# Growth, Development and Reproduction

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# Contents

# Growth and development

## By the end of this chapter you should be able to:

1 discuss the meaning of the term *growth*;

2 explain how cell division and cell enlargement lead to growth;

3 explain that development is a progressive series of changes which includes the specialisation of cells;

4 distinguish between *absolute growth*, *absolute growth rate* and *relative growth rate;*

5 understand the use of different types of growth curve to represent growth and explain patterns of growth;

6 describe techniques for measuring growth in a representative range of living organisms, namely plants, insects and microorganisms;

7 appreciate the problems of measuring growth;

8 measure the growth of a plant from seed;

9 explain the use of microorganisms as a simple model of population growth.

The fundamental activities of living organisms can be summarised as nutrition, growth, reproduction, respiration, excretion, sensitivity and, for some, locomotion. Two of these activities, namely growth and reproduction, are the theme of this book. Growth is usually accompanied by development, so it is usual to study both together.

## What is growth?

In its usual sense, the word **growth** simply means 'getting larger'. It is something we associate with both living and non-living things. For example, crystals can grow in size, and even abstract things, like the economy, can grow.

All living things show growth and, since all living things are made of cells, growth must involve cells getting larger or increasing in number. Individual cells get larger after they have divided as they grow back to full size. Individual multicellular organisms grow in size as their cells grow in number and size. It is estimated that the average adult human contains about 50 million

million cells, all of which have grown from one original cell, the zygote. The largest organism of any kind ever to have existed on this planet is the blue whale, which may grow to over 30 m in length (*figure 1.1*). This must also grow from one cell, the zygote, a programmed increase in size of



● **Figure 1.1** A blue whale, the largest living thing ever to have existed. Like all multicellular organisms produced by sexual reproduction, it has grown and developed from a single cell, the zygote.

astronomical proportions. Populations of organisms can grow in size too. The global human population reached 6000 million for the first time in 1999 and is expected to grow to at least 10 000 million before it stabilises. Starting from one cell, some bacterial populations can grow to 6000 million in just half a day given ideal conditions.

So far then, we have thought of growth as an increase in *size*. For biologists though, this definition can be improved upon. What, for example, do we mean by size? This is important to know when we want to *measure* growth. There are various measurements which could be made, as we shall see later in this chapter. Three common examples are height, length and mass. Growth of humans, for example, is often measured as increase in height. However, a person may grow in size without increasing in height simply, for example, by developing more fat, larger muscles, or a larger uterus and breasts during pregnancy. A plant may grow more leaves or shoots without growing taller. Bearing factors like this in mind, biologists consider that, overall, the most appropriate measure of growth is increase in *mass*.

### SAQ 1.1
Why is growth normally associated with an increase in mass?

Biologists tend to view growth as part of a planned programme of development. Imagine a potato plant producing the potatoes that we commonly eat as vegetables. The potatoes grow underground as tubers. A tuber grows in mass as the number of its cells increases. But imagine if the soil around the tuber becomes dry. The tuber could lose water by evaporation from its surface and lose mass as a result. Then, if the soil becomes wet again, the tuber could 'grow' back to its normal mass as the cells take up water by osmosis. Would these changes in mass be signs of genuine growth? Such changes in water content are common in plant cells and can happen in any cells depending on their environment. Biologists prefer not to think of such changes as genuine growth because they are *reversible* and they are not part of programmed development. The definition of growth that most biologists prefer is that

> growth is an irreversible increase in dry mass of living material.

(Dry mass is mass after removal of water.)

### SAQ 1.2
Consider the following situations and suggest why the 'growth' described might be regarded as an exception to the definition of growth given above.
**a** A zygote (a cell formed by the fusion of two gametes) can divide to form a ball of smaller cells with no increase in mass.
**b** A germinating seedling shows a net loss in dry mass (mass after removal of water) until it starts to photosynthesise. By this time much develop-ment, including that of a primary root and shoot, has taken place, accompanied by an increase in size, cell numbers and fresh mass (mass including water).

The two exceptional examples in SAQ 1.2 show that growth is a complex process for which it is difficult to give a precise definition. Although the two examples appear to contradict the definition of growth, common sense suggests that they should still be regarded as growth.

## What is development?
As already mentioned, growth and development usually go hand in hand. We can say that

> development is a progressive series of changes, which includes the specialisation of cells.

In biology, development is genetically programmed and may be modified by the environment.

Multicellular organisms, such as humans or plants, grow from single cells, so growth and development must involve cell division. As each new cell is produced, it must grow to its mature size and become specialised for its essential functions. This process of specialisation is called **differentiation**. Thus growth and development typically involve three separate processes, namely:
- **cell division** leading to an increase in cell numbers;

- **cell enlargement** i.e. an increase in cell size;
- **cell differentiation** leading to cell specialisation.

*SAQ 1.3*
What type of cell division is responsible for growth?

*SAQ 1.4*
What is the genetic significance of mitosis?

## Cell differentiation

In any multicellular organism, all the cells derived from the zygote by mitosis are genetically identical. In humans, for example, this would be all the diploid cells, that is all the cells apart from the sex cells. Therefore a liver cell, for example, contains the same set of genetic instructions as a kidney cell. The question therefore arises, how can the two cells have developed differently when they have identical DNA? It has been shown by cloning new plants and animals from differentiated cells that these cells have not lost any information as they mature. They still contain all the instructions needed to make a whole organism. We cannot say therefore that a liver cell has lost the DNA needed to become a kidney cell. Instead, as cells differentiate, different genes are 'switched' 'on' or 'off'. In a liver cell the 'liver genes' are switched on and other genes are switched off; in a kidney cell the 'kidney genes' are switched on and other genes are switched off. The study of how this differing 'behaviour' of cells is controlled may lead to new techniques for treating a variety of medical disorders using **stem cells**. These are undifferentiated cells, found in e.g. young embryos, able to develop into any of the organism's cell types.

At any one time, a particular cell will have a variety of genes switched on or off in response to its environment without losing its identifying characteristics. For example, a fully mature pancreas cell is still a pancreas cell whether or not it is secreting insulin.

## Growth and development in plants

The location of growth and development within an organism differs between animals and plants. In animals, cell division can occur throughout the body. In complex animals the body develops systems made up of organs, and all these organs contain cells capable of dividing. In plants, however, cell division is much more localised. In fact, it can only occur in particular regions called meristems (from the Greek *merizein*, to divide). Growth in *length* occurs from **apical meristems**, found at the tips of roots and shoots ('apical' comes from 'apex', meaning tip). Growth in *width* occurs from **lateral meristems**, which are found along the length of roots and shoots.

> A meristem is a region of unspecialised plant cells from which new cells arise by cell division.

When a meristem cell divides, one of the cells produced remains **meristematic**. The other cell gives rise to one or more specialised cells. All the cells, tissues and organs of a plant are derived from meristems.

The three stages of growth mentioned previously, namely cell division, cell enlargement and cell differentiation, are all shown particularly clearly in the apical regions of roots and shoots because they are separated in time and place. (This will be explained below.) In animals, these three phases of growth also occur, but it is usually much harder to locate the exact position of the growing cells and to follow the sequence of events clearly. It is therefore useful to study plant root tips and shoot tips as 'models' of growth.

## Root tips

Root tips are responsible for the increase in length of the roots. *Figure 1.2a* is a diagram of a longitudinal section (LS) through a root tip and shows three **zones**. Moving back from the tip, these are the zones of cell division, then cell enlargement and then cell differentiation. This is a time sequence, with the youngest cells being the dividing cells near the tip and the most mature being the differentiated cells furthest away from the tip. *Figure 1.2b* shows a photograph of a section through a root tip. The **zone of cell division** in the root tip (*figure 1.2c*) keeps producing new cells while the root is growing. In the **zone of cell enlargement**, the new cells get larger by taking up water by osmosis and synthesising new

a  Simplified diagram of LS of a root tip.

