Field methods for rodent studies in Asia and the Indo-Pacific

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Contents

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CHAPTER 1

Why study rodent populations?

Introduction

Rodents are a dominant group of mammals. There are more than 2700 species of rodents worldwide; in fact, 42% of all the mammal species on Earth are rodents. Two-thirds of living rodent species belong to just one family, the Muridae, and most of the rodents found in Asia, both pests and non-pests, also belong to this family.

Rodents occupy a wide range of natural habitats, including forests and grasslands, as well as the human world of agricultural landscapes, villages and townships. Most rodents are prolific breeders and they often represent a significant amount of the animal biomass in forests and other natural ecosystems. As such, they play an important role in the food web, both as consumers of plants and fungi, and as a food resource for many of the larger predators. They are also important environmental engineers, helping to spread pollen and seed, aerating the soil through their digging and burrowing activities, and in extreme cases (e.g. beavers), changing the whole nature of the landscape. These ecological benefits are sometimes called 'ecosystem services'.

A relatively small number of rodent species have adapted successfully to the human environment of gardens, fields, villages and towns. Unfortunately, the people who created this environment generally view the successful rodents in a different light. Indeed, in almost all societies, the rodent species found around houses and in fields are viewed as 'pests' or even as 'vermin'. And often with just cause—the rodents consume and spoil crops in the field and in storage bins, they damage household possessions and even buildings and roads, and they play an often overlooked but highly significant role in the transmission of various diseases.

Rodents as pest species

Rodents affect rural families in three main ways: they eat agricultural crops in the field; they eat, spoil and contaminate stored food; and they carry diseases of humans and their livestock. In the Asia–Pacific region, rodents are one of the most important constraints to agricultural production. This region contains two-thirds of the World's poor—approximately 800 million people in 2001—and the majority of these people live in rural areas. Management of rodent pests in agricultural regions is therefore a high priority for reducing poverty.

The losses caused by rodents to rice crops in Asia provide a graphic example of their impact. Rodents typically cause annual preharvest losses to rice of between 5% and 10% of production. However, in some areas, episodic outbreaks of rodents cause heavier losses or even the complete destruction of crops. Postharvest losses in some areas may match or exceed the preharvest damage, and reports of 20% losses caused by rodents to grain after harvest are not unusual. Some 90% of the world's rice is grown and consumed in Asia. If we were able to reduce rodent losses by only 5%, then there would be enough rice to feed the population of Indonesia for one year (210 million people who rely on rice providing 65% of their daily calories)!

Rodents as beneficial species

For decades, the literature on integrated pest management of insects has emphasised that not all insects are pests. Indeed, there has been much scientific effort in identifying non-pest species and those that are described as 'beneficial' insects because they provide benefit through preying upon, or competing with, pest species of insects, or play a significant role in the pollination of crop and other plant species. We have reviewed the available literature on rodents and found that for any particular region, only 5–10% of rodent species are major agricultural pests (Table 1.1). Hence, rather than developing general methods that will control most rodent populations, we should try to minimise the effect of control on species of rodents that are not pests. Indeed, the conservation of non-pest species of rodents should always be of concern in any control program. To illustrate this issue, a rare species of tree rat (*Chiromyscus chiropus*; Fea's tree rat) is sometimes captured at the edge of upland rice fields in Laos (Lao People's Democratic Republic). If farmers conduct non-specific rodent control around the rice fields, then these animals may be affected.

The importance of conserving non-pest species of rodents is not an easy concept to promote in developing countries. Many farmers have a long cultural tradition of battling the depredations of rodents; it is understandable if from their perspective 'the only good rat is a dead rat'. We may be able to change this perspective, but to do so will require some very clear examples of the benefits that non-pest rodent species provide.

The high diversity of rodent species in many agro-ecosystems may also provide an opportunity to identify species that can indicate whether the ecosystem is in poor condition (degraded landscape) or in good condition (sustainable production is likely). Such species are known as 'indicator species'. The indicator species concept has been widely adopted using certain bird species as a measure of the health of a landscape. In agricultural landscapes,

Table 1.1 The number of species belonging to the Order Rodentia in various geographical regions that are considered significant pests of agriculture, and those whose conservation status is of concern (endangered, critical or vulnerable) or insufficient is known to assess the risk. The conservation status data are from the International Union for Conservation of Nature and Natural Resources (IUCN) website (<http://www.redlist.org/>). (Based on Singleton et al. 2003a.)

Continent or country	Number of rodent species	No. of rodent species that damage crops	No. of significant pest species in cropping systems	Conservation status	
				No. of species at risk	Little known
Africa	381	77	12–20	60	11
Australia	67	7	4	14	1
Europe	61	16	5	4	
India	128	18	12 (5 wide distribution 7 restricted distribution)	21	1
Indonesia (not incl. Papua)	164	25+	13	11+	28+
Laos	53	12+	4–8	4	14+
New Guinea (PNG + Papua; not incl. Island Melanesia)	73	10+	6	0	9+

rodents and other more sedentary animals may be better indicators of environmental health at a local to regional scale.

Ecologically based rodent management

Ecologically based management of rodent pests is a concept that has developed a strong following in developing countries since the late 1990s. The concept aims to combine basic and applied research on rodents through focusing on the population ecology of rodents and developing management directed at the agro-ecosystem level. The concept is appealing because it promotes actions that facilitate sustainable agriculture and have minimum environmental impact. However, developing an effective integrated management plan requires a good understanding of the basic ecology of individual rodent pest species. This in turn is dependent on access to field methodologies that enable us to understand the population dynamics and field ecology of rodents.

In our experience, the process of developing effective, ecologically based rodent management is a learning cycle that involves phases of observation, formulation and testing of hypotheses, and further observation or experimentation, with each round of activities leading to better understanding. This flexible and responsive process is appropriate to the complex nature of the ecological problems that we face in dealing with rodent pests, and to the equally complex socioeconomic context presented by the diverse political and cultural systems of the Asia–Pacific region.

Despite the cyclic nature of the learning process, we believe that it is useful to distinguish three distinct phases in any investigation of rodent problems. These phases, described below, can provide a useful framework for designing a long-term rodent management study, or as a means of assessing the current state of knowledge for any given region. Indeed, a good way to begin is to ask the question, Where do we currently fall in relation to the three phases?

Phase 1: problem definition

Although rodents are frequently mentioned as a major cause of damage to both field crops and stored foodstuffs, there is often little in the way of hard data on crop losses or on other economic or social impacts. Rodent control activities always cost money and time, so before launching into any kind of control activity, it is a good idea to first define the scale of the problem. This usually involves the following steps:

- confirming that rodents are genuinely the cause of the problem
- identifying the species of rodents involved
- estimating the amount of damage to field crops and stored food.

Identifying the major rodent pest species is a useful part of problem definition because it allows the researcher to make use of the results of prior ecological studies and to learn from previous attempts to control the same species. For example, finding that *Rattus rattus* is the major field pest in an area would immediately alert the fieldworker to the likelihood that this highly adaptable species will need to be controlled in all local habitats, including around human habitation.

A preliminary assessment of health issues, perhaps based on local clinic or hospital records and some focus group meetings, might also be informative at this stage.

The problem definition phase might also be called the 'question definition' phase, for it is during this period that we should be trying to identify the key factors that influence rodent numbers and activity, and the level of risk that they pose to crops, stored food and human health. Such questions might be, Are we dealing with a localised problem or one that occurs over large areas? Do rodents cause substantial losses every year (chronic problem) or is the damage much heavier in some years than others (episodic acute problem)? Are periods of high crop damage due to increases in rodent numbers or due to a shift in the focus of their activities? If the former, is the

population increase due to rapid breeding within the fields at certain times of year, or is it due to migration of rodents from other habitats? Issues of this kind are fundamental to the design and implementation of ecologically based rodent management—where the goal is to manipulate the ecological system in ways that reduce the opportunities for rodents and thus improve human livelihoods.

Other important questions might relate to the history of rodent problems for a particular region: Have rodents always damaged crops in the area, or have their impacts increased in recent years? What changes in land use or cropping systems might have taken place at the same time?

Local knowledge is, of course, fundamental to framing many of these questions. Although some information might be contained in reports or other documentary sources, the richest and most direct source of information on the scale and extent of the problem invariably comes from members of the farming community itself. Various methods can be used to gain access to this wealth of information, many of them drawn from the realm of farmer participatory research (see Chapter 10).

Phase 2: ecological and historical studies

During this phase, we try to find answers to particular questions or test particular hypotheses that we identified during phase 1. In many cases, this means carrying out basic ecological studies on: changes in population size; the timing and location of breeding activity; patterns of habitat use and movement; and the timing and pattern of damage within both the cropping systems and the habitation areas.

An important part of ecological research is to decide upon an appropriate spatial and temporal scale for the studies (see Chapter 2). How large an area do we need to study and how long does our study need to last? These are particularly important questions where the primary objective is to develop options for ecologically based rodent pest management. This is because rodent management actions generally will need to be implemented over large areas and in a coordinated and sustained fashion if they are to be effective.

Before starting any ecological studies, it is sensible to learn as much as possible from any previous studies of the same species or similar cropping systems. Much of the information currently available is summarised in Chapter 11 for the major pest species, with the relevant literature sources provided at the end of each species account. Where basic biological information is known for a particular species from earlier studies (e.g. average litter size, preferred location of nesting sites), it may be sufficient to do a small study only—just enough to test whether the species has a similar basic biology in your local population. This book contains information on many of the basic field techniques required to carry out ecological studies of this kind.

To answer historical questions, it is sometimes possible to obtain information from written sources such as agricultural records of crop production or pest problems. In some countries, these records are detailed and extensive, and span many decades. These can provide valuable insights into the history of rodent problems and it is usually worthwhile investing some time and effort into extracting the useful information. For many areas, records of this kind do not exist. In such situations, it may be possible to piece together a history of the rodent problem by conducting interviews with farmers and extension personnel. While gathering this information, we would also recommend asking questions about changes in cropping patterns and rodent management methods (e.g. poison use), and in general lifestyle factors such as the size and location of villages. By building up an overall picture of the historical changes, it may be possible to identify some of the key factors that have led to increased rodent problems—and hopefully then use these insights to reverse the trend.

Phase 3: designing and testing management options

Options for the management of rodent pests in any particular agro-ecosystem should develop in the first instance out of the improved ecological knowledge of the system. However, this knowledge in itself may not be a sufficient basis for designing management options. The other essential component is an understanding of what we might term 'the human factor'.

The human factor has many dimensions, including diverse cultural beliefs relating to rodents and the wider environment, variable systems of social organisation that influence the willingness or ability of people to work together in particular ways, and complex economic considerations that determine local priorities for allocating money and labour. It is also expressed at a variety of scales, from individual differences between members of one community, to more structured variations based on factors such as gender and wealth.

The complex interaction of ecological, cultural, social and economic factors needs to be given careful consideration when designing rodent management options. This is particularly so in areas where the agricultural community consists of smallholder farmers who are perhaps more used to making individual decisions and less familiar with the concept of broad-scale and coordinated actions.

The issue of sustainability is also vitally important. Because it is rarely, if ever, possible to completely eradicate a rodent pest (except perhaps from small islands), a lapse in management actions, even for a short period, may lead to a rapid resurgence of rodent populations and associated problems. In most situations, a high level of ongoing community commitment and involvement is therefore fundamental to effective pest rodent management.

The most direct way to find management options that may be appropriate for any given location is to adopt a participatory approach at all stages of project design and implementation. This involves working closely with communities that are representative of the potential long-term users of the management options. Once we have identified some management options that are ecologically appropriate, culturally acceptable, and both socially and economically sustainable, we then need to perform further tests to see how well they will perform in the real world. In many cases, their performance will need to be judged against a range of criteria, including their immediate economic benefit, their social implications, and their longer-term environmental impact. Some of these parameters may be difficult to measure; hence wide community consultation may be needed to gain a comprehensive and balanced view of how a particular management strategy is likely to perform in the longer term.

Despite these complexities, whenever we test a management option, we need to keep in mind that we are conducting an experiment. This is a critically important point. Field or village-level trials that are not conducted according to the principles of experimental design very often fail to deliver any truly interpretable results. This is not to say that an experimental approach will automatically guarantee good management options. Rather, good experimental design should allow a researcher or manager to understand why a particular management option has failed, and to design new trials or experiments accordingly, thus continuing the cycle of learning.

Purpose and scope of this book

We have written this book as a resource for anyone who is intending to conduct field studies of rodents in Asia or the Pacific. However, given the current, strong interest in reducing the impact of rodent pests on rural livelihoods across the region, we expect that the majority of users of this book will be agricultural scientists, extension personnel and students working in the context of management projects. For this reason, we will focus on methods that are appropriate for the study of 'pest' rodents and of the damage to crops that they cause. Nevertheless, many of the same methods would be appropriate for

the study of forest rodents (and with some minor adaptation, other small mammals) and in different geographical regions.

Wherever possible, we have avoided the use of specialised ecological and anatomical terminology; a glossary is provided at the end of the book to explain the technical terms that are used. Throughout the text we use scientific names rather than 'common' names for the main rodent pests. The reasons for this are explained in Chapter 4, and we encourage all users to become familiar with the scientific names of at least the main pest species in their area.

The methods that we describe in this book are ones that we have found especially useful in studies of pest rodents in Australia, Bangladesh, Indonesia, Laos and Vietnam. The coverage is by no means exhaustive and we freely acknowledge that there are many alternatives to the methods presented here. While we do not wish to be prescriptive, we do believe that there are advantages to be gained by other researchers adopting the methods recommended here, at least as a basic set. Most importantly, the use of common methods will facilitate the rapid growth of ecological data for the main pest rodents of the Asia–Pacific region. This will hopefully reduce the need to acquire basic ecological data in each new study area, and will also allow everyone involved in ecologically based rodent management to learn directly from each other's experiences. Rapid advances in this field will depend to a large degree upon the free sharing of information, experiences and ideas.

Further reading

Buckle, A.P. and Smith, R.H. 1994. Rodent pests and their control. Cambridge, UK, Cambridge University Press, 405 p.

Dickman, C.R. 1999. Rodent–ecosystem relationships: a review. In: Singleton, G.R., Hinds, L.A., Leirs, H. and Zhang, Z., ed., Ecologically-based management of rodent pests. ACIAR Monograph No. 59. Canberra, Australian Centre for International Agricultural Research, 113–133.

Macdonald, D. 2001. The new encyclopaedia of mammals, 2nd edition. Oxford, UK, Andromeda, 961 p.

Narayan-Parker, D. 1997. Toward participatory research. World Bank Technical Paper No. 307. Washington, DC, World Bank, 265 p.

Okali, C., Sumberg, J. and Farrington, J. 1994. Farmer participatory research: rhetoric and reality. London, Intermediate Technology on behalf of the Overseas Development Institute, 159 p.

Singleton, G.R. 2003. Rodent impacts on rice production in Asia. IRRI Occasional Paper No. 45. International Rice Research Institute, Los Baños, Philippines, International Rice Research Institute, 30 p.

Singleton, G.R., Brown, P.R., Jacob, J., Aplin, K. and Sudarmaji 2003. Unwanted and unintended effects of culling. In: Harris, S. and Lavigne, D., ed., Culling of mammals. Cambridge, UK, Cambridge University Press (in press).

Singleton, G.R., Hinds, L.A., Krebs, C.J. and Spratt, D.M. (ed.) 2003. Rats, mice and people: rodent biology and management. ACIAR Monograph No. 96. Canberra, Australian Centre for International Agricultural Research, 564 p.

Singleton, G.R., Hinds, L.A., Leirs, H. and Zhang, Z. (ed.) 1999. Ecologically-based management of rodent pests, ACIAR Monograph No. 59. Canberra, Australian Centre for International Agricultural Research, 494 p.

Stenseth, N.C., Leirs, H., Skonhoft, A., Davis, S.A., Pech, R.P., Andreassen, H.P., Singleton, G.R., Lima, M., Machang'u, R.S., Makundi, R.H., Zhang, Z., Brown, P.R., Shi, D. and Wan, X. 2003. Mice, rats and people: the bio-economics of agricultural rodent pests. Frontiers in Ecology and the Environment, 1(7), 367–375.

CHAPTER 2

Design of field studies

Introduction

Field studies of rodents can be highly rewarding but also immensely time-consuming. Many species are difficult to catch and it is often necessary to set large numbers of traps over many months before any general pattern will emerge. Environmental data, such as measurements of crop damage caused by rodents, can be obtained much more easily, but fieldwork and subsequent analysis of the large datasets are also very time-consuming. Before we start any field activities, we need to be sure that our work will not only be done accurately and precisely, but also that the activities fit into a framework with a good experimental design. The aim of this chapter is to explain and illustrate some of the general principles of ecological experimental design for field studies on rodents.

General principles of experimental design

Experimental design is a term describing the logical structure of an experiment. An **experiment** is an attempt to test a **hypothesis**—an explanation for one or more observations made in the field or laboratory (see below). Rodent ecologists typically make many different kinds of observations and they frame many different kinds of hypotheses. Throughout this chapter, we use two hypotheses to illustrate our key points. These are:

- hypothesis 1—rice-field rats are more abundant in fields near refuge habitat, such as a large canal
- hypothesis 2—providing barn owl nest boxes will reduce rat damage to paddy rice.

These examples illustrate that there are two broad types of experiments—mensurative and manipulative.

- **Mensurative experiments** involve making some measurements of rodents and their habitat. The ecologist does not take any specific action against the rodents but measures what currently happens under current conditions. For example, to test hypothesis 1, we could measure the abundance of rats in fields near canals and in fields more distant from canals.
- **Manipulative experiments** involve taking some action either directly against the rodents or that somehow modifies their habitat. At least two sets of plots or manipulations are required. For example, to test hypothesis 2, we might 'treat' four fields by installing barn owl nest boxes and leave four similar fields without nest boxes as 'controls' (see below).

Both kinds of experiments share many properties and require that certain essential design features are met. The most important of these are:

- identification of the key factors under investigation
- use of experimental units of an appropriate size and duration
- inclusion of a baseline or control to distinguish non-random from random events
- replication to estimate causal linkages and experimental error
- randomisation and interspersion to avoid bias.

