Asking Questions in Biology

A Guide to Hypothesis-testing, Experimental Design and Presentation in Practical Work and Research Projects

Fifth edition

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Preface

Science is a process of asking questions, in most cases precise, quantitative questions that allow distinctions to be drawn between alternative explanations of events. Asking the right questions in the right way is a fundamental skill in scientific enquiry, yet in itself it receives surprisingly little explicit attention in scientific training. Students being trained in scientific subjects, for instance in sixth forms, colleges and universities, learn the factual science and some of the tools of enquiry such as laboratory techniques, mathematics, statistics and computing, but they are taught little about the process of question-asking itself.

The first edition of this book had its origins in a first-year undergraduate practical course that we, and others since, ran at the University of Nottingham for many years. The approach adopted there now also forms the basis for a more advanced second-year course. It is also the approach for FdSc- and BSc-level research methods courses at Cornwall College Newquay. The aim of all these courses is to introduce students in the biological sciences to the skills of observation and enquiry, but focusing on the process of enquiry – how to formulate hypotheses and predictions from raw information, how to design critical observations and experiments, and how to choose appropriate analyses – rather than on laboratory, field and analytical techniques per se. This focus is maintained in the fifth edition. However, as in previous editions, we have responded to a number of positive suggestions from people who have used the book, either as teachers or as students, which we think enhance further its usefulness in teaching practical biology generally.

Again, the largest change has been with respect to the presentation of statistical tests. In the fourth edition we replaced the previous boxes based on the procedures and output of the Statistical Package for the Social Sciences (SPSS®) with the freeware package 'R®', obtainable from www.r-project.org**.** This collaborative project of biologists and statisticians worldwide is now pretty much the industry standard in biology. It also has the enormous benefit of an extensive user base who can provide advice rapidly when you hit a snag.

Thus in the fifth edition we have decided to base the analyses on R® alone, to encourage undergraduates to use it. Unlike most modern programs, R® is not a 'point-and-click' process, but you have to write the commands in yourself. This means you need to know what you are doing, otherwise you will not get any meaningful results. Several colleagues have suggested that this will be a barrier to modern undergraduates who have become used to simpler ways of obtaining output from such programs. However, our experience teaching it to undergraduates shows that they learn it almost as quickly as any other package, and so the 'barrier' is not as large as many think. And using R® will be much more useful to students in the longer term.

The book website (www.pearsoned.co.uk/barnard) contains instructions for using SPSS®, Minitab®, Genstat® and the Excel®-based AQB package from the fourth edition, as well as data files. The formulae and hand calculations remain because, as previously, we consider it important that the underlying arithmetic of the tests is understood.

The range of tests remains as in the fourth edition, i.e. it includes, among other things, repeated-measures designs, analysis of covariance, multiple regression and principal components analysis. This is a wider spectrum of designs than is likely to be encountered in a first course in experimental design, but caters for many kinds of data collected in field-course and final-year projects. The Test Finder, Quick Test Finder and Help sections should enable students to find what test they need to carry out, while simultaneously underlining the principles involved.

With increasing emphasis on the wider communication and public understanding of science, we have retained the sections on presenting information. We include giving talks or presenting posters at scientific meetings, and writing for broader non-specialist readers such as newspapers and magazines. To the sections on using online literature databases such as the World of Knowledge, we have added some practical tips on how to extract the information you require from the literature such searches throw up. We have retained sections on the ethical implications of working with biological material, now an essential consideration in any study as legal regulation of biological experiments in both teaching and research becomes ever more stringent.

The book looks at the process of enquiry during its various stages, starting with unstructured observations and working through to the production of a complete written report. In each section, different skills are emphasised and a series of main examples runs through the book to illustrate their application at each stage.

The book begins with a look at scientific question-asking in general. How do we arrive at the right questions to ask? What do we have to know before we can ask sensible questions? How should questions be formulated to be answered most usefully? Chapter 1 addresses these points by looking at the development of testable hypotheses and predictions and the sources from which they might arise.