Labels on diagram a:
- epidermis
- root hair
- cortex (parenchyma)
- pith (parenchyma)
- mature xylem
- mature phloem
- immature xylem – first vessels beginning to differentiate
- immature phloem – first sieve tubes beginning to differentiate
- apical meristem – maximum rate of cell division
- root cap (for protection)
- zone of cell differentiation
- zone of cell enlargement
- maximum rate of cell enlargement
- zone of cell division

● **Figure 1.2**
**a** Simplified diagram of LS of a root tip.
**b** Photomicrograph of LS root tip of *Vicia faba* (broad bean) ( 100).
**c** Cells from zone of cell division ( 250).
**d** Cells from zone of cell enlargement ( 1500).
**e** Cells from zone of cell differentiation.
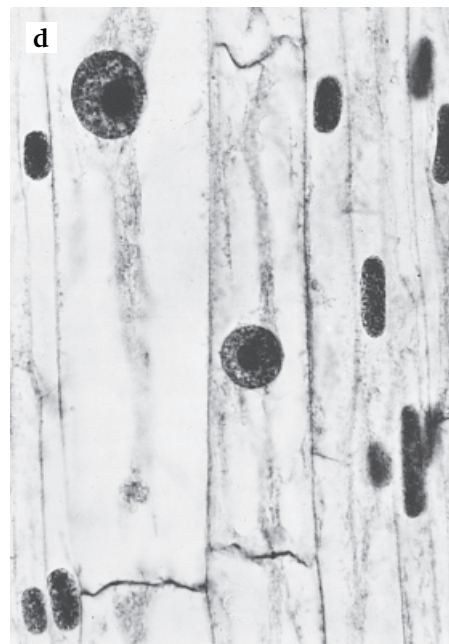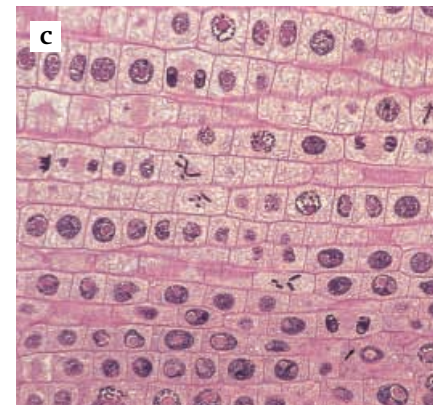








materials, often becoming much longer (*figure 1.2d*). In the **zone of cell differentiation**, the cells become specialised for particular functions and develop specialised structures. Three examples are:

■ **xylem**, made from cells called xylem vessel elements which fuse together as they differentiate to form long, dead tubes specialised for transporting water and mineral salts over long distances (*figure 1.2e* and *Biology 1*, chapter 10);

■ **phloem,** containing long, living tubes called sieve tubes, again made by cells (called sieve tube elements) fusing together. Sieve tubes are specialised to transport organic solutes such as sucrose around the plant (*Biology 1*, chapter 10);

■ **epidermis**, the outermost layer. Epidermal cells have a protective function. In roots they may grow extensions, the root hairs, to increase the surface area for water absorption.

Remember that all these cells contain identical DNA and therefore identical sets of genes. The control of differentiation involves the switching on and off of different genes in different cells at
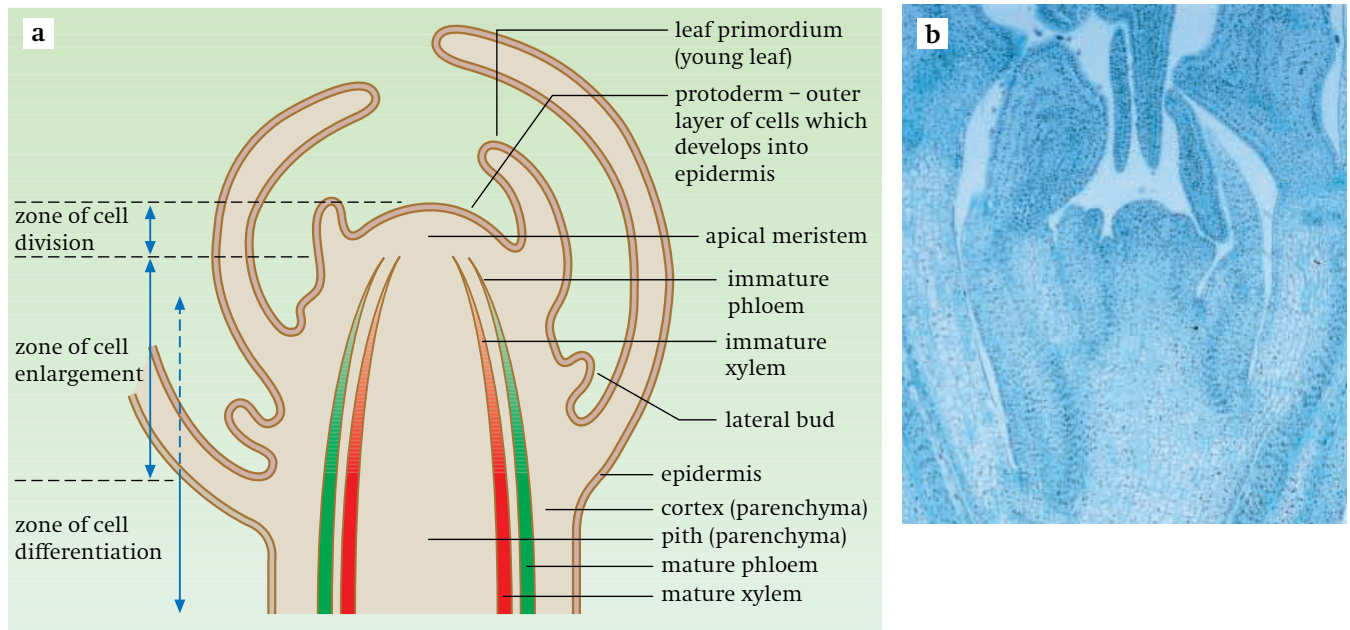
● **Figure 1.3  a** Simplified diagram of LS of a shoot tip.   **b** Photo of LS of a shoot tip.

different times. Cells that become xylem vessel elements, for example, must synthesise the strengthening material lignin, which reinforces their walls. They must therefore have enzymes that control the synthesis of lignin. The genes that control production of these enzymes must therefore be switched on in cells that become xylem vessel elements.

We can now summarise how the root tip provides a good example of growth and development. Growth involves an irreversible increase in size and this is brought about by cell division in the apical meristem followed by cell enlargement. The process is irreversible because new materials and structures are added. Examples of these are proteins, such as enzymes, and entire new organelles inside the cells, and lignin in the cell walls of xylem vessel elements. We know that development is a progressive series of changes that includes the specialisation of cells. This is shown clearly here by the changes that cells undergo, from small meristematic cells in the zone of cell division to the range of cells seen in the zone of differentiation. We also saw earlier that development is genetically programmed and may be modified by the environment. This is also shown by the root. The root has evolved to respond to certain environmental stimuli such as gravity, moisture and light. Roots usually grow downwards in response to gravity, and towards water. They tend

to grow away from light. These are just a few of the many ways in which environment helps to determine the development of organs such as roots. The root tip thus illustrates general principles of growth and development which apply to *all* multicellular organisms.

## Shoot tips

The shoot tip is a more complex structure than the root tip because it also grows leaves and buds. Each leaf starts growth as a small swelling called a leaf primordium. As well as this, a bud develops between the leaf and the stem, known as a lateral bud or axillary bud depending on its position. The bud has the potential to form a new branch.

Despite the shoot tip being more complex than the root tip, their growth shows the same principles. Zones of cell division, cell enlargement and cell differentiation can be recognised, as shown in *figure 1.3*. This is particularly well illustrated by the development of the vascular tissue (the xylem and phloem).

# Types of growth curve

Before we look in detail at ways of measuring growth, we shall consider how best to describe growth and show it in the form of graphs. Graphs showing growth are known as **growth curves**.

Growth is usually recorded in one of three ways:

- absolute growth;
- absolute growth rate;
- relative growth rate.

For each of these a growth curve can be constructed.

## Absolute growth curves

**Absolute growth is increase in size or mass with time**. Absolute growth is also known as actual growth. A graph which shows absolute growth has time on the *x* axis and size on the *y* axis, and is called an absolute growth curve. It is the simplest way of showing growth.

Absolute growth curves are useful for showing:

- the overall pattern of growth;
- how much growth has taken place.

