PAULO INACIO K.L. PRADO

ASKING QUESTIONS IN BIOLOGY

Design, Analysis and Presentation in Practical Work

Chris Barnard, Francis Gilbert
& Peter McGregor

Department of Life Science
University of Nottingham

Longman Scientific & Technical

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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.

This book has its origins in a first-year practical course we have run at Nottingham over the past few years. The aim of the course is to introduce students in the life 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 analysis – rather than on laboratory, field and analytical techniques *per se*. Statistics and the use of computer packages are introduced simply as aids at the appropriate stage of enquiry. We thus emphasize their role and the information they provide rather than the theory behind them and the mechanics of their use. Provided students know which test to use and how to interpret its result, statistics can be regarded as a useful 'black box'.

The book takes this approach by looking 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 emphasized and a series of main examples runs through the book to illustrate their application at each stage. We have deliberately used behaviour as a vehicle for the main examples because of its flexibility and scope for open-ended investigation and because it is cheap and easy to lay on as practical material for large numbers of students. However, as should be readily apparent from the range of other examples used, the vehicle itself is immaterial to the aims of the book, which is concerned with asking questions across the field of biology.

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? The section addresses these points by looking at the development of testable hypotheses and predictions and the sources from which they might arise.

The next section 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 section introduces plots and summary statistics as useful ways of identifying interesting patterns on which to base hypotheses. The section 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 the third section 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 basic but 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 the final section, the book considers the presentation of information. Once hypotheses have been tested, how should the outcome be conveyed for greatest effect? The section discusses the use of tables, figures and other modes of presentation and shows how a written report should be structured. The points made in the section are then illustrated in a complete written report based on one of the main worked examples.

At the end of the book is a number of appendices. These provide some self-test questions and answers based on the material in the book, some worked examples of significance tests and some statistical tables for use in significance testing.

We said that the book had its inception in our introductory practical course. However, the practical course itself was developed in response to an increasingly voiced need on the part of students to be taught how to ask and answer questions. Both the practical course and the book have benefited immensely from a constant and pleasurable interaction with our undergraduates over the years. Their enquiries and insights continue to hone the way we teach and have been the guiding force behind all the discussions in the book. Without them it simply wouldn't have been written.

CJB, FSG, PKM

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DOING SCIENCE

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 formalized speculation backed up (or otherwise) by equally formalized observation and experimentation. In its broadest sense most of us 'do science' all the time.

SCIENCE AS ASKING QUESTIONS

Science is often regarded by those outside it as a 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, it 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 by students to satisfy their own curiosity. However, the skills it emphasizes are just as appropriate to more limited set-piece practicals. Perhaps a distinction – admittedly over-generalized – 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.

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 recognize 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.

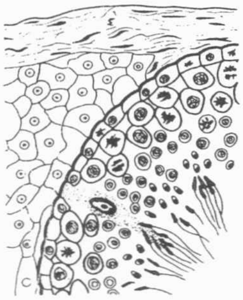
Of course, there are several ways in which such knowledge can be acquired: e.g. textbooks, specialist journals, asking an expert or simply finding out for yourself through observation and exploration. Whichever way is most appropriate, however, a certain amount of background preparation is usually essential, 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 generalized and often highly stylized 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. 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 in 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. 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

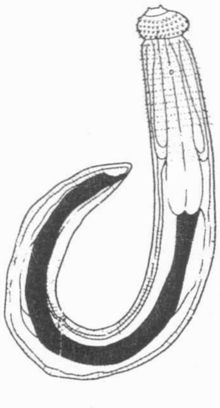
Can the material of interest be studied usefully under laboratory conditions or will unavoidable constraints or manipulations so affect it that any conclusions will have only dubious relevance to its normal state or functions?



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 the growth and developmental fate of certain cells 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 28–32 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.

Todas essas questões estão relacionadas às premissas?

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

Question

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.

Has the material been prepared properly?