Identification of hypotheses and key factors

As a field biologist, you will start making observations from the very first day of a new project. These observations will lead to ideas about how the various rodent species are distributed across the various local habitats, how the rodent populations are likely to respond to the changes in food availability through the natural and agricultural cycles, and how the different species will respond to possible management options. As the body of observations and information grows, each of these ideas will develop in substance and sophistication. At an early stage in a new project, it is a good idea to write out a number of general hypotheses about the position and role of rodents in the local environment. Each of these hypotheses will probably lead to a number of more specific hypotheses that can serve as the basis for an experimental design.

A **hypothesis** is distinguished from a simple observation in various ways. One distinguishing feature is that a hypothesis can be tested by further observations or by an experiment. This means that it is capable of either being supported or proven incorrect by further observation. Testing of a hypothesis often leads to a refinement of ideas and a new hypothesis that incorporates the new evidence and insights.

A clearly stated hypothesis will include mention of one or more **key factors**. Using the two examples introduced above, hypothesis 1—rats are more abundant in fields near canals—identifies *distance to a canal* as a potential key factor in determining the local abundance of rats in any given field. As indicated above, an obvious way to test this is to compare rat numbers in fields located at different distances from a canal.

Hypothesis 2—owls reduce rat damage—identifies *the presence of owls* as a potential key factor in controlling rat damage in rice fields, although in this case, it does not specify whether this is because owls will reduce rat numbers or because they will modify rat behaviour in some way that makes them less likely to damage rice. This hypothesis might also be made more explicit by specifying that the number of owls might be important, rather than just their presence or absence.

In general, the more explicit we can make our hypotheses, the more likely we are to have good experimental design and ultimately come up with satisfactory answers.

Size of experimental units

The concept of an **experimental unit** is critical for understanding the design of all ecological experiments because it determines the scale of the study. An experimental unit is defined as *the smallest division of the experimental material such that any two units may receive different treatments*.

Before defining the experimental unit for your study, it is necessary to think very carefully about the biology of the situation. In the case of the owl example, if our hypothesis is that the *presence* of an owl will reduce crop damage, then clearly the experimental unit cannot be any smaller than the area hunted over by an individual owl. However, if our hypothesis is that the *abundance* of owls will influence the *intensity* of crop damage, then the experimental unit for a mensurative experiment could be smaller than one owl's hunting range, assuming that the ranges overlap and that we can measure differences in owl abundance between locations. For experiments that involve agricultural damage, the size

of the experimental unit will often be determined by the size of the average crop field or plot.

If the owl experiment is manipulative, as suggested by the example of installing nest boxes in some fields but not others, then the experimental unit will be the area influenced by the installation of nest boxes. If the nest boxes are spread evenly through an entire 10 ha area of rice paddy, bounded by non-paddy habitat, then the experimental unit will be the 10 ha area. However, if the 10 ha area of paddy is surrounded by other paddy fields, the experimental unit will extend beyond the 10 ha in which nest boxes are installed, out to some point where the influence of the increased number of owls is no longer felt. Judgment is very important in deciding on the size of the experimental units and, wherever possible, this judgment should be based on sound biological knowledge or, in the absence of biological information, on conservative estimates of critical parameters (such as how far owls might fly). Many ecological experiments have suffered from using too small experimental units. In particular, rodent management experiments will often need to use large experimental units if they are to demonstrate differences in crop protection. Rats, like owls, often move much larger distances than you might think when they are searching for food or a mate.

Experimental units can also be too large or, more commonly perhaps, they can be located too far apart. The key problem here is that the experimental units should be as similar to each other as possible. Typical problems that might come from having overly large or widely spaced experimental units might be differences in soil types or hydrology, or differences in the variety of crops planted or in their time of planting. Uncontrolled sources of variation in an experiment may seriously reduce our ability to identify the role of the key factor or factors.

Duration of an experiment

Experiments need to be run over appropriate time periods. In testing hypothesis 1, measurements of rat abundance at various distances from a canal should probably be taken over an entire 12-month period. Most rodent populations undergo marked seasonal fluctuations in abundance and it is likely that any differences in abundance would be expressed at certain times of year but not at others. In almost any study of rodent ecology, once-off measurements may produce a result but they are unlikely to produce any real, meaningful insights.

Rodent researchers involved in management studies often attempt to determine the impact of a specific 'treatment' applied to a population. A simple illustration of why it is important to think about the duration of such an experiment before you begin is shown in Figure 2.1. Suppose that you are the manager of a rice farm and you wish to determine if adding barn owl nest boxes on the farm will reduce the abundance of rats. If you do a single measurement before and after the addition of nest boxes, you might observe the data shown in Figure 2.1a. These results by themselves might encourage

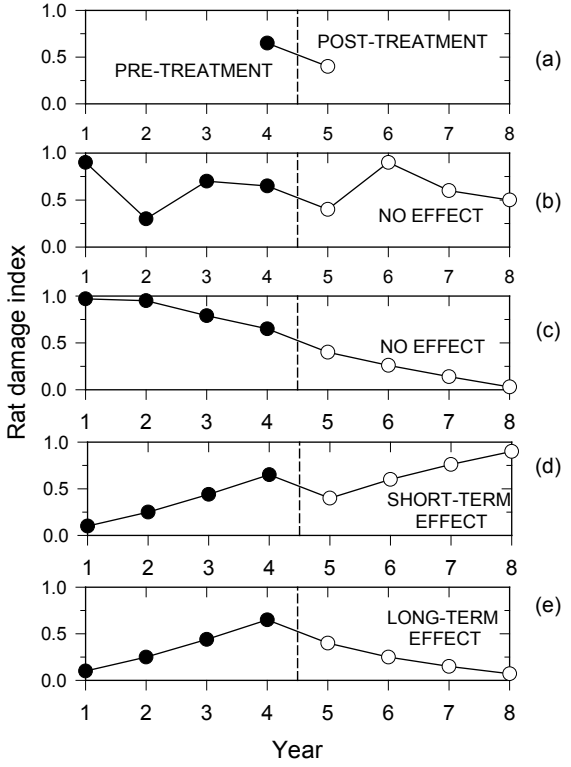

Figure 2.1 Why ecologists need to think about experimental design in field experiments. A manipulation such as putting up owl nest boxes is carried out between years 4 and 5 (dashed line): (a) a single observation before and after with no control—this result is impossible to interpret; (b) to (e) illustrate four possible scenarios if additional data before and after the manipulation are available.

you to jump to the conclusion that the treatment reduces rat damage. But by collecting data for a longer period, both before and after the addition of nest boxes, you would be in a much stronger position to draw the correct inference. As illustrated in Figure 2.1b–e, you might observe no effect, a temporary effect, or a long-term effect of the manipulation.

Inclusion of controls

The need for a 'control' is a general rule of all scientific experimentation. Quite simply, if a control is not present, it is impossible to conclude anything definite about an experiment.[1] For manipulative experiments, such as the owl experiment, a **control** is defined as *an experimental unit that has been given no treatment* (an unmanipulated site). For mensurative experiments, a control is defined *as the baseline against which the other situations are to be compared*. For the canal experiment, the baseline situation would come from fields that are so distant from a canal that the canal has no influence on the rats. Again, sound judgment is needed in such cases as to what distance from the key factor is far enough away. In this case, the relevant biological parameters are the distance that individual rats might move from the canal, the total distance that one season's progeny from canal-dwelling rats might disperse, and the distance away from the canal that any 'knock-on' or 'ripple' effect might be felt (e.g. through displacement of other individuals).

For the owl nest box experiment, the control would be a nearby farm that is similar to the treated one but does not have any owl nest boxes added. If the treatment site showed a long-term effect of the kind shown in Figure 2.1e but the control site showed either no change in rat damage or only random change through the experimental period (e.g. Figure 2.1b), then the case for adding nest boxes would be even more compelling. However, in the event that both treated and control areas showed similar long-term patterns of change, then you would have to conclude that some other, entirely different factor was responsible for the observed changes. Changes in climatic conditions would be worth considering or perhaps changes in the abundance of some other predator.

Although the exact nature of the controls will depend on the hypothesis being tested, a general principle is that the control and the treatments should differ in only the key factor being studied. For example, if you wish to measure rat damage in paddies near to a canal and distant from a canal, you should use experimental units that are planted with the same variety of rice and that were planted at the same time. In ecological field experiments, there is often so much year-to-year variation in communities and ecosystems that you should always do the entire experiment at the same time. You should not measure the controls in 2003 and the treatments in 2004, for example.

Replication

Replication means the *repetition* of the basic experiment. There are two reasons why experiments **must** be repeated and one other reason why it should be. The most important reason for replication is that any experimental outcome might be due to chance. Repeating the experiment will allow us to distinguish a chance or random outcome from a genuine or non-random outcome. The more times we repeat an experiment and observe the same or similar outcomes, the more certain we can be that our hypothesis has identified a genuine causal factor.

The second essential reason for repeating experiments is that replication provides an estimate of **experimental error**. This is a fundamental unit of measurement in all statistical analysis, including the assessment of statistical significance and the calculation of confidence limits. Increased replication is one way of increasing the precision of any experimental result in ecology.

In addition, replication is a type of insurance against the intrusion of unexpected events on ecological experiments. Such events are one of the major sources

[1] In some experiments, two or more treatments (like fertilisers) are applied to determine which one is best. Unless an unfertilised control is included, this experiment will not allow you to say whether either treatment would give a better outcome than using no fertiliser at all.

of interference or 'noise' in field ecology. They are most troublesome when they impinge on one experimental unit and not on the others. As an example, let us assume in our study of rat numbers close to and distant from canals that we have three **replicates** (i.e. three fields close to the canal, three distant from the canal). During the course of our study, one of the plots close to the canal is accidentally flooded. The flooded site would be omitted from the final analysis, but because we have sufficient replication, we can still obtain meaningful results from the other sites.

These considerations mean that every experiment should be repeated at least once, giving two replicates. When this requirement is added to the need for a control or baseline, it is clear that field experiments should include at least two treatment areas and two control or baseline units. However, two is a minimum number of replicates and statistical power will increase if you have three replicates or more. Each additional replicate gives more statistical power to the experiment, but each replicate also represents an additional cost in terms of labour, resources etc.

The decision about how many replicates are needed is a fundamental one in experimental design. In essence, it can be seen as a trade-off between benefit and cost—the benefit of additional statistical power and confidence in the results, but gained at the cost of extra fieldwork, and extra data processing and analysis. Statisticians can advise you on optimal number of replicates for any given experiment, but they will need to know many details concerning the cost of obtaining data, the likely sources of variation, and the risk of chance events (e.g. the flood example) intruding on your experiments.

Randomisation and interspersion

There are three main sources of variability that can cloud the interpretation of experimental results (Table 2.1). Some of these sources of confusion can be reduced by the use of controls, and by replication, as discussed already. However, two other important methods remain—these are called **randomisation** and **interspersion**.

Randomisation

One kind of randomisation involves the random selection of individuals from within a population of animals or of field plots from large areas of uniform habitat (e.g. for measurement of crop damage). A second kind involves the random allocation of experimental units to treatment or control categories. This second type is an important consideration in experimental design. Randomisation by categories insures against bias that can inadvertently invade an experiment if some subjective procedure is used to assign treatments and controls. Randomisation of treatments and controls also helps to ensure that observations are independent—that what happens in any one of the experimental units does not affect what happens in the others. This is especially important where the data will be subject to statistical significance testing, because most such tests are invalid unless experimental units are independent.

In many ecological situations, complete randomisation is not possible. Study sites cannot be selected at random if not all land areas are available for ecological research. Within areas that are available, patterns of land ownership or access will often dictate the location of study sites. The rule of thumb to use is simply to *randomise whenever possible*. Where this is not possible, statistical tests should be applied with caution.

Table 2.1 Potential sources of error in an ecological experiment and features for minimising their effect.

Source of error	Features of an experimental design that reduce or eliminate error
Temporal changes	Treatments with a control or baseline 'Before and after' experimental designs
Experimenter bias	Randomised assignment of experimental units to treatments 'Blind' procedures[a]
Initial or inherent variability among experimental units	Replication of treatments Interspersion of treatments

[a] A 'blind' procedure is one where the researcher is unaware of whether a particular test animal or site is part of a 'treatment' group or a 'control' group. This removes any possibility of bias in the experimental procedure. However, it is usually only possible in laboratory studies, such as in feeding trials.

Interspersion

Where should experimental and control plots be placed in relation to one another? This is a critical problem in field experiments, and the general principle is to avoid spatial segregation of treatment plots. Randomisation does not always ensure that experimental units are well interspersed; there is still a chance that all the treatments will be 'bunched'. Hence, after randomly assigning treatments, you should check that they have not been grouped by chance—for example, with all treatment plots north of a village and all control plots south of a village. Such a design would not be desirable if there is some kind of systematic differences between the sites, such as a soil nutrient or moisture gradient. **Interspersion** means getting a good spatial mixture of treatment and control sites. Avoiding bias of any kind is one of the main goals of good experimental design.

Summary

The general principles of experimental design are often overlooked in the rush to set up ecological experiments. The first step in designing a good experiment is to develop one or more testable *hypotheses*. Each hypothesis should clearly identify the *key processes* or *factors* under investigation and should also include a definition of appropriate *experimental units*. *Baselines* or *controls* need to be established for any measurement or treatment plot. *Replication* is needed to estimate experimental 'error', the measure of statistical significance. The experimental units must be sampled *randomly* to satisfy the assumption that all observations are independent and to reduce bias. Treatments and controls should be *interspersed* in space and in time to minimise the possibility that chance events will affect the results of the experiment. If interspersion is not used, replicates may not be independent and statistical tests will be invalid.

Checklist for experimental design

1. What is your hypothesis?
2. What are the experimental units?
3. What measurements or treatments will you undertake?
4. Have you established appropriate baselines or controls?
5. How many replicates of these units do you need?
6. Have you randomised your measurements or treatments?
7. Are your measurements or treatments segregated or interspersed?

Further reading

Dutilleul, P. 1993. Spatial heterogeneity and the design of ecological field experiments. Ecology, 74, 1646–1658.

Heffner, R.A., Butler, M.J. and Reilly, C.K. 1996. Pseudoreplication revisited. Ecology, 77, 2558–2562.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs, 54, 187–211.

Rice, W.R. and Gaines, S.D. 1994. 'Heads I win, tails you lose': testing directional alternative hypotheses in ecological and evolutionary research. Trends in Ecology and Evolution, 9, 235–237.

Underwood, A.J. 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbances. Ecological Applications, 4, 3–15.

Walters, C.J. 1993. Dynamic models and large-scale field experiments in environmental impact assessment and management. Australian Journal of Ecology, 18, 53–62.

CHAPTER 3

Capture and handling of rodents

Introduction

Rodents are generally difficult to observe directly in the field. Most species are nocturnal in habit and they are often extremely wary of all potential predators, including humans. Under some circumstances, indirect signs of rodent activity, such as footprints, faeces or burrows, may provide a good measure of rodent numbers and activity patterns. However, methods of this kind will first need to be calibrated against more conventional measures of abundance and activity. All field studies of rodents thus begin with a phase of trapping, sampling and identification of the rodents themselves. In this chapter, we describe some basic methods for the capture and handling of rodents. Chapter 4 is devoted to the process of identifying captured rodents.

It is important to be aware that some countries have laws governing the capture and handling of wild animals. In some cases, these laws even cover introduced or pest animals. Depending on the country where the study is being undertaken, you may need to obtain permits before you start to trap animals. Furthermore, in some countries, you may need to obtain animal ethics approval for any study involving the capture and handling of live animals.

Capture methods

Human ingenuity has come up with many different ways of catching rodents. Many groups of people have developed specific traps and snares that either kill or capture any rodent that ventures too close. These are usually either set in a place that shows signs of regular rodent activity, such as across a rodent pathway, or are baited with a substance that acts to attract rodents from the surrounding area. Sometimes traps are used in combination with low fences that guide the rodents towards the trap (e.g. Figure 3.1).

Figure 3.1 A traditional dead-fall trap set in a low fence in the uplands of Laos.

In many places, rodents are actively hunted. This is either done at night while the rodents are active, or during the day by digging into their burrow systems or flushing them from their hiding places. Dogs are often used to help locate rodents in their daytime retreats.

Poisoned baits are used extensively in many parts of the world. Use of baits is not considered here as a capture method because there is no certainty that any animals killed by poisons will be recovered. Nevertheless, rodents killed through the application of poisons should not be neglected as a possible source of biological information, especially during the early part of a study, when even the most basic questions may need to be answered (e.g. Which species are found in my study area? When do they breed?).

Major types of trap

The four main kinds of traps are:

- single-capture live-traps
- single-capture kill-traps and snares
- multiple-capture live-traps
- pitfall traps.

Any of these trap types can be used in combination with a drift fence that directs the rodents towards the trap. However, this method is most commonly used with multiple-capture live-traps and pitfall traps and is discussed under those headings.

Care should be taken to ensure that all traps are well maintained and set to optimum sensitivity. A poorly set trap is a waste of precious time and resources—and it will bias your trapping results. Whenever a trap is set for the first time in a trapping period, it should be test-fired to ensure that all parts are functioning correctly. If a trap fails to fire or seems insufficiently sensitive, it should be fixed on the spot if possible, or taken back to a workshop for repair.

Single-capture live-traps

There are two main types of single-capture live-traps: **cage-traps** made of open material such as wire mesh (Figure 3.2) or perforated sheet metal, and **box-traps** with fully enclosed sides. Box-traps offer protection for the captured animals and are favoured in many parts of the world, especially where overnight conditions are very cold or wet. Some box-trap designs are covered by patents—Longworth and Sherman traps are perhaps the best-known examples. Cage-traps are used more often in Asia. They are cheaper and simpler to make than box-traps, and they are often manufactured locally and sold in markets.

All single-capture live-traps work on the principle that an animal enters the trap and then releases a trigger which allows the door to close behind it. In some cases, the trigger is released when the animal pulls on a bait. In other variants, the trigger is released when the animal steps on a treadle.

Single-capture live-traps must be made of strong material and have reliable functioning components. The captured animal must not be able to break through the sides of the trap or push open the door once it has closed. The trap must be large enough and strong enough to comfortably hold the largest rodent that is likely to be caught. In most parts of South and Southeast Asia, this is probably an adult *Bandicota indica* (body weight of approximately 500–1000 g). We have captured this species in Vietnam in traps measuring approximately 400 × 150 × 150 m.