### The sigmoid curve

Absolute growth curves often have a simple mathematical form such as a straight line or, more commonly, a sigmoid shape (S-shape) (*figure 1.4a*). Some common examples of sigmoid-shaped growth are:
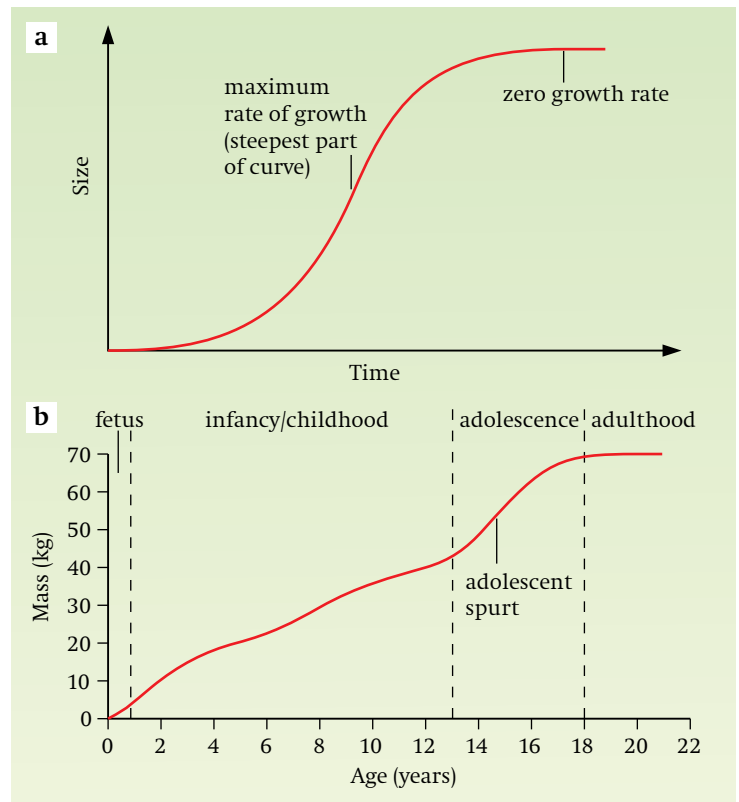
- **Figure 1.4** Absolute growth curves.
- **a** Idealised S-shaped (sigmoid) absolute growth curve.
- **b** Absolute growth curve for a sample of humans. Growth is measured as increase in mass. Four phases of growth are shown.
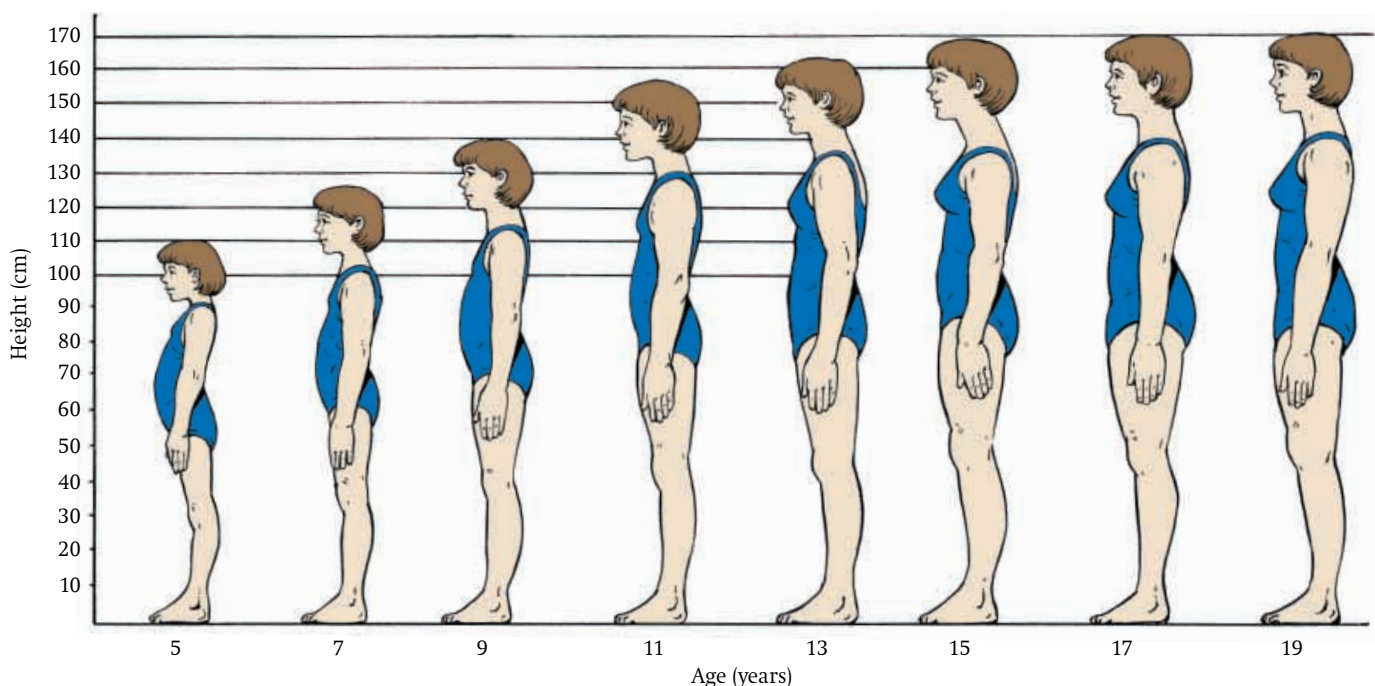
- **Figure 1.5** The growth in height of an average human female from age 5 to 19 years. Note: there is a great deal of variation between individuals.

- many multicellular organisms, e.g. annual plants, insects, birds, mammals (including humans);
- some parts of organisms, e.g. leaves and fruits;
- the population growth of microorganisms and many other natural populations.

Figure 1.4a shows an idealised sigmoid growth curve. Growth is slow at first but, as the size of the organism or population increases, the rate of growth increases and this forms the steep part of the graph. The larger the organism or population, the faster it grows, until a maximum growth rate is reached. Then growth slows down until it stops and the graph levels off, completing the S shape. At this point the maximum size has been reached.

Figure 1.4b shows the increase in mass with age of a sample of humans. This approximates to a sigmoid curve, although it is complicated by small spurts of growth, particularly during adolescence. Figures 1.5 and 1.6 show respectively the growth and development of a human female and a human male from age 5–19 years. The absolute growth curves could be drawn by drawing a line from head to head in the equally spaced diagrams.

### SAQ 1.5

The positions of two cells in the growing root of a maize plant were noted at different times. The positions were measured as distance from the end of the root tip. The results are shown in figure 1.7. The zone of cell division occupied the bottom 2 mm of the root tip. The zone of cell enlargement extended from 2–10 mm from the end of the tip.

a  How far was cell 1 from cell 2 at the start of the experiment?

b  How far was cell 1 from cell 2 after 5 hours?

c  Explain the difference between your answers to **a** and **b**.

d  How far back from the end of the root tip would you first expect to find mature xylem?

e  Explain why the distance from the end of the root tip of both cells 1 and 2 increases with time.

f  Explain why the distance of cell 2 from the end of the root tip increases most rapidly after about 7 hours.

g  If the root continues to grow at the same rate, what shape would the curves show between 20 and 40 hours?
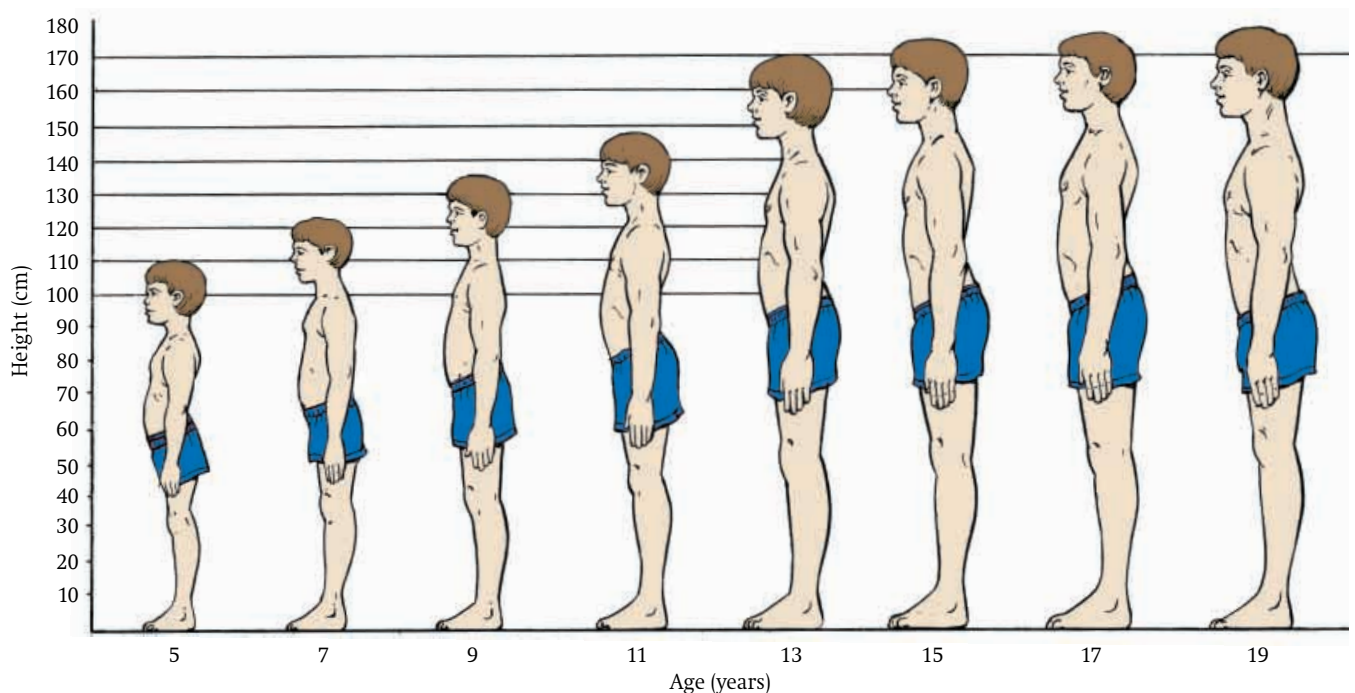
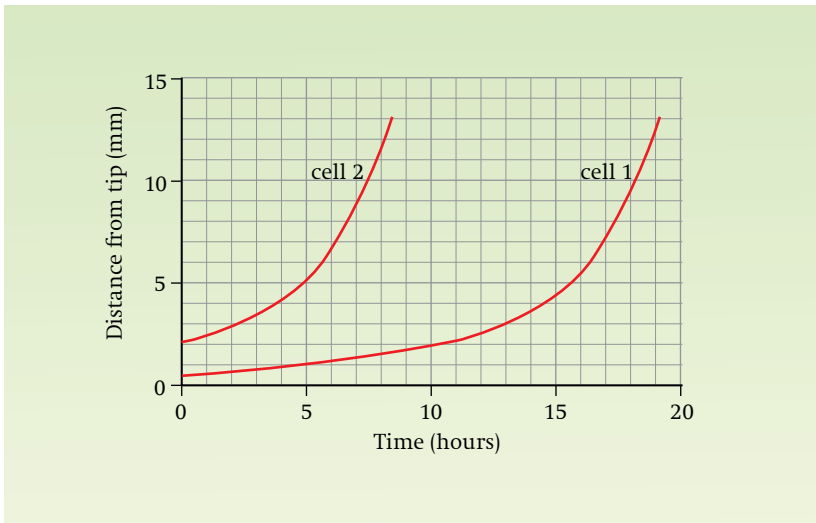h  At what rate is the root growing through the soil?