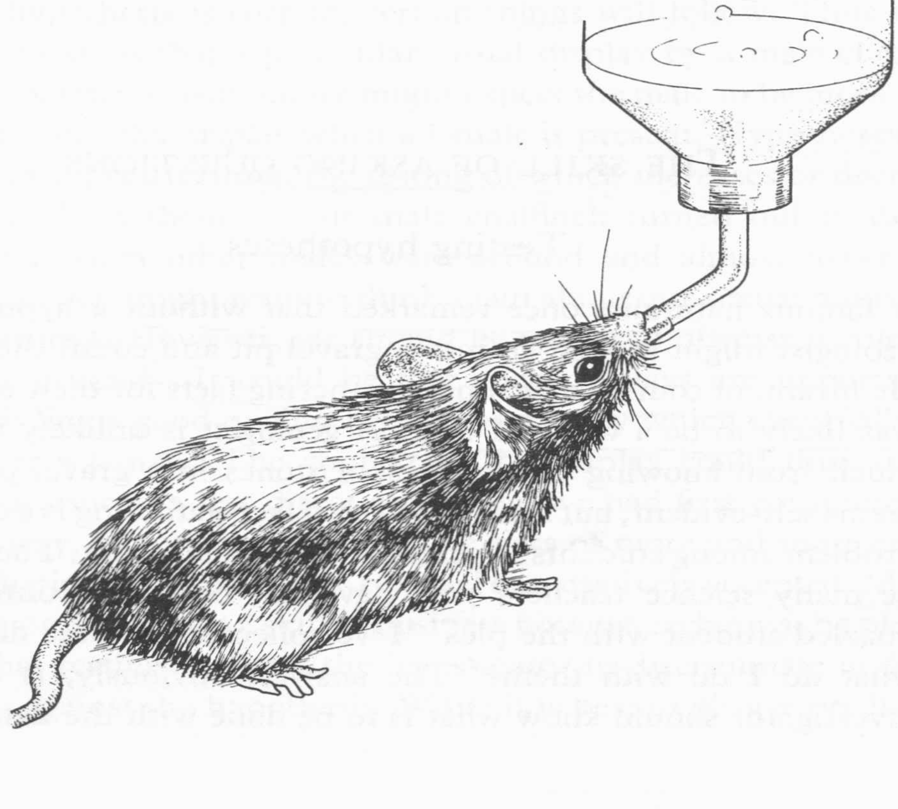
Question

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?

Does the investigation make demands on the material that it is not designed to meet?

Question

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 true; 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.

THE SKILL OF ASKING QUESTIONS

Testing hypotheses

A famous naturalist 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 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 the naturalist well knew, what gives data collection direction is a working **hypothesis**.

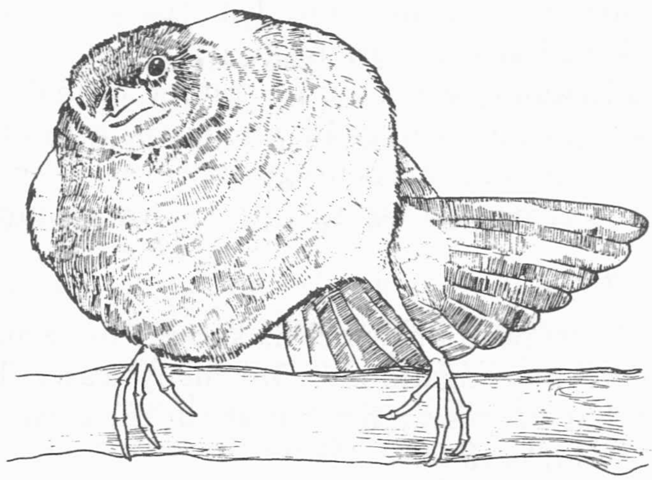
The word 'hypothesis' sounds rather formal and, indeed, in some cases hypotheses may be set out in a tightly constructed, formal way. In more general usage, however, its meaning is a good deal looser. Verma and Beard (1981), for example, define it as simply

> 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

A hypothesis, then, can be little more than an intuitive feeling about how something works, or how changes in one factor will relate to changes in another, or about any aspect of the material of interest. However vague it may be, though, it is formative in the purpose and design of investigations because these set out to test it. If at the end of the day the results of the investigation are at odds with the hypothesis, the hypothesis may be rejected and a new one put in its place. As we shall see later, hypotheses are never proven, merely rejected if data from tests so dictate, or retained for the time being for possible rejection after yet further tests.