Figure 3.2 Metal, single-capture live-traps (cage-traps). Each trap has a door at one end with hinges at the top of the trap. The door can be locked open with a pin that connects to a trigger device holding some bait. When a rodent touches the bait, the pin holding the door open is released and a spring mechanism is used to close the door firmly.

Single-capture live-traps are always baited. The bait is either attached to the trigger device or placed behind the treadle. In either case, the bait should be firmly attached so that it cannot be easily stolen. Ideally, only one type of bait should be used in all traps. However, where the rodent community contains a range of species with different preferences, it may be necessary to use several different baits. These might be alternated between traps, or placed together in the same trap. The most important point is that the type of bait or combination of baits should not be altered during the course of a study, or it will be difficult to assess whether changes in capture rates are due to bait preference or to other factors. An experimental design for selecting suitable baits is discussed below.

Certain kinds of bait play a second role in that they provide food for captured animals to protect them from starvation or dehydration. This is particularly important in population studies where we must be careful that the period spent in the trap does not have any serious impact on the health of the individual. Where the primary bait will not satisfy the basic food and water requirements of the target species, you should consider whether or not to add some other moist food, such a block of cassava or sweet potato.

Traps are often set under cover, such as low vegetation or under a house. Where cage-traps are set in exposed positions, it may be necessary to provide some shade so that the animals do not become heat-stressed. This can be as simple as placing rice straw or large leaves on top of the trap.

Single-capture kill-traps or snares

These traps also work on a trigger mechanism, but they are designed to kill the rodent rather than catch it alive. Kill-traps offer a number of advantages, including the fact that they are often very cheap and readily available, allowing very large numbers to be set. In some circumstances, they also are more effective than live-traps. In many parts of Asia, locally produced snares made of bamboo or wire are highly effective in catching rodents, having been perfected over many generations of use.

Kill-traps are obviously only useful where the experimental design specifies that all captured animals will be sacrificed, such as for studies of diet and breeding activity. This is not the case in many ecological studies, where animals will be marked and released as a way of estimating population density or to study patterns of survival, habitat use and movement. Another disadvantage of using kill-traps is that the specimens are often damaged by the trap's mechanism or by ants.

Multiple-capture live-traps

A disadvantage of all single-capture live-traps is that once triggered (either with or without a successful capture), they are no longer effective. This can be a serious issue where rodent numbers are high relative to the number of traps, such that all available traps have caught a rodent early in the evening, or in situations where heavy rain or interference by other animals causes the triggers of many traps to be fired without capturing a rodent.

Multiple-capture live-traps are similar in general design to the single-capture models, but instead of having a trigger mechanism, they have a 'one-way' entrance that allows rodents in, but not out. The most common entrance of this kind is a funnel, as shown in Figure 3.3. However, a doorway that is opened by a treadle mechanism is also effective.

Figure 3.3 Multiple-capture live-trap with a cone-shaped funnel leading from the entrance of the trap.

There are several variations on the standard multiple-capture live-trap. One type, developed in Vietnam, is divided into two compartments by an internal partition, but joined by a second funnel. Captured

rats tend to move into the second compartment in their bid to escape. The rationale for this design is that rats may be deterred from entering the trap if any prior captives are moving around too close to the fence. Experimental results show a higher capture rate for the two-funnel version compared with the standard trap. Another variant on this concept includes a 'false wall' that stops rats from huddling against the fence.

As with single-capture live-traps, each multiple-capture live-trap should be provided with moist food, such as blocks of cassava or sweet potato. Provision of food will maintain captured animals in better health and may also provide further incentive for rats to enter the traps. Traps should be covered with rice straw or other loose vegetation to protect captured animals from the sun. In addition, a small amount of rice straw or similar material should be placed inside the traps. This will allow animals to hide and may reduce the chance of fighting between adults or between different species.

Trap–barrier systems

Multiple-capture live-traps are generally set at openings along a fence or 'barrier system' (Figure 3.4). When rodents encounter a barrier, instead of jumping or climbing over, most will run along it until they find a way through. Traps are usually placed opposite regularly spaced holes in the fence. The linear trap–barrier system (LTBS) has been used to good effect in several field sites in Southeast Asia. Here, we describe the method as used in lowland rice fields in Java, Indonesia.

The LTBS was implemented in Indonesia after initial studies using single-capture live-traps, break-back traps and various designs of multiple-capture live-trap gave poor capture rates for the major rodent species, *Rattus argentiventer*. This species is often extremely abundant, but notoriously 'trap-shy'. Studies on the use of different bait types showed that choice of bait could increase the success of trapping, but only before the booting stage of the rice and after the harvest of rice crops. The reduced capture rate between these two stages was probably due to the general availability of high-quality food in the fields.

The LTBS has proven to be a successful alternative to conventional trapping for population studies in Indonesia. Placing a LTBS across the path of regular movements of rodents, such as between burrow sites and feeding areas, often leads to large numbers of rats being captured. Importantly, because the system does not depend on bait to lure rats into the trap, the effectiveness of LTBS is not influenced by the availability of alternative foods in the field.

Figure 3.4 A linear trap–barrier system with a multiple-capture live-trap, set through dense streamside vegetation in the uplands of Laos.

The system used in Indonesia comprises eight multiple-capture live-traps set along a plastic barrier fence which is 180 m long (Figure 3.5). Alternate traps are set facing opposite directions and are spaced 20 m apart. The traps are checked early every morning. Other animals caught in the traps, such as lizards, frogs and snails, are either released or destroyed (e.g. pests such as the golden apple snail).

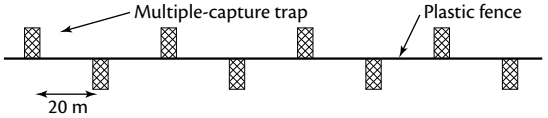

Figure 3.5 Layout of a linear trap–barrier system for trapping rats for population studies.

The multiple-capture live-traps used in Indonesia measure 600 × 240 × 240 mm. The funnel attached to the opening of the trap allows rats to enter but not to exit. A door at the other end of the trap allows access to captured rats. This door is held closed by a pin or wire. All components of the trap are checked to be in working order before each trap is set. After installation, the traps are loosely covered with rice straw to provide shelter from the sun for captured animals.

The fences are made from heavy-duty (woven) plastic sheeting approximately 500 mm high (Figure 3.6). The fence is supported by bamboo or wooden stakes every 1 m, and tension is provided by thick string running along the top of the fence. Holes are made in the fence at the appropriate spacing. Each trap is held tightly against its hole, so that rats cannot squeeze between the fence and the trap. Each trap is held in place with a stick or small piece of bamboo. The bottom of the fence is anchored by burying the base of the plastic in mud or soil, to stop the rats from digging underneath (Figure 3.7). This is easy to do in mud, but more difficult in dry ground.

LTBSs are particularly effective when set up in shallow water, such as in a flooded rice field. In this situation, the rats will be swimming along the fence in search of a way through. They can be encouraged to enter the traps by placing an entry ramp that leads up to the hole (Figure 3.8).

Regular maintenance of the fence is important for the success of the LTBS. Any holes chewed by rats should be quickly repaired or countered by the addition of another trap at the place of the hole. The fence must be kept vertical and taut, and grass or other vegetation must be kept clear of the fence. Construction of a LTBS represents a significant investment of time and resources, so it is important to keep it working at peak efficiency.

Pitfall traps

Pitfall traps work on the principle that animals will either fall or jump into a hole in the ground. Although this sounds unlikely, many animals have no concept of being unable to climb or jump out of a hole. If the pit is very deep, or if it has smooth or overhanging sides, captured animals will be unable to escape. A traditional variety of pitfall trap, with steeply overhanging sides, is used to catch rats in several regions of Southeast Asia. These traps are sometimes covered by a framework of interlaced sticks and a layer of straw. This apparently encourages rats to enter the structure and drop into the underlying pit. In other cases, the pit has sloping margins with a covering of loose sand or gravel that is said to cause the rats to slide into the pit.

Figure 3.6 Plastic fence supported by bamboo posts.

Figure 3.7 Anchoring the fence by burying the bottom of the plastic in the mud.

Pitfall traps are used to great effect in ecological studies in many parts of the world. In most cases, they are used in combination with a plastic barrier of the kind already described for the trap–barrier system that leads the animals towards the pits. The pits most often consist of plastic buckets or short sections of polyvinyl chloride (PVC) piping set into the ground (Figure 3.9).

Figure 3.8 A small mound of mud at the entrance to a trap functions as an entry ramp.

Pitfall trapping is easiest in dry areas with sand or soft soil, as illustrated in Figure 3.9. It is less practical where the ground is waterlogged, because plastic buckets or piping either tend to float out of the ground or fill up with water. It is important to ensure that the captured animals do not drown, so it might be necessary to place a small piece of wood or polystyrene foam that will float if the trap is partially filled with water.

Figure 3.9 A polyvinyl chloride (PVC) pitfall trap used in combination with a fence made of light fly wire or plastic can be an effective way of catching small rodents.

Checking and cleaning traps

All types of traps should be checked and cleared of rats as early as possible each morning. This is most critical for population studies where it is assumed that the trapping method does not affect the animal's normal behaviour (see Chapter 5). However, it is important also for reasons of animal welfare and to ensure that no harm comes to non-target captures, such as other small mammals, frogs or snakes.

In studies involving rodents, it is important to use water only when cleaning traps. Avoid using detergents because the odour may deter a rodent that might otherwise have entered the trap. After washing traps, mix them up with unwashed traps (interspersion!) so that any odours can be masked and to ensure that any impact is randomly distributed across the trapping grids.

Comparing trap and bait efficacy

Just about any combination of trap and bait will eventually catch some rodents. However, it is in your interest to maximise the capture rate. **Trap success**, defined as the number of rodents caught divided by the total number of traps set, is influenced by many factors. It will differ between trap types, depending on the behaviour of the rodent species in your area. It will also vary according to how well the traps were set, where they were set, the reliability of the trap mechanism, the age and sex composition of the population, and the weather conditions during the trapping period. Where baits are used as an attractant, trap success also will reflect the general availability of food in the vicinity of the traps. As noted earlier, single-capture traps may have an overall low success rate at times when abundant food is available in surrounding fields.

At the start of a new study, we recommend that you carry out a small trial to test the effectiveness of the available trap types to see which works best at your particular location. You might also test different baits at the same time, but it is important to remember not to make the experiment so complex that adequate replication is not achieved. You can try almost anything as bait, provided that it is attractive to the rodents, and it is also possible to use a combination of bait types. Some types of bait for rodents that have been used successfully in Southeast Asia are:

- vegetables (e.g. sweet potato, cassava)
- fruits (e.g. apple, banana)
- dried or cooked meat (e.g. crab, fish, snail)
- grain (e.g. wheat, rice), usually wrapped in a small piece of cloth or netting
- vegetable oil (coconut, peanut), soaked into cloth.

In studies where the captured animals will be marked and released, the bait will need to sustain the animal in good condition. Cloth soaked in vegetable oil would not be suitable in this case, but could still be used in combination with something less attractive.

Habitat surveys

During the problem definition phase of a study (see Chapter 1), you should set traps in positions that will maximise the chances of sampling the full local diversity of species and habitats in the study area. Set the traps directly alongside burrows or on obvious rodent pathways to maximise the capture rate.

After you have developed some preliminary ecological hypotheses, you need to carry out trapping in a systematic way to ensure that data are comparable between habitats and trapping periods. Systematic trapping is usually carried out on **trap-lines** or **trapping grids**. In both methods, the traps are set at equal spacing as a way of standardising the trapping effort per unit distance or area. The spacing of traps should reflect the expected size and abundance of the target species. Under most conditions, with expected rodent densities of tens to hundreds of animals per hectare, you would probably want to place your traps about 10–20 m apart. However, in some situations, you may want to place the traps closer together or further apart—an example would be if you are trapping specifically for a large, highly mobile species where each animal may occupy a territory of several hectares.

Trap-lines are usually set by walking through a habitat and placing traps after a standard distance (e.g. every 10 or 20 m). It is important to determine the number of paces per standard distance for each person involved in setting traps; for example, some people take 10 paces for 10 m, others take up to 15 paces. The course taken may be a straight line but it can also be a loop that ends back at the point of origin. Trapping grids are more structured arrangements, with traps set in parallel lines that ensure an even density of traps per unit area. Trapping grids also allow the population density to be calculated, by multiplying the number of animals caught by the area trapped (see Chapter 5 for details).

The choice of whether to use trap-lines or trapping grids will be influenced by the diversity of habitat types available. If there is a uniform habitat type (e.g. large wheat fields), then grids may be appropriate. If a range of crops and other habitats are present (e.g. a mixture of rice fields with vegetable crops and villages—as found in many parts of Southeast Asia), then trap-lines are usually more appropriate. A combination of trap-lines and grids can be used, provided, of course, that the same method is used for each habitat and trapping period.

In village habitat, it may be impractical to set either trap-lines or trapping grids. An alternative is to set one or more traps per house, most often taking a random selection of houses.

Trapping effort and frequency

After deciding on whether to use trap-lines or trapping grids for a habitat survey, the next issues to think about are how many lines or grids should be set up per site, how many traps should be allocated to each unit, and for how many nights each trapping period should run. A good way to think about this is in terms of **trapping effort**.

Trapping effort is usually expressed as the number of effective **trap-nights**. In the simplest case, this is calculated by multiplying the number of traps by the number of nights of trapping (e.g. 100 traps set for 4 consecutive nights = 400 trap-nights). However, traps that have been triggered without making a capture (sometimes called 'null traps') should really be subtracted from the total. In this case, total trapping effort is calculated as the sum of non-null traps for each night (e.g. 95 + 92 + 99 + 97 = 383 trap-nights).

Trapping effort can be increased either by increasing the number of traps or by trapping over a longer period. In theory, this means that a large number of traps could be set for only one night. However, there are good reasons to spread the trapping effort over a *minimum trapping period of three consecutive nights*. One reason is that variable weather conditions may mean that rodents are far more active on some nights than on others—an extended trapping period is obviously less likely to be affected by this kind of variation. But an even more important reason is that many rodents are **neophobic**, which means that they are naturally wary of any new object in their environment. Neophobia often results in low capture rates on the first night, followed by better results on the subsequent nights as animals lose their initial fear of the traps. In most of our studies, we have found a trapping period of 3–4 nights to be adequate. A good way to decide on the most cost-effective trapping period is to plot the capture rate for each day. If you see the capture rate start to decline then the trapping should be stopped. This will happen most often

where the captured animals are being killed, but it can also be due to learned avoidance of the traps by animals that have been captured once and released. Long periods of continuous trapping should also be avoided in some population studies because multiple captures can have an impact on the health of the animals (e.g. captures of pregnant or lactating females may affect survival).

To decide how many traps to set per line or grid, you should first work out how many traps can be set in total per site and how many consecutive nights of trapping can be done. These are often limited by very practical considerations including the budget available to purchase traps and the availability of people to check the traps. The total number of effective trap-nights should then be allocated across the different habitats selected for trapping. For example, with a total of 100 traps set over 5 nights (500 effective trap-nights), you could set up 10 trap-lines of 10 traps in each of 10 habitats (giving 50 trap-nights per habitat), or 5 trap-lines of 20 traps in a subset of five habitats (e.g. the most important ones; giving 100 trap-nights per habitat). The decision is obviously a trade-off between numbers of habitats and the intensity of sampling, i.e. more habitats but fewer traps in each, or fewer habitats but each with more traps. This is never an easy decision but a good way to start is to think about whether you are interested primarily in statistical testing of particular hypotheses or in getting a general overview of the ecological system.

Another factor that you should take into account when thinking about trapping effort is the abundance of the target animals. If they are likely to be very abundant and easily captured, such that almost every trap can be expected to catch a rat, then 10 traps per habitat, set over 3–4 nights, may be quite enough. However, because our experience in agricultural contexts in Asia suggests that capture rates of around 5–10% are more typical, we would recommend a minimum number of 20 traps per trap-line or grid, giving a trapping effort of 60–80 trap-nights per habitat. Other issues to do with the allocation of trapping effort are discussed in Chapter 5.

Where statistical power is critical, another factor to take into account is the need for replication of habitats. In particular, you should ask whether it is sufficient to replicate the most common habitats between two or more different localities (e.g. rice fields in each of two treatments and two control sites). Perhaps the habitat also should be replicated within each village? Remember, as a general rule of thumb, you should replicate the sampling of all experimental units (in this case, a specific habitat).

Trapping **frequency** will depend on the aims of the study. In many studies, trapping is carried out at regular intervals (e.g. every two weeks or once a month). More frequent trapping sessions will provide better data on population dynamics (e.g. survival of marked animals, changes in breeding condition) and may be especially valuable during the initial phase of a new study, when basic ecological research is needed. However, as the dynamics of the ecosystem become better known, it may be appropriate to trap at specific periods in relation to the ecological cycles which, in agricultural landscapes, are often linked to the cropping cycles. For example, trapping may be timed for the period immediately before planting, before the reproductive phase of crop growth, just before harvest, and then during a fallow period when food is limited.

Handling a captive rodent

Safe handling methods are important both for captured rodents and for fieldworkers. Whatever methods are used, they should minimise stress to the animals and should also minimise the risk of injury or disease transmission to the handler. Handling live animals is normally only required for population studies where captured animals need to be examined closely to allow taxonomic identification, determination of age, sex and reproductive status, the taking of measurements, and the marking or tagging of an animal before release. Even very competent handlers should not handle captive animals any more than is absolutely necessary.

The first step in handling a captured rodent is to extract it from a trap. This is usually done by placing a cloth bag around the opening of the trap and waiting for the animal to move into the bag. Be patient:

shaking the trap usually just causes the animal to panic and generally does not speed up the process. Once the animal is in the bag, gently move it to a bottom corner and wait until its nose is in the corner of the bag. You can then hold the bag around the body with one hand, while your other hand enters the bag to take hold of the body. Alternatively, hold the animal within the bag, then peel away the bag to expose parts of the animal for marking, measuring or assessment.