Chapter 2 looks at how hypotheses and predictions can be derived from unstructured observational notes. Exploratory analysis is an important first step in deriving hypotheses from raw data, and the chapter introduces plots and summary statistics as useful ways of identifying interesting patterns on which to base hypotheses. The chapter concludes by pointing out that although hypotheses and their predictions are naturally specific to the investigation in hand, testable predictions in general fall into two distinct groups: those dealing with some kind of *difference* between groups of data, and those dealing with a *trend* in the quantitative relationship between groups of data.

The distinction between difference and trend predictions is developed further in Chapter 3, which discusses the use of confirmatory analyses. The concept of statistical significance is introduced as an arbitrary but generally agreed yardstick as to whether observed differences or trends are interesting, and a number of broadly applicable significance tests are explained. Throughout, however, the emphasis is on the use of such tests as tools of enquiry rather than on the statistical theory underlying them. Having introduced significance tests and some potential pitfalls in their use, the book uses the main worked examples to show how some of their predictions can be tested and hypotheses refined in the light of testing.

In Chapter 4, the book considers the presentation of information. Once hypotheses have been tested, how should the outcome be conveyed for greatest effect? The chapter discusses the use of tables, figures and other modes of presentation, and shows how a written report should be structured. The chapter then moves on to consider the presentation of material in spoken and poster paper formats, and how to recast written reports of results for a general, rather than specialist, readership.

At the end of the book are a number of appendices. These provide expanded self-test questions and answers sections based on the material in the previous chapters and some statistical tables for use in significance testing.

We said that the book had its inception in our introductory practical course. This course was developed in response to an increasingly voiced need on the part of students to be taught how to formulate hypotheses and predictions clearly and thus design properly discriminating experiments and observations. As we descend into ever more neurotically prescribed teaching and assessment procedures, the need for students to be given clear guidance on such aspects of their work becomes correspondingly greater. As always, both our practical teaching and the book have continued to benefit enormously from our ongoing and enjoyable interaction with our undergraduates. Their insights and enquiries continue to hone the way we teach, and have been the guiding force behind all the discussions in the book.

Finally, we should like to thank all the people who have commented on the book since its first appearance, and encouraged us to think about the further amendments we have made in this present edition. We particularly thank Kelly Haynes and Angus Jackson and the 2013 and 2014 cohorts of BSc Applied Zoology students at Newquay for input to the 'Getting key information from papers quickly' section. In particular, we thank Tom Reader for the generous amount of time he has spent discussing the book with us, commenting on drafts of many of the amendments and giving freely of his experience in and enthusiasm for the business of communicating science. Rufus Curnow at Pearson Education encouraged us to produce a fifth edition, and guided us as to how it should be modified. James Gilbert and Lucy Browning made useful changes to the R® descriptions.

Francis Gilbert
Peter McGregor
January 2016

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Figures

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Dedicated to the memory of Chris Barnard

Chris Barnard succeeded in completing the third edition before his untimely death in June 2007 at the age of 55. We dedicate this latest edition to his memory: his talents as a scientist, educator and communicator of science, raconteur, poet, artist and musician are greatly missed.

1 Doing science

Where do questions come from?

You're out for a walk one autumn afternoon when you notice a squirrel picking up acorns under some trees. Several things strike you about the squirrel's behaviour. For one thing it doesn't seem to pick up all the acorns it comes across; a sizeable proportion is ignored. Of those it does pick up, only some are eaten. Others are carried up into a tree, where the squirrel disappears from view for a few minutes before returning to the supply for more. Something else strikes you: the squirrel doesn't carry its acorns up the nearest tree but instead runs to one several metres away. You begin to wonder why the squirrel behaves in this way. Several possibilities occur to you. Although the acorns on the ground all look very similar to you, you speculate that some might contain more food than others, or perhaps they are easier to crack. By selecting these, the squirrel might obtain food more quickly than by taking indiscriminately any acorn it encountered. Similarly, the fact that it appears to carry acorns into a particular tree suggests this tree might provide a more secure site for storing them.

While all these might be purely casual reflections, they are revealing of the way we analyse and interpret events around us. The speculations about the squirrel's behaviour may seem clutched out of the air on a whim, but they are in fact structured around some clearly identifiable assumptions, for instance that achieving a high rate of food intake matters in some way to the squirrel and influences its preferences, and that using the most secure storage site is more important to it than using the most convenient site. If you wanted to pursue your curiosity further, these assumptions would be critical to the questions you asked and the investigations you undertook. If all this sounds very familiar to you as a science student, it should, because, whether you intended it or not, your speculations are essentially scientific. Science is simply formalised speculation backed up (or otherwise) by equally formalised observation and experimentation. In its broadest sense most of us 'do science' all the time.