How is a hypothesis tested?

If a hypothesis is correct, certain things will follow. Thus if our hypothesis is that a particular visual display by a male chaffinch is sexual in motivation, we might expect the male to be more likely to perform the display when a female is present. Hypotheses thus generate **predictions**, the testing of which increases or decreases our faith in them. If our male chaffinch turned out to display mainly when other males were around and almost never with females, we might want to think again about our sexual motivation hypothesis. However, we should be wrong to dismiss it solely on these grounds. It could be that such displays are important in defending a good quality breeding territory which eventually will attract a female. The context of the display could thus still be sexual, but in a less direct sense than we had first considered. In this way, hypotheses can produce tiers of more and more refined predictions before they are rejected or tentatively accepted. Making such predictions is a skilled business because each must be phrased so that testing it allows the investigator to discriminate in favour of or against the hypothesis. While it is best to phrase predictions



as predictions (thus: *males will perform more of display* y *in the presence of females*), they sometimes take the form of questions (*do males perform more of display* y *when females are present?*). The danger with the question format, however, is that it can easily become too woolly and vague to provide a rigorous test of the hypothesis (e.g. *do males behave differently when females are present?*). Having to phrase a precise prediction helps counteract the temptation to drift into vagueness.

Hypotheses, too, can be so broad or imprecise that they are difficult to reject. In general the more specific, mutually exclusive hypotheses that can be formulated to account for an observation the better. In our chaffinch example, the first hypothesis was that the display was sexual in motivation. Another might be that it reflected aggressive defence of food. Yet another that it was an anti-predator display. These three hypotheses give rise to very different predictions about the behaviour and it is thus, in principle, easy to distinguish between them. As we have already seen, however, distinguishing between the 'sexual' and 'aggressive' hypotheses may need more careful consideration than we first expect. We shall look at the development of hypotheses and their predictions in more detail later on.

WHERE DO QUESTIONS COME FROM?

As we have already intimated, questions do not spring out of a vacuum. They are triggered by something. They may arise from a number of sources.

Curiosity

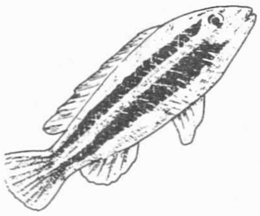
Questions arise naturally when thinking about any kind of problem. Simple curiosity about how something works or why one group of organisms differs in some way from another group can give rise to useful questions from which testable hypotheses and their predictions can be derived. There is nothing wrong with 'armchair theorizing' and 'thought experiments' as long as, where possible, they are put to the test. Sitting in the bath and wondering about how migratory birds manage to navigate, for example, could suggest roles for various environmental cues like the sun, stars and topographical features. This in turn could lead to hypotheses about how they are used and predictions about the effects of removing or altering them. By the time the water was cold, some useful preliminary experiments might even have been devised.

Casual observation

Instead of dreaming in the bath, you might be watching a tank full of fish, or sifting through some histological preparations under a microscope. Various things might strike you. Some fish in the tank might seem very aggressive, especially towards others of their own species, but this aggressiveness might occur only around certain objects in the tank, perhaps an overturned flowerpot or a clump of weed. Similarly, certain cells in the histological preparations may show unexpected differences in staining or structure. Even though these aspects of fish behaviour and cell appearance were not the original reason for watching the fish or looking at the slides, they might suggest interesting hypotheses for testing later. A plausible hypothesis to account for the behaviour of the fish, for instance, is that the localized aggression reflects territorial defence. Two predictions might then be: (a) *on average, territory defenders will be bigger than intruders* (because bigger fish are more likely to win in disputes and thus obtain a territory in the first place) and (b) *removing defendable resources like upturned flowerpots will lead to a reduction in aggressive interactions*. Similarly, a hypothesis for differences in cell staining and structure is that they are due to differences in the age and development of the cells in question. A prediction might then be: *younger tissue will contain a greater proportion of* (what are conjectured to be the) *immature cell types*.

