limited by 0 and 1.

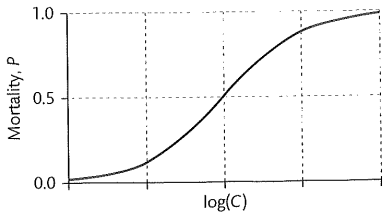


Fig 8.21 Sigmoid curve

The data in Fig 8.19(a) can be modelled by the mathematical *sigmoid* curve in Fig 8.21, which is given by the logistic function

$$P = \frac{e^x}{e^x + 1} = \frac{1}{1 + e^{-x}}$$

where P is the proportion (= P%/100) and x is a value proportional to log(C).
There are two transformations that can be used to linearize the data in Fig 8.19(a):

Logit(P) is the actual inverse of the above logistic function:

$$\text{logit}(P) = \ln\left(\frac{P}{1-P}\right) \tag{8.1}$$

which gives a value of 0 when $P = 0.5$ (50%).

Probit(P) is an alternative transformation that was developed for this type of problem, with values derived from a set of probit tables. However the values can also be calculated by using the inverse of the normal distribution in Excel:

$$\text{Probit}(P) = 5 + \text{NORM.S.INV}(P) \tag{8.2}$$

which gives a value of 5 for $P = 0.5$ (50%). The '5' was included to avoid negative values (as occur with Logit) which were thought to cause confusion.

The logit transformation has a more widespread use in software analysis, but the probit analysis has been developed for quick dose–response calculations of LC50/LD50 using published tables. We can compare their use with the following analysis given in Fig 8.22.

In the experiment, initial measurements were made by diluting a standard drug solution giving concentrations of 0, 1%, 5%, 10%, and 20%, but, when it was observed that the LC50

	A	B	C	D	E	F	G	H
1	Conc, C%	log(C%)	Alive	Dead	Prop, P	Corr, P	Logit(P)	Probit(P)
2	0		95	1	0.010			
3	1	0.00	88	2	0.022			
4	5	0.70	82	0	0.000			
5	8.3	0.92	67	23	0.256	0.248	-1.11	4.32
6	10	1.00	59	42	0.416	0.410	-0.37	4.77
7	12.5	1.10	29	81	0.736	0.734	1.01	5.62
8	20	1.30	0	92	1.000			

Fig 8.22 Numbers of nematodes killed by different concentrations of a drug

value falls between the 5% and 20% values, further measurements were made at 8.3% (1/12) and 12.5% (1/8).

The proportion dead (mortality) at each concentration is calculated in column, E, e.g.

Mortality proportion: [E2] = D2 / (C2 + D2)

The proportion, P_0, for zero concentration (in E2) is used as a 'control' for the nematodes that die independently of the treatment. The values in column F then give the corrected proportion using Abbott's formula which includes the 'control' data:

$$\text{Corrected, } P = 100 \times \frac{P - P_0}{100 - P_0}. \tag{8.3}$$

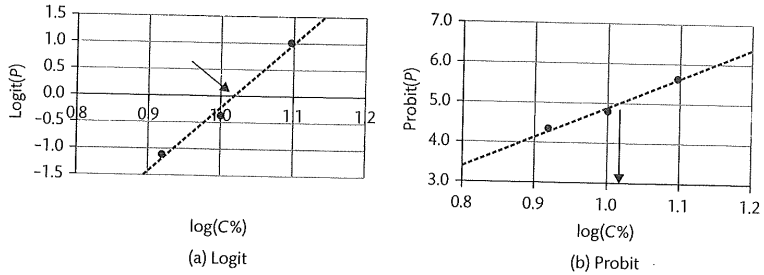


Fig 8.23 Logit and probit transformations

The two transformations, Eqn 8.1 and Eqn 8.2, give the results in columns G and H respectively, which now both give best-fit *straight* lines in Fig 8.23. The intercepts at both logit(P) = 0, and probit(P) = 5, give the log of the LC50 concentration as 1.02 from which $LC50 = 10^{1.02} = 10.5\%$.

It is important to note that, for the type of problem in this case study, proportion values close to 0 (0%) or 1.0 (100%) will be dependent on the states of a very few individuals, alive or dead, which can lead to large *relative uncertainties* at these limits. For this reason we have only used proportions between 25% and 75%.

Binary regression: Minitab analysis for Fig 8.24. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

8.3.4 Binary regression

In 8.3.3 we used a data transformation to help us predict, given a specific drug concentration, whether the probability of nematodes being alive or dead was greater or less than 50%. We now develop this into a more sophisticated regression model that enables us to predict actual *probability* values.

Case study: Screening test / 3. Binary regression

–continued from 9.1.4, leading to 8.3.5

In 9.1.4 we use principal component analysis to derive the elements of the principal components, PC1, PC2, etc. that can describe the separation between the two subject groups, *A* and *B*. These groups are given in Fig 8.20, together with the values of the variables, *v2*, *v5*, *v6*, and *v9*, which are identified as the elements of the main principal component, PC1. We wish to develop a mathematical model in which we can use these values to predict the state of a given *individual* subject.

The first step in modelling binary outcomes, is to develop a regression equation that relates the binary *probability* to the values of one or more predictor variables. Section 2.3 introduces the use of transformations to *linearize* a regression equation, and we meet the concept of *multiple regression* in 9.1.6 in which several variables are used to predict the value of a single outcome. Combining these techniques we start by using the logit transformation from Eqn 8.1 for the probability, *P*, and expressing this in a similar way to Eqn 9.1:

$$\text{logit}(P) = \ln\left(\frac{P}{1-P}\right) = B_0 + B_1x_1 + B_2x_2 + B_3x_3 + \cdots . \tag{8.4}$$

The coefficients of the equation, B_0, B_1, B_2, B_3... define the relative contributions from each *x* variable.

When combining probabilities it is useful to use the concept of odds, the values of which are given by the ratio P/(1-P), and we can express the equation in odds simply by taking the exponential of both sides of the equation. The overall odds are then the *product* of power factors:

$$\text{Odds} = \frac{P}{1-P} = e^{B_0+B_1x_1+B_2x_2\cdots} = e^{B_0} \times \left(e^{B_1}\right)^{x_1} \times \left(e^{B_2}\right)^{x_2} \cdots \tag{8.5}$$

The process of regression calculates the values of the coefficients in the equation that produce the best fit to the experimental data. A binary *regression* uses an iterative maximum likelihood procedure (2.4.2) to get the best-fit of a model to the data and calculate the coefficients in Eqn 8.4.

In the following analysis we choose *not* to use variable *v1*, because this is not identified (9.1.4) as one of the cluster of variables that best predict the subject group probability. We compare our results here with *v1* in Fig 8.27.

Minitab

Minitab > Stat > Regression > Binary Logistic Regression > Fit Binary Logistic Regression Model ...

▼ *Response in binary response / frequency format*

Response: *Group*

Response event: *A* (makes *A* the value of *Group* for probability = 1.0)

Continuous predictors: *v2 v5 v6 v9*

> Options...: Choose link function, e.g. **Logit**

> Storage...:

☑**-Fits (event probabilities)** - stores the probabilities for each subject in a new variable FITS1 (EPRO1 in Minitab 16)

☑**-Delta deviance** - stored as new variable DDEV1

→ Output: Fig 8.24

```
Link Function: Logit

Response Information
Variable  Value  Count
Group     A         50  (Event)
          B         50
          Total    100

Logistic Regression Table

                                                    Odds    95% CI
Predictor       Coef    SE Coef      Z      P      Ratio  Lower  Upper
Constant    -12.7234    2.90043  -4.39  0.000
v2        -0.0573760  0.0431040  -1.33  0.183   0.94   0.87   1.03
v5         0.0368401  0.0643224   0.57  0.567   1.04   0.91   1.18
v6          0.190982  0.0782711   2.44  0.015   1.21   1.04   1.41
v9         0.0591785  0.0352363   1.68  0.093   1.06   0.99   1.14

Log-Likelihood = -33.027
Test that all slopes are zero: G = 72.576, DF = 4, P-Value = 0.000
```

Fig 8.24 Extract of binary regression results (Minitab)

This first part of the results in Fig 8.24 confirms that the *link function* is given by the logit function. It then gives the observed numbers in each of the two categories *A* and *B* and shows that *A* has been defined as the 'event' related to a probability of 1.0.

The results then give the coefficients $B_0 = -12.72$, $B_1 = -0.0574$ etc. for the logit(*P*) equation:

$$\text{logit}(P) = \ln\left(\frac{P}{1-P}\right) = -12.72 - 0.0574 \times v_2 + 0.0368 \times v_5 + 0.1910 \times v_6 + 0.0592 \times v_9$$

If we now consider the first subject, *RecNo* = 1 in Fig 8.20, we can use the logit(*P*) equation to predict its group. We enter the variable values of *v2* = 41.2, *v5* = 58.1, etc. into the above equation and we get:

$$\text{logit}\,(P) = 1.303$$

It is then possible to calculate the probability, P, that this particular subject is in group A by evaluating

$$P = \frac{e^{\text{logit}(P)}}{1+e^{\text{logit}(P)}} = 0.786$$

Hence our model gives a 78.6% probability that subject 1 is in group A, which matches the fact that subject 1 is known to be group A.

Within the analysis, we requested that the 'event probabilities' be stored, and in a new column under the default title *FITS1* (or *EPRO1* in Minitab 16), we see that, for subject 1, the result 0.785 in Fig 8.20 agrees, within rounding errors, with our calculation above.