There are various techniques that can be used to hold an animal directly. Different methods are appropriate for smaller or larger animals. Whatever technique you use, take care not to hold the animal too tightly and to allow the animal to breathe easily. For a rat-sized animal, we recommend the following technique: place your first and second fingers on either side of the animal's head, creating a firm hold of the head (Figure 3.10). Ensure that there is no undue pressure from your fingers on the skull and that your fingers are not on the animal's neck, as this will cause suffocation. Hold the body gently with your thumb and remaining two fingers. An alternative method for rat-sized animals is to place your first finger on top of the animal's skull, between the ears and position your second finger and thumb on either side of the head. Hold the body with your third and fourth fingers.

For smaller animals (juvenile rats and mouse-sized rodents), it is usually possible to 'scruff' the animal by gently pinching the loose skin along the back of the neck and upper back between the thumb and first finger.

The grip shown in Figure 3.10 is still suitable for a very large rodent, such as an adult *Bandicota indica*,

Figure 3.10 The recommended method of holding a rat.

but it may be necessary for a second person to control the hind-limbs (and their claws).

Whatever method you are using, take the initial grip inside the confines of the bag. When your hold is comfortable, peel the cloth bag away to expose the animal. If it struggles and your hold is no longer secure, put the animal back in the bag, have a short break and start again.

An alternative to free handling methods is to use a specially designed, funnel-shaped observation bag. This has straps along the length of the bag that can be tightened to restrict the animal's movements. Mesh along the underside of the bag allows the researcher to sex the animal and take basic external measurements such as body and tail lengths.

Methods of euthanasia

Some studies require the humane killing or **euthanasia** of captured animals. This may be necessary to obtain reference specimens for taxonomic studies, to obtain detailed information on breeding activity or diet, or for parasite and other disease studies. Our general objective when euthanasing animals should be to deliver a rapid death with minimal distress and a rapid loss of consciousness before death. A number of standard techniques are available but their appropriateness depends on the experience of the field personnel

and the equipment available. See Further reading for sources of information on a variety of methods.

Asphyxiation

Asphyxiation methods have many advantages. They generally result in rapid death and do not require any direct handling of the animals. Provided a large enough container or bag is available, multiple animals can be killed simultaneously. The two most commonly used methods involve carbon dioxide or carbon monoxide.

Using carbon dioxide

This is probably the best method for euthanasia as it leads to rapid death and poses no threat to people. Carbon dioxide (CO_2) gas cylinders are typically fitted with valves and a pressure gauge (Figure 3.11). The gas is fed by hose into a sealed chamber such as a plastic bucket with a close-fitting lid. Two small holes should be cut in the lid, one for the gas hose and the other to release excess air. Because CO_2 gas is heavier than air, once the chamber is filled, excess gas will spill onto the ground and disperse. Do not use this method in a tightly closed room.

Procedure

- Before putting the animal in, pre-charge the chamber with CO_2 for 30 seconds. The pressure dial on the regulator should read no higher than 138 kPa (20 psi). Close the adjustment valve.
- Place the animal in the chamber and close the lid. The animal can be still inside a bag or even in a cage.
- After 1–2 minutes, check the animal briefly. At this stage, it should be losing balance or becoming sleepy. Open the adjustment valve again for 1 minute to replenish the CO_2.
- After approximately 3–5 minutes, check the animal again for any signs of life. The eyes should be fixed and dilated.

The animal is not dead if:

- its heart is still beating—check this by feeling the chest between your thumb and forefinger
- it blinks when you touch its eyeball.

Pressurised CO_2 gas is available in most countries. However, the large size of most CO_2 cylinders makes this method most useful in a laboratory setting and generally impractical in the field.

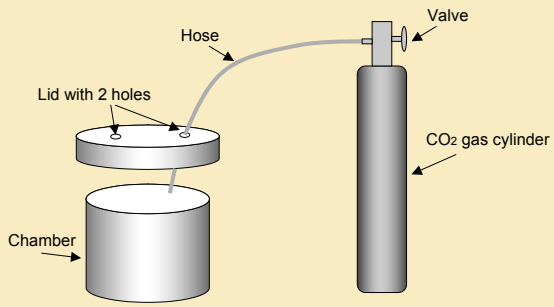

Figure 3.11 Equipment needed for euthanasia with carbon dioxide (CO_2) gas.

Using carbon monoxide

Vehicle exhaust fumes contain carbon monoxide (CO) and this can be used to euthanase animals where CO_2 is not available. (However, for safety reasons, we strongly recommend the use of carbon dioxide wherever possible.) The basic method is similar to that described above for CO_2 gas, except that the source is a running vehicle (car or motorbike) that runs on petrol. A diesel-powered vehicle is *not* suitable.

Procedure

- Cut a small hole into the corner of a large plastic bag. This allows excess air and fumes to escape.
- Place the animals into the large plastic bag (inside cloth bags or cage).
- Place a collar (rubber tubing or cloth) around the exhaust pipe of the vehicle, then wrap the plastic bag around the rubber collar—so that the plastic bag does not melt onto the exhaust pipe, and so that the person holding the bag does not get burnt. Once the bag is in place, start the vehicle engine. The whole operation should be performed in a well-ventilated place so that the person holding the plastic bag does not get exposed to the vehicle fumes.
- It should take approximately 1.5–2 minutes for an adult rat to die using this method.

Cervical dislocation

This technique is useful for small (mouse-size) animals only. It requires experience to conduct this method quickly and effectively. The technique involves grasping the head and the body in each hand and pulling quickly and firmly so that you feel the neck dislocate. This severs the spinal cord and death occurs very rapidly. This technique is not recommended if the animal will be used for taxonomic assessment as it may cause damage to the cranium.

Safety issues

Anyone working with wild rodents should be aware that many species carry diseases and parasites that can be transmitted to people. However, the risk of transmission can be minimised by following some simple guidelines:

- avoid being bitten—handle animals as little as possible, use secure methods, and avoid causing them distress or injury
- cover open wounds, scratches or cracked skin on hands or wrists before handling rodents—apply bandaids (adhesive dressings) or bandages to affected areas (applying a barrier cream to hands during field work may help prevent cracked skin and therefore lessen the chance of infection)
- avoid placing your hands near your eyes, mouth or nose while handling rodents
- wash your hands thoroughly as soon as possible after handling rodents or traps etc., using soap, nail brush and hot water and then an alcohol lotion, if available
- wear surgical gloves when conducting dissections/autopsies.

Diseases transmitted to humans by rats and mice

There are more than 200 pathogenic micro-organisms, helminths and arthropods described from the three main commensal rodents—*Mus domesticus*, *Rattus rattus* and *Rattus norvegicus*. Some of these micro-organisms may be pathogenic to humans. We have a good knowledge from our recent studies of the range of helminths and arthropods that occur in *Mus domesticus* in Australia and this species also has been screened for antibodies to various micro-organisms. In contrast, our knowledge of pathogens carried by *Rattus* species both in Australia and Southeast Asia is poor.

Some human pathogens that can be transmitted by rodents are *Leptospira* (reactions vary from asymptomatic to fatal disease; responds rapidly to antibiotic treatment), the arbovirus family (arthropod-borne viruses such as Ross River virus), the reovirus family (associated with the respiratory and enteric tract of humans), Hantaan virus, plague (again, responds well to antibiotic treatment), rat typhus and lymphocytic choriomeningitis virus (LCMV; symptoms vary from influenza-like to severe meningitis). The plague (225 cases detected in rodents in Java in 2001), leptospirosis (more than 14,000 human cases with 365 deaths in Thailand in 2000), Hantaan virus (sero-positive rodents reported in Indonesia and Thailand) and rat typhus (5000 human cases and 9 deaths in Thailand in 2001) are present in Asia. Further information on the importance and impact of rodent-borne diseases is given in Chapter 8.

Further reading

Leung, L.K.-P. and Sudarmaji 1999. Techniques for trapping the rice-field rat, *Rattus argentiventer*. Malayan Nature Journal, 53, 323–333.

National Health and Medical Research Council 1997. Australian code of practice for the care and use of animals for scientific purposes, 6th edition. Canberra, Australian Government Publishing Service, 73 p. Also available on the Internet: <http://www.health.gov.au/nhmrc/research/awc/code.htm>.

National Health and Medical Research Council 2003. Animal ethical issues. On the Internet: <http://www.health.gov.au/nhmrc/issues/animalethics.htm>.

Research Animal Resources 2003. Euthanasia guidelines. University of Minnesota. On the Internet: <http://www.ahc.umn.edu/rar/euthanasia.html>.

Singleton, G.R, Smythe, L., Smith, G., Spratt, D.M., Aplin, K.P. and Smith, A.L. 2003. Rodent diseases in Southeast Asia and Australia: inventory of recent surveys. In: Singleton, G.R., Hinds, L.A., Krebs, C.J. and Spratt, D.M., ed., Rats, mice and people: rodent biology and management. ACIAR Monograph No. 96. Canberra, Australian Centre for International Agricultural Research, 25–30.

Smith, A.L., Singleton, G.R., Hansen, G.M. and Shellam, G.R. 1993. A serological survey for viruses and *Mycoplasma pulmonis* among wild house mice (*Mus domesticus*) in southeastern Australia. Journal of Wildlife Diseases, 29, 219–229.

Stewart, A.P. 1979. Trapping success in relation to trap placement with three species of small mammals, *Rattus fuscipes*, *Antechnius swainsonii* and *A. stuartii*. Australian Wildlife Research, 6, 165–172.

Tanton, M.T. 1965. Problems of live-trapping and population estimation for the wood mouse, *Apodemus sylvaticus* (L.). Journal of Animal Ecology, 34, 1–22.

Weber, W.J. 1982. Diseases transmitted by rats and mice. Fresno, California, USA, Thomas Publications, 182 p.

CHAPTER 4

Rodent taxonomy and identification

Introduction

South and Southeast Asia and the main island of New Guinea support some of the richest rodent communities of anywhere in the world. Even in heavily modified agricultural land, it is not uncommon to find six or seven different species living together in one community. In upland regions, with their complex mosaic of forest, gardens and regrowth, this number may reach 15 or 20, with the addition of a suite of primarily forest-dwelling species that make occasional forays into adjoining cropping areas.

Naturally, it is important to be able to accurately identify the various rodent species in these complex communities. To properly understand the ecology, information on abundances, breeding activity or movements will need to be collected separately for each species, and any misidentifications may result in a confused picture. Good species identification is also necessary to ensure that rodent control activities do not have an adverse effect on any non-target species that may be either neutral or beneficial to agriculture, or rare and of conservation concern.

Unfortunately, rodents are often quite difficult to identify to species level. This is especially true of members of the family Muridae, the group that includes nearly two-thirds of living rodents, and almost all of the major pest species. Three factors contribute to this situation. The first is the remarkable ability of murid rodents to undergo major shifts in ecological adaptation with only minor changes in morphology. For example, *Rattus rattus* (the house rat) and *R. argentiventer* (the rice-field rat) are so similar in appearance that a trained eye is needed to tell the two apart, even when they are lain side by side. However, *R. argentiventer* is entirely terrestrial and lives in burrows, while *R. rattus* is an excellent climber and often occupies arboreal nests. The second complicating factor is that all murid species go through quite pronounced changes through life in body proportions, fur texture and colouration. This means that juveniles, subadults and adults of one species often differ more from each other than do the same growth stage of different species. And finally, some rodent species are highly polymorphic—that is, they show a lot of morphological variation within populations. For example, many populations of *R. rattus* contain adult individuals with pure-white, brown or grey belly fur and these variants are often mistaken for separate species. High levels of variation in turn provide prime material for natural selection—with the result that many murid populations can undergo rapid

morphological changes, over only a few generations, to suit local conditions.

The science of taxonomy tries to make sense of all of this variation and to identify the basic species that exist in nature. It also attempts to provide diagnostic criteria whereby the species can be distinguished from each other. In this chapter, we will start by introducing some of the basic concepts and principles that underpin the 'science' of taxonomy and the 'art' of rodent identification. We then review some of the more important morphological features that are useful in distinguishing between different rodent species and provide instructions on how to collect voucher specimens and tissues for genetic analysis. A key to the pest rodents of Southeast Asia and the Pacific region is given in Chapter 11.

Basic taxonomic concepts

The meaning of scientific and common names

All species have two-part scientific names that should be written in italics (e.g. *Mus musculus*) or may be underlined instead (e.g. Mus musculus). The first part always begins with a capital letter and signifies the name of the **genus**. The second part always begins with a lower case letter and is the **specific epithet** (specific name). Together, the two parts denote the proper species name. If the name includes a third part (also all lower case), this denotes a subspecies (e.g. *Mus musculus castaneus*). A scientific name is sometimes followed by a name and a date, e.g. *Rattus argentiventer* (Robinson and Kloss, 1916). This is the name of the person or people who first described the species and the date of the publication in which the name was first used; the combination of name and date is known as the **authority**.

The application of scientific names is governed by a set of very precise rules set down by the International Commission for Zoological Nomenclature. One of these rules states that the earliest available published name must be used for each currently recognised species or subspecies. Various checklists of rodent names are available but it can still be difficult to navigate through the plethora of different names and combinations (see Box 4.1). In Chapter 11 we list some of the more commonly used alternative scientific names for each of the major rodent pests.

Common or 'vernacular' names are not bound by any equivalent set of rules. This means that there is no such thing as a 'correct' common name and each person can use whatever term they prefer. For example, the wild progenitor of the domesticated laboratory rat, *Rattus norvegicus*, is variably called the brown rat, sewer rat or Norway rat in English, and it is *chuot cong* ('tunnel rat') in Vietnamese. None of these names is any more correct than the others. Indeed, almost all common names are sometimes misleading if they are taken as genuinely descriptive terms. *Rattus norvegicus* is not always brown, it does not always inhabit sewers, and the species most certainly did not originate in Norway!

Box 4.1 Why taxonomic names sometimes change

Many species of rodents have been known by a variety of different scientific names and this can make it difficult to use some of the earlier literature. These name changes can reflect a variety of past taxonomic actions and decisions, including:

- the lumping of various geographical populations into a single, more widespread species
- the splitting of one species into two or more individual species, based on new studies
- the movement of a species from one genus to another; e.g. *Gunomys bengalensis* became *Bandicota bengalensis* when the genus *Gunomys* was placed under *Bandicota*
- the discovery of an earlier name in a previously obscure publication.

Units of classification

The basic biological unit of the natural world is a **population**—a group of individuals that occupy a single locality and among which all members of one sex could potentially interbreed with all members of the opposite sex (however breeding is often

constrained by social structures). In theory, genetic and morphological variation should be more or less randomly distributed among individuals within a single population (although preferential mating systems may cause some non-random effects, as may very strong local selection).

A **species** (plural also 'species') is a more abstract concept. It is a group of populations from different geographical areas that would be able to interbreed freely if they were all placed together. These populations are thought to share their reproductive compatibility because of a shared ancestry—a common point of origin from whence they spread to occupy their present geographical range. Members of different species are generally unable to breed with each other. This is usually on account of genetic incompatibilities. However, in some cases, the separateness of the species is maintained by behavioural differences and this may break down when individuals of different species are placed together in captivity or in an unnatural environment such as around human habitation. Interbreeding between members of two different species is called **hybridisation**.

Where a species has come to occupy a large geographical area, different local populations often differ from each other in subtle ways. This may have occurred through random genetic changes in isolated populations (e.g. on islands) or through natural selection to better suit local environmental conditions. These morphologically distinct local populations are sometimes identified as different **subspecies**. Subspecies names are also sometimes used for different variants within a single population (e.g. white-bellied *Rattus rattus* are sometimes called *Rattus rattus arboreus*). However, this is an incorrect use of the category and should be discouraged. Another undesirable practice is the use of subspecies names to distinguish geographically isolated populations that do not otherwise differ in morphology (e.g. many island populations).

The **genus** and **family** categories are even more abstract than the species. In the past, a genus (plural 'genera') was most often used to draw together a group of species that were basically similar to each other in appearance and habits. Likewise, a family pulled together a group of similar genera. More recently, both of these categories have been given an evolutionary meaning—a genus is group of species that are believed to have evolved from a common ancestral species; and a family is a still larger group of related genera.

Morphological and genetic approaches to distinguishing species

Rodent species are most often distinguished on the basis of morphological characteristics, including differences in body size and shape, fur texture and colour, and details of the teeth and skull. This has sometimes included the statistical analysis of large numbers of measurements, making the taxonomy somewhat more repeatable and hence more 'scientific'.

In recent years, the application of genetic methods has produced a revolution in taxonomy (see Box 4.2 for notes on collecting samples for genetic analysis). At the species level, genetic analysis can be used to directly quantify the amount of interbreeding that is occurring within and between populations. Hybridisation between species is easily detected genetically and its potential impact on each species can be estimated. Genetic methods can also be used to recover the history of dispersal of species across a landscape and to estimate the relative (and to some extent, the absolute) timing of key events such as water-crossings or other causes of range fragmentation. At the genus and family levels, the evolutionary history of groups of species also can be reconstructed with increasing levels of precision, thereby removing much of the guesswork that previously surrounded these categories.

Genetic studies are currently under way for several groups of Asian rodents. The results of this work will almost certainly require some changes in the taxonomy of several groups including some of the major pest species. However, in the long term, the application of these methods will result in a more stable and scientifically based classification, as well as many valuable insights into the evolutionary history of the group.

Collecting voucher specimens

You can preserve voucher (reference) specimens either as dry or wet specimens. In either case, it is essential that you label these with details of the place and date of collection, the collector's name and any specimen number or code that links the voucher back to tissue samples. The label should be durable, securely tied to the specimen and written in pencil if the specimen and label are to be placed in ethanol (as most inks are alcohol soluble will thus disappear). Wherever possible, you should also preserve a piece of soft tissue (ideally, liver) for future DNA analysis (see Box 4.2).

Wet specimens

Wet specimens first need to be **fixed** in an appropriate solution. Formalin or ethanol are the two most commonly used fixatives. Each has advantages and disadvantages and you should think carefully before deciding which one to use.