1.1 Science as asking questions

Science is often regarded by those outside it as an open-ended quest for objective understanding of the universe and all that is in it. But this is so only in a rather trivial sense. The issue of objectivity is a thorny one and, happily, well beyond the scope of this book. Nevertheless, the very real constraints that limit human objectivity mean that use of the term must at least be hedged about with serious qualifications. The issue of open-endedness is really the one that concerns us here. Science is open-ended only in that its directions of enquiry are, in principle, limitless. Along each path of enquiry, however, science is far from open-ended. Each step on the way is, or should be, the result of refined question-asking, a narrowing down of questions and methods of answering them to provide the clearest possible distinction between alternative explanations for the phenomenon in hand. This is a skill, or series of skills really, that has to be acquired, and acquiring it is one of the chief objectives of any scientific training.

While few scientists would disagree with this, identifying the different skills and understanding how training techniques develop them are a lot less straightforward. With increasing pressure on science courses in universities and colleges to teach more material to more people and to draw on an expanding and increasingly sophisticated body of knowledge, it is more important than ever to understand how to marshal information and direct enquiry. This book is the result of our experiences in teaching investigative skills to university undergraduates in the life sciences. It deals with all aspects of scientific investigation, from thinking up ideas and making initial exploratory observations, through developing and testing hypotheses, to interpreting results and preparing written reports. It is not an introduction to data-handling techniques or statistics, although it includes a substantial element of both; it simply introduces these as tools to aid investigation. The theory and mechanics of statistical analysis can be dealt with more appropriately elsewhere.

The principles covered in the book are extraordinarily simple, yet, paradoxically, students find them very difficult to put into practice when taught in a piecemeal way across a number of different courses. The book has evolved out of our attempts to get over this problem by using open-ended, self-driven practical exercises in which the stages of enquiry develop logically through the desire of students to satisfy their own curiosity. However, the skills it emphasises are just as appropriate to more limited set-piece practicals. Perhaps a distinction – admittedly over-generalised – that could be made here, and which to some extent underpins our preference for a self-driven approach, is that with many set-piece practicals it is obvious *what* one is supposed to do but often not *why* one is supposed to do it. Almost the opposite is true of the self-driven approach; here it is clear why any investigation needs to be undertaken, but usually less clear what should be done to see it through successfully. In our experience, developing the 'what' in the context of a clear 'why' is considerably more instructive than attempting to reconstruct the 'why' from the 'what' or, worse, ignoring it altogether.

1.2 Basic considerations

Scientific enquiry is not just a matter of asking questions; it is a matter of asking the *right questions* in the *right way*. This is more demanding than it sounds. For a start, it requires that something is known about the system or material in which an investigator is interested. A study of mating behaviour in guppies, for instance, demands that you can at least tell males from females and recognise courtship and copulation. Similarly, it is difficult to make a constructive assessment of parasitic worm burdens in host organisms if you are ignorant of likely sites of infection and can't tell worm eggs from faecal material.

Of course, there are several ways in which such knowledge can be acquired: e.g. the Internet/World Wide Web, textbooks, specialist academic journals (mostly now available electronically through licensed subscribers like universities and colleges, or free on the Internet), asking an expert, or simply finding out for yourself through observation and exploration.

These days, the first choice for browsing information is often the Internet/ World Wide Web. The advantages of such 'online' searching in terms of speed and convenience hardly need detailing here, but there *are* dangers, as we indicate later. A good way of accessing reliable scientific information like this is to use one or more of the professional Web-based literature databases, such as the Web of Knowledge (http://wok.mimas.ac.uk), PubMed (www.ncbi.nlm.nih.gov/pmc/), Google Scholar (http://scholar.google.co.uk) or BIOSIS. These search the peer-reviewed (and therefore quality-controlled) academic journals for articles containing information relevant to your request. Each of these provides tips on how best to use them, but a handful of basic ones is given in Box 1.1.