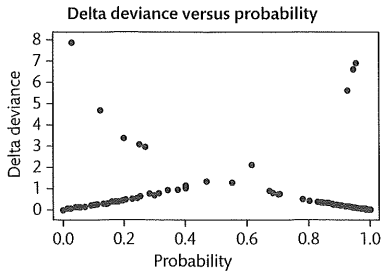


Fig 8.25 Deviance when using the variables *v2*, *v5*, *v6*, and *v9* as predictors for group *A* or *B* (Minitab)

The other data stored was delta deviance, which we have plotted in an x–y graph against the event probability in Fig 8.25. (This graph can be requested directly in Minitab 16.)

Deviance is a measure of the goodness of fit of the model:

$$D = -2\ln\left(\frac{\textit{Likelihood of fitted model}}{\textit{Likelihood of perfect fit}}\right) \tag{8.6}$$

An overall value for D provides a similar role to R^2 for the goodness of fit for analyses using the least squares method. Values of D can be calculated for individual points and Fig 8.25 shows the variation of D with the individual subject probabilities. If the probability of a subject being in group A is close to 1 and it is in group A (correct), then this will give very low deviance as shown by the data values in the *bottom right* of the plot, but, if a subject with a probability close to 1 is actually in group B, then this is an incorrect prediction and the point will have a high deviance as shown by the few points leading to the *top right* of the diagram. Points from group A form the line from the top left to bottom right and those from group B the line from bottom left to top right. The few points where the prediction fails to put the subjects in the correct groups are those in the top branches of the two lines.

ROC curves: SPSS analysis for Fig 8.27. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

8.3.5 Binary probabilities and ROC plots

In 8.3.4, we developed the mathematics of calculating the *probabilities* of binary values, and it is clear from Fig 8.25 that *decisions* based on those probabilities can produce a percentage of wrong choices. We now introduce a graphical presentation of the process of making choices based on two overlapping probabilities, and the name of the plot, receiver operating characteristics (ROC) derives from its early use to describe the properties of signal detectors in recording either a *true* or *false* signal.

We will illustrate the calculations by considering the medical diagnostic case where the relevant test results, v, of a large survey of *healthy* adults (no disease, $\bar{D}$) are shown to have a mean value of 20 and a standard deviation of 4.0, whereas the test results of another large survey of adults with a *disease* condition, D, have a mean value of 30 and a standard deviation of 5.0. These probability distributions are shown in Fig 8.26(a). This is an idealized example because real diseased states would often show a much larger standard deviation than the healthy state and probably with a pronounced positive skewness.

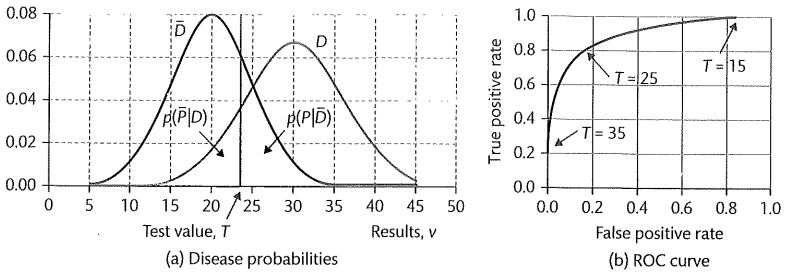


Fig 8.26 Disease probabilities and the associated ROC curve

The aim is to use this data to develop a decision criterion, by which it is possible to put a person into a specific category depending on their personal test result, v. We need to decide on a test value, T, such that we classify the result according to the simple choice:

- If $v > T$ we classify the result as positive (D) and decide that the person *does* have the disease.
- If $v < T$ we classify the result as negative ($\bar{D}$) and decide that the person does *not* have the disease.

It is clear that both distributions have 'tails' that overlap the test value, and, in each case the shaded region represents an *incorrect* classification. This leads to two key performance criteria for a binary test:

- **Sensitivity** of the test is the probability of recording a *positive* result, P, if the person *does* have the disease, $p(P|D)$.
  Sensitivity is the probability, between 0 and 1.0, of a *true positive.*
- **Specificity** of the test is the probability of recording a *negative* result, $\bar{P}$, if the person does *not* have the disease, $p(\bar{P}\,|\,\bar{D})$.
  Specificity is the probability, between 0 and 1.0, of a *true negative.*

It is useful to note that the probability of a *false* positive is given by $p(P\,|\,\bar{D}) = 1.0 - \text{Specificity}$.

We can trace the characteristics of the test in a ROC curve shown in Fig 8.25(b), where we plot the *true positive rate* (sensitivity) against the *false positive* rate (1.0 – specificity) for different chosen values of T.

If the value of T is set low (e.g. $T = 15$) then most measured values of v will result in positive decisions, making the probabilities of *true* positives and *false* positives both close to 1.0. As the value of T is increased the probability of a *false* positive initially drops more quickly than that of a *true* positive, drawing out the line in Fig 8.26(b). At the other extreme, a large value of T (e.g. $T = 35$) will result in most decisions being negative, with low probabilities for both *true* positives and *false* positives.

The optimum choice of test value will aim to maximize the probability of a true positive without unduly increasing the rate of false positives. In the ROC plot, this optimum situation will be a position on the graph towards the top left of the plot, with an intermediate value of T, e.g. $T \approx 25$. The choice of the optimum value for the decision criterion, T, will depend on the relative *consequences* of recording false positives and negatives.

The overall quality of a test is greatest if the curve fits closely into the top left-hand corner of the plot. In this case the area under the curve would approach 1.0. If the test has no diagnostic quality, the characteristic line would be a straight diagonal with a cumulative area of 0.5.

Case study: **Screening test / 4. Binary classification**

–continued from 8.3.4

Referring to the data in Fig 8.20, we now compare the abilities of the variable, *v1*, and the probability, *EPRO1*, derived in 8.3.4 as a combination of *v2*, *v5*, *v6*, and *v9*, to predict the group, *A* or *B*, of a specific subject.

In practice, the characteristics of a diagnostic test are usually developed, not through theoretical modelling as above, but through the analysis of experimental results. Fig 8.27 compares the effectiveness the two variables *v1* and *EPRO1* in diagnosing whether each subject is in group *A* or *B*.

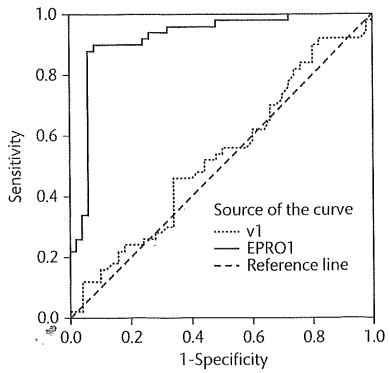


Fig 8.27 ROC curves for *v1* and *EPRO1*

Using SPSS

SPSS > Analyze > ROC curve...
Test variable: *v1 EPRO1*
> State variable: *Group*
Value of State Variable: Identify the 'true' level of the state being tested, e.g. *A*
☑**-ROC curve**
☑**-With diagonal reference line**
→ Output: Fig 8.27

We find in Fig 9.6 that the variable *v1* does not appear in the principal components, PC1 or PC2, and thus has little predictive capability for identifying the group *A* or *B*. This is confirmed in Fig 8.27 in that it lies close to the *neutral* reference line with an area (to the bottom right) of 0.531. However, the calculated probabilities, *EPRO1*, for predicting the group *A* or *B*, produce a line which passes close to the top left-hand corner of the ROC plot with an area of 0.922. This confirms that the probability variable, *EPRO1*, is a good predictor for identifying the group of an individual subject.

The area is not the only important factor, as the relative importance of sensitivity and specificity can depend on the *consequences* of false positives or false negatives, making the *shape* of the curve important.

Multiple variables

Introduction

This chapter considers situations in which the investigation produces several measured variables, which can be confusing for students who have only been introduced to the univariate ANOVA, the two sample *t*-test, and linear regression between two variables. The first section develops techniques of data reduction through principal component analysis, and data modelling through cluster analysis and multiple regression. The second section takes the example of questionnaire data, which could also represent multiple experimental measurements, and identifies a wide range of possible analytical techniques, together with specific references to analyses developed previously. This final section could be used as a review of the analyses and techniques that have been introduced throughout the book.

Section 9.1 develops the statistical analyses in which a data set of *multiple input variables* can be rationalized to create a *model* that predicts the state of a scientific system.

Section 9.2 uses a data set with multiple experimental variables as an example to consider the different analytical *questions* that could be asked, particularly in reference to questionnaire data.

9.1 Modelling multiple variables

As the ability to measure and record multiple variables with greater ease and accuracy continues to increase, we are being faced with very large data sets from which we wish to draw out hidden information. A general type of approach is to investigate whether different *groups of variables* provide related information and then whether it is possible to represent the actions of many variables with a *small number* of composite variables. This is a process of *data reduction*, and we investigate this using both graphical and numerical representations.

9.1.1 Example data

Referring to the screening data in Fig 9.1, other investigations producing a similar structure could include:

- interpreting questionnaire responses by looking for common patterns in the opinions of respondents on nine issues. In this case the data values would probably be on an ordinal scale.
- identifying common survival traits by comparing the occurrences of nine species in different environments. In this case the data values may be counts or frequencies.
- developing a method to identify and classify bacteria using hyperspectral imaging at nine possible wavelengths.

Case study: **Screening test / 1. Clustering of variables and subjects (overview)**

The data in Fig 9.1 shows the measurements of nine variables relating to each of 100 subjects. These were recorded by gas chromatography as part of an investigation to develop a screening sensor to detect human abnormalities by 'sniffing' a range of aromatic compounds released from saliva. Fifty 'normal' subjects are identified in set A and 50 subjects with the abnormal condition in set B. We start in 9.1.3 by using cluster analysis to identify groups of variables and subjects within the experimental data.