Formalin is the best fixative if you intend to use specimens for detailed anatomical or histological studies (examining the tissues microscopically). Formalin is usually purchased as formaldehyde, mixed as a 37% solution. You will need to dilute this with water to 10% of its original concentration to give a **10% formalin** solution. If the specimens are going to remain in this solution, the formalin should be buffered to a pH of 7 (one option is to use 4 g monobasic sodium phosphate and 6 g dibasic sodium phosphate per litre of 10% formalin). Without buffering, the bones of specimens stored in 10% formalin will soon decalcify and the flesh will harden.

The two main disadvantages of using formalin as a fixative are:

- it causes extensive damage to the deoxyribonucleic acid (DNA), making specimens fixed in this way unsuitable for genetic studies
- it is a severe irritant (especially to eyes and the respiratory tract) and a poison. Formalin should only be mixed and used in well-ventilated spaces.

Ethanol is not recommended as a fixative for anatomical studies. However, it is much safer to use than formalin and has the extra advantage that it gives good preservation of DNA. Ethanol is also more readily available than formaldehyde in many countries. If ethanol is not available, methanol (another alcohol, usually sold as methylated spirit) can be used instead, but only as a last resort. Ethanol is usually diluted to 70% concentration. Higher concentrations are good for DNA preservation but will dehydrate tissues and make a specimen very hard and inflexible.

Regardless of whether you use alcohol or formalin for fixation, it is always best to slit open the belly (taking care not to cut into the intestine or damage any embryos) to allow the fixative to enter the body cavity. If a needle and syringe are available, you should also inject the fixative into each of the major muscle masses (shoulders, thighs, neck) and into the chest cavity. Injection is especially important when using ethanol as a fixative. After injection, place the specimen in at least five times its own volume of fixative. It is usually necessary to leave the specimen for at least 3 days, or until the big muscle masses feel firm (but not hard) to the touch.

Specimens that are fixed in formalin are usually transferred to alcohol for long-term storage. Before placing a formalin specimen into alcohol it must be rinsed thoroughly in water. If you have used ethanol or methanol for fixation, replace the fluid after 5–7 days. Small specimens will take less time to fix than larger ones.

Keep specimens stored in ethanol/methanol in airtight containers, out of direct sunlight. Check the fluid level occasionally and top up if necessary. Specimens stored in ethanol/methanol can remain essentially intact for many decades, or even centuries. They can be rinsed and stored for one or two days in water for use in training sessions, but they should be returned to ethanol as soon as possible after use.

Wet voucher specimens can take up a lot of storage space and consume large quantities of fixative. A good way to conserve space and materials is to fix and preserve only the skin. This involves carefully removing the skin from the body, leaving only the

head, hands, feet and tail inside the skin. A skin will be well fixed after only 1 day in formalin or 3–4 days in ethanol. A skin fixed in this way can be made up into a dry specimen at a later date (see below).

Dry specimens

If it is not possible to preserve and store wet specimens, the next best option is to prepare the skull as a voucher specimen. If possible, before you do this, photograph the living or freshly killed specimen as this provides a valuable source of supplementary information to accompany a cleaned skull. Carefully label the photograph with the same details as the skull.

To clean the skull, remove the skin and then boil the head until the muscles and other tissues are soft enough to be picked away without damaging the bones. You can also soften the flesh using a weak solution of sodium perborate. Alternatively, place the skulls in a location where ants can consume the flesh (but away from the attention of dogs or chickens) or put them in a fine mesh bag (wire or nylon) and submerge them in a pond or paddy field where aquatic organisms will do the job.

After cleaning, label the skulls individually and tie or wire the lower jaw to the cranium. Skulls should be stored in plastic or glass vials, or in sturdy cardboard boxes to protect them from damage.

The preparation of dry 'museum-style' skins is a specialist task that is not recommended unless a permanent reference collection is needed. In this case, a special collection area has to be established—somewhere that can be kept dry and free of insect pests. Insects will rapidly destroy any dry specimens left unprotected. Unless moisture is excluded, fungus will also invade and eventually destroy dry specimens. Long-term storage of dry specimens requires similar conditions as storage of dry insect or plant collections.

Box 4.2 Collection of tissues for DNA analysis

Good DNA sequences can be obtained from small pieces of animal tissue preserved in ethanol. Almost any tissue can be used, but some suggestions are given below for the tissues that give the best results.

If an animal is to be sacrificed, take a tissue sample as soon as possible after death. The most widely used tissue is the liver, but other organs such as lung, kidney, spleen etc can also be used. Muscle either from the heart or from the chest or thigh can also give good results. The most important thing in all cases is to fix the tissue soon after death. This is particularly critical in the case of organs such as liver and kidney that contain many destructive enzymes. If an animal has been dead for some time (e.g. from a kill-trap or a road kill), it is best to collect a sample of muscle tissue from the part of the body that shows the least obvious decomposition. Also, pluck some hairs from the body and include them with the muscle sample.

Place a 5 mm cube of the chosen tissue immediately into a 3–5 mL tube of 70–90% ethanol, then cut the tissue into smaller pieces (approx. 1 mm cubes) with a new scalpel blade or clean fine-pointed scissors. This assists with penetration of the ethanol and improves fixation of the DNA.

Label the tube clearly and carefully. If the tube is likely to leak, write the labels in pencil or scratch them into the tube (as most inks are soluble). The information on the tube must be sufficient to allow the collector to determine the date and place of collection, and the identity of the sampled animal. This latter information might be a numbered voucher specimen or it might be a reference to measurements in a field notebook or to a photograph. The most useful samples are those associated with a voucher specimen, because this allows the DNA results to be linked back to the physical characteristics of the sampled animal.

Keep the samples stored in ethanol out of direct sunlight in as cool a place as possible. Storing them at 5–7°C in a refrigerator is ideal, but not essential for good results.

Major groups of Asian rodents

Four major groups of rodents are represented in Southeast Asia and the Pacific region (Figure 4.1). The major attributes of each are listed below. Here we will be concerned primarily with the Muridae, the group that includes all of the major pest species. Some good general sources on squirrels and other groups of rodents are indicated under Further reading.

Family **Hystricidae** (porcupines)—chunky build; very long, stiff, sharp spines project through fur

Family **Rhizomyidae** (bamboo rats)—chunky build; tail is short, unscaled and almost hairless

Families **Sciuridae** (ground squirrels) and **Petromyidae** (flying squirrels)—variable build; tail is heavily furred to tip

Family **Muridae** (rats, mice etc.)—mostly slender build; tail is generally sparsely furred and has distinct scales arranged in concentric rings.

The family Muridae includes more than 1350 species, the majority of which are found in Eurasia, Africa and Australia. It includes many of the world's most familiar rodents, such as the house rats and house mice, and some of the most destructive of all agricultural pests. However, it also includes many hundreds of other species that play important roles in landscape ecology at all scales and that should be protected and conserved.

Identifying murid rodents

The process of identifying unknown rodent specimens can be made simpler and more reliable if the following basic steps are followed:

- determine the age and sex of the specimens (see below)
- set any juveniles aside and **work first with adults**
- work each through the key provided in Chapter 11 to obtain a provisional identification
- check the notes on geographical distribution and morphological features given in Chapter 11
- if the specimen does not fall within or close to the known geographical range or does not match the description, try working through the key again
- if a convincing identification cannot be obtained, consider taking a voucher specimen and a DNA sample (see Box 4.2).

The reason why determining age and sex is so important is that rodents change greatly in appearance through their growth and development. This is most notable in the texture of the fur but it also affects their body proportions (e.g. relative tail length). Age and sex can be determined by examining the external reproductive condition, as described below. Young rodents are often very difficult to identify. This is best done by first identifying some

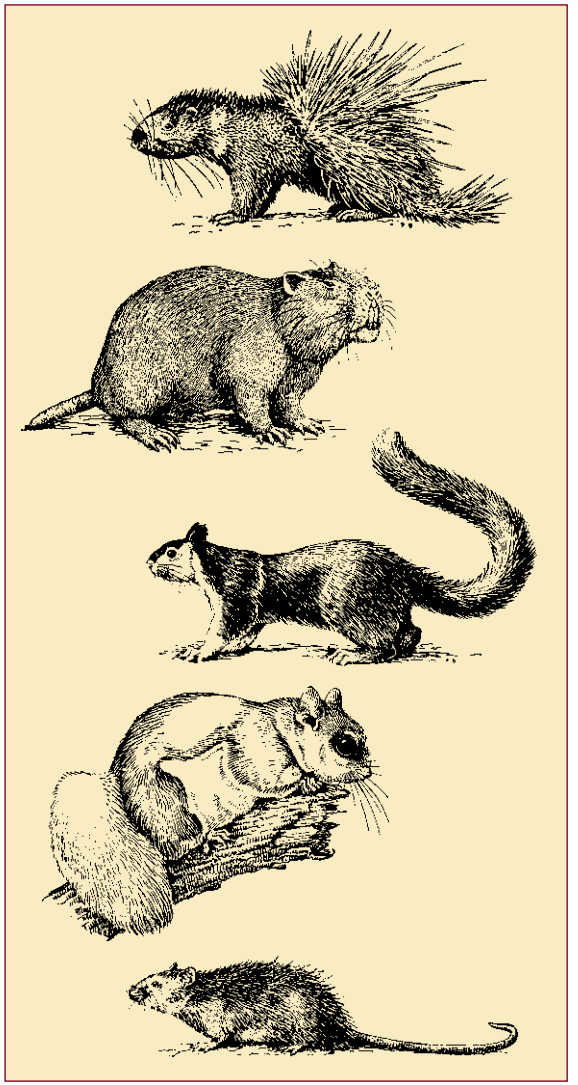

Figure 4.1 Examples of each major group of Asian rodents. From top to bottom: a porcupine; a bamboo rat; a ground squirrel; a flying squirrel; and a rat (after Grassé and Dekeyser, 1955).

sexually mature specimens and then attempting to match juveniles with adults by comparing their features directly. Digging of breeding burrows can result in young animals sometimes being captured together with their parents; these can be used as reference specimens.

Determining the age and sex of a rodent

To determine the age and sex of a captured rodent, hold the animal so that the belly faces you and the head is pointed away from you. The opening at the base of the tail is the **anus**. Both sexes have a **genital papilla** that covers the penis in males and the clitoris in females.

In juvenile male rodents, the **testes** are initially located inside the body, in an **abdominal** position. As the animal matures, the testes enlarge and descend to adopt a scrotal position, inside a hairy **scrotal sac**. In a fully adult rodent, the scrotal sac often projects behind, and hence obscures, the anus (Figure 4.2). The skin at the back of the scrotal sac is often hairless and darker than the surrounding skin. This houses a sperm storage organ called the **epididymis**.

Figure 4.2 Comparison between juvenile (abdominal testes; left) and adult (descended testes; right) males.

In females, the anus and genital papilla are close together and the skin between them is bare or thinly furred. The vagina should be visible just behind the genital papilla. In juvenile rodents, the vagina is sealed off by a thin, shiny layer of skin, the hymen. This condition is known as an **imperforate vagina** (Figure 4.3). As the animal reaches sexual maturity, the vaginal covering breaks down and the vagina is open or **perforate** from then on. The vagina will be widely open if the animal has recently mated or given birth. It is smaller (but never fully closed off) if the animal is mature but has never mated, or not recently mated.

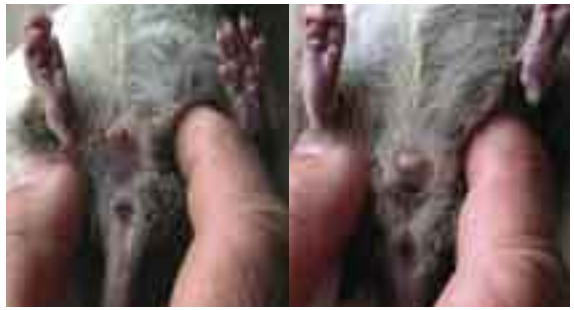

Figure 4.3 Comparison between juvenile (closed or imperforate vagina; left) and adult (open or perforate vagina; right) females.

Female rodents also have teats associated with subcutaneous mammary glands. These are arranged down either side of the body (Figure 4.4). The teats are prominent and should be easy to locate in sexually mature females, especially in those that have had young. However, they can be very difficult to locate in juveniles and the presence or absence of teats should not be used as a means of determining the sex of an individual. For classification purposes, pairs of teats, or **mammae**, are counted in three groups; pectoral, postaxillary and inguinal. For the rodent shown in Figure 4.4, for example, the number of teats would be given as 1+2+2.

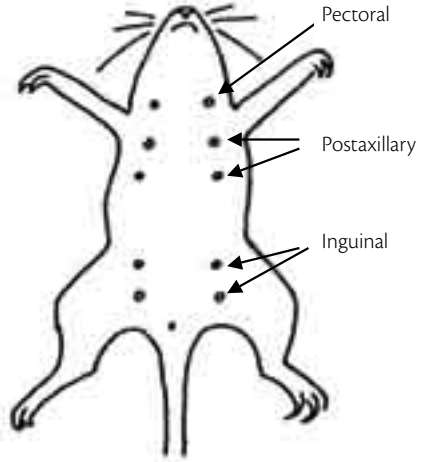

Figure 4.4 Arrangement of mammae on an adult female *Mus domesticus*. In this species, there is one pair of pectoral teats, two pairs of postaxillary teats, and two pairs of inguinal teats (denoted as 1+2+2).

It is more difficult to determine the sex of very young individuals, such as thinly furred pups or recently weaned juveniles. The best way is to compare the distance between the anus and the genital papilla. This will be much greater in a juvenile male than in a juvenile female. In addition, there should be a distinct unfurred line between the vagina and the anus in juvenile females.

Taking measurements

Body measurements are quite useful in limiting the range of possible identifications for an unknown rodent. For example, a fully adult rodent weighing only 10 to 20 g is almost certainly a species of *Mus*, while a body weight of 400 g eliminates all but handful of possibilities. Routine recording of body measurements also provides a good check on field identifications, and may help to highlight any records that might be in error.

Fieldworkers usually take a standard set of external measurements. These are explained and illustrated below. The linear measurements should be taken to the nearest millimetre; any greater precision is probably not repeatable, especially if the measurement is taken on a squirming live rodent! It is best to use a good-quality plastic ruler which has the end trimmed to set the zero mark at the very edge of the ruler.

If you are working in a group, ensure that everyone in the group takes measurements in exactly the same way. This minimises variation occurring between researchers. If anyone is inexperienced, they should practice by taking measurements on an individual already measured by another person; the external measurements should be repeatable to ± 1 mm for the ear and hind-foot lengths, and ± 3 mm for the head+body and tail lengths.

Head+body length

The combined length of the rodent's head and body is known as the 'head+body' length. Take the head+body measurement in a straight line along the animal's vertebral column, from the tip of the nose to the distal end of the anus (with the animal lying on its back) (Figure 4.5). Live rodents rarely cooperate in this exercise, hence the head+body measurement is often less precise than those taken of the tail, foot and ear.

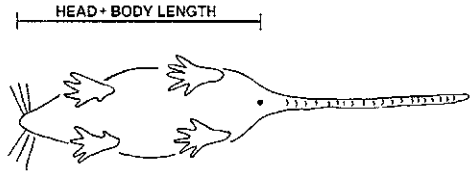

Figure 4.5 Measure the head+body length along the spine of the rodent from the tip of the nose to the middle of the anus.

Tail length

Measure the tail along a straight line from the middle of the anus to the tip of the tail (Figure 4.6). Do not suspend the animal by its tail to take this measurement—the tail will stretch and the measurement will be too long.

Only take the tail measurement on complete, undamaged tails. A damaged tail will terminate in a short, pale section that lacks hairs and scales. If the tail is incomplete, note this on your data sheet.

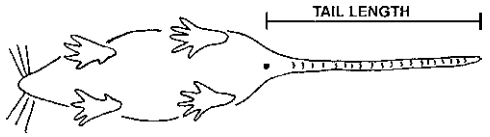

Figure 4.6 Measure tail length from the middle of the anus to the tip of the tail.

Pes length

Measure the **pes** (hind-foot) from the heel to the tip of the central (longest) toe, but without including the claw (Figure 4.7). For live animals, the end of the ruler can usually be hooked under the claw, allowing the foot to be gently flattened against the ruler.

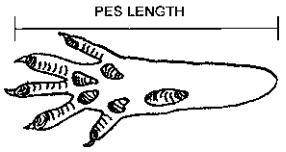

Figure 4.7 Measure the pes length from the base of the heel to the end of the toe pad on the longest toe (not including the claw).

Ear length

Measure the ear from the bottom of the notch of the ear to the furthest point along the rim (Figure 4.8). Do not take the measurement if the margin of the ear is damaged as a result of fighting.

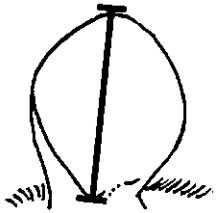

Figure 4.8 Measure the length of the ear from the bottom of the ear notch to the furthest point along the rim.

Body weight

Rodents and other small mammals are usually weighed using a calibrated spring balance (such as a Pesola spring balance; Figure 4.9). Such balances are available in various sizes. Be sure to use an appropriately sized balance for the individual rodent and hold the balance by the swivel ring at the top. Suspend dead animals by a foot or the tail.

Check balances before each session to make sure that they are calibrated to zero (or to the correct mark if it has been adjusted to allow for error).

Live animals are generally weighed inside a cloth bag. Tie a knot in the top of the bag and take the weight of the rodent plus the bag. After the animal is removed, weigh the bag by itself. The weight of the rodent will be the difference between the two measurements. Make sure that you use an appropriately sized bag—do not weigh a 5 g mouse in a rice sack!

Figure 4.9 Weighing a rodent using a Pesola spring balance. If possible, two people should read the balance to avoid any misreading.

Diagnostic characteristics

Only a few species of rodents possess uniquely diagnostic features, such that they are instantly recognisable. More typically, rodents are distinguished from each other by unique *combinations* of features.

The following list indicates the kinds of external characteristics that will be useful for identification:

- general body proportions
- colour and texture of the fur on the belly, flanks and back
- size, shape and hairiness of the external ears
- colour and length of the vibrissae (whiskers) on the face
- size and colour of the incisor teeth
- detailed patterning and hairiness of the tail
- colour and overall shape of the manus and pes (fore- and hind-feet, respectively)
- size and shape of the pads and claws on the manus and pes
- size and shape of the scrotal sac in males
- number and distribution of teats in females.