● **Figure 1.6** The growth in height of an average human male from age 5 to 19 years.
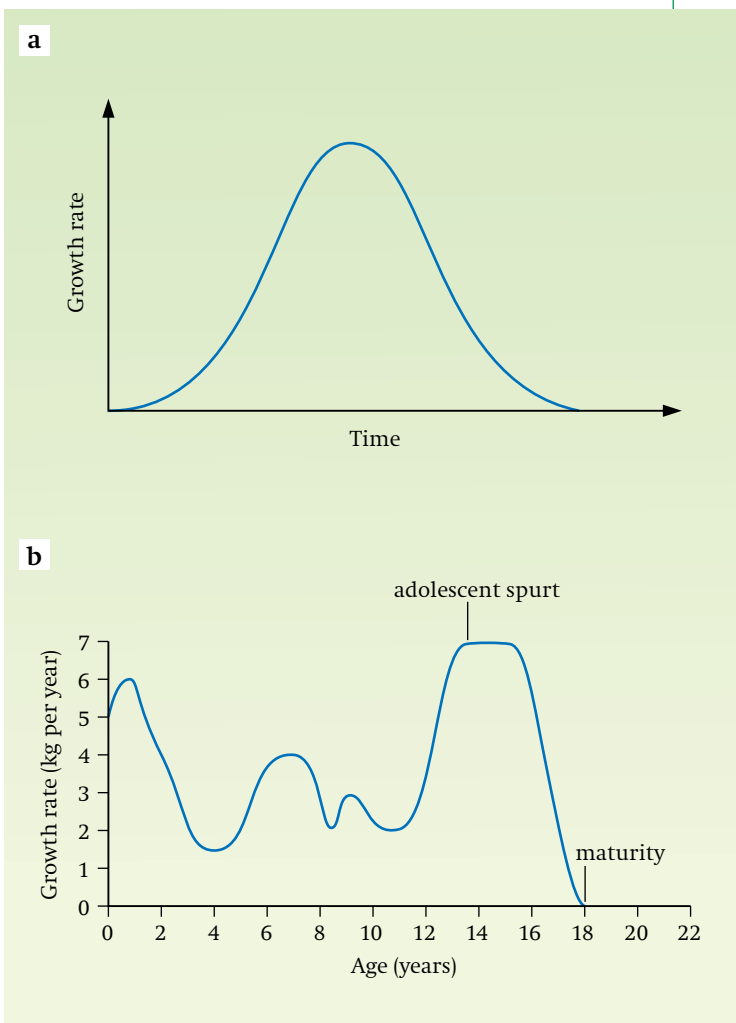Note: there is a great deal of variation between individuals.

● **Figure 1.7** Changes in the distance of two cells from the end of a growing maize root tip.

**a**



**b**



● **Figure 1.8** Absolute growth rate curves.
**a** Absolute growth rate curve derived from the sigmoid curve shown in *figure 1.4a*.
**b** Absolute growth rate curve for a sample of humans measured as rate of increase of mass against time (age).

## Absolute growth rate curves

**Absolute growth rate** is the absolute growth in a given time period, e.g. an increase in height of 4 cm per year. An absolute growth rate curve has time on the *x* axis and growth rate on the *y* axis.

Absolute growth rate curves are useful for showing:
■ when growth is most rapid (the peak of the curve);
■ how the rate of growth changes with time (the slope of the curve).

### The bell-shaped curve

When a sigmoid curve showing absolute growth is converted to an absolute growth *rate* curve it shows a typical 'bell' shape, as in *figure 1.8a*. The peak of the bell shape represents the highest rate of growth. This corresponds to the steepest part of the sigmoid curve in *figure 1.4a*. The final growth rate in the bell-shaped curve is zero. This corresponds to the plateau at the top of the sigmoid curve in *figure 1.4a*.

*Figure 1.8b* is an absolute growth rate curve of a sample of humans, measured as increase in mass per year at different ages. Note that a fall in the graph indicates a slowing of growth, not a loss of mass. Note also that a horizontal line does not mean that growth has stopped, but that the rate of growth is constant (see adolescent spurt). At maturity the absolute growth rate is zero.

### SAQ 1.6
*Figure 1.9* shows absolute growth rate curves for boys and girls based on height.
**a** Describe the difference in growth between boys and girls as shown by the curves.
**b** Which sex is taller on average at the beginning of the adolescent spurt? Explain your answer.
**c** At what age is the maximum growth rate for (i) girls and (ii) boys in the sample?
**d** What is the growth rate for (i) girls and (ii) boys at the peak of the adolescent spurt?
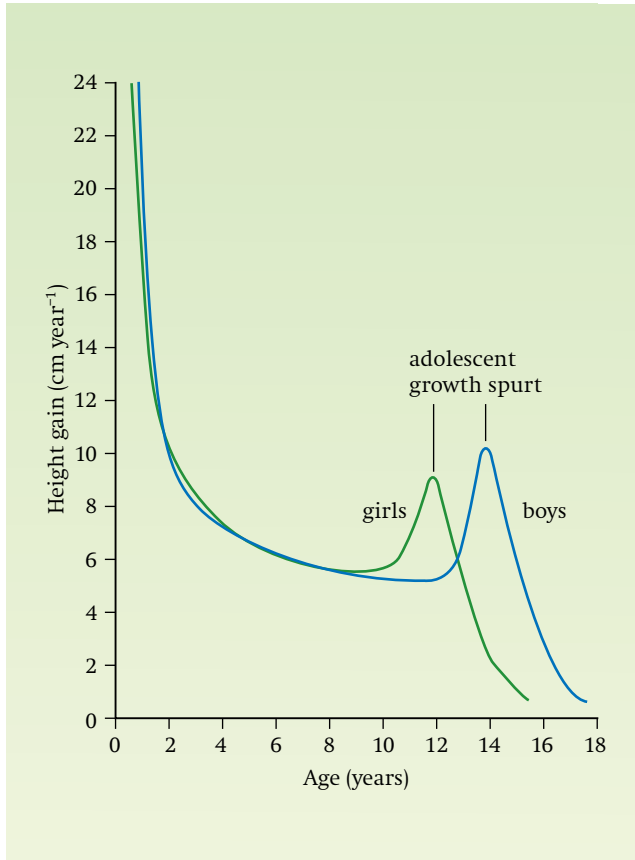
- **Figure 1.9** Absolute growth rate curve for boys and girls based on height.



## Relative growth rate curves

If a boy of two years old and one of 14 years old are both growing at a rate of 10 cm per year their absolute growth rates are the same. However, if the sizes of the two boys are taken into account, the two-year-old boy is growing relatively faster because 10 cm is a greater *relative* increase in height for him. The relative sizes of individuals can be taken into account by calculating the **relative growth rate**.