9.1.4 / 2. Principal component analysis: Uses PCA to derive the combination of variables that best discriminates between the two main groups of subjects.

8.3.4 / 3. Binary regression: Uses multiple regression to predict the probability (odds) of each individual being in a specific state.

8.3.5 / 4. Binary classification: Develops the ROC curve as a graphical indication of the sensitivity and specificity of a binary choice prediction.

	A	B	C	D	E	F	G	H	I	J	K	L
1	*RecNo*	*Group*	*v1*	*v2*	*v3*	*v4*	*v5*	*v6*	*v7*	*v8*	*v9*	Cluster
2	1	A	74.7	41.4	66.6	30.2	58.1	68.2	69.7	17	20.8	
3	2	B	89.5	30.1	49	21.7	39.7	51.1	47.7	12.5	23.8	
4	3	A	61.7	22.9	46.6	22	48.5	67.9	58	21.7	49.2	
5	4	A	77.2	35.7	72.8	35.1	57.3	64.4	81.7	18.8	50.2	
6	5	B	70.5	14.8	85.3	43.5	41.8	46.5	96.3	13.5	9.3	
7	6	A	72.6	47	59.8	30.9	64.5	70.5	69.1	15.6	48.9	
8	7	A	73.7	44.2	54.7	16.1	54.7	64.9	57.4	20.5	46.4	
101	100	B	89	45.5	67	31.7	49.6	62.2	80.2	20.6	25.5	

Fig 9.1 100 subjects in groups *A* or *B* each recording nine variables, *v1* to *v9* (Rows 9 to 100 are 'hidden' in the worksheet)

We also treat factors and their interactions as multiple variables in the case study:

Boxing performance

9.1.6 / 2. Multiple regression: Uses stepwise regression and general regression to identify the significant factors in a best-fit model.

9.1.2 Analytical options

Data reduction. The overall aim of many investigations is to develop a mathematical model that describes how an observed response variable can be predicted with just a *subset* of a large number of possible input variables. For example, we might know that

a range of symptoms are related to the existence of a disease, but it would be valuable to develop a model which identifies a specific combination of a few symptoms as being strongly predictive of the existence of the disease. There are several steps in the process:

1. Quantifying the relationships between possible input and output variables.
2. Identify *relationships* (e.g. correlations) between variables that are possible predictor variables.
3. Develop a practical model which maximizes the accuracy in *predicting* the output with a *minimum* of input variables.

Cluster analysis (9.1.3) is a technique that enables us to *visualize* how input variables might be grouped together, and in so doing help in simplifying our understanding of the system. It is also possible to use clustering techniques to identify similarities and differences between the *subjects* being measured, and to act as a diagnostic test between different subject states, e.g. between well and ill patients.

Principal component analysis, PCA, (9.1.4) is a technique that is used to both simplify the number of variables that are used to predict the output and also to optimize their relative inputs to the model.

Factor analysis (9.1.5) is a development of PCA that performs additional calculations ('rotations') to derive mathematical 'factors' that are new combinations of the original variables. The advantage of these new factors is that they can provide a more effective way of summarizing the whole data set.

Multiple regression (9.1.6) expresses the response variable in relation to multiple predictor variables, and the analyses in SPSS and Minitab will add and/or reject possible inputs depending on whether they have a significant effect on the response variable.

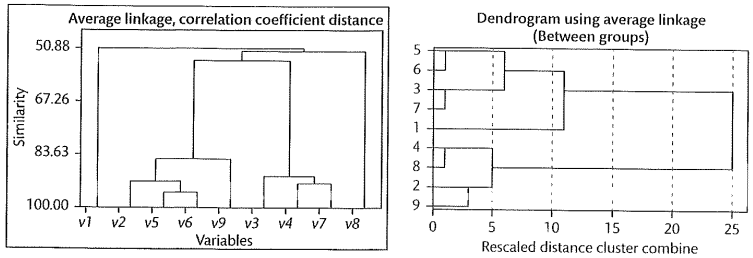
9.1.3 Cluster analysis

Cluster analysis: Minitab and SPSS analyses for Fig 9.2. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

It is very difficult to see any patterns within a table of *numbers*, but, if these numbers can be converted into suitable *graphics*, then the human brain is very good at picking out visual patterns. Clustering is a useful method of identifying and displaying groups within the data, occurring in two main forms:

- Hierarchical clustering looks for groupings either between different experimental *variables* or between different *records* (also called *subjects*, *cases* or *observations*) in the data set.
- *K*-means clustering looks for *groupings* of *records* in an 'area' defined by the calculated components or factors.

We use the data in Fig 9.1 to introduce hierarchical clustering, with the immediate objective of identifying which *combinations* of the nine compounds respond in similar ways. Using analytical software, the *hierarchical cluster analysis* analyses the relationships between variables, and produces the dendrogram (from the Greek, *dendron*, for tree) in Fig 9.2 which shows the *similarity* between different variables.



(a) Minitab (b) SPSS

Fig 9.2 Variables cluster dendrogram

Minitab

Minitab > Stat > Multivariate > Cluster Variables...
Variables: *v1-v9*
Linkage method: Try different methods to improve discrimination of variables.
☑ **Show dendrogram**
→ Output: Fig 9.2(a)

SPSS

SPSS > Analyze > Classify > Hierarchical Cluster...
Variables: *v1-v9*
Cluster: Choose to cluster either ◉**-Cases** (subjects, see below) or ◉**-Variables**
Plots...: ☑**-Dendrogram**
> Method...: Try different methods to improve discrimination of variables
→ Output: Fig 9.2(b)

In Fig 9.2(a) all the variables are separated along the 100% baseline, showing that there are *no* variables that are 100% similar. At about 85% similarity, the variables *v2*, *v5*, *v6*, and *v9* form one similar group, and *v3*, *v4*, and *v7* form a second group, but variables *v1* and v8 show little similarity with any other variables.

The dendrogram suggests that variables, *v2*, *v5*, *v6*, and *v9*, are similar measures for one main *component* and *v3*, *v4*, and *v7* are similar measures for a *second* main component, with *v1* and v8 unrelated variables. This interpretation is developed further in the next section.

The two dendrograms in Fig 9.2, although in different orientations, are derived from the same data, using Minitab and SPSS respectively. The reason for the different grouping of variables is due to different default choices in the way in which the statistics links and groups the data values. Fig 9.2(a) uses the Minitab default 'correlation' calculation and Fig 9.2(b) uses the SPSS default 'squared Euclidean distance' calculation. In practice, it is often useful to try different methods to see if they are more effective at grouping the variables. If the correlation calculation is used in SPSS, it produces the same grouping as for Minitab.

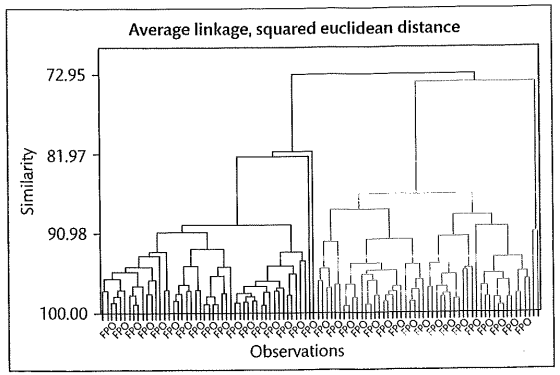


Fig 9.3 Dendrogram separation of subjects into clusters

We can also investigate if there is any clustering of the *subjects* (*records* or *observations*) in the data. Using Minitab we find a good separation into two clusters is achieved in Fig 9.3 by using 'average linkage' with 'squared Euclidean distance'.

Minitab

Minitab > Stat > Multivariate > Cluster Observations...
Variables: *v1-v9*
Linkage method: *Average*
Distance measure: *Squared Euclidean*
Specify final partition by: ◉ **Number of clusters:** Choose 2 in this example
☑ **Show dendrogram**
> Storage...: Enter new column to receive the cluster allocation for each subject
→ Output: Fig 9.3

Fig 9.3 shows the individual subjects along the horizontal axis, but with 100 subjects it is not possible to identify separate labels in this diagram. Nevertheless, we can see from the plot that the subjects split into two clear 'clusters' separated at about 73% similarity.

In SPSS, selecting ◉ **Cases** under 'Hierarchical Clusters' gives the same diagram but it is turned through 90 degrees and stretched vertically to separate the subjects.

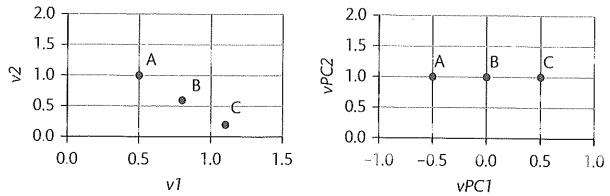
Using the option **> Storage**, Minitab allows us to record the 'cluster' allocation, '1' or '2', for each subject in a new column. We find that 43 group A subjects were correctly allocated to cluster '1' and 44 group B subjects to cluster '2', leaving only seven group A and six group B subjects misclassified.

Principal component analysis: Minitab analysis for Figs 9.6 and 9.7. Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

9.1.4 Principal component analysis

The aim of principal component analysis (PCA) is to describe as much *variability* in the data as possible by using as *few variables* as possible. We introduce the *concept* of PCA with

reference to a very simple data rotation example, using three points, A, B, and C, on the x–y diagram in Fig 9.4(a) which are described by the related variables *v1* and v2 with values (0.5, 1.0), (0.8, 0.6), and (1.1, 0.2) respectively.