The following notes are provided as a guide to the kinds of features to look for when examining a rodent specimen.

Body proportions

Murid rodents do not vary much in basic body proportions. The most striking difference between species relates to the relative length of the tail, which ranges from less than 50% of head+body length to more than 200%. Some murid rodents have a distinctly chunky body form with strongly muscled shoulders and neck, while others have proportionally longer or shorter heads; however, such variations are difficult to quantify and are thus of little diagnostic

value. Keep in mind that species with long, thick fur will tend to look more heavily built than those with short, sleek fur.

As in most other groups of organisms, body proportions in murid rodents change during the course of individual growth. As illustrated in Figure 4.10 for *Rattus losea*, the ears and feet undergo a period of early, rapid growth, while the tail grows more steadily through life. Young rodents thus appear to have proportionally larger ears and feet than adults of the same species, and this can sometimes lead to them being identified as different species. Tail length shows a more constant proportional relationship to head+body length.

Fur texture

Mammal fur consists of a number of different hair types and it is variation in the length, thickness, form and frequency of each type that give the fur of each species a particular look and 'feel'. The main hair types found in murid rodents are:

- **contour hairs**—these make up the bulk of the externally visible fur. They are usually morphologically unspecialised but are often 'banded' in colour
- **spines**—specialised contour hairs with a flattened (often grooved) midsection. Fur with large and abundant spines often feels quite 'stiff' and will stay in position when brushed forward (Figure 4.11). Usually confined to the back and flanks, but also present on the belly in some species
- **underfur hairs**—short, fine hairs that can only be seen by parting the contour hairs. Dense underfur will give the fur a 'woolly' texture
- **guard hairs**—long and often quite thick hairs that project some distance (sometimes several centimetres) beyond the contour hairs. These are generally longest down the centre of the back, especially on the lower back.

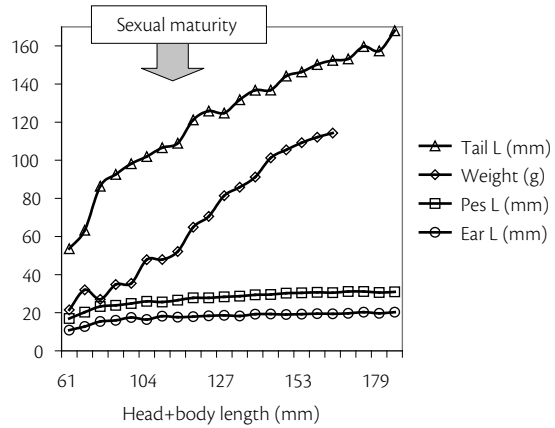

Figure 4.10 Changes in body proportion during growth in male *Rattus losea*, illustrated by plotting each of tail length, body weight, pes length and ear length against head+body length. The pes and ear attain their final size at an early stage of growth, while the tail continues to grow well past the time of sexual maturation (at around 45–50 g).

In juvenile rodents, the outer fur consists of contour hairs and short guard hairs, and it is always soft. Spines only emerge following completion of one or more **moults**, and the guard hairs also only become conspicuous with increasing maturity. Moults occur as 'waves' of hair replacement running backwards and upwards from the shoulders and lower flanks, and they take place throughout life. Early moults are quite orderly and may be visible on the flanks and back of juveniles and subadults as bands of different fur colour or texture. The moulting process in adults is more erratic and is generally difficult to detect. However, it is worth noting that the brightness or 'freshness' of the fur colour will vary according to the moult stage of the individual.

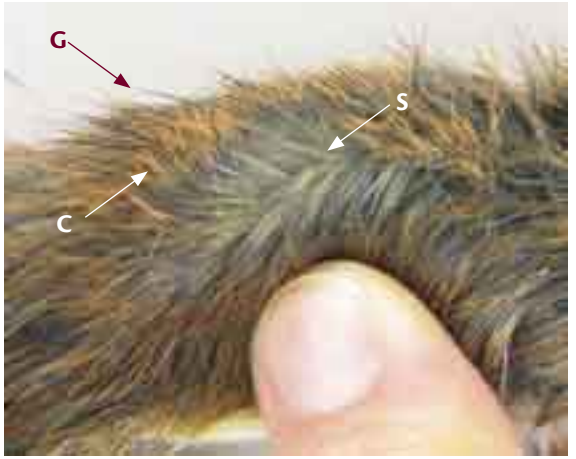

Figure 4.11 Dorsal fur of a species of *Niviventer*, illustrating three of the four hair types: guard hair (G); contour hair (C); and spine (S). The underfur is visible as very fine fibres among the spines and contour hairs.

Fur texture may vary greatly within a species, especially where one species spans a broad altitudinal or latitudinal range. Not surprisingly, populations living in regions with a cool climate tend to have denser, woollier fur than those living in hotter regions.

Colouration

Fur colour in rodents is a complex feature that requires some specific terms if it is going to be useful for identification. This is because the different kinds of hairs (described above) often differ in colour, while some kinds of hairs are commonly 'banded', with different zones of colour along individual hairs. In addition, the fur of most murid rodents is coloured differently on their back (**dorsum**) and belly (**venter**). These colours may blend gradually on the flanks, or they may be sharply demarcated (see Figure 4.12). In a few species, a third, distinct band of colour is present on the flanks.

In describing the fur colour of a rodent, it is often useful to note its 'overall colouration', as might be observed by holding it at arm's length. Hence, a species might be described as 'overall, dark grey above, with a sharply demarcated cream venter' or ' overall, dorsum reddish brown, merging into a buff venter'. However, it is often necessary to be far more specific. When describing banded hairs on the dorsum or venter, it is usual to distinguish the outermost colour (called 'tipping' or a 'wash') from the deeper, basal colour, e.g. 'fur on belly grey-based, with cream tipping'. The colour of individual hair types on the back is often noted, e.g. 'contour hairs reddish-brown with dark-grey bases, guard hairs clear or with short, black tips'. Where the different hair types are strongly contrasting, the overall colour may be described as 'peppered', e.g. 'dorsum orange–brown, peppered with black'.

In taxonomic descriptions, fur colour is sometimes specified more carefully, using various colour standards taken from soil science or other sources. This level of detail is generally not helpful for field identifications.

Two regions of the body deserves special mention in regard to fur colour, namely the head and the pectoral region or 'chest'.

In most rodents, fur colour on the head is a continuation of that on the body, with the belly colour typically extending onto the throat and chin. However, a significant number of species show more complex fur colouring on the face (see Figure 4.13). The most common elements are:

- an **eye ring**—usually a narrow band of dark hairs encircling the eye
- a **facial mask**—a more extensive strip of dark fur running through the eye and onto the side of the snout
- a **cheek patch**—usually made up of pale hairs and situated below the eye, sometimes extending onto the lower part of the muzzle
- a **preauricular patch**—consisting of a narrow fringe of distinctly coloured hairs along the anterior margin of the ear.

Figure 4.12 An example of sharp demarcation in fur colour between the dorsum and venter, in *Leopoldamys sabanus*.

Figure 4.13 Examples of facial patterning among murid rodents. An orange cheek patch and dark eye ring in *Chiromyscus chiropus* (top left); a white cheek patch and lower muzzle in *Mus terricolor* (top right); and an orange preauricular patch in *Rattus argentiventer* (bottom).

The chest in rodents sometimes bears a distinctly coloured patch of fur. This may be cream or white set against a darker background colour, or it can be a darker patch (or mid-ventral line) of fur set against an otherwise pale venter. In some species, this patch is reddish-brown and has the appearance of a 'stain'. This may be due to the presence of skin glands in this region (found in some murids and in many other groups of mammals), but little is known about this phenomenon in Asian rodents.

In *Nesokia indica*, the head and shoulders are more brightly coloured than the rest of the dorsum (see Chapter 11). This degree of patterning on the body is common in other groups of rodents (e.g. squirrels) but it is quite rare among Asian murids. One very unusual group of murids (*Chrotomys* spp.), that is sometimes trapped in rice fields and gardens in the Philippines, is distinguished by the presence of dark longitudinal stripes along the dorsum.

A final word on colouration concerns intraspecific variation and 'aberrant' patterns. Coat colour variation in *Rattus rattus* has been noted earlier. The venter is particularly variable in this species, with many populations showing a mixture of pure-white and grey-based ventral fur colours. This variation is under simple genetic control and strong natural selection can lead to some segregation of these colour forms by habitat. As a general rule, dark-bellied forms seem to be more common around villages where white-bellied individuals might be easier to observe and kill.

Variation in dorsal fur colour (various shades of browns to black) is also found in *R. rattus*, and the best known example is the melanistic form (the true 'black rat') that is common in Europe and some other parts of the world. Melanism is rare among Asian populations of *R. rattus*, but it has been observed in various other species, including *Rattus norvegicus* and *R. losea*. Other species of rodents generally show less variation in dorsal and ventral fur colour within any one population, but there are many examples where fur colour differs between populations, especially where one species occupies a range of habitats.

Aberrant colour patterns include individuals with one or more, randomly positioned spots of contrasting colour, or in some cases, with a 'saddle' of pale fur that runs up from the belly on both sides and may even encircle the whole body. These aberrant patterns may occur in low frequency in all species and in some cases they reflect previous injuries (e.g. burns or torn skin). Albino individuals presumably occur in all species, but these would be unlikely to survive for long in the wild.

Vibrissae

Vibrissae (often called whiskers) are specialised hairs that are connected to special sensory nerves. In murid rodents, they are found only on the head and lower forelimbs. The head vibrissae are arranged in seven or more groups, the placement of which is fairly constant within and between species (Figure 4.14). The most obvious and functionally most important group are the **mystacial vibrissae** that occupy either side of the snout. These are highly mobile and are used in orientation and movement. The other groups are used mainly for orientation or, in the case of those clustered around the mouth, in the positioning and protection of the lips during gnawing, digging and food ingestion.

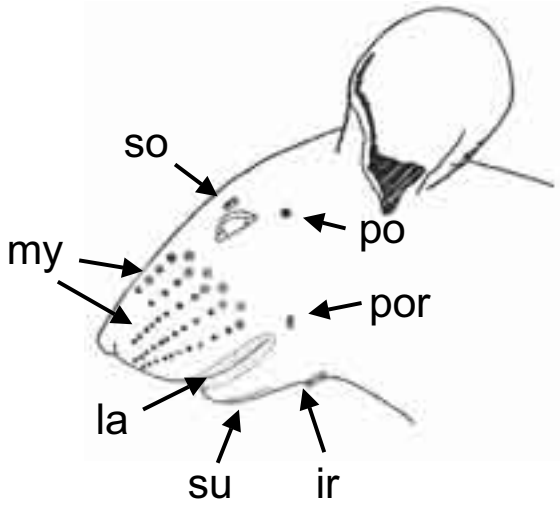

Figure 4.14 Terminology of vibrissal groups on the head of a murid rodent: interramal (ir); labial (la); mystacial (my); postorbital (po); postoral (por); supraorbital (so); submental (su).

All vibrissae grow out of specialised follicles; hence their position is constant through life. Worn vibrissae are replaced by a new shaft that grows from the same follicle. Because the old and new vibrissae can coexist for some time, the exact number of vibrissae is quite variable.

Although all murid rodents probably share the same basic set of vibrissal groups, the vibrissae themselves can vary greatly in thickness, colour and length in different species. As a rule, the vibrissae appear to be especially thick and long in the more highly arboreal species (e.g. *Leopoldamys* spp.), and noticeably short and fine in some of the more terrestrial or fossorial forms (e.g. *Bandicota* spp.). Notably, however, there is no obvious difference in vibrissal thickness or length between the arboreal *Rattus rattus* and its terrestrial, burrowing relatives *R. argentiventer* and *R. losea*.

External ears

Murid rodents have very simple external ears (Figure 4.15). However, the ear flap or **pinna** varies in both length and relative breadth between species. It also varies in the degree of pigmentation, from relatively pale to quite heavily pigmented and dark.

Figure 4.15 The external ear or pinna of two murid rodents showing a difference in the degree of hairiness of the ear between *Leopoldamys sabanus* (left) and *Bandicota indica* (right).

In all species, the inner and outer surfaces of the pinna are covered with fine hairs. These are more conspicuous in some species than others, and in a few species they form a delicate fringe around the margin of the pinna (Figure 4.15).

The ears of unweaned pups are very small and fleshy. Rapid growth of the ears usually starts towards the end of the second week of life.

Incisors

All rodents have only one incisor in each side of the upper and lower jaws. These teeth grow continuously and the animals must gnaw on hard material regularly to stop them from overgrowing. Enamel is restricted to the front and outer surface of each tooth.

The upper incisors of Southeast Asian murid rodents vary in relative width and orientation, and in the colour of the enamel. The widest incisors, with a combined upper incisor width greater than 3 mm, are found in the species of *Bandicota* and *Nesokia*. *Nesokia indica* is unique in having the paired lower incisors wider than the paired upper incisors.

Incisor enamel in murids is usually a dark orange on the upper pair and slightly paler on the lower pair. Some species have much paler enamel—perhaps best described as pale yellow or cream coloured (Figure 4.16).

The upper incisor tips point vertically downwards or even slightly backwards in most murids (Figure 4.17). However, species that excavate extensive burrow systems often use their incisors to dig and transport fragments of soil and rock. In these species, the upper incisors usually point slightly forward. This is best seen in *Bandicota bengalensis* and *Berylmys berdmorei*, both of which are strong diggers. In contrast, other

Figure 4.16 Incisor teeth of two murid rodent species, showing differences in relative width and in the colour of the enamel. *Berylmys berdmorei* (left) has relatively narrow incisors with pale enamel; *Bandicota indica* (right) has broad incisors with dark orange enamel.

Figure 4.17 Differences in upper incisor orientation among murid rodents; curved backward in *Rattus rattus* (left) compared with forward pointing in *Berylmys berdmorei* (right).

burrowing species like *Bandicota indica* and *Rattus argentiventer* have unspecialised incisors.

In some *Mus* species (e.g. many *M. musculus*), the cutting edge of the upper incisors bears a distinct notch. This is produced through wear against the lower incisors and may not be present in all members of a population.

Tail

The degree of hairiness and scaliness of the tail clearly distinguishes each of the four major groups of rodents found in Southeast Asia (differences noted on page 38). Among murid rodents, the tail also provides a suite of useful diagnostic characters, including:

- its length relative to the body
- the form, size and colour of its scales
- the number, length and colour of its hairs
- the presence of a terminal hair tuft or, less commonly, of a lateral hair fringe.

Tail length is variable in all species and should be used with caution in identification. More highly arboreal species generally have longer tails than terrestrial forms, presumably reflecting the use of the tail as a balance organ. Tail length may be under strong selective pressure in populations of some species that occupy a range of different habitats (e.g. *Rattus rattus*). Relative tail length is most usefully expressed as a proportion of head+body length. As noted above, the tail grows at approximately the same rate as the head+body in rodents, hence relative tail length is not greatly affected by individual age.

Although the tail is scaled in all murids, the size and shape of the **scales** varies between species (Figure 4.18). The size of scales is usually expressed as the number of rows that occupy a 1 cm section, as measured one-third of the way down from the tail base. While this value is highly correlated with overall body size (larger species tend to have lower counts), there are significant differences in mean counts between species of similar body size (e.g. between *Rattus rattus* and *R. argentiventer*; the latter having larger tail scales and lower scale counts).

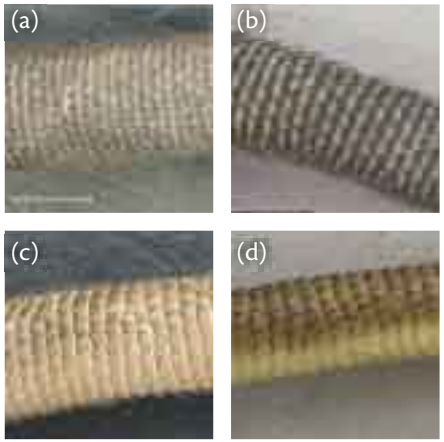

Figure 4.18 Variation in tail morphology among four murid rodents: (a) *Bandicota indica*; (b) *Bandicota bengalensis*; (c) *Chiromyscus chiropus*; (d) *Niviventer* sp. Examples (a) and (b) are 'hairier' than the other two. Examples (c) and (d) are 'bicoloured'. The latter has a sharp boundary between the upper and lower surfaces.

Individual tail scales are essentially rectangular in shape in all Asian pest murids. However, they vary in the extent to which the posterior margin of each scale is prolonged to overlap the scale behind. The extent of overlap is also indicated by the amount of pale skin that is visible between the scale rows (contrast Figure 4.18a with b). 'Strongly overlapping' scale rows are typical of *Rattus* and *Berylmys* species, and some *Bandicota* species. 'Non-overlapping' or 'weakly overlapping' scale rows are found in *Mus* species and in various genera of forest rats (e.g. *Niviventer*; Figure 4.18d).

All arboreal murids use their tail to grasp onto branches or foliage while climbing, but only a few show any obvious morphological specialisation. In a few highly arboreal groups (e.g. the New Guinean *Pogonomelomys* spp.), the upper surface of the very tip of the tail bears a patch of smooth skin—a specialised grasping organ.

Tail colouration in murid rodents is often characterised as being either 'unicoloured' or 'bicoloured'. In a typical **unicoloured** tail—such as occurs in all *Bandicota* and *Nesokia* species, in most *Rattus* species, and in some *Mus* species (e.g. *M. musculus*)—the tail scales are heavily and evenly pigmented at all points on the tail (Figure 4.18a–b). In *Berylmys berdmorei*, the tail is also unicoloured but the scales are weakly pigmented in juveniles and seem to be largely free of pigment (thus 'flesh-coloured') in adults. In a typical **bicoloured** tail

(Figure 4.18d), the scales on the upper half of the tail contain dark pigment while those on the lower half are unpigmented or contain white pigment. The boundary between the upper and lower portions of a bicoloured tail is usually sharp; however, it is diffuse in a small number of taxa, including some *Rattus* species (e.g. *R. norvegicus* and *R. nitidus*) and *Chiromyscus chiropus* (Figure 4.18c).