The relative growth rate for height can be calculated as:

$$\frac{\text{change in height in one year}}{\text{height at beginning of year}}$$

or

$$\frac{\text{absolute growth rate}}{\text{height at beginning of year}}$$

So a relative growth rate curve has time on the *x* axis and relative growth rate on the *y* axis. (Other measures of growth, such as mass, can be used instead of height.) Relative growth rate curves are useful for showing how efficiently an organism is growing at different ages. A high relative growth rate indicates a high efficiency of growth, because the growth rate is high relative to the size of the organism.

### Shape of curve

When a bell-shaped curve showing absolute growth rate is converted to a relative growth rate curve, the shape is as shown in *figure 1.10a*. A relative growth rate curve for a sample of humans is shown in *figure 1.10b*. Note the very high relative growth rate early in human life.

### SAQ 1.7

From what you have studied so far in this chapter, suggest at least three ways in which growth could be measured.
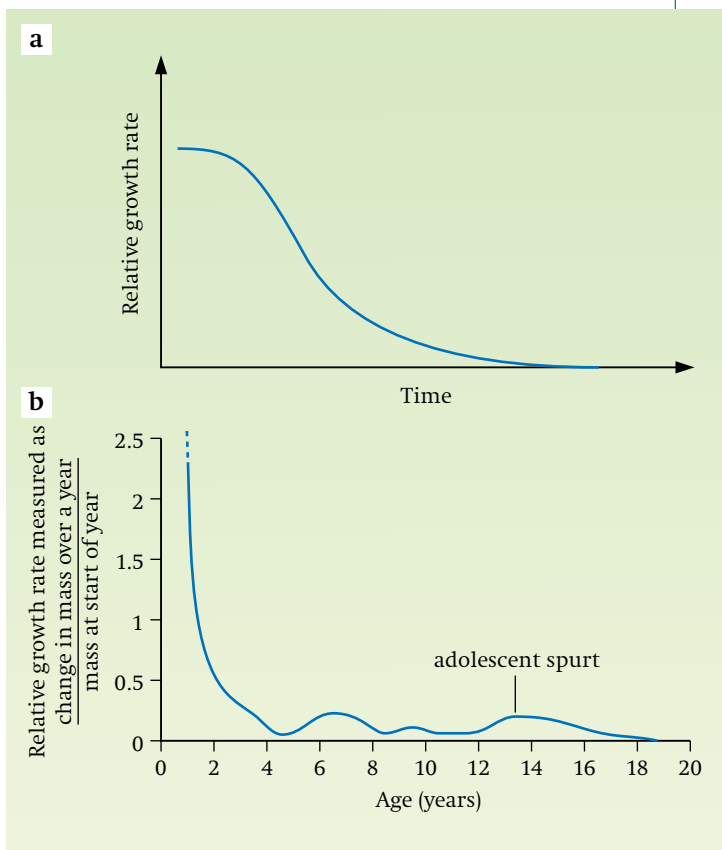
- **Figure 1.10**
  **a** Relative growth rate curve derived from *figure 1.8a*.
  **b** Relative growth rate curve for a sample of humans.

# Measuring growth in plants

Having looked at growth curves, we will now consider the various ways in which growth can be measured. Being able to measure and analyse growth has a number of important applications. For example, the growth of crops and domestic livestock such as cattle is of obvious commercial significance if not a matter of survival. If techniques are available for measuring growth, the effects of important variables such as light, water, mineral availability, sowing density for crops, and diet and shelter for livestock, can be investigated. Optimum (ideal) growth conditions can then be found. With plants, experiments are commonly carried out in special growth rooms in which environmental variables can be controlled and one variable at a time investigated (*figure 1.11*). Growth in plants is most commonly measured by increase in height, length, dry mass, or fresh mass. Each method has its advantages and disadvantages, but let us first look at the techniques themselves.

● **Figure 1.11** A typical growth room.

# Measuring growth by increase in length or height

It is relatively easy to measure the length of structures such as leaves or fruits, or to measure the overall height of a plant from a convenient point such as soil level. A more complex experiment to measure growth of root tips is described below.

## Experiment to measure growth in length of root tips

A well-established method of measuring plant growth is to mark a growing root tip at equal intervals, about 1–2 mm apart, with Indian ink and then measure the distances between the marks at 24-hour intervals (*figure 1.12*). Peas or runner beans have suitably large and straight primary roots (first roots). Starting from the tip, a primary root about 2 cm long can be marked with an ink-soaked cotton thread stretched taut between the ends of a bent piece of wire. The peas, runner beans or other large seeds can be pinned near the top of pieces of softboard covered with filter paper. The base of each board is then placed in a beaker of water and the whole covered with polythene. Overall changes in length, and percentage increases in length, for each interval marked on the primary root can be plotted on graphs. The various types of growth curve already discussed on pages 8–9 can then be plotted.

Since dividing cells are found only in the root tip, enlarging cells just behind the tip and differentiating cells still further from the tip, the marks in the enlargement zone move further apart and those in the differentiation zone stay approximately the same distance apart (*figure 1.12*).

Note that a full definition of growth should include both cell division and cell differentiation as well as cell enlargement, and this technique only measures cell enlargement. More elaborate techniques involving microscopy are used to measure the numbers of cells and their degree of differentiation.

main zone of cell differentiation
main zone of cell enlargement
root cap
main zone of cell division
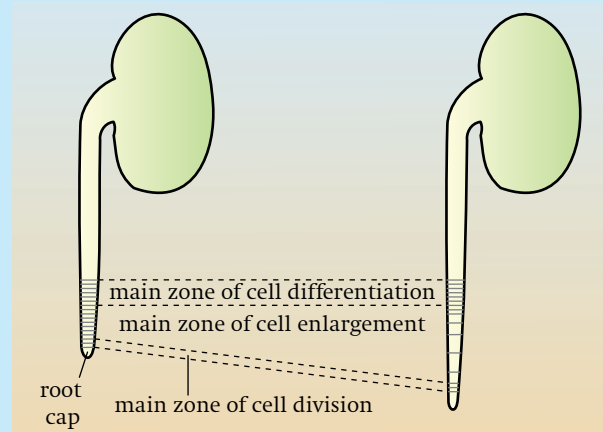
● **Figure 1.12** Growth in a root tip.

## SAQ 1.8

*Table 1.1* provides data on the growth of oat plants from seed.

**a** Draw a graph of height against time.

**b** What type of growth curve have you drawn?

**c** What other types of growth curve could be obtained from the data?

| Time from sowing (days) | Mean height (cm) |
|---|---|
| 0 | 0 |
| 2 | 0 |
| 4 | 1.5 |
| 6 | 5.0 |
| 8 | 10.8 |
| 10 | 19.3 |
| 14 | 28.8 |
| 21 | 43.2 |
| 28 | 55.2 |
| 35 | 62.6 |
| 42 | 74.8 |
| 49 | 89.3 |
| 56 | 96.7 |

● **Table 1.1** Growth in height of oat plants from seed.

| Time from sowing (days) | Mean total dry mass (mg) | Mean dry mass of endosperm (mg) | Mean dry mass of embryo (mg) |
|---|---|---|---|
| 0 | 43 | 41 | 2 |
| 2 | 41 | 39 | 2 |
| 4 | 38 | 30 | 8 |
| 6 | 34 | 18 | 16 |
| 8 | 33 | 9 | 24 |
| 10 | 34 | 4 | 30 |
| 14 | 38 | | |
| 21 | 50 | | |
| 28 | 108 | | |
| 35 | 201 | | |
| 42 | 432 | | |
| 49 | 865 | | |
| 56 | 1707 | | |
| 63 | 2765 | | |

● **Table 1.2** Changes in dry mass of oat plants during germination and early growth.

## Measuring growth by increase in mass

It is more difficult and time-consuming to measure changes in mass than changes in length or height but there are advantages (see later) and the method is as follows.

In order to standardise conditions when carrying out experiments, (see later) large numbers of seeds of the chosen plant, for example pea or wheat, can be germinated in a commercial compost or in a sterile non-soil medium such as vermiculite, and watered with a standard culture solution containing the required mineral salts. Fresh mass (that is, including water content) can be measured after blotting dry a sample of the plants to remove excess liquid. (Various other measure-ments may also be recorded at the same time, such as leaf length, the distance between leaves (internode) and root length, plant height, and number or area of leaves.) Dry mass can subsequently be determined by drying in a warm area for 24 hours, weighing, and repeating the procedure every 24 hours until the mass is constant.

Efforts must be made to make the samples truly representative, which ideally means taking the mean value of at least 30 individual plants and ensuring that, as far as possible, all plants grow under

identical conditions. A suitable procedure might be as follows.

Start with 300 seeds, and soak them in water for 24 hours. Take a sample of 30 for fresh and dry mass determinations and plant the remainder. Further samples of 30 can then be taken at, for example, two-day intervals. When the seedling stage is reached the masses of whole seedlings, including roots, should be determined. Graphs of mean mass against time can be plotted for the first 20 days of growth.