(a) Using initial variable values, *v1* and *v2*
(b) Using derived components, *vPC1* and *vPC2*

Fig 9.4 Three data points, A, B, and C, defined by values of the variables, *v1* and v2

	A	B	C	D	E	F	G	H	I	J	K	L
1		*v1*	*v2*		Variable	PC1	PC2	giving:			*vPC1*	*vPC2*
2	A	0.5	1		*v1*	0.6	0.8	*vPC1* = 0.6×*v1* + (-0.8)×*v2*		A	-0.5	1
3	B	0.8	0.6		*v2*	-0.8	0.6	*vPC2* = 0.8×*v1* + 0.6×*v2*		B	0	1
4	C	1.1	0.2							C	0.5	1
5												
6	Initial variables				Transforming variables from *v1/v2* to *vPC1/vPC2*					Principal components		

Fig 9.5 Deriving principal components

We can choose, in Fig 9.5, to calculate two *new* variables, *vPC1* and *vPC2*, called *principal components*, derived from the original variables, *v1* and *v2*, by using the equations in H2 and H3 respectively. These equations are based on the *coefficients* in the table in shaded cells E1:G3. The calculated values of *vPC1*/*vPC2*, equivalent to *v1*/*v2* for each of the three data points, are then given in cells K2:L4.

For example, the value of *vPC1* for the first data point is given by:

$$vPC1 = 0.6 \times 0.5 - 0.8 \times 1.0 = 0.3 - 0.8 = -0.5$$

and calculated in Excel with

[K2] = F$2 * $B2 + F$3 * $C2.

If we now use *vPC1* and *vPC2* to plot the three data points, we produce Fig 9.4(b). We can see that, in this very simple example, the *variability* in *both v1* and *v2* in the original data is now described just by the *variability* in a *single* new variable, *vPC1*, with the other variable, *vPC2*, remaining constant. The use of the new principal components, as combinations of the previous variables, has identified and *simplified* the description of the key *pattern* within the data.

The mathematical operation in the example was a simple rotation of coordinates in *two* dimensions. However, the concept is the same for more than two variables, in that the 'rotation' occurs in a multi-dimensional mathematical space.

Case study: **Screening test / 2. Principal component analysis**

–continued from 9.1.1, leading to 8.3.4

Referring to the data in Fig 9.1, we now endeavour to find up to nine *principal components* that can simplify the description of the variability in the data set.

We apply principal component analysis (PCA) to the data in Fig 9.1:

Minitab > Stat > Multivariate > Principal Components...

Variables: *v1-v9*

> Graphs...: ☑ **Scree plot** and ☑ **Score plot for first 2 components**

> Storage...: Identify (perhaps four) empty columns to receive the PC coefficients as given in Fig 9.6.

Variable	PC1	PC2	PC3	PC4
v1	0.047	0.066	**-0.725**	**0.654**
v2	**0.466**	0.171	0.040	0.128
v3	0.241	**-0.509**	-0.094	0.016
v4	0.201	-0.541	-0.042	-0.045
v5	**0.484**	0.183	-0.029	0.004
v6	**0.482**	0.199	0.017	-0.059
v7	0.178	-0.561	**-0.006**	**-0.006**
v8	0.025	-0.065	**0.673**	**0.721**
v9	0.429	0.153	0.086	**-0.176**

Fig 9.6 Coefficients of the first four principal components

The output gives results in Fig 9.6, similar in format to cells E1:G3 in Fig 9.5, except with up to nine new principal components (although we have only stored the first four, PC1, PC2, PC3, and PC4).

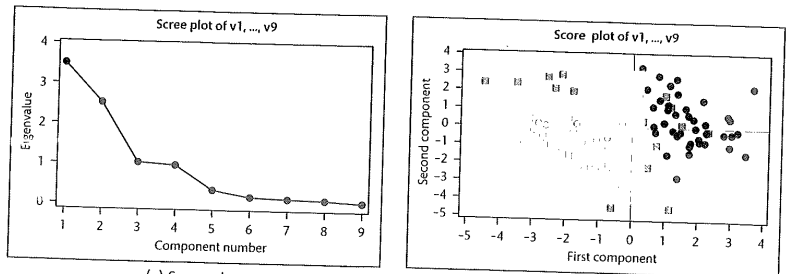
The more significant coefficients in Fig 9.6 are shown in bold print for each of the four principal components, and we see that

- PC1 is mainly a combination of *v2, v5, v6,* and *v9,* and
- PC2 is mainly a combination of *v3, v4,* and *v7.*

which is consistent with the two main groups of variables that we detected by cluster analysis in Fig 9.2. PC3 and PC4 are mainly combinations of the remaining variables, *v1* and *v8.*

The **scree plot** in Fig 9.7(a) shows the contribution (recorded as 'eigenvalues') that each component makes to the *variability* in the overall data, and for this data set we can see that most of the variance can be described by the first two components, PC1 and PC2. This is an example of effective data reduction in which the scree plot drops quickly within the first two or three components before levelling off under an eigenvalue of '1.0'.

The other important plot produced by principal components analysis is the **score plot** shown in Fig 9.7(b) which gives the position of each record (subject) in the data set, plotted using its values of *vPC1* and *vPC2* as the *x–y* coordinates, centralized around zero. It is possible to edit the score plot such that different groups are identified by different markers, giving a good visual picture of differentiation within the data. Although not very clear in Fig 9.7(b), the round markers are from group A and the square markers are from group B, which allows us to see that *most* of group B are to the left of the median value and *most* of group A are to the right, with only a small number misplaced in opposite groups. The first principal component *vPC1* proves to be successful in predicting the group of each subject.



(a) Scree plot (b) Score plot

Fig 9.7 Plots for principal component analysis

The set of values in each principal component can be calculated in the same way as in Fig 9.5. For example, to calculate the values *vPC1* for the first component, we can use

Minitab > Calc > Calculator...

as shown in Fig 9.8 to store the values in a new column which we could call *vPC1.* For each row of data, the expression multiplies the PC1 coefficient in row 1 by the *v1* value, PC1(1)**v1*, and adds the multiplication of the PC1 coefficient in row 2 by the *v2* value, PC1(2)**v2*, and so on.

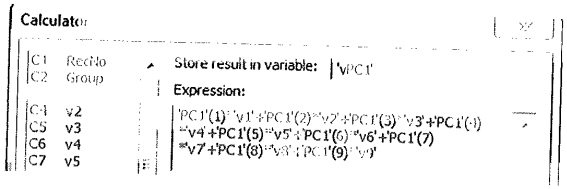


Fig 9.8 Calculation of *vPC1* values using the coefficients of PC1

9.1.5 **Factor analysis**

We introduced the concept of principal component analysis by giving the simple example of rotating axes in Fig 9.4. The technique of factor analysis performs a more complex mathematical 'rotation' which ensures that the variability in the original data is shared out *equally* between the new factors.

Factor analysis in data analysis software usually offers different types of 'rotation', but the 'varimax' rotation is often a suitable choice as a default starting point. However, in the particular example above, we would find that the effect of the data 'rotation' in factor analysis makes relatively little difference in differentiating between groups.

Multiple regression (Minitab): Analysis for Fig 9.10(a). Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

9.1.6 Multiple regression

The general concept of regression in *statistics* mirrors the desire in many *scientific* investigations to describe how one (or more) variables are dependent on, and may be predicted by, other variables. For this reason, we see regression at the core of many statistical models used to describe our scientific world.

Multiple regression develops an equation that models the output (response) variable based on *several* input (predictor) variables. The software analyses have the ability to 'test' the significance of a given input variable and either include or reject it as a useful addition to the overall model.

For **basic linear regression** see Chapter 2, with the development of the statistics with which an independent variable, x, is assumed to *predict* the value of the dependent variable, y. These statistics find application in a very wide range of situations in science, e.g. calibration.

We see (2.1.1) that the behaviour of y as a simple function of x can be written as

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

and that the process of linear regression calculates the best-fit values for the coefficients of slope, m or b, and intercept, c or b_0. If a system responds to more than one factor variable x, we can express y using a linear combination of x_A, x_B etc.:

$$y = b_0 + b_A x_A + b_B x_B + b_{AB} x_A x_B + b_C x_C \ldots \quad (9.1)$$

Multiple regression (SPSS): Analysis for Fig 9.10(b). Scan here to watch the video or find it via www.oxfordtextbooks.co.uk/orc/currell/

where the $x_A x_B$ term represents an *interaction* (3.3.2) between the variables x_A and x_B. Multiple regression is then the process of calculating the best-fit values for b_0, b_A, b_{AB}, etc.

However, it is not always necessary or beneficial to include all possible factor terms in the model. If it is known that a specific factor is not a significant term, then it is appropriate to exclude it from the model. The process of multiple regression tests whether, or not, the inclusion of specific factors improves the goodness of the fit.

For a system with several input factors/variables there are different *approaches* to identifying a final model:

- *Force all factors* to be included, in which case each factor will have an associated p-value relating to its significance in that particular model.
- *Forward selection* starts with *no* factors included and allows the analysis to *include* factors, one at a time, for which their p-values are less than a defined significance level, α, (typically 0.1). Several steps may be required, with the procedure stopping when *no new* factor would have $p < \alpha$.
- *Backward elimination* starts with *all* the factors and allows the analysis to *reject* any for which their p-values are greater than a defined significance level, α, (typically 0.1). Note that as one factor is rejected, the remaining p-values may change and further steps may be required. The procedure stops when *all* factors have $p < \alpha$.
- *Stepwise* regression allows the analysis to include *or* reject factors, forwards and backwards, as required to move towards the best-fit.