Whichever mode of acquiring information is preferred, however, a certain amount of background preparation is usually essential, even for the simplest investigations. In practical classes, some background is usually provided for you in the form of handouts or accompanying lectures, but the very variability of biological material means that generalised and often highly stylised summaries are poor substitutes for hard personal experience. Nevertheless, given the inevitable constraints of time, materials and available expertise, they are usually a necessary second best. There is also a second, more important, reason why there is really no substitute for personal experience: the information you require may not exist or, if it does exist, it may not be correct. The Internet/World Wide Web is a particular hazard here because of the vast amount of unregulated information it makes available, often dressed up to appear professional and authoritative. *Such material should always be treated with caution and verified before being trusted.* Where academic information is concerned, a first step might be to check the host site to see whether it is a recognised institution, like a university or an academic publisher; another might be to look for other research cited in the information, for instance in the form of journal citations (*see* section 4.3.1), which can be cross-checked. Entering the author's name into the search field of one of the web-based professional literature databases (Box 1.1) to see whether this person has a published research track record can be another approach. Using general-purpose search engines, like Yahoo! or

BOX 1.1 Searching online literature databases

Searchable online literature databases, like the Web of Knowledge, Google Scholar, BIOSIS or PubMed, allow you to search for articles by particular authors, or on particular topics, or according to some other category, such as a journal title or research organisation. An example of the kind of search fields on offer, in this case for the Web of Knowledge, is shown in Fig. (i).

The key to using the search fields effectively lies in the precision with which you specify your terms: too general and you will be swamped with articles that are of little or no interest; too narrow and you will wind up with only one or two and miss many important ones. To help with this, the search fields provide various means of linking terms so that searches can be focused (the AND, OR, NOT options – called 'operators' – in Fig. (i)). However, the process inevitably involves some compromises.

For example, suppose you were interested in steroid hormone secretion as a cause of immune depression in laboratory mice. You might start, seemingly reasonably, by typing '*steroid hormone* AND *immune depression* AND *laboratory* mice' into the 'Topic' search field in Fig. (i) and hitting the 'Search' button. Disappointingly, and rather to your surprise, this yields nothing at all – apparently nobody has published anything on steroid hormones and immune depression in mice. At the other extreme, a search for '*immune* AND *mice'* yields over 45,000 articles, a wholly unmanageable number, of which many can be seen at a glance to be irrelevant to your needs. Clearly, something between the two is what required.