## SAQ 1.9

*Table 1.2* provides further data on the growth of the same oat plants as *table 1.1*. (The **endosperm** is the food store in cereal seeds and the **embryo** grows into the new oat plant.)

**a** Draw absolute growth curves to show changes in total dry mass, dry mass of endosperm and dry mass of embryo over the first 10 days from sowing. Draw the three graphs on the same pair of axes so that they can be compared easily.

**b** Describe and try to account for the changes in (i) dry mass of the endosperm and (ii) dry mass of the embryo over the first 10 days after sowing and (iii) total dry mass over the first eight days after sowing.

**c** Draw a further graph on another set of axes to show the changes in total dry mass over the first 63 days (nine weeks) after sowing.

**d** Describe and suggest reasons for the changes in total dry mass between eight days and 63 days after sowing.

## SAQ 1.10

*Figure 1.13* shows changes in dry mass and rate of growth of a plant over a period of 40 days from the start of germination. Rate of growth was calculated by measuring the change in dry mass per day.

**a** Which graph, A or B, represents dry mass and which graph represents rate of growth?

**b** State one important way in which you would expect a graph showing increase in shoot length to differ from graph B.

**c** What types of growth curve are A and B?

**d** Describe the shape of graph B.

**e** Why is there a negative growth rate immediately after germination?
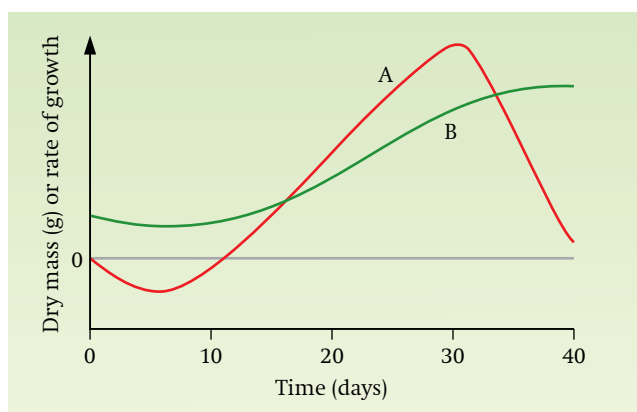


● **Figure 1.13** Growth of a plant.

# Measuring growth in animals

Measuring the growth of animals presents a new set of problems compared with plants. Much of our detailed knowledge has come from humans or from farm and laboratory animals because trapping wild animals and taking measurements from them is difficult. Insects, however, are useful animals for growth studies as they are convenient to keep and grow relatively quickly. They also show an interesting split into two groups with very different growth characteristics.

## Insect life cycles and growth

Insects belong to a large and successful group of animals known as the arthropods, which also includes crustaceans, centipedes, millipedes, scorpions and spiders. One of the distinguishing features of the arthropods, and one reason for their success as a group, is that they all possess an **exoskeleton** ('exo' means 'outside'). This is a tough, fairly rigid layer which covers the body and is secreted by the epidermis. It functions as a skeleton because it provides support and muscles are attached to it. It can be thin and light, as in insects, or thick and hard for protection, as in crabs. It also has a waterproof layer to help prevent desiccation (drying out).

Having an exoskeleton imposes some limitations on arthropods, however. For example, the exoskeleton cannot grow in size. Arthropods therefore grow by shedding their exoskeletons at regular intervals, a process known as **ecdysis** (the Greek word for 'moulting'). A new, soft exoskeleton is grown before the old one is shed. After moulting, this stretches, partly due to the pressure of new growth that has already occurred since the last moult, and partly due to the uptake of water or air by the animal. The volume occupied by this water or air can be replaced with new tissue after the new exoskeleton has hardened until, in its turn, the new exoskeleton becomes too small. The period between each moult is known as an **instar** (a Latin word meaning 'form'). Growth therefore appears to take place in a series of steps, with a sudden increase in size at each step. In insects, the number of instars is usually pre-determined and the adult does not moult any further.

### Metamorphosis

Before considering the effect of regular moulting on growth curves, it is important to recognise that most insects also undergo metamorphosis during growth. **Metamorphosis** is a change in form from a larval stage to an adult stage during the life cycle. It is also characteristic of amphibians, as in the change from tadpole (larva) to frog (adult). Insects show two types of metamorphosis, known as complete and incomplete metamorphosis (*figure 1.14*).

■ **Complete metamorphosis.** A total change in form takes place with the result that the adult is physically very different from the larva and so usually has a completely different way of life and food source. For example, the adult mosquito flies but its larva is aquatic. Good examples of insects with complete metamorphosis are butterflies and moths, where the
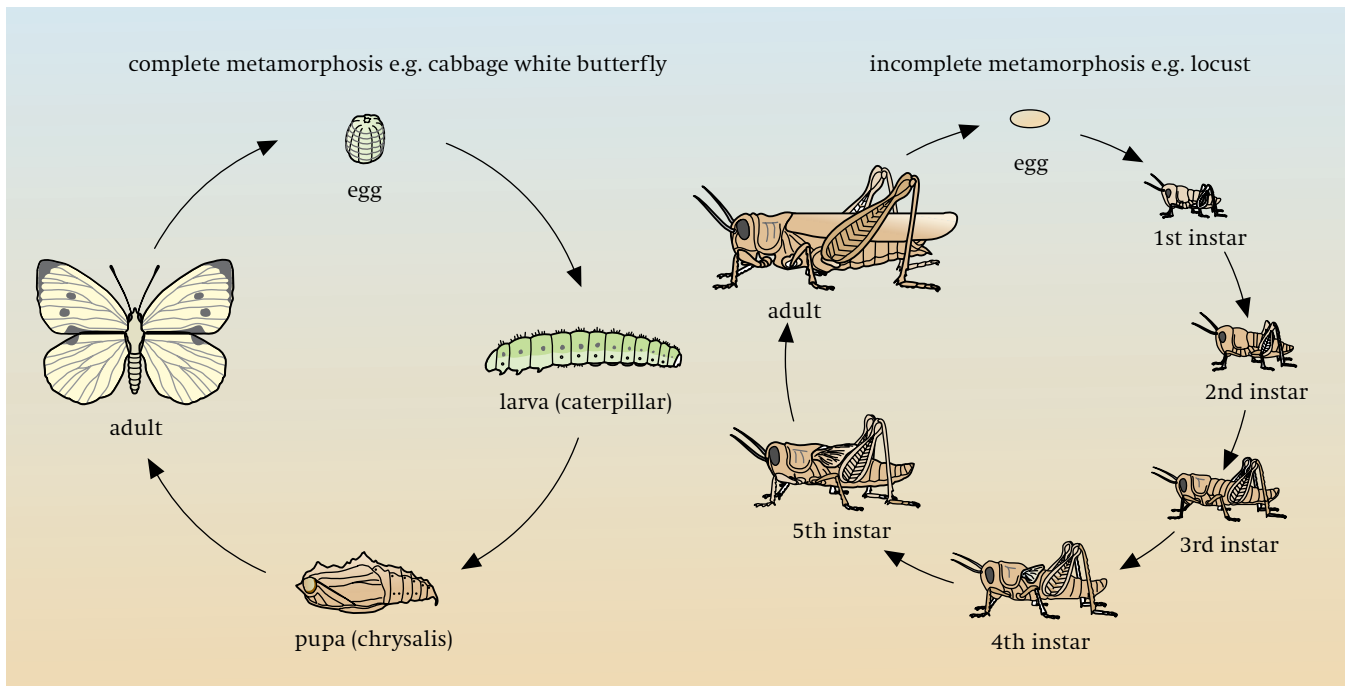
● **Figure 1.14** The two types of life cycle in insects, complete and incomplete metamorphosis.

larva is a caterpillar, and flies, where the larva is a maggot. Other examples are beetles, bees, wasps, ants and fleas. There are four stages in the life cycle:

1 **egg**
2 **larva** – undergoes a series of moults as it grows. Wings develop internally but are not visible.
3 **pupa** – the body of the larva is broken down and reorganised into the adult form.
4 **adult (imago)** – winged.

■ **Incomplete metamorphosis**. A gradual change in form takes place from larva to adult and there is no pupa stage. The larva is often referred to as a **nymph** (or **naiad** if aquatic). As with complete metamorphosis, the larva undergoes a series of moults as it grows. Each successive stage is larger and more like the adult, though only the adult has functional wings. Examples of insects with incomplete metamorphosis are locusts, grasshoppers, cockroaches, dragonflies, mayflies, stoneflies, aphids, earwigs and bugs. The nymphs of locusts and grasshoppers are sometimes called 'hoppers' because they cannot fly.