The operation of multiple regression is illustrated here for Minitab and SPSS, using the 'Boxing' case study.

Case study: Boxing performance / 2. Multiple regression

–continued from 6.4.1

Fig 9.9 reproduces the data from Fig 6.33, showing the numbers of punches recorded over six rounds (two bouts) by each of six amateur boxers for two levels of hydration, *E* and *D*, with the objective of testing for the effect of hydration on performance.

	A	B	C	D	E	F	G	H	I
1	RecNo	Punches	Subject	Hydrat	HydratN	Round	Bout	H*R	H*B
2	11	131	e	E	1	2	1	2	1
3	3	129	c	E	1	1	1	1	1
4	62	117	b	D	2	5	2	10	4
5	42	127	f	D	2	1	1	2	2
6	24	135	f	E	1	4	2	4	2
7	16	150	d	E	1	3	1	3	1
8	55	147	a	D	2	4	2	8	4
9	51	132	c	D	2	3	1	6	2
10	40	132	d	D	2	1	1	2	2
11	59	122	e	D	2	4	2	8	4
12	31	159	a	E	1	6	2	6	2

Fig 9.9 'Boxing performance' case study data

In Fig 9.9, the data set *HydratN* gives the levels of hydration, *E* and *D*, coded with the scale values 1 and 2. We wish to include possible *interactions* (3.3.2) within the model, and, for the purposes of this regression model, these have been coded as *additional factors*:

HxR— *hydration* and *round*, coded by multiplying the values of *HydratN* and *Round*.
HxB— *hydration* and *bout*, coded by multiplying the values of *HydratN* and *Bout*.

Software analyses can be performed using:

Minitab

Mintab > Stat > Regression > Regression > Fit Regression Model...
Response: *Punches* **Continuous predictors:** *HydratN, Round, Bout, HxR, HxB*
Method: ▼ **Choose method from:** *Stepwise, Forward selection, Backward elimination*
Potential terms: *HydratN, Round, Bout, HxR, HxB*
☑ **Display the table of model selection details**
▼ *Include details of each step*

SPSS

SPSS > Analyze > Regression > Linear...
Dependent: *Punches* **Independent(s):** *HydratN, Round, Bout, HxR, HxB*
Method: Select method from the drop down menu

Step	1	2	3
Constant	89.42	82.33	82.33
HydratN	41	45	47
T-Value	2.33	2.75	2.88
P-Value	0.023	0.008	0.005
Round	7		
T-Value	0.71		
P-Value	0.482		
Bout	38	59	59
T-Value	1.11	3.63	3.64
P-Value	0.272	0.001	0.001
HxR	-6.0	-1.7	
T-Value	-0.94	-0.85	
P-Value	0.351	0.396	
HxB	-26	-38	-43
T-Value	-1.18	-3.22	-4.22
P-Value	0.241	0.002	0.000
S	22.0	21.9	21.8
R-Sq	33.19	32.68	31.95
R-Sq(adj)	28.13	28.66	28.95
Mallows Cp	6.0	4.5	3.2

(a) Minitab 16

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	89.417	27.769		3.220	.002
	HydratN	40.931	17.563	.795	2.331	.023
	Round	7.083	10.029	.470	.706	.482
	Bout	37.972	34.256	.738	1.108	.272
	HxR	-5.958	6.343	-.745	-.939	.351
	HxB	-25.625	21.665	-1.085	-1.183	.241
2	(Constant)	82.333	25.798		3.191	.002
	HydratN	45.181	16.438	.878	2.749	.008
	Bout	59.222	16.316	1.151	3.630	.001
	HxR	-1.708	1.998	-.214	-.855	.396
	HxB	-38.375	11.934	-1.625	-3.216	.002
3	(Constant)	82.333	25.747		3.198	.002
	HydratN	46.889	16.284	.911	2.880	.005
	Bout	59.222	16.284	1.151	3.637	.001
	HxB	-43.500	10.299	-1.842	-4.224	.000

a. Dependent Variable: Punches

(b) *SPSS*

Fig 9.10 Extracts from multiple regression output using backward elimination (The output for Minitab 17 provides the same data in a different layout)

Using the *backward elimination* method, multiple regression gives the results in Fig 9.10, with (a) Minitab proceeding in three *steps* from left to right, and (b) SPSS proceeding from top to bottom through three *models*.

Starting with all possible factors, *HydratN*, *Round*, *Bout*, *HxR*, and *HxB*, the *Round* factor is the first to be removed, followed by *HxR* in the next step. The model concludes that hydration, bout, and the interaction between them are the appropriate predictors of boxing performance. A knowledge of the round does not add significant information to the model. If we review the quality of fit, we find that with all factors, the R^2 values (4.4.1) are $R^2 = 33.19$ and $R^2(\text{adj}) = 28.13$, and the removal of two 'factors' actually reduces the *total* fit of the model and R^2 drops to 31.95. However, with *fewer* factors, the $R^2(\text{adj})$ *increases* to 28.95, giving a more *efficient* model for describing the data. We can also see in Fig 9.11 that a general regression calculation follows the same analytical logic.

The results in Fig 9.10 include the coefficients, *b*, (B in SPSS) related to each factor plus the constant, b_0, and, from the above results, the equation to describe the number of punches becomes

$$n = 82.33 + 46.9 \times HydratN + 59.2 \times Bout - 43.5 \times (H \times B)$$

where $H \times B$ is the interaction term of *HydratN* and *Bout*.

The *forward selection* method arrives at the same model for the data. However, it is possible using *stepwise selection* with different *starting* factors in Minitab for the regression process to stop with a different, and less optimal, model. For example, starting with *Round*, *Bout* and *HxR*, the *Bout* factor provides little additional input and is rejected and then *HydratN* is included. However, the final model in this sequence is a slightly less efficient fit to the data as $R^2(\text{adj}) = 28.76$ as compared to 28.95 for the backward elimination model.

We could also use the general regression option in Minitab, entering the factors and interaction as in the model below:

Minitab > Stat > Regression > Regression > Fit Regression Model...
Response: *Punches* **Continuous predictors:** *HydratN, Round, Bout*
> Model...
Highlight *HydratN* and *Bout* in **Terms in the model**
Cross predictors and terms in the model: Add, giving
Terms in the model: *HydratN, Round, Bout, HydratN*Bout*
(Delete any other cross-terms that may appear)
→ Output: Fig 9.11

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Regression	4	15402.5	15402.5	3850.63	7.9898	0.000025
HydratN	1	6068.3	3957.4	3957.42	8.2113	0.005556
Round	1	818.1	165.0	165.02	0.3424	0.560410
Bout	1	0.9	5648.4	5648.38	11.7199	0.001059
HydratN*Bout	1	8515.1	8515.1	8515.13	17.6682	0.000080
Error	67	32290.4	32290.4	481.95		
Lack-of-Fit	7	1060.5	1060.5	151.50	0.2911	0.954930
Pure Error	60	31229.8	31229.8	520.50		
Total	71	47692.9				

Fig 9.11 Output from general regression using Minitab

The output in Fig 9.11 agrees with the results in Fig 9.10 by showing that *HydratN, Bout,* and the interaction *HydratN*Bout* are all significant factors ($p < 0.05$).

These results also show the effect of *sequencing* the analysis as discussed in 3.4.5. When *first introduced* into the model, *Round* was identified as contributing $SS_{SEQ} = 818.1$ and *Bout* only $SS_{SEQ} = 0.9$ to the variability in the data, which suggests a greater significance for *Round*. However, when these values were adjusted, taking into account all other factors, the analysis reduces the contribution from *Round* to $SS_{ADJ} = 165.0$ and identifies the major contribution of *Bout* with $SS_{ADJ} = 5648.4$.

9.2 Multiple questions

Section 9.1 considered how multiple predictor variables could be used to model the state of a system. In this section, we consider a variety of different variables recorded in relation to a 'system', both before and after a possible intervention, and the different types of 'questions' that can be asked in the analysis. This naturally applies to the analysis of questionnaires, but similar data sets could be produced by measuring multiple laboratory and field variables and factors. We have already met elsewhere most of the analyses that can be applied to this data set, but we will develop here a general overview with links to the relevant techniques.

9.2.1 Example data

Fig 9.12 gives the questionnaire responses for the Forensic Questionnaire case study, but it can also be viewed as an example of a data set that provides information about any scientific

	A	B	C	D	E	F	G	H	I	J
1	Grouping		Background			Responses				
2	*Grp*	*Year*	*T1*	*Q1*	*T2*	*Q2*	*Q3*	*Q4*	*Q5*	*Q6*
3	G2	3	88	N	87	N	Y	Y	1	0
4	G1	3	57	Y	56	Y	Y	Y	0	-2
5	G2	3	68	N	67	N	N	N	-2	-1
6	G2	2	36	Y	33	N	Y	Y	0	1
7	G2	3	81	Y	78	N	N	N	0	-2
8	G1	2	42	N	38	N	N	N	0	2
9	G1	3	60	N	63	Y	Y	N	2	2
10	G1	3	71	N	77	Y	N	N	0	2

Fig 9.12 Multiple variables data set

Case study: **Forensic questionnaire / 1. Multiple variables (overview)**

Fig 9.12 shows the questionnaire responses for eight out of 66 randomly selected students who took part in a forensic science investigation. The students were either in year 2 or 3 of their course and were further identified as belonging to one of two cohort groups *G1* or *G2* relating to different programmes being followed.