A different type of tail patterning, sometimes also referred to as 'bicoloured', features a contrasting white or cream-coloured terminal portion (Figure 4.19). This condition is occasionally found as a variant in *Rattus* and *Bandicota* species—but with the white tip usually not more than 5% of total tail length. However, it is common, or even represents the typical condition, in some forest murids, such as some species of *Maxomys*. In some cases, the two forms of tail patterning are found in combination: dorso-ventral distinction combined with an all-white tail tip (e.g. Figure 4.19).

Figure 4.19 A pale tail tip in *Leopoldamys sabanus.*

In all species, tail colouration tends to be more intense and the boundaries more sharply defined in juveniles and subadults than in adults.

In all murids, small **hairs** emerge from under the posterior margin of each scale (Figure 4.18). There are usually three hairs per scale (occasional scales may have five), but in some species this is reduced to a single, very short hair per scale. The hairs also vary in length between species, ranging from less than a scale length to more than two scale lengths. In most species, the hairs become longer towards the end of the tail, and it is not uncommon for the tail to end in a distinct 'tuft' of hairs (Figures 4.19–4.20). However, in some species (e.g. *Bandicota* spp.) the reverse is true and the terminal portion of the tail is almost naked.

Figure 4.20 A strong terminal tail tuft in *Chiromyscus chiropus.*

The tail hairs also vary in colour between species, ranging from clear to white or black (contrast Figure 4.18b with 4.18c). Species with bicoloured tails usually have dark hairs along the upper surface and white hairs below; however, there are exceptions in which the hairs are dark against both pale and dark surfaces.

In a few Asian murid species (e.g. *Chiropodomys* spp.), the lateral tail hairs are elongated and project outwards to form a distinct **lateral tail fringe**.

Fore-limb

The fore-limbs of rodents are used in many tasks including locomotion, climbing, digging, grooming, sexual grasping, and the manipulation of food items. Perhaps because of this multifunctionality, they are very conservative in morphology and show only slight variations in proportions and detailed form, even in species with quite specialised patterns of locomotion (e.g. hopping rodents).

Small vibrissae (carpal group) are found near the wrist in all groups of rodents including murids. Murids lack a second group of vibrissae (anconeal group) that are located near the elbow in some other rodents.

The **manus** or fore-foot of murid rodents, also sometimes referred to as the 'fore-paw' or 'hand', has four well-developed digits, each with a sharp claw. A fifth digit (the innermost one) is reduced to a small nubbin with a flattened nail. The claws tend to be larger and more elongated in species that spend much of their time digging, but smaller and sharply recurved in arboreal species. More generalised terrestrial species tend to resemble the arboreal group in the size and shape of their claws.

The palmar surface of the manus has five fleshy pads in all species (Figure 4.21). These tend to be smaller and more discrete in terrestrial species, but larger and grouped closer together in the more arboreal forms.

In murids, the underneath of each digit bears a series of well-defined transverse ridges called **subdigital lamellae**. These are absent in members of the family Rhizomyidae, and replaced by smooth or randomly creased skin (Figure 4.21).

Figure 4.21 The manus (fore-foot) of a murid rodent (*Rattus rattus*; left) and a rhizomyid rodent (*Rhizomys pruinosis*; right). Note the subdigital lamellae under the toes of the murid rodent only.

The pattern of fur colouring on the fore-limb and manus varies somewhat among the murid rodents. In the most common condition, the general fore-limb colour extends onto the upper surface of the wrist, giving way on the lower wrist and digits to a contrasting zone of white or transparent hairs (Figure 4.22a). In a few species (e.g. *Bandicota* spp.), the fore-limb fur colour extends over the manus to part-way along the digits (Figure 4.22b). Even less often, as seen in *Rattus nitidus*, the white fur of the manus extends partway up the fore-limb, forming a more elongated 'glove' (Figure 4.22c). A final variant, found in species of *Leopoldamys*, has a well-defined strip of dark fur extending down the centre of the wrist (Figure 4.22d).

Figure 4.22 Variation in the patterning of the manus (fore-foot) among murid rodents: (a) *Rattus rattus*; (b) *Bandicota indica*; (c) *Rattus nitidus*; and (d) *Leopoldamys sabanus*.

Hind-limb

The hind-limb of rodents is used more exclusively for locomotion and it shows more obvious patterns of specialisation. Among the Southeast Asian murids, this is most clearly expressed in the morphology of the **pes** or hind-foot. As a general principle, terrestrial rodents have long, narrow feet that enhance running speed, while arboreal rodents have short, broad feet that provide better purchase and are also better suited for grasping (Figure 4.23)

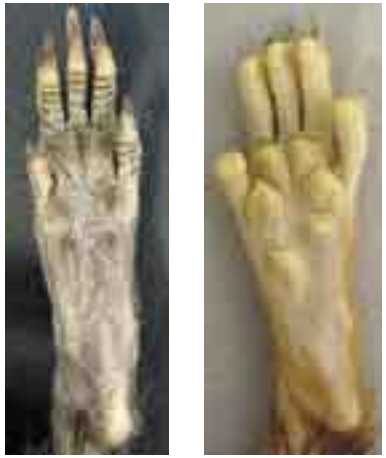

Figure 4.23 Pes (hind-foot) shape among murid rodents, contrasting a highly terrestrial species (*Bandicota savilei*; left) with a highly arboreal one (*Chiromyscus chiropus*; right). Note differences in relative digit versus heel lengths, claw size and shape, and plantar pad size.

The pes of murid rodents has five distinct digits, the innermost digit being the shortest (Figure 4.24). All digits have subdigital lamellae and apical pads as described for the manus. The number of subdigital lamellae (as counted on the central digit) is relatively constant (±1–2) within each species but differs

between them. This partly reflects the length of the digits (e.g. low counts of 4–6 in the short-toed diggers such as *Bandicota* spp.; counts of 7–8 in most other species). However, some species also seem to have unusually small and numerous lamellae, with very high counts obtained in some species of *Maxomys* and *Chiromyscus*.

In most species, well-formed claws are present on all five digits (see Figure 4.24). Some of the more highly arboreal forms have a small, flattened nail on the innermost digit only (see Figure 4.25)—or, in one species, *Vandeleuria olearea*, on both the innermost and outermost digits. The form of the apical pads and claws mirrors that seen in the manus—specialised diggers tend to have small apical pads with large, forward-projecting claws; while arboreal and more generalised terrestrial forms have prominent apical pads and sharp, recurved claws.

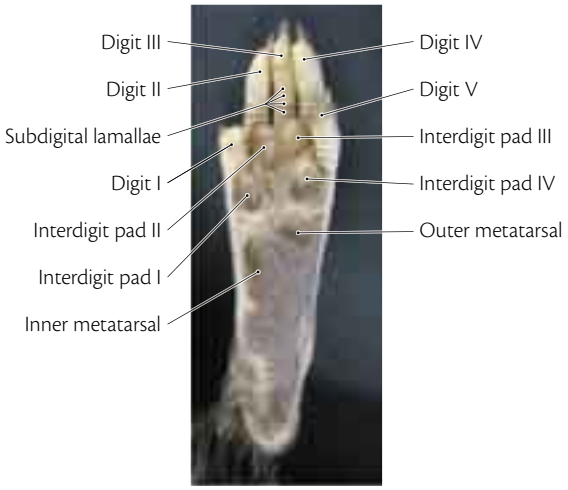

Figure 4.24 Left pes (hind-foot) of *Leopoldamys sabanus*, illustrating the major morphological features of the plantar surface. Note that all digits bear sharp claws.

The plantar surface of the pes usually has six large fleshy pads—four **interdigital pads** arranged in an arc at the base of the digits, and two **metatarsal pads** ('inner' and 'outer') situated further back on the sole (Figure 4.24). In many species, the two outermost interdigital pads have a small accessory pad fused to their outer margin, and this sometimes gives them an upside-down U-shaped appearance (e.g. *Chiromyscus chiropus*; see Figure 4.23). The **inner metatarsal tubercle** in many murids is elongated and curved posteriorly, giving it a comma-like shape (Figures 4.24 and 4.26). In a few groups of murids (e.g. *Mus* spp.), the skin between the primary plantar pads is covered in fine tubercles; more normally, it is smooth (Figure 4.26).

Figure 4.25 Left pes (hind-foot) of *Chiromyscus chiropus*, showing the flattened nail on the first digit of this species.

The plantar pads in terrestrial species (especially those that habitually dig) tend to be relatively small and low, and their surfaces generally appear smooth or weakly striated. In contrast, the pads of arboreal forms are usually larger, more prominent and more obviously striated (see Figure 4.23). These adaptations present obvious advantages for climbing.

Figure 4.26 Left pes (hind-foot) of *Rattus norvegicus* (left) and *Mus cookii* (right) illustrating contrastings of (i) inner metatarsal tubercle—elongated in *R. novergicus* versus rounded in *M. cookii*; and (ii) the skin between the interdigital pads—granular in *M. cooki* versus smooth in *R. norvegicus*.

The skin on the upper surface of the pes is covered in very fine, scale-like structures and it is also sparsely covered in a layer of fur, with hairs extending onto the toes. The skin varies in colour from essentially transparent (flesh-coloured) to white and dark brown or grey. It often appears to be speckled with colour due to the presence of scattered, pigmented scales.

The fur on the pes is sometimes completely dark (e.g. *Bandicota indica*) or pure white (e.g. *Rattus nitidus*), but more often it consists of both pale and dark hairs. These may be randomly mixed, giving the upper surface of the foot a grizzled appearance (e.g. *Bandicota bengalensis*), or they may be segregated into a distinct pattern. The most common pattern is a narrow band or wedge of dark hairs extending forward from the ankle, along the outer side of the pes (Figure 4.27). In some species, the dark hairs are concentrated on the front of the pes, around the bases of the digits. As in the manus, the toes are usually clothed in white or clear hairs; however, even these hairs are dark in some examples of *Bandicota indica*. A few species have pale, gingery fur on the upper surface of the pes (e.g. *Chiromyscus chiropus*, some *Rattus rattus*), sometimes in combination with dark brown or black hairs.

Figure 4.27 Upper surface of the left pes (hind-foot) of *Rattus sikkimensis*, illustrating the common patterning of a wedge of dark hairs extending forward from the ankle.

Scrotal sac

In adult males of most murid rodent species, the **testes** are held in a prominent scrotal sac that overhangs the base of the tail and hides the anus from view (Figure 4.28; see also Figure 4.2). The scrotal sac is most prominent in the smaller species, such as *Mus* spp., in which the testes are largest relative to body size. However, some much larger-bodied species (e.g. *Rattus* spp.) also have quite large testes (length in adult 20–30 mm) that occupy a prominent scrotal sac. In contrast, the species of *Bandicota* and *Nesokia* have relatively small testes (rarely more than 25 mm in length) and these occupy a poorly developed scrotal sac that barely projects past the anus. The more protected location of the testes in these species may be related to their burrowing habits.

The **epididymal pouch** (see Figure 4.28) is a small posterior extension of the scrotal sac that houses the paired **cauda epididymes**, the organs in which mature sperm are stored. The epididymal pouch is prominent and darkly pigmented in most murids. In contrast, it is poorly developed and weakly pigmented in the species of *Bandicota* and *Nesokia*.

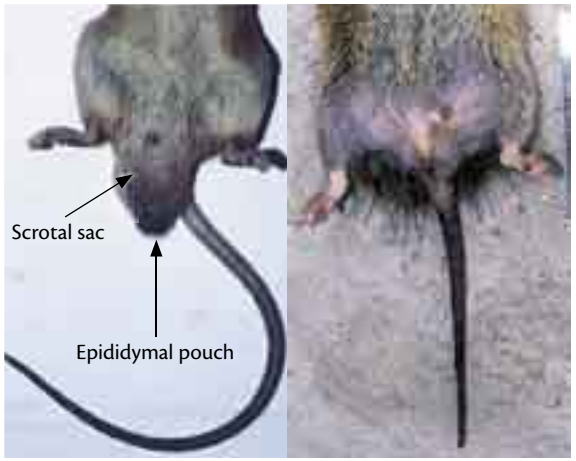

Figure 4.28 Scrotal region of two adult male murid rodents with proportionally very different sized testes: *Rattus exulans* (left) with relatively large testes, has a large scrotal sac and prominent epididymal pouch; *Nesokia indica* (right) with relatively small testes, has a small scrotal sac and indistinct epididymal pouch.

Mammae

The number of teats differs between some genera and species of murid rodents (Table 4.1). This makes the mammae useful for taxonomic diagnosis, but generally only for adult females. As mentioned earlier, the mammary formula is usually expressed as the sum of three parts: pectoral + postaxillary + inguinal (e.g. 1+2+2 for *Mus domesticus*; see Figure 4.4). Although this system is adequate for most species, some individuals of *Bandicota bengalensis* have numerous teats in more or less continuous series along either side, sometimes as many as 18 on one side alone (Figure 4.29). This is best expressed as a total count.

Some other species also show individual variation in teat number. However, in most cases this variation affects only the postaxillary teats. For example, *Rattus rattus* may have one or two teats in this position, sometimes with different numbers on opposite sides of the same individual. A variable mammary formula can be written as 1+1/2+3.

Cranial features

Rodents can also be identified from features of the skull and teeth. However, this is really a specialist task and it is beyond the scope of this book to review all of the diagnostic characters.

Anyone who is seriously interested in conducting taxonomic research on rodents should prepare some representative skulls, using the methods described on page 37. You will also need to learn the complex terminology used by rodent taxonomists to identify all of the individual features of the molar teeth and the cranium. Some useful introductory references are given under Further reading.

Figure 4.29 Adult female of *Bandicota bengalensis* illustrating the unusually large number of teats that often occurs within this species.

Table 4.1 Distribution of the major pest rodent species according to mammary formula.

Mammary formula	Species included
0+1+2	some *Rattus steini*
0+2+2	some *Rattus steini, R. mordax, R. praetor*
1+1+2	*Berylmys bowersi, Cannomys badius, Nesokia indica, Rattus exulans*
1+2+2	*Berylmys berdmorei,* all Southeast Asian *Mus* spp., *Mus musculus* Group
1+0+3	some *Rhizomys pruinosis*
1+1+3	*Rattus losea,* some *R. rattus, R. tiomanicus,* some *Rhizomys pruinosus, Rhizomys sinensis, Rhizomys sumatrensis*
1+2+3	some *Bandicota bengalensis, B. indica, B. savilei, Rattus argentiventer, R. nitidus, R. norvegicus,* some *R. rattus, R. sikkimensis, R. turkestanicus*
numerous	some *Bandicota bengalensis*

Further reading

Aoki, B. and Tanaka, R. 1938. Biostatistical research on *Rattus losea* (Swinhoe, 1870), a Formosan wild rat, with special reference to its diagnostic characters for taxonomy. Memoirs of the Faculty of Science and Agriculture, Taihoku Imperial University, 23, 1–74.

Grassé, P.P. and Dekeyser, P.L. 1955. Ordre des rongeurs. In: Grassé, P.P., ed., Traité de Zoologie. Anatomie, systématique, biologie. Tome XVII, 2nd fascicle. Paris, Masson et c^ie^, 1321–1573.

Lunde, D. and Nyuyen Trong Son 2001. An identification guide to the rodents of Vietnam. New York, American Museum of Natural History.

Mahoney, J.A. and Richardson, B.J. 1988. Muridae. In: Walton, D.W., ed., Zoological catalogue of Australia, volume 5, Mammalia. Canberra, Bureau of Flora and Fauna, 54–192.

Marshall, J.T. 1977. Family Muridae. In: Lekagul, B. and McNeely, J.A., ed., Mammals of Thailand. Bangkok, Kurusapha Press, 397–487.

Musser, G.G. 1981. Results of the Archbold Expeditions. No.105. Notes on systematics of Indo-Malayan murid rodents, and descriptions of new genera and species from Ceylon, Sulawesi and the Philippines. Bulletin of the American Museum of Natural History, 168, 225–334.

Musser, G.G. and Carleton, M.D. 1993. Family Muridae. In: Wilson, D.M. and Reeden, D.M., ed., Mammal species of the world, 2nd edition. Washington DC, Smithsonian Institution Press, 501–755.

Musser, G.G. and Newcomb, C. 1983. Malaysian murids and the giant rat of Sumatra. Bulletin of the American Museum of Natural History, 174, 327–598.

Musser, G.G. and Newcomb, C. 1985. Definitions of Indochinese *Rattus losea* and a new species from Vietnam. American Museum Novitates, No. 2814, 1–32.

Sokolov, V.E. and Kulikov, V.F. 1987. The structure and function of the vibrissal apparatus in some rodents. Mammalia, 51, 125–138.

CHAPTER 5

Population studies

Introduction

Rodent population studies attempt to document and explain variation or changes in the abundance of one or more species. These studies form the basis of any ecologically based rodent management system, as they help us to understand the major factors that control or regulate the population growth of pest rodent species and to identify the vulnerable points in the system that might allow effective intervention.

The population abundance of any individual species is determined by the numbers of births and deaths in a given area, and by the number of animals moving into (immigration) and out of (emigration) that area (see Figure 5.1). Each of these factors may be influenced by seasonal or longer-term climatic cycles, fluctuations in the abundance of food or predators, or changes in land-use patterns. The population abundance of other species that compete for food or space may also be important.

The most basic type of population study is one that simply documents changes in animal abundance through time and space. This information can be obtained by taking a census of population size at various localities or at various times. Methods for carrying out a population census are described in this chapter.

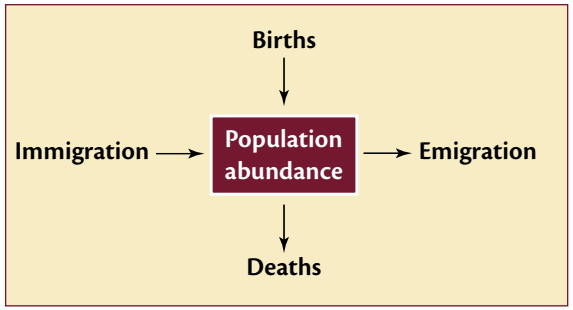

Figure 5.1 Simple conceptual model of the four factors that determine population size in a given area.