## Measuring growth of insects

If you were trying to measure the growth of an insect showing incomplete metamorphosis, such as locust or grasshopper, an obvious method might be to measure body length at regular intervals. However, growth curves based on length have an unusual appearance (*figure 1.15*) because growth appears to take place in a series of spurts, with no growth in-between. This is very misleading, because a growth curve based on the increase in dry mass of the insect is a smoother, typically S-shaped, or sigmoid curve (as in *figure 1.4a*). Such growth starts rapidly then begins to slow down and finally ceases. Thus the *true* growth of the insect, based on irreversible increase in dry mass, is continuous and does not have the interrupted pattern shown in *figure 1.15*. The reason for the stepped growth curve for changes in body length is that the exoskeleton is too rigid to allow expansion. Growth in length is therefore confined to brief periods after moulting of the old exoskeleton, when a new exoskeleton is forming and is still flexible.
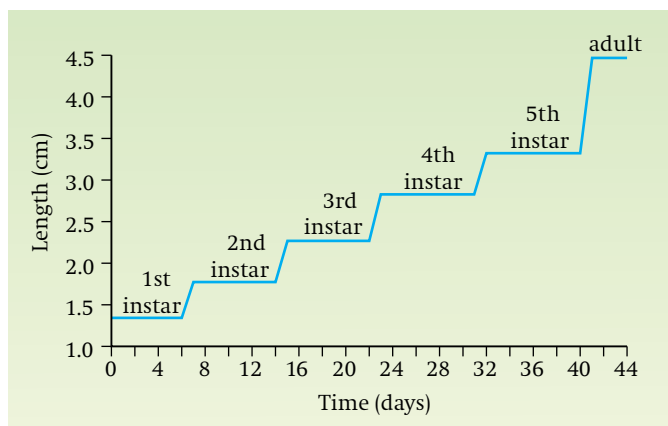
● **Figure 1.15** Growth curve based on body length for the short-horned grasshopper.

## SAQ 1.11

Suggest two ways in which the daily growth of an insect could be measured, other than by determining increase in body length or dry mass.

## SAQ 1.12

A representative sample of locust instars and adults was collected and the lengths of the head, tibia (part of the hind leg) and wings were accurately measured. Mean lengths revealed the following features of growth and development:

- head – maximum rate of growth early in development;
- tibia – steady rate of growth throughout development;
- wings – maximum rate of growth late in development.

How might the changes observed be related to the life cycle of the insect?

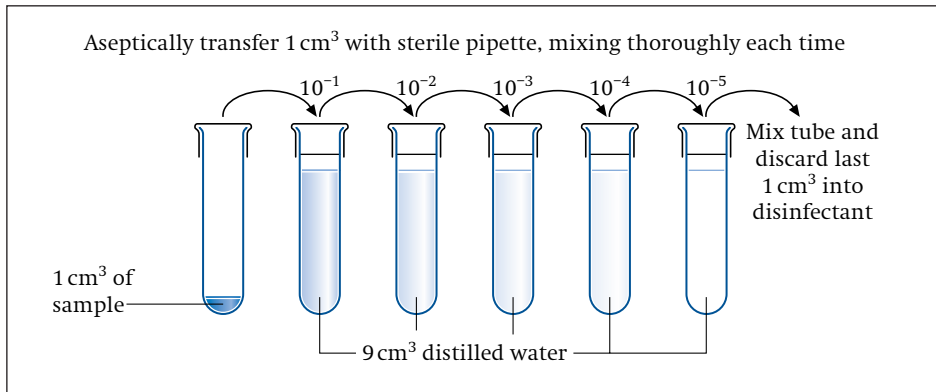# Measuring growth in microorganisms and populations

Growth can be studied at any level of biological organisation, from cells, organs and organisms to populations and communities. You may remember from *Biology 1,* chapter 7 that a **population** is a group of organisms of the same species living together in a given place at a given time that can interbreed with each other. The term could refer, for example, to all the badgers living in an oak wood, or to all the bacteria living in a test-tube. Studying the growth of populations is important for a number of reasons. In agriculture, for instance, it is useful to know about the growth of pest populations. This might allow the more effective timing of spraying with pesticides. The growth of human populations is another major concern. If we are to control human population growth, for example, we need to understand what factors are important in regulating the growth.

Very simple single-celled organisms such as yeasts and bacteria are a useful starting point when we wish to study population growth. Because large numbers can be grown relatively quickly, they can be used to model population growth under ideal conditions, and to investigate some of the factors which can limit population growth. In a typical experiment, a small number of such microorganisms are introduced (inoculated) into a suitable nutrient medium and their population size monitored over a period of time. The nutrient media used will generally allow the growth of a wide range of microorganisms, so contamination must be prevented. It is therefore usual to use aseptic techniques, which involve using sterilised apparatus and materials.

It is outside the scope of this book to describe in detail how to carry out experiments using aseptic techniques. The techniques are highly specialised and must be conducted with regard to the possible health hazards involved in handling bacteria. Reference should therefore be made to more specialised books, such as *Microbiology and Biotechnology* in this series, if practical work is to be carried out. In this chapter we shall look at some of the principles involved in measuring growth of populations of unicellular microorganisms. Outline descriptions of some procedures used and some of the problems commonly encountered also follow.

In order to measure population growth, the number of individuals in representative samples has to be counted at regular intervals. Two types of count may be used, namely **viable counts**, which include living cells only, and **total counts**, which include all cells, living plus dead.

Aseptically transfer $1\,cm^3$ with sterile pipette, mixing thoroughly each time

$10^{-1}$   $10^{-2}$   $10^{-3}$   $10^{-4}$   $10^{-5}$

Mix tube and discard last $1\,cm^3$ into disinfectant

$1\,cm^3$ of sample

$9\,cm^3$ distilled water

● **Figure 1.16** Making a serial dilution.

## Serial dilution

In practice, a sample may contain too many cells to count easily using the techniques described below. In this situation it is usual to prepare a series of dilutions of the sample so that one of the dilutions will prove to have a suitable concentration of cells. A correction factor can then be applied to allow for the dilution once the count has been made. A common technique for serial dilution is as follows. (Remember, all procedures must be carried out under aseptic conditions.)

$1\,cm^3$ of the culture in a liquid medium is taken in a sterile pipette and mixed thoroughly with $9\,cm^3$ of sterile distilled water, making a total of $10\,cm^3$. This dilutes the original by 10 times, a $10^{-1}$ dilution. A $1\,cm^3$ sample of the $10^{-1}$ dilution is then added to $9\,cm^3$ sterile distilled water as before to produce a $10^{-2}$ dilution. This procedure can be repeated to produce a dilution series down to an appropriate concentration such as $10^{-6}$ or $10^{-12}$ (*figure 1.16*).

## Viable counts

Viable counts are used when it is important to know the number of *living* microorganisms. For example, the effect of pasteurising milk could be investigated by finding the total number of living bacteria in samples of milk taken before and after pasteurisation. The most common method of viable counting of bacteria is based on the basic principle that, given a suitable medium in which to grow, each bacterium in a sample will multiply over one or two days to produce one visible colony.

A typical procedure is as follows:

1 Add a known small volume (for example, $0.1\,cm^3$) of each sample to molten nutrient agar jelly in a separate petri dish. Setting up two or more identical cultures (**replication**) for each sample and averaging the results ensures more accurate results and gives an indication of how much variation can occur merely as a result of the technique.

2 Rotate to mix, and allow the agar to set.

3 Set up a **control** nutrient agar dish with no bacteria. No bacteria should grow in this. This is a check that the technique is not allowing contamination by other bacteria.

4 Incubate the cultures as appropriate for the particular organism, for example at $30\,^{\circ}C$ for 24 hours. Then count the number of colonies in each dish. In theory, this should equal the number of bacteria in the original sample added to the dish (*figure 1.17*).

5 In some instances there are so many colonies in the petri dish or tube that it is impossible to count them. In such cases, a dilution series should be prepared as described in the previous section.