Initially they were given a short test of multiple choice questions to define their background understanding of a specific topic, with the percentage result, *T1*. They were also given the evidence presented in an imaginary trial and asked the binary question, *Q1*, whether they considered that the defendant was guilty. There was then an 'intervention' in the form of specially prepared video tutorials on the chosen topic, and they were then asked to repeat the test, giving result, *T2*, and asked whether they now believed, *Q2*, the defendant to be guilty. They were also asked two other binary questions, *Q3* and *Q4*, and two questions with Likert scale responses, *Q5* on a scale of –2 to +2 and *Q6* on a scale of –3 to +3.

Section 9.2 Gives an overview of the analysis of the data in Fig 9.12.

8.2.1 / 2. Crosstabs and contingency table. Develops the statistics and technique for analysing related categorical data.

4.4.5 / 3. McNemar's test and Cochran's Q. Develops the statistics for measuring the agreement between related binary variables.

6.2.1 / 4. Ordinal and binary responses. Uses nonparametric analyses for testing for differences between ordinal and binary variables.

system for which each row represents sets of measurements on different 'subjects'. In a very broad classification, it can be useful to group the variables into three main groups:

- Categorical variables, e.g. *Grp* and *Year* that identify **specific groups** within the rows of subjects.
- **Background** variables, e.g. *T1* and *Q1*, that provide further information about each measurement or subject.
- **Response questions** following the **effect of an intervention**.

The variables in Fig 9.12 are summarized in Table 9.1.

The variability for the *T1* and *T2* test data is not initially known. Although the scores of a moderately homogenous group of students sitting a well-structured examination are often

normally distributed, this is not the situation here and we can have no expectation that these variables will show a normal distribution.

Table 9.1 Variable characteristics (5.1.2)

Variable	Action	Type	Levels	Variability
Grp	Input	Nominal	*G1* / *G2*	Fixed
Year	Input	Ordinal	2 / 3	Fixed
T1	Input	Interval	*Continuous* 0–100	Not normal ?
Q1	Input	Binary	*Y* / *N*	
T2	Output	Interval	*Continuous* 0–100	Not normal ?
Q2 to *Q4*	Output	Binary	*Y* / *N*	–
Q5	Output	Ordinal	Likert scale: -2 to +2	–
Q6	Output	Ordinal	Likert scale: -3 to +3	–

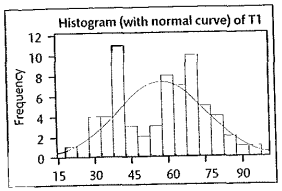
The label of a variable as an 'input' or 'output' can depend on the analysis being performed (1.2.1). If we measure the correlation between *T1* and *T2*, then neither are inputs, but a best-fit straight line could be drawn to predict the value of *T2* (output) based on the value of *T1* (input). In this example, the variables *T1* and *Q1* are recorded as evidence of the respondents initial state and, in general, we can see them as *inputs* into an analysis of the effect of the interaction from which the other variables, *T2*, *Q2*–*Q6*, are *outputs*.

9.2.2 **Describing the data**

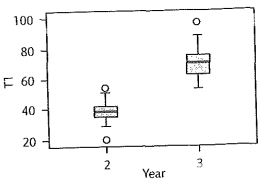
The first step is to get a good overall understanding of the data, both *numerically* and *graphically*. It is useful to summarize numerical values to check for any obvious differences, e.g. mean, median, standard deviation for *numeric* data, and frequencies and proportions for *categorical* and *binary* data. Graphs and data plots also provide a valuable visualization within which it is often possible to see patterns that are hidden in lists of numbers.

Various methods are given for *individual* variables in 6.1.3, with, for example, the bimodal distribution of the histogram in Fig 9.13(a) suggesting two distinct groups in the *T1* data. Often of greater interest however is the *simultaneous* display of data from *two or more* variables or groups, developed in 6.2.3, 6.3.3, and 6.4.3. For example, Fig 9.13(b) gives separate boxplots with quite different median values for the two *Year* groups within the *T1* data, which suggests that these two cohorts are responsible for the overall *bimodal* distribution in *T1*. We can also see a datum value of 20 visible as an outlier in both Fig 9.13 (a) and (b).

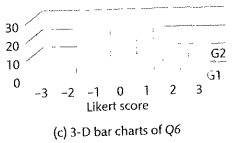
For categorical data we can plot the frequencies of occurrences within different categories and Fig 9.13(c) gives the numbers of respondents from Fig 8.17 recording values for *Q6*, separated into the two groups, *G1* and *G2*. This provides an immediate suggestion of a possible difference in distribution between the groups which is tested in 8.2.7.



(a) Histogram of *T1* (Minitab)



(b) Boxplots of *Year* groups within *T1* (SPSS)



(c) 3-D bar charts of *Q6* for groups *G1* and *G2* (Excel)

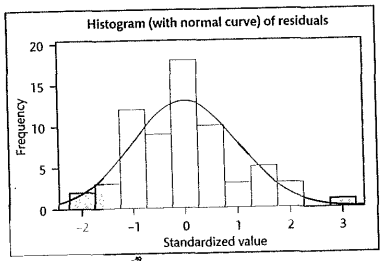
Fig 9.13 Describing *T1* and *Q6* values

9.2.3 Testing for normality and homoscedasticity

One of the earliest questions that students learn to ask is 'Is my data normally distributed?' The best approach to this is to consider the *scientific* factors (5.4.3) that may, or may not, cause the random variations to follow a normal distribution. It is then possible, provided that the samples are not too small, to carry out a normality test for an individual variable (8.1.6) and also test for the equality in variance (homoscedasticity) between two sets (6.2.4). However, if we consider *T1*, it is quite clear from Fig 9.13(a) that the overall distribution of values is *not* normal due to the two separate year groups.

The next step is to consider the distribution of residuals after *fitting* an analytical model, e.g. after deriving the best-fit straight line or using an ANOVA calculation (Sections 5.4, 6.3.4, 6.4.5). If we perform a one-way ANOVA analysis on *T1* and produce a model which includes the effect of the two year groups, we can save the residuals as a new data set. The distribution of these residuals is given in Fig 9.14(a), showing that they can now be treated as a normal distribution, with $p = 0.444$ from the Anderson–Darling normality test (Minitab, 5.4.5).

Similarly we can test for a difference in variance between data sets *within* the main statistical analysis. For example, SPSS gives $p = 0.081$ for Levene's test, Fig 9.14(b), when performing either an independent sample *t*-test (6.2.5) or a one-way ANOVA (6.3.5) for a difference between year groups in *T1*, confirming that there is no significant difference in variance and that the choice of parametric test was valid.



(a) Normality *T1* residuals

Levene's Test of Equality of Error Variances[a]

Dependent Variable: T1

F	df1	df2	Sig.
3.134	1	64	.081

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Year

(b) Equality of *T1* variance

Fig 9.14 Testing for normality and homoscedasticity

9.2.4 Analysing an individual variable

We may wish to compare a measured variable with specific expectations:

- Is the mean of *T1*, or the median of *Q6*, different from an expected value? One sample *t*-test, 6.1.4 or Wilcoxon test, 6.1.5.
- Is the proportion of *Y* answers in the binary questions different from expected proportions? One proportion test, 6.1.7.
- Is the distribution of values in *interval* data, e.g. *T1*, different from an expected distribution? Kolmogorov–Smirnov test, 6.1.6.
- Is the distribution of *categorical* data, e.g. Q5 or Q6, different from an expected distribution? Chi-squared 'goodness of fit test', 8.1.5.
- Is the order of values random? Runs test, 6.1.6.

9.2.5 Dependence of specific factors

The next level of analysis for interval variables is often to ask:

- Are the values affected by a grouping defined by a separate categorical variable, e.g. do the groups *G1*, *G2* in *Grp* affect the value of *T1*?

For the effect of just *two levels* (e.g. *G1*, *G2*) we may be testing for a difference in means (*t*-test, 6.2.5), medians (Mann–Whitney test, 6.2.6) or variance (*F*-test, Levene's test, 6.2.4). For example, a *t*-test for the different *Grp* mean values of *T1* gives the non-significant result, $p = 0.790$. More than two levels requires an ANOVA, normally conducted through the GLM (6.3.5), or the nonparametric Kruskal–Wallis test (6.3.7).
A more complicated question involves the effects of more than two categorical variables:

- Are the values affected by groupings and interactions defined by two or more categorical variables, e.g. How do *Grp* and *Year* affect the value of *T1*?

In this case the analysis becomes a multifactorial ANOVA (6.4.4), with which it can be possible to test *separately* for the effects of each factor and a possible *interaction* (3.3.2) between them. For example, the GLM/ANOVA results for *T1* give:

$p = 0.000$ (i.e. < 0.0005) for *Year*, showing the very significant difference between the years that we have already seen in Fig 9.13(b).

$p = 0.790$ for *Grp*, showing that there is no significant difference between groups, *G1* and *G2*, agreeing with the results of the *t*-test above.

$p = 0.263$ for *Year*Grp*, showing that there is no significant interaction (3.3.2) between the groups *Year* and *Grp*.

These results can be viewed graphically in the interaction plot (6.4.3) for *T1* in Fig 9.15, which plots the mean values of the four possible combinations of *Year* and *Grp*. There is a clear difference in *T1* values between years 2 and 3, but there is no vertical difference between the lines for *G1* and *G2*, and the fact that the two lines are nearly parallel suggests that there is no significant interaction between the factors.

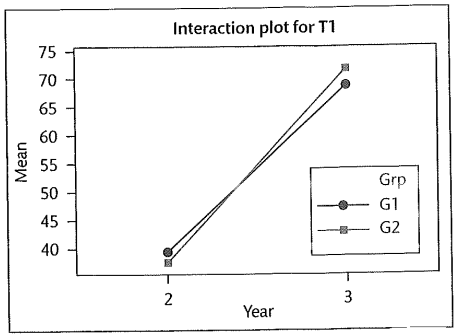


Fig 9.15 Interaction plot for *T1* against *Year* and *Grp*

For ordinal/binary variables, we may wish to ask:

- Are the values affected by a grouping defined by a separate categorical variable, e.g. do the groups *G1* and *G2* in *Grp* affect the value of *Q5*?