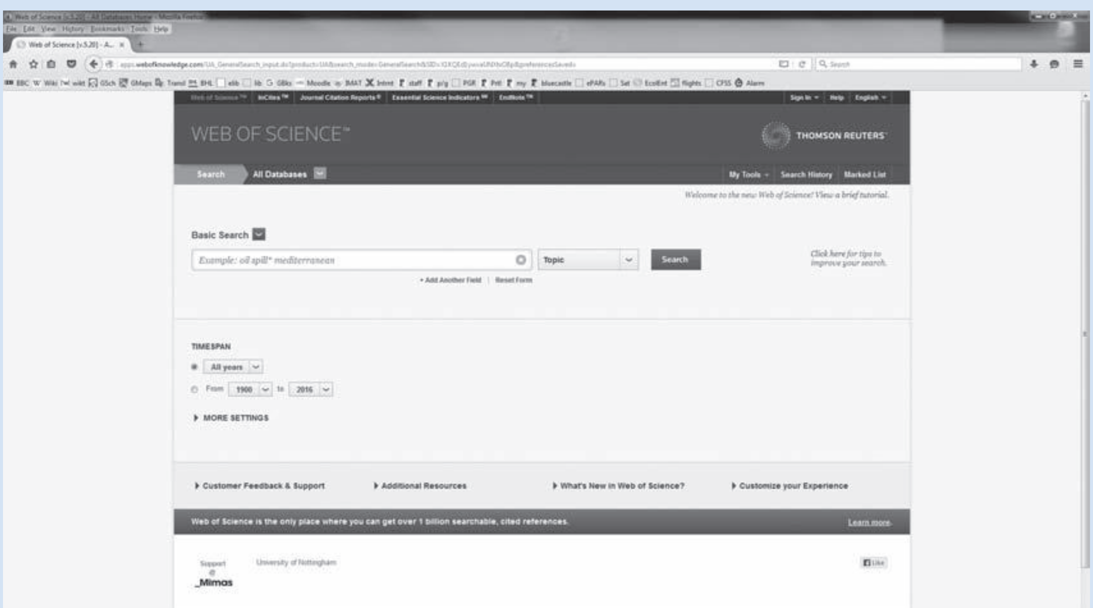


Figure (i) A screen capture from the Web of Knowledge as it appeared in 2016. Like other similar sites, it is regularly updated, so the exact appearance of the search field screen may change.

The reason the first search turned up nothing is not, of course, because nobody has published anything on the topic, but because the search term was restricted to a very specific combination of phrases. It could well be that people have published on the effects of steroid hormones on immune depression in mice but didn't use the precise phrases selected. For instance, they may have reported 'depressed immune responsiveness' or 'depressed immunity', rather than 'immune depression', and referred to specific hormones, such as testosterone or cortisol, rather than the generic term 'steroid'. There are various ways of catering for this. In the Web of Knowledge, the form '*immun** SAME *depress** AND *mice'* in the 'Topic' search field allows the system to search for any term beginning with 'immun' or 'depress', such as 'immune', 'immunity', 'immunocompetence', 'depression', 'depressed' and so on, thus picking up all the variants. The term 'SAME' ensures similar combinations of phrase are recognised, in this case, say, 'immune depression', 'depressed immunity' or 'depressed immune response'. Running the search again in this form yields around 450 articles, much better than zero or 45,000, but with quite a lot of them still redundant. If the search is specified a little more tightly as '*immun** SAME *depress** AND *mice* AND *hormone',* however, it turns up around 40 articles, and all much more on target.

All the searchable databases use these kinds of approaches for refining searches, some very intuitive, some less so. One thing you will quickly notice, though, is that exactly the same search can turn up a different number and selection of articles depending on which database you are using – BIOSIS, for example, manages to find something under the initial over-specific search that drew a blank on the Web of Knowledge. For this reason, it is good practice to run searches on a selection of databases.

Google, can often turn up information from the professional literature too, but just as often you're likely to get information from unregulated personal websites, or other sources of uncertain provenance. Taking received wisdom at face value can be a dangerous business – something even seasoned researchers can continue to discover, the famous geneticist and biostatistician R®. A. Fisher among them.

In the early 1960s, Fisher and other leading authorities at the time were greatly impressed by an apparent relationship between duodenal ulcer and certain rhesus and MN blood groups. Much intellectual energy was expended trying to explain the relationship. A sceptic, however, mentioned the debate to one of his blood-group technicians. The technician, for years at the sharp end of blood-group analysis, resolved the issue on the spot. The relationship was an artefact of blood transfusion! Patients with ulcers had received transfusions because of haemorrhage. As a result, they had temporarily picked up rhesus and MN antigens from their donors. When patients who had not been given transfusions were tested, the relationship disappeared (Clarke, 1990).

Where at all feasible, therefore, testing assumptions yourself and making up your own mind about the facts available to you is a good idea. Indeed, science is often characterised as systematic scepticism – a demand for evidence for every assertion. It is impossible to draw up a definitive list of what it is an investigator needs to know as essential background; biology is too diverse a subject, and every investigation is to some extent unique in its factual requirements. Nevertheless, it is useful to indicate the kinds of information that are likely to be important. Some examples might be as follows:

Question

Can the material of interest be studied usefully under laboratory conditions or will unavoidable constraints or manipulations so affect it that any conclusions will have only dubious relevance to its normal state or functions?

For instance, can mating preferences in guppies usefully be studied in a small plastic aquarium, or will the inevitable restriction on movement and the impoverished environment compromise normal courtship activity?

Or, if nutrient transfer within a plant can be monitored only with the aid of a vital dye, will normal function be maintained in the dyed state or will the dye interfere subtly with the processes of interest?

Question

Is the material at the appropriate stage of life history or development for the desired investigation?

There would, for instance, be little point in carrying out vaginal smears on female mice to establish stages of the oestrous cycle if some females were less than 28 days of age. Such mice may well not have begun cycling.