Census data can provide useful insights into the relationship between population abundance and some potential causal factor such as variations in rainfall or temperature, or between population abundance and crop damage. However, census data alone generally will not provide any real insight into the underlying ecological dynamics of the system. To understand why population abundance varies in time and space, it is necessary to not only study the changes in population abundance, but also study each of the main factors—breeding activity, mortality (including predation) rates, and movements. In Chapters 6 and 7, we describe methods for studying reproduction and movement of rodents, respectively. We do not cover methods used to estimate mortality rates, or to study the impact of predators. However,

these topics are covered in some of the general references listed under Further reading at the end of this chapter.

There are two main approaches to studying the abundance of animals in the environment. The first approach is to estimate the actual population size or **population density** (number of animals per unit area). If these estimates are taken simultaneously at different locations or repeatedly at one location, you will be able to study variations in population size through time or space. However, methods for estimating actual population density are laborious (see below) and before embarking on such a study, it is wise to ask first whether such data are really needed. For comparative studies, the alternative approach of taking **relative estimates of abundance** may be both adequate and far more cost effective. These simpler methods will be described first.

Relative estimates of abundance

Relative estimates of abundance do not give any absolute value for population size but they do allow you to make comparisons between localities or between time periods. One of the simplest measures of relative abundance is **trap success**, already introduced in Chapter 3. However, other relatively simple and inexpensive methods such as the use of tracking tiles, census cards, visual surveys and active burrow counts are also worth considering, especially if these methods are used in combination. Each of these methods is described briefly in the following pages.

Trap success

Trap success is usually calculated for single-capture traps (either live- or kill-traps) as the number of rodents captured divided by the total number of traps set. This value is usually multiplied by 100 to give percentage trap success. For example, if trapping occurred for 3 consecutive nights with 50 traps set each night, and the number of rats caught on each night was 5, 7 and 3, respectively, then the total trap success is (15 rats/150 traps) × 100 = 10% trap success.

Various adjustments are sometimes made to the raw trap-success figure. As mentioned in Chapter 3, one adjustment that is commonly made is to subtract the number of 'null' traps, i.e. traps that were sprung without making a capture, from the total number of traps set. A similar, but more sophisticated, adjustment takes account of the impact of occupied traps on overall trap success. Every time an animal is caught, there is one trap fewer available to make more captures. The number of active traps thus reduces progressively throughout the night. Caughley (1977) recognised that this situation reflects a simple frequency–density relationship approximated by the equation:

$$\text{Adjusted trap success (ATS)} = \ln\left(1 - \frac{\text{animals caught}}{\text{number of traps}}\right) \times (-100)$$

A simple, step-by-step method for use with a calculator is as follows:

- divide number of animals caught by number of traps, e.g. 22/52 = 0.423 (unadjusted or raw trap success)
- store the answer in the calculator's memory
- subtract memory from 1 (i.e. 1 minus recall memory), 1 – 0.423 = 0.577
- take the natural log (ln) of that = −0.55
- convert that to a percentage, −0.55 × −100 = 55% (adjusted trap success or ATS).

Tracking tiles

Tracking tiles are flat squares of metal (Figure 5.2), ceramic, vinyl or wood (usually around 250 × 250 mm) that are covered with a layer of grease or mud and placed in positions where rodents are likely to be moving during the night. The following morning, the tiles are inspected for signs of rodent activity. This may take the form of complete footprints, a tail swipe, or just the marks of the rodent's claws. It is generally not possible to identify individual footprints to species. It is usually also

difficult to tell how many rodents have visited the tile, so most people just record the activity as 'yes' or 'no'.

In rice fields, an alternative to tracking tiles is to smooth a set length (e.g. 1 or 2 m) of mud along a bund between rice fields. The number or length of rodent track-ways can be taken as a measure of activity. To be effective, the mud needs to be moistened and smoothed late in the afternoon or early in the evening, after the sun has fallen. This method is not suitable for use during periods of heavy rain. At such times, the use of grease is preferable as it is unaffected by rain.

Tracking tiles are sometimes used in combination with single-capture traps, with tiles and traps interspersed along a trap-line or within a trapping grid. Although neither method gives an absolute estimate of abundance, the combination does provide a useful independent measure of the effectiveness of the single-capture traps.

Figure 5.2 Squares of metal (250 × 250 mm) with grease (tracking tiles). Footprints can be seen on one tile, on the right.

Census cards

Census cards are used to estimate relative abundance of the house mouse in grain-growing areas of Australia (Figure 5.3). Squares of paper (100 × 100 mm) are marked with a grid and then soaked in vegetable oil (or canola oil), which is attractive to mice. The paper squares are pegged to the ground with metal wire.

Census cards are set out in lines of 10 or 15, with 10 m between each card. Lines should be set along a range of habitats such as channel banks, small banks, large banks and along edges of paths or roads. The following morning, the number of squares consumed by the mice is recorded. The average percentage of each card consumed is calculated as an index of relative abundance. Census cards tend to be consumed more when there is little alternative, high-quality food available. The method is thus subject to some of the same limitations as the use of baited single-capture traps. Census cards cannot be used during periods when heavy rainfall is expected.

Figure 5.3 Census cards, before and after one night. Approximately 40% of the card on the right was consumed by mice.

Burrow counts

The number of rodent burrows in a given area is a useful index of the relative abundance for many ground-dwelling species. It is obviously of no use for tree-dwelling species or those that build grass or leaf nests on the ground. In some cases, the burrows of different species can be identified from their size or morphology, but this will depend on the number and variety of species found in any area.

In taking burrow counts, it is important to distinguish active from abandoned burrow systems, and to distinguish rodent burrows from those excavated by crabs or other creatures. A technique used in Indonesia involves locating all burrows along a transect of a given length. Each burrow entrance is plugged with a thin layer of mud (Figure 5.4). It is important to mark the location of all burrows so that they can be found the next day, or to draw an accurate map. The following day, the number of freshly reopened entrances is recorded. Footprints made by the rodents are often seen in the mud. In the Mekong Delta in Vietnam, dry grass is used instead of mud to seal burrow entrances.

The number of reopened burrows does not tell you exactly how many rodents are present along

the transect. In many species, the burrow systems may have multiple entrances and only some may be reopened. Some rodents may have chosen to remain within the burrow, while some burrows may house more than one rodent, especially during the breeding season. Many studies have shown strong seasonal changes in the average number of animals per burrow system. Excavating a set number of burrows per sampling period to estimate the occupancy rate can reduce this uncertainty.

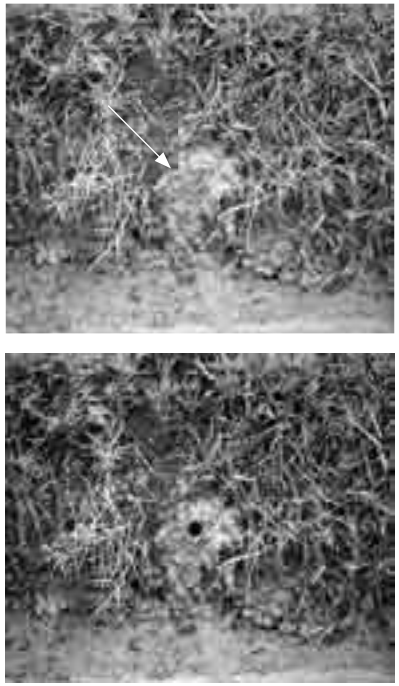

Figure 5.4 (Top) Rat burrow covered with mud (arrow). (Bottom) Rat burrow that has been sealed with mud and reopened.

Visual surveys

Under some conditions, it is possible to count the number of rodents that are active at night in a particular location. This is usually done with a torch—an activity that is known as 'spotlighting'. The basic method involves walking at a constant pace along a transect and counting the number of active rodents that are detected either from their movement or by their eye shine (usually glowing red). With experience, it may be possible to identify different rodent species from sightings of this kind.

For spotlighting to be an effective and useful tool, the method must be standardised. The observer, the observer's pace, the route taken, the time of night and the strength of the torch must all be kept as constant as possible. Factors that may interfere with the ability to see or hear the animals, such as rain or dense plant cover, should also be recorded for each survey period.

Calibrating relative estimates of abundance

All relative estimates of abundance can be made more useful if they are 'calibrated' against estimates of actual population densities, as suggested above in the case of burrow counts. However, it is also important to realise that the appropriate calibration factor may vary between seasons or stages in a cropping cycle. For example, methods that rely on baits (single-capture traps and census cards) will almost certainly have a lower relative success rate during periods when the local environment contains abundant alternative food.

One approach worth considering is to use a variety of these methods for estimating relative abundance in combination. During the course of a full year's cycle, each method can be expected to provide different kinds of information that, when added together, might give a better overall picture of the relative intensity of rodent activity through time and across a range of different habitats.

Estimates of population size

With rodents, it is generally not possible to count all of the animals in a population. The next best thing is to estimate the number of animals in a given area, using one or more of the following methods.

One way of estimating population size is to convert relative abundance data obtained from trapping or from visual surveys into population density values. To do so, you will need to make some fairly large assumptions. For trapping data, you will need to estimate the trappability of each species—that is, the proportion of a population that you would expect to enter the traps each night. As mentioned earlier, this value may vary seasonally, depending on both the availability of other foods around the traps and

on the activity pattern of the animals (which in turn might reflect breeding, dispersal activity etc.). For visual survey data, you will need to estimate the proportion of a population that you would expect to be active at any one time, the likelihood of observing an individual animal, even if it is active, and the width of the transect (i.e. the distance of reliable detection). Visual surveys are often used to estimate population densities of large, easily spotted animals. For rodents, the error factor is probably too large to make the method useful, except perhaps in very open habitat.

Capture–mark–release methods are the most commonly used technique for estimating population size. As the name suggests, these methods all require that captured animals are marked in some way and then released at the point of capture. After one or more nights, the locality is trapped again. Population size is then estimated by comparing the number of recaptures with new captures in the sample. If the trapping is continued over several nights, the proportion of new captures can be compared with the numbers of animals caught once before, twice before etc. Various methods are available for estimating population size from the recapture data. However, before moving on to these, we will review some of the basic equipment and methods used in a capture–mark–release study.

Equipment

When collecting data for a capture–mark–release study, the following equipment is required (Figure 5.5):

- a large bag to hold the rodent; this can be plastic or cloth—a cloth pillow case is ideal
- rulers to take length measurements; transparent plastic rulers are good because it is easier to see what you are measuring; steel rulers are easy to disinfect and will last longer
- a balance for weighing rodents; a spring balance (e.g. Pesola) is best, but any balance which is portable and hardy will be sufficient (ensure the weight range is suitable for the animals you are trapping; in many regions you may need two or more Pesola balances, one for mice (to 60 g), one for rats (to 300 g) and one for *Bandicota* (to 1 kg)
- individually numbered ear-tags or an ear-punch for marking animals; an applicator is also useful
- a simple taxonomic key for species identification in the field (see Chapter 11)
- data sheets, data codes, pencils, pens; data must be recorded in a systematic, logical and consistent way. Standard data sheets and codes **must** be used—this way, no information will be forgotten and comparisons can be made with other sites and countries. A sample data sheet is provided in Appendix 1.

Figure 5.5 Equipment needed for a live-trapping study, clock-wise from top: field manual with codes and taxonomic key, field data sheets, pencil, plastic ruler, ear-tags, ear-tag applicator, Pesola spring balance and cloth bag for holding rodents.

It is a good idea to carry duplicates of essential equipment. We find that it is best to carry everything in a small bag that attaches around the waist.

If possible, two people should work together to collect population data. One person handles the rodents and takes measurements, while the other person records the data.

Marking techniques

Most capture–mark–release studies require that every captured animal is assigned a unique number. This number must either be attached to the animal in some way, or else encoded into a marking system that can be applied to the animal. The numbers or coded marks must remain visible on the animal for

the duration of the study and also must have little or no impact on the animals' behaviour, fitness or survival. Three alternative methods for marking are described here.

Ear-tagging

Some capture–mark–release studies use metal ear-tags that are imprinted with a four-digit number (0001 to 9999) (Figure 5.6). The tags have one short side with a point and two longer sides, one with the imprinted numbers and one with a slot (for attachment). The tags are easy to apply, once the correct technique has been demonstrated, and they are easy to read on subsequent captures.

Figure 5.6 Ear tag in a mouse.

The pointed side of the tag is pushed through the base of the ear, just under the fold of cartilage. It is best to have someone show you the correct location as the animal can easily rip the tag out if it has not been applied correctly. The point of the tag is then fed through the slot and flattened to reduce the risk of anything catching under the tag and causing the ear to rip. If ear-tags are applied in the correct position and with care, they will generally stay in place for many months and have no effect on the animal's behaviour.

Ear-punching

This method is of limited use for capture–mark–release studies because of the limited number of individual marks that can be applied (Figure 5.7). However, the method is mentioned here because it is sometimes useful to mark groups of animals with a single type of mark. Examples would be a study involving trapping every second month, where animals are marked according to the census period in which they were first captured (which will provide information on survival rate between trapping periods) and a study in which animals are marked according to the habitat in which they are first and subsequently captured (to analyse patterns of movement between habitats).

Figure 5.7 Right ear-punch in a mouse.

Ear-punches should be made with a good-quality ear-puncher of the kind used to mark laboratory animals. Ear-punches are less obvious than some other marking techniques and they probably have little impact on fitness. However, natural wounds to the ears can sometimes lead to incorrect identification.

The codes given in Table 5.1 and illustrated in Figure 5.8 show how an ear-punch numbering system works. For example, for census or habitat number 4, you would ear-punch all animals in the lower position of the left ear.

Table 5.1 Combinations of ear markings.

Position on ear	Number
Lower right	1
Upper right	2
Upper left	3
Lower left	4
Lower right + upper left	5

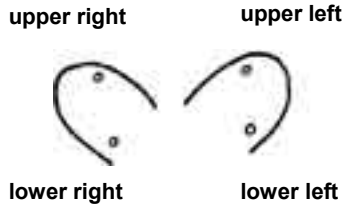

Figure 5.8 Positioning of ear-punches.

More complex combinations of punches can be used to increase the number of codes. However, care must be taken to ensure that the codes can be read without introducing errors.

Other methods

A range of alternative methods are available that could be used on rodents in field studies. These include ear slits, colour markings, tattoos, and shavings. Some references are provided at the end of this chapter (Further reading).

Calculating population size from capture–mark–release data

A wide range of methods is available to estimate population size from capture–mark–release data. Many of these methods are very sophisticated and they all rely on critical assumptions, including:

- the population is closed to additions (births or immigration) and deletions (deaths or emigrants)
- all animals are equally likely to be captured in each sample
- marks are not lost and are not overlooked by the observer.

The second assumption is often called the assumption of 'equal catchability'. This assumption is unlikely to hold true for many wild populations of animals, where the probability of capture is likely to be influenced by age, sex, social status, trap placement in relation to individual territories, and prior history of capture (e.g. 'trap-shy' versus 'trap-happy' individuals). A trap-happy animal becomes easier to catch after being caught once; a trap-shy animal becomes more elusive.

Many of the available methods also depend on high recapture rates (>20%). In our experience, recapture rates for Southeast Asian rodent populations are typically very low (often less than 1%), hence these methods will not produce useful population estimates. One of the simpler methods, called the Petersen Estimate, is explained in Box 5.1. This method is only appropriate where recapture rates exceed 5%.

To convert estimates of population size into a population density, we need to include some estimate of the area that is effectively sampled by the trapping grid. For a very sedentary species, this area my not be very much larger than the trapping grid itself. However, for more mobile species, the effective trapping area may be considerably larger. To convert a population estimate into an estimate of population density, we therefore need some information on the movement patterns of the particular species. Methods for studying movement patterns of rodents are described in Chapter 7.

Box 5.1 The Petersen Estimate for calculating population size

This is one of the simpler methods for estimating population size (number of animals per unit area). The calculations can be done on a calculator or using a spreadsheet program such as Excel on a computer. The Petersen Estimate can be calculated easily following the steps below:

- 1st trapping—mark animals caught and released (M)
- 2nd trapping—capture marked (m) and unmarked animals (total = n)
- Calculate proportion of population marked (Y):

$$Y = \frac{\text{Number marked } (m)}{\text{Number caught } (n)}$$

- Estimate population size:

$$N = \frac{\text{Size of marked population } (M)}{\text{Proportion of population marked } (Y)}$$

There are some important assumptions for this method:

- marked and unmarked animals are captured randomly
- marked animals are subject to the same mortality rate as unmarked animals
- marks are not lost or overlooked.

Here is a simple example to illustrate this method. Trapping with 50 traps set in a grid produced 15 rats caught, marked and released on the first night. On the second night, 13 rats were caught, including 5 marked animals. For this example, $M = 15$, $m = 5$ and $n = 13$, hence:

$$Y = \frac{m}{n} = \frac{5}{13} = 0.385$$

$$N = \frac{M}{Y} = \frac{15}{0.385} = 39$$

The estimated population size is 39 rats.

A range of more sophisticated methods is available free from the Internet (see Further reading).

Further reading

Caughley, G. 1977. Analysis of vertebrate populations. New York, John Wiley and Sons, 234 p.

Chitty, D. and Shorten, M. 1946. Techniques for the study of the Norway rat (*Rattus norvegicus*). Journal of Mammalogy, 27, 63–78.

Gurnell, J. and Flowerdew, J.R. 1994. Live trapping small mammals—a practical guide. Occasional Publication No. 3. London, The Mammal Society, 36 p.

Krebs, C.J. 1999. Ecological methodology. Menlo Park, CA, Benjamin Cummins, 620 p.

Pollock, K.H., Nichols, J.D., Brownie, C. and Hines, J.E. 1990. Statistical inference for capture–recapture experiments. Wildlife Monographs, 107, 1–97.

Seber, G.A.F. 1986. A review of estimating animal abundance. Biometrics, 42, 267–292.

Smith, M.H., Chew, R.M. and Gentry, J.B. 1969. New technique for baiting snap traps. Acta Theriologica, 14, 271.

Tanaka, R. 1960. Evidence against reliability of the trap-night index as a relative measure of population in small mammals. Japanese Journal of Ecology, 10, 102–106.

White, G.C. and Burnham, K.P. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study, 46 (Supplement), 120–138. Also available on the Internet: <http://www.cnr.colostate.edu/~gwhite/mark/mark.htm>. This website provides software for analysis of capture–mark–release data.