In 6.2.6 we use the nonparametric Mann–Whitney test, which records $p = 0.031$, from which we accept that there is a difference between the *median* values of *Q5* for the two groups *G1* and *G2*. This is consistent with Fig 9.13(c) with the *G1* group recording the higher median value.

9.2.6 Comparing variables as unrelated data

The distinction between *related* and *unrelated* variables is developed in Section 6.2. As presented in Fig 9.12, all the variables are related because of the horizontal links through the subject/record rows, and, if we were to treat them as unrelated, we would be ignoring important analytical information.

If the *T2/Q2* results were obtained from a *different sample* of respondents than the *T1/Q1* results, then we would have to ask the *unrelated* questions:

- Is there is an overall difference between two variables, e.g. *T1* and *T2* or *Q1* and *Q2*?

We have to perform the nonparametric Mann–Whitney test between *T1* and *T2* because we know that *T1* is not normally distributed, and this gives $p = 0.90$ which shows no significant difference between the *median* values. To compare *Q1* and *Q2* we test for a difference in the *proportion* of *Y* values between *Q1* and *Q2*, and, in 6.2.9, we obtain the value $p = 0.053$. Neither of these differences are significant when treated as *unrelated* variables, but we see below that there are subtle differences when analysed as *related* variables.

9.2.7 Modelling interrelated variables

We may wish to model a mathematical relationship between two *interrelated* interval variables. This is very common in experimental science, usually described by the familiar *x–y* graph and often leading to the slope and intercept of a best-fit straight line

(Section 2.1), a defined nonlinear relationship (Sections 2.3 and 7.2) or more general patterns of behaviour (Section 7.3). Typically these examples are relating the variation of two *different* quantities, e.g. absorbance and concentration in spectrophotometry, radioactivity decay and time, etc.

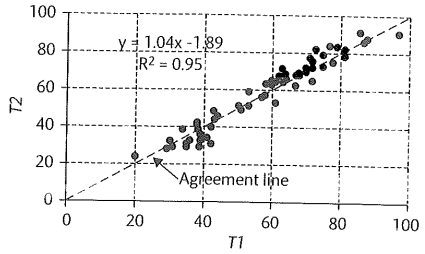


Fig 9.16 Linear regression of *T2* against *T1*

If we plot the interrelationship of two variables measuring the *same* quantity we can display the amount of *agreement* between them. Fig 9.16 plots the values of *T2* as a function of *T1*, with each point showing the responses of one individual. For *perfect* agreement between *T2* and *T1* every point would lie on the agreement line drawn with a slope of 1.0, an intercept of 0.0, and with an R^2 measure of agreement (4.4.1) equal to 1.00.

The actual relationship has the equation:

$$T2 = 1.04 \times T1 - 1.89$$

with $R^2 = 0.95$, which shows fairly good overall agreement. However, we can see that the year 2 students (with *T1* about 30 to 50) tend to give lower values for *T2*, appearing to the lower right of the agreement line, and most year 3 students (with *T1* about 60 to 80) recording an increase to the upper left of the line. This confirms the result of the repeated measures analysis below.

The data in Fig 9.12 does not include any extensive *x-y* relationships, but these can often appear in experimental data results, and we consider a range of possibilities in Section 7.3, including:

- *convolution* (7.3.5) to create a 'best-fit' smoothed line through a whole data set, by using local 'averaging' of data at each point, and convolutes to produce a smoothed 'differential' (7.3.6) of the curve, giving the slope of the curve at each point, and
- *autocorrelation* (7.3.7) and *spectral analysis* (7.3.8) techniques to analyse periodic frequency components hidden within the data.

9.2.8 Comparing related variables

A common example of *repeated* measurements is a 'before' and 'after' situation:

- Does the tutorial intervention cause a difference in the test results *T1* and *T2* of subjects, and is any difference dependent on a categorical grouping of the subjects, e.g. years 2 and 3?

For just two variables the question becomes a *paired* test for means (paired t-test 6.2.7) or medians (paired Wilcoxon test, 6.2.8), but for three or more variables we use *repeated measures* (6.3.8, 3.6.2).

In 6.3.8 a repeated measures analysis of *T1* and *T2* with the *Year* as a factor confirms the different *Years* as being highly significant ($p = 0.000$), and, although there is no overall difference between *T1* and *T2* ($p = 0.927$), there is an interaction between the differences, *T2-T1*, and the year group ($p = 0.016$) which shows that the two groups react differently to the intervention of additional videos (see Fig 9.16 above). The lack of *overall* difference between *T1* and *T2* is confirmed using a simple paired t-test with $p = 0.670$. We can use the *parametric* analysis for the *paired* test, because the *difference values*, *T2-T1*, can be considered to be sufficiently close to a normal distribution with $p = 0.076$ for the Anderson–Darling test, even though *T1* itself is not normal.

Alternatively we may have a number of different assessments which might be expected to give the same result, e.g. different assay methods might be used to estimate the same bacterial mortality, or several 'experts' give their opinions on the quality of different wines. We have tested above for agreement between the interval values of *T1* and *T2* by using repeated measures analysis and linear regression, and we now develop the general analysis of agreement between ordinal variables in Section 4.4, involving correlation, Kendall's coefficient of concordance, Kappa, McNemar's test, and Cohen's Q.

In relation to the data in Fig 9.12, we can test for agreement between the *binary* answers.

- Do the binary answers to questions *Q1* to *Q4* agree?

In 4.4.5 we use McNemar's test and Cochran's Q to test for agreement between *Q1*, *Q2*, *Q3*, and *Q4*, finding a significant difference just between *Q1* and *Q2* with $p = 0.031$.

9.2.9 Ordinal responses

Choosing the number of categories to use for ordinal responses (e.g. the range Likert style for *Q6*) can be difficult. If there are *too few*, then almost all of the respondents may give the same answer, e.g. on a scale of 1 to 4, everyone might reply '3', making it impossible to do detailed analysis. However, if you have *too many* levels, then the *frequency* of responses in individual categories can become too small for useful analysis. Ideally a pilot study would reveal the ranges of answers that could be expected in a final questionnaire, allowing you to design the questions more sensitively, but this is not always possible for a final year project.

The frequency of responses to levels in *Q6* are given in Fig 9.13(c) in which it can be seen that responses, –3, –2, and +3 all give low numbers. The analytical comparison of the answers from the two groups *G1* and *G2* to *Q6* is given in 8.2.7 which introduces two methods for dealing with low expected frequencies: consolidation of categories and using a Monte Carlo analysis.

9.2.10 Multiple variables

Section 9.1 introduces the analysis of multiple variables to answer the questions:

- Are there any relationships between the variables?
- Is it possible to develop a model that predicts outcomes using multiple input variables?

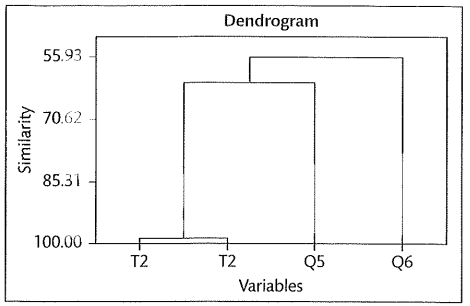


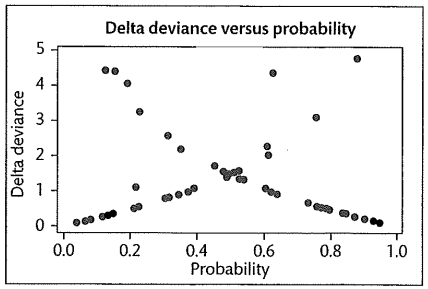
Fig 9.17 Clustering variables dendrogram (Minitab)

Fig 9.17 uses the technique of data clustering (9.1.3) to investigate relationships between variables. It shows the strong similarity in the values of *T1* and *T2*, but a lack of similarity between *Q5* and *Q6*. The binary variables are not included here because they have been entered into the data as text variables '*Y*' or '*N*', and would need to be coded numerically, e.g. 0 and 1, for inclusion in this analysis.