Likewise, it would be fruitless to monitor the faeces of infected mice for the eggs of a nematode worm until a sufficient number of days have passed after infection for the worms to have matured.

Question

Will the act of recording from the material affect its performance?

For example, removing a spermatophore (package of sperm donated by the male) from a recently mated female cricket in order to assay its sperm content may adversely affect the female's response to males in the future.

Or, the introduction of an intracellular probe might disrupt the aspect of cell physiology it was intended to record.

Question

Has the material been prepared properly?

If the problem to be investigated involves a foraging task (e.g. learning to find cryptic prey), has the subject been trained to perform in the apparatus and has it been deprived of food for a short while to make it hungry?

Similarly, if a mouse of strain X is to be infected with a particular blood parasite so that the course of infection can be monitored, has the parasite been passaged in the strain long enough to ensure its establishment and survival in the experiment?

Question

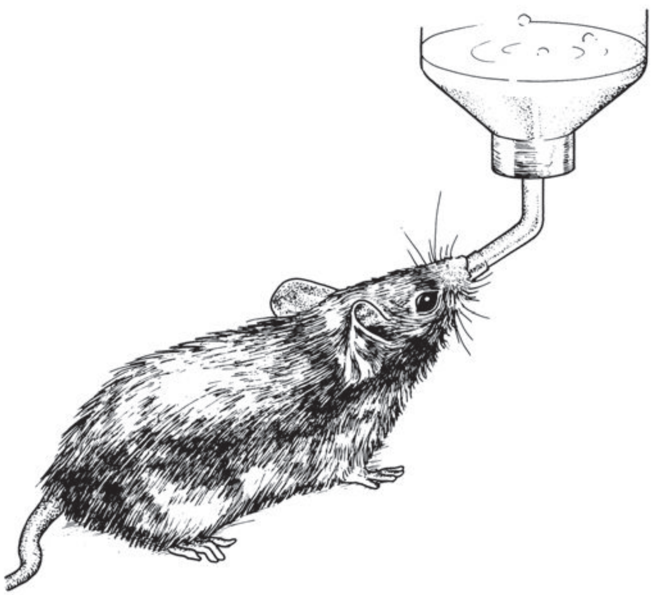
Does the investigation make demands on the material that it is not capable of meeting?

Testing for the effects of acclimation on some measure of coping in a new environment might be compromised if conditions in the new environment are beyond those the organism's physiology or behaviour have evolved to meet.

Likewise, testing a compound from an animal's environment for carcinogenic properties in order to assess risk might not mean much if the compound is administered in concentrations or via routes that the animal could never experience naturally.

Question *Are assumptions about the material justified?*

In an investigation of mating behaviour in dragonflies, we might consider using the length of time a male and female remain coupled as an index of the amount of sperm transferred by the male. Before accepting this, however, it would be wise to conduct some pilot studies to make sure it was valid; it might be, for instance, that some of the time spent coupled reflected mate-guarding rather than insemination.



By the same token, assumptions about the relationship between the staining characteristics of cells in histological sections and their physiological properties might need verifying before concluding anything about the distribution of physiological processes within an organ.

The list could go on for a long time, but these examples are basic questions of practicality. They are not very interesting in themselves but they, and others like them, need to be addressed before interesting questions can be asked. Failure to consider them will almost inevitably result in wasted time and materials.

Of course, even at this level, the investigator will usually have the questions ultimately to be addressed – the whole point of the investigation – in mind, and these will naturally influence initial considerations. Before we develop this further, however, there is one further, and increasingly prominent, issue we must address, and that is the *ethics* of working with biological material.

1.2.1 Ethical considerations

Because biological material is either living, or was once living, or is derived from something that is or was living, we are sensitive to the possibility that another living organism may be harmed in some way as a result of what we are doing. Of particular concern is the possibility that our activity might cause such an organism to suffer, physically or psychologically. We try very hard to avoid suffering ourselves because, by definition, it is extremely unpleasant, so the question arises as to whether we should risk inflicting it on another living being simply because we are interested in finding something out about it. This is not an easy question to answer, not least because of the difficulty of knowing whether species very different from ourselves, such as invertebrates, are capable of experiencing anything that might reasonably be called suffering in the first place. However, good science is mindful of the possibility, and works to various guidelines and codes of practice, some enforced by law, to give organisms the benefit of the doubt. While minimising the risk of suffering is important in itself, there is also a straightforward practical reason why we should take care of the organisms we use, whatever they may be, since any results we obtain from them could be affected if the organism is damaged or in some way below par.