Exploratory observations

It may be that you already have a hypothesis in mind, say that a particular species of fish will be territorial when placed in an appropriate aquarium environment. What is needed is to decide what an appropriate aquarium environment might be so that suitable predictions can be made to test the hypothesis. Obvious things to do would be to play around with the size and number of shelters, the position and quality of feeding sites, the number and sex ratio of fish introduced into the tank and so on. While the effects of these and other factors on territorial aggressiveness among the fish might not have been guessed at beforehand, such manipulations are likely to suggest relationships with aggressiveness which can then be used to predict the outcome of further, **independent** investigations. Thus if exploratory results suggested aggressiveness among defending fish was greater when there were ten fish in the tank compared with when there were five, it would be reasonable to predict that aggressiveness would increase as the number of fish increased, *all other things being equal*. An experiment could then be designed in which shelters and feeding sites were kept constant but different numbers of fish, say 2, 4, 6, 8, 10 or 15, were placed in the tank. Measuring the amount of aggression by a defender with each number of fish would provide a test of the prediction.



Previous studies

One of the richest sources of questions is, of course, past and ongoing research. This might be encountered either as published literature or 'live' as research talks at conferences or seminars. A careful reading of most published papers, articles or books will turn up ideas for further work, whether at the level of alternative hypotheses to explain the problem in hand or at the level of further or more discriminating predictions to test the current hypothesis. Indeed, this is the way most of the scientific literature develops. Some papers, often in the form of mathematical models or speculative reviews, are specifically intended to generate hypotheses and predictions and may make no attempt to test them themselves. At times, certain research areas can become overburdened with hypotheses and predictions, generating more than people are able or have the inclination to test. If this happens, it can have a paralysing effect on the development of research. It is thus important that hypotheses, predictions and tests proceed as nearly as is feasible hand in hand.

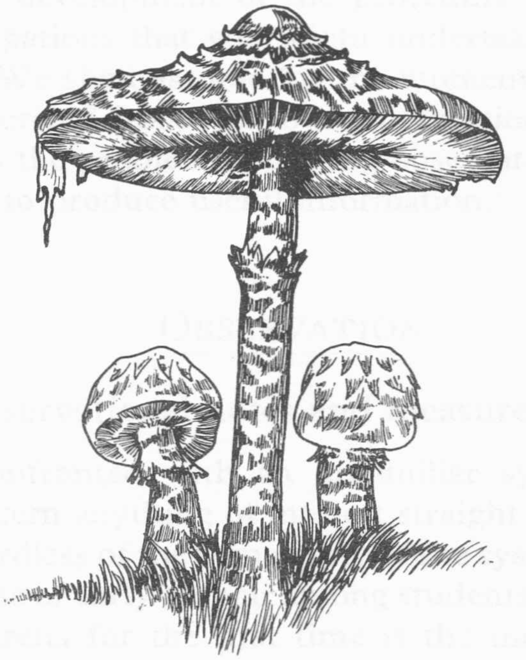
WHAT THIS BOOK IS ABOUT

Okay, we've said a little about how science works and how the kind of question-asking on which it is based can arise. We now need to look at each part of the process in detail because while each may seem straightforward in principle, some knotty problems can arise when it is put into practice. In what follows, we shall see how to:

1. frame hypotheses and predictions from preliminary source material;
2. design experiments and observations to test predictions;
3. analyse the results of tests to see whether they show anything interesting; and
4. present the results and conclusions of tests so that they are clear and informative.

The discussion deals with these aspects in order so that the book can be read straight through or dipped into for particular points. A summary at the end of each section highlights the important take-home messages and the questions at the end show what you should be able to tackle after reading the book.

Remember, the book is about asking and answering questions in biology – it is not a biology textbook or a statistics manual and none of the points it makes are restricted to the examples that illustrate them. At every stage you should be asking yourself how what it says might apply in other biological contexts, especially if you have an interest in investigating them!



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