If we now code the group *Grp* numerically as *GrpN* with values '1' and '2', and the binary answers as *B1*, *B2*, *B3*, and *B4* respectively with values '0' and '1', we could develop a mathematical model using multiple regression to predict *GrpN* values. Fig 9.18(a) shows the result of a stepwise regression (9.1.6), which identifies only *Q6* and *B2* as highly significant predictors for *GrpN* ($p = 0.002$ each) with *B1* included because $p = 0.058$ is less than the default rejection criterion of 0.1. However, since *GrpN* is a binary value we can use *binary* regression (8.3.4), with, for example, *Q6*, *B2*, *B1*, and *T1* as predictor variables. The results again identify *Q6* and *B2* as highly significant ($p = 0.005$), with *B1* giving $p = 0.089$ and *T1* 'not significant' with $p = 0.734$. The ability to predict the correct group can be displayed with the deviance vs probability graph in Fig 9.18(b), which identifies the two groups as two curves (8.3.4), with correct predictions towards the lower ends of both curves and misclassifications in the upper branches.

```
Response is GrpN on 8 predictors, with N = 66

Step            1       2       3
Constant    1.516   1.677   1.616

Q6         -0.131  -0.132  -0.114
T-Value    -3.61   -3.82   -3.25
P-Value     0.001   0.000   0.002

B2                  -0.30   -0.35
T-Value             -2.80   -3.25
P-Value              0.007   0.002

B1                           0.23
T-Value                      1.93
P-Value                      0.058

S           0.461   0.438   0.429
R-Sq        16.94   26.11   30.32
R-Sq(adj)   15.65   23.77   26.94
Mallows Cp   8.0     2.2     0.7
```

(a) Stepwise regression



(b) Deviance vs probability curve for binary regression

Fig 9.18 Regression modelling of *GrpN* (Minitab)

APPENDIX I

Videos available in the Online Resource Centre

Number	Title	Description	Section
1	**Combining uncertainties**	Excel analysis for Fig 1.13	1.4.4
2	**Propagation of errors**	Excel analysis for Fig 1.14	1.4.4
3	**DIY dice**	Excel analysis for Fig 1.15	1.4.6
4	**Sample statistics and confidence intervals**	Excel analysis for Fig 1.16	1.5.1
5	**Samples and population**	Excel analysis for Fig 1.17	1.5.3
6	**Statistics of linear regression**	Excel analysis for Fig 2.2	2.1.1
7	**Calibration uncertainty 1**	Excel analysis for Fig 2.7. See also 7.1.5	2.2.1
8	**Exact x/y intercepts**	Excel analysis for Figs 2.9 (b) and 2.10 (b)	2.2.2
9	**Weighting data**	Excel analysis for Fig 2.12	2.2.4
10	**Using Solver**	Excel analysis for Fig 2.18	2.4.1
11	**Nonlinear regression**	Excel analysis for Fig 2.20. See also 7.2.3	2.4.3
12	**Difference in slopes**	Excel analysis for Fig 3.1	3.1.1
13	**Two sample *t*-test and *F*-test**	Excel analysis for Fig 3.3	3.1.3
14	**Analysis of variance**	Excel analysis for Fig 3.4	3.2.2
15	**One Way ANOVA**	Minitab and SPSS analyses leading to Fig 3.8	3.2.3
16	**Multi-factorial ANOVA (Minitab)**	Minitab analyses for the data in Figs 3.11 and 3.15	3.3.1
17	**Multi-factorial ANOVA (SPSS)**	SPSS analyses for the data in Figs 3.11 and 3.15	3.3.1
18	**ANCOVA 1**	Excel and Minitab analysis for Fig 3.20. See also 6.4.8	3.3.3
19	**General linear model**	Excel analysis for Fig 3.24	3.4.2
20	**General regression (Minitab)**	Minitab analysis for Fig 3.26 data. See also 7.2.5	3.4.3
21	**Generalized linear model (Poisson loglinear)**	SPSS analysis leading to Fig 3.35. See also 6.4.7	3.4.7
22	**Repeated measures 1**	SPSS analysis leading to Figs 3.41, 3.42 and 3.43. See also 6.3.8	3.6.2
23	**Chi-squared 'goodness of fit'**	Excel analysis for Fig 3.46. See also 8.1.5	3.7.2
24	**Contingency table test for association**	Excel analysis for Fig 3.47. See also 8.2.4	3.7.4

25	**One proportion**	Excel and Minitab analysis for Fig 3.48. See also 6.1.7	3.8.2
26	**Two proportions**	Minitab analysis leading to Fig 3.51. See also 6.2.9	3.8.3
27	**Resampling t-test and Mann-Whitney test**	Excel analysis for Fig 3.52	3.9.2
28	**Resampling chi-squared**	Excel analysis for Fig 3.53	3.9.3
29	**Parametric and nonparametric correlation**	Excel analysis for Fig 4.2	4.1.1
30	**Bivariate and partial correlation**	Analysis for Fig 4.7	4.1.4
31	**Fisher's exact test**	Excel analysis for Fig 4.12	4.2.3
32	**MKT analysis**	Excel analysis for Fig 5.9	5.2.5
33	**Transforming data**	Minitab and SPSS transformations in Fig 5.10	5.3.2
34	**Analysing residuals (Minitab)**	Analysis for Fig 5.14 data. See also 6.4.5	5.4.6
35	**Analysing residuals (SPSS)**	Analysis for Fig 5.14 data. See also 6.4.5	5.4.6
36	**Transforming for normality**	SPSS analysis leading to Fig 5.18 and Table 5.3. See also 5.3.2	5.4.7
37	**Describing sample data (Excel)**	Descriptives and graphs	6.1.3
38	**Describing sample data (Minitab)**	Descriptives and graphs	6.1.3
39	**Describing sample data (SPSS)**	Descriptives and graphs	6.1.3
40	**One sample tests (Minitab)**	Analysis for *t*-test and Wilcoxon test	6.1.4
41	**One sample tests (SPSS)**	Analysis for *t*-test and Wilcoxon test	6.1.4
42	**Nonparametric tests (SPSS)**	Nonparametric tests. See also 6.4.6	6.1.6
43	**One proportion (SPSS)**	Analysis for Fig 6.1 data. See also 3.8.2	6.1.7
44	**Two sample tests (Minitab)**	Analysis of variance, means and medians	6.2.5
45	**Two sample tests (SPSS)**	Analysis of variance, means and medians	6.2.5
46	**Paired tests (Minitab)**	Analysis for paired *t*-test and Wilcoxon test	6.2.7
47	**Paired tests (SPSS)**	Analysis for paired *t*-test and Wilcoxon test	6.2.7
48	**Two proportions (SPSS)**	Analysis for Fig 6.22. See also 3.8.3	6.2.9
49	**GLM/ANOVA (Minitab)**	Analysis leading to Figs 6.27(a), 6.28 and 6.30	6.3.5
50	**GLM/ANOVA (SPSS)**	Analysis leading to Figs 6.27(b), 6.29 and 6.31	6.3.5
51	**Repeated measures 2**	SPSS analysis leading to 6.32. See also 3.6.2	6.3.8
52	**Factor plots (Minitab)**	Analysis leading to Figs 6.35(a), 6.36(b)	6.4.3
53	**Factor plots (SPSS)**	Analysis leading to Figs 6.35(b), 6.36(a)	6.4.3
54	**Multifactorial GLM/ANOVA (Minitab)**	Analysis leading to Fig 6.37	6.4.4
55	**Multifactorial GLM/ANOVA (SPSS)**	Analysis leading to Fig 6.37	6.4.4
56	**Normality and homoscedasticity (Minitab)**	Analysis of Boxing, case study. See also 5.4.6	6.4.5
57	**Normality and homoscedasticity (SPSS)**	Analysis of Boxing, case study. See also 5.4.6 and 6.3.4	6.4.5
58	**Nonparametric ANOVA (Minitab)**	Analysis leading to Fig 6.39(b)	6.4.6
59	**Nonparametric ANOVA (SPSS)**	Analysis leading to Fig 6.39(a). See also 6.1.6	6.4.6
60	**Generalized linear model (ordinal logistic)**	SPSS analysis leading to Fig 6.40. See also 3.4.7	6.4.7
61	**ANCOVA 2**	SPSS analysis leading to Figs 6.41 and 6.42. See also 3.3.3	6.4.8
62	**Calibration uncertainty 2**	Minitab and SPSS analysis. See also 2.2.1	7.1.5
63	**Nonlinear regression (Minitab)**	Analysis for Fig 7.6(a). See also 2.4.3	7.2.3
64	**Nonlinear regression (SPSS)**	Analysis for Fig 7.6(b). See also 2.4.3	7.2.3
65	**Deriving the model**	Excel analysis for Fig 7.7	7.2.4
66	**General regression (Minitab)**	Analysis for Fig 7.8. See also 3.4.3	7.2.5
67	**Using convolutes**	Excel analysis for Figs 7.13 and 7.14	7.3.5
68	**Spectral analysis**	SPSS and Minitab analyses for Figs 7.16 and 7.18	7.3.7
69	**Frequency data (Minitab)**	Descriptives and graphs	8.1.3
70	**Frequency data (SPSS)**	Descriptives and graphs	8.1.3
71	**Chi-squared goodness of fit (Minitab)**	Analysis for Fig 8.6. See also 3.7.2	8.1.5
72	**Chi-squared goodness of fit (SPSS)**	Analysis for Fig 8.7. See also 3.7.2	8.1.5
73	**Testing distributions (Minitab)**	Analysis for distributions and Fig 8.8(b)	8.1.6
74	**Testing distributions (SPSS)**	Analysis for distributions and Fig 8.8(a)	8.1.6
75	**Crosstabs and contingency tables (Minitab)**	Analyses for Figs 8.12/13/14/15. See also 3.7.4	8.2.4
76	**Crosstabs and contingency tables (SPSS)**	Analyses for Figs 8.12/13/14/15/16. See also 3.7.4	8.2.4
77	**Logit and probit**	Excel analysis for Fig 8.22	8.3.3
78	**Binary regression**	Minitab analysis for Fig 8.24	8.3.4
79	**ROC curves**	SPSS analysis for Fig 8.27	8.3.5
80	**Cluster analysis**	Minitab and SPSS analyses for Fig 9.2	9.1.3
81	**Principal component analysis**	Minitab analysis for Figs 9.6 and 9.7	9.1.4
82	**Multiple regression (Minitab)**	Analysis for Fig 9.10(a)	9.1.6
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APPENDIX II

Case studies used throughout this book

The case studies are listed in alphabetical order and then stage order within each case study. Note that, in some cases, the stage order does not follow linearly within the book.

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Forensic questionnaire	4. Ordinal and binary responses	6.2.1
Forensic questionnaire	5. Repeated measures	6.3.1
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