Suffering may not be the only potential ethical concern. If material is coming from the field, for example, there could be conservation issues. Is the species concerned endangered? Is the habitat it occupies fragile? Are there unwelcome consequences for populations or habitats of removing material and/or returning it afterwards? Questions like this can lead to acute dilemmas. For instance, the fact that a species is becoming endangered may mean there is a desperate need for more information about it, but the very means of acquiring the information risks further harm.

As awareness of these issues increases, ethical considerations are beginning to play a more explicit role in the way biologists approach their work, not just in terms of taking greater care of the organisms they use, and being better informed about their needs, but at the level of how investigations are designed in the first place. Take sample size, for instance. Deciding on a suitable sample size is a basic problem in any quantitative study. It might involve an informal judgement on the basis of past experience or the outcome of other studies, or it might depend on power tests (*see* section 3.4.1) to calculate a sample size statistically. Where there are ethical concerns, a power test would arguably be better than 'guesstimation' because it would provide an objective means of maximising the likelihood of a meaningful result while minimising the amount of material needed (a smaller sample would risk the outcome being swamped by random noise, while a larger one would use more material than necessary). But, of course, the ideal sample size indicated by the power test might demand more material than can be sustained by the source, or involve a very large number of animals in a traumatic experimental procedure. The value of proceeding then has to be judged against the likely cost from an ethical perspective, a task with considerable room for debate. Detailed discussion of these issues is beyond the scope of this book, but a good idea of what is involved can be found in Bateson

(1986, 2005), who provides a digestible introduction to trading off scientific value and ethical concerns, and the extensive ethical guidelines for teachers and researchers in animal behaviour published by the Association for the Study of Animal Behaviour (ASAB) and its North American partner, the Animal Behavior Society (ABS) (see www.asab.org or www.animalbehaviorsociety.org .uk or each January issue of the academic journal *Animal Behaviour*). It is also well worth looking at the website of the UK National Centre for the 3Rs (www .nc3rs.org; the three Rs stand for the Replacement, Refinement and Reduction in the use of animals in research), a government-funded organisation dedicated to progressing ethical approaches to the use of animals in biology. For discussion of more philosophical issues, see, for example, Dawkins (1980, 1993) and Barnard & Hurst (1996). It is important to stress that, tricky as these kinds of decision can be, ethical considerations should *always* be part of the picture when you are working with biological material.

1.3 The skill of asking questions

1.3.1 Testing hypotheses

Charles Darwin once remarked that without a hypothesis a geologist might as well go into a gravel pit and count the stones. He meant, of course, that simply gathering facts for their own sake was likely to be a waste of time. A geologist is unlikely to profit much from knowing the number of stones in a gravel pit. This seems self-evident, but such *undirected* fact-gathering (not to be confused with the often essential descriptive phase of hypothesis development) is a common problem among students in practical and project work. There can't be many science teachers who have not been confronted by a puzzled student with the plea: 'I've collected all these data, now what do I do with them?' The answer, obviously, is that the investigator should know what is to be done with the data before they are collected. As Darwin well knew, what gives data collection direction is a working *hypothesis.* Theories and hypotheses are absolutely vital to science, otherwise 'we shall all be washed out to sea in an immense tide of unrelated information' (Watt, 1971). With them, 'the enormous ballast of factual information, so far from being about to sink us, is used to reveal patterns and processes so that we need no longer to record the fall of every apple' (Dixon, 2000).

The word 'hypothesis' sounds rather formal and, indeed, in some cases hypotheses may be set out in a tightly constructed, formal way. In more general usage, however, its meaning is a good deal looser. Verma & Beard (1981), for example, define it as simply:

> a tentative proposition which is subject to verification through subsequent investigation. In many cases hypotheses are hunches that the researcher has about the existence of relationships between variables.