Another method of viable counting is to measure a product of metabolism such as a gas (for example, carbon dioxide from



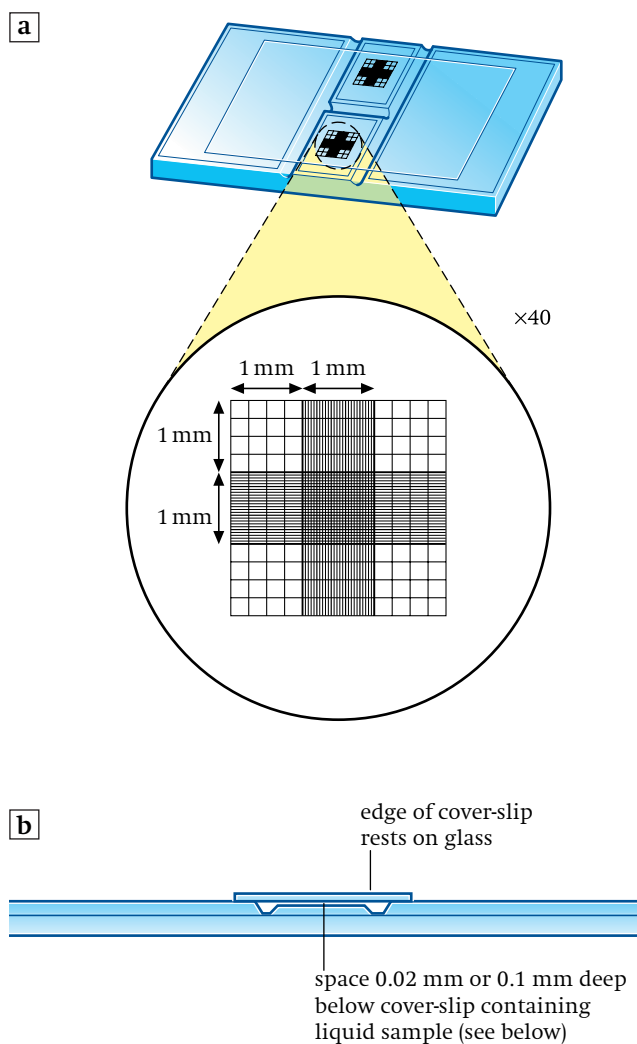● **Figure 1.17** Colonies in an agar dish.

respiration) or an acid. Such a method could be used for yeasts as well as bacteria.

There are several problems associated with viable counting.

- Aseptic (sterile) procedures require special apparatus and techniques. Contamination occurs easily.
- If more than one type of bacterium is present in a sample, as in milk, the culture conditions will not favour them all equally.
- Bacteria are rarely distributed evenly throughout a sample. They are often found in clumps of variable numbers, so a single colony could be derived from many bacteria. It is therefore difficult to obtain reproducible results. However, high levels of accuracy are not often needed because the numbers are so large.
- Some bacteria are pathogenic (cause disease). Care is therefore required with handling, and there are restrictions on the bacteria that can be grown in schools or colleges.

## Total counts

Unlike viable counts, which measure only living microorganisms, total counts measure both living *and* dead cells in the sample. A number of methods may be used to obtain total counts. They can be used for bacteria and yeasts. **Direct counting** of the number of cells in a known volume is possible using a microscope and a special slide known as a **haemocytometer slide** (*figure 1.18a*). It is designed so that a known volume of sample covers a ruled grid. A representative sample of cells can thus be counted and estimates can be made of the number in the total sample. Again, it is useful to prepare a dilution series. The central section of the slide is thinner than the rest of the slide so that when a coverslip is placed over this section, a space, usually 0.1 mm deep, is left below it (*figure 1.18b*). When the coverslip is in place, a small volume of the sample is added under the coverslip by holding a pipette against the side of the coverslip. This fills the space with an exact volume of liquid. The number of cells in a representative sample of grid squares is counted, and using the dilution factor the number in the original sample is calculated. At least 600 cells should be counted. Where cells overlap the grid lines they are usually judged as in the

×40

1 mm 1 mm

1 mm

1 mm

edge of cover-slip rests on glass

space 0.02 mm or 0.1 mm deep below cover-slip containing liquid sample (see below)

● **Figure 1.18** Haemocytometer slide.

square on two sides, e.g. top and left, and out of the square on the other two sides, rather than trying to count parts of cells, or risk counting the same cell twice. (Also see page 31, *Microbiology and Biotechnology* in this series.)

In the case of yeasts, the use of a stain which only stains dead cells, such as methylene blue, may allow a viable count to be made. Direct counting has several disadvantages.

- The technique requires practice.
- The slide must be scrupulously clean.
- It is difficult to distinguish bacteria from other small particulate matter.
- Bacteria are often not evenly distributed throughout a sample, so small samples may result in large errors.

Another common method for obtaining a total count depends on the fact that the more bacteria there are in a solution, the more turbid (cloudy) it appears. **Turbidity methods** measure the amount of light that is transmitted through a suspension of the bacteria using a colorimeter. If necessary, *actual* numbers can be obtained by comparing the turbidity of unknown samples with the turbidity of samples containing known numbers.

## Bacterial population growth curve

The growth curve of a population of bacteria was examined in detail in *Biology 2*, pages 49–50. You may remember that the curve could be divided into the following phases: **lag phase**, **log phase** (exponential phase), **stationary phase** and **decline phase** (death phase).

It was also shown that the growth curve is sigmoid up to the beginning of the decline phase (*figure 1.19*). Note that the vertical axis in *figure 1.19* is logarithmic, so the exponential phase of growth appears as a straight line if the theoretical maximum rate of growth is achieved.

**SAQ 1.13**
Starting with one bacterium, how many bacteria would be present after five hours assuming exponential growth and the ability of the bacteria to grow and divide every 30 minutes?

**SAQ 1.14**
Why does a lag phase often precede the log phase?

**SAQ 1.15**
State three factors which could cause the growth rate to start to decline.

**SAQ 1.16**
At which stage does **a** the rate of dying equal the rate of production of new cells, **b** the rate of dying exceed the rate of production of new cells?

We can learn some important principles from laboratory studies of microorganisms. However, understanding the population growth of organisms under *natural* conditions is often far more difficult. This is because many more factors are important variables. Such factors include
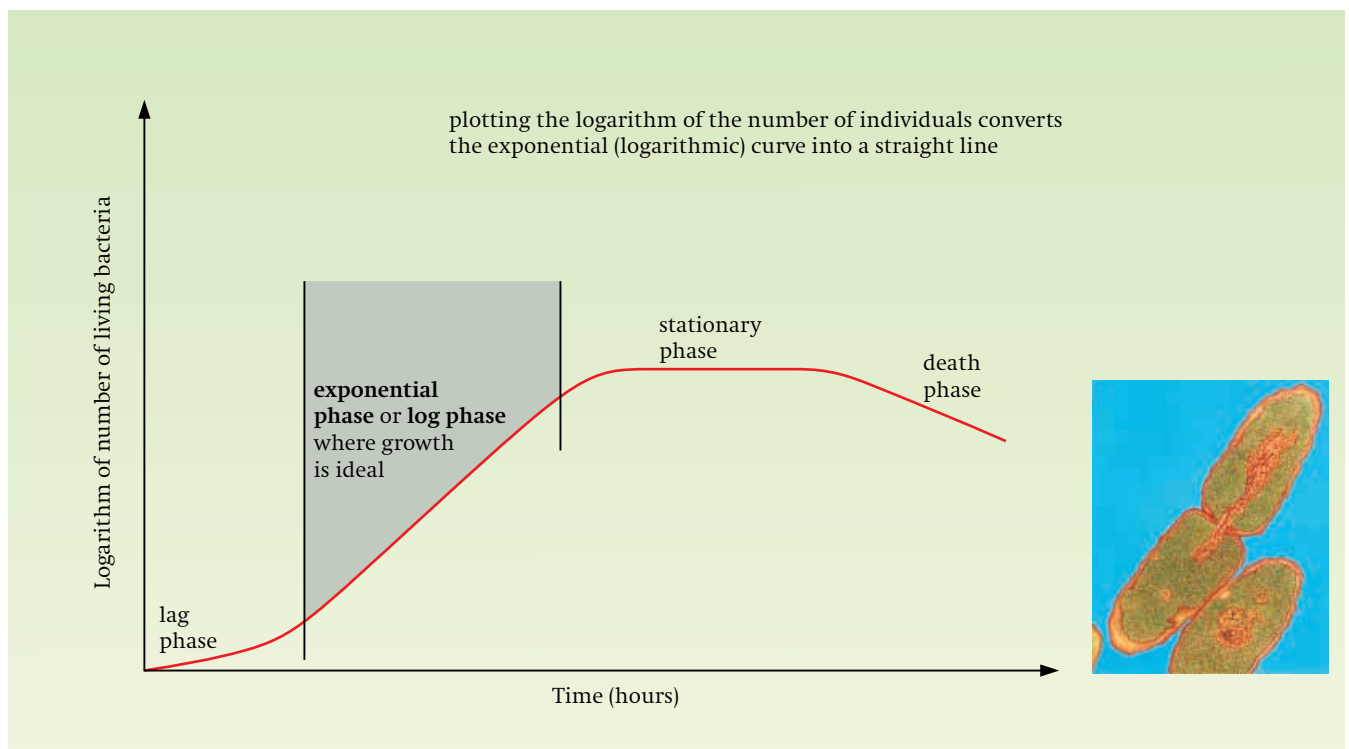


● **Figure 1.19** Typical growth curve of a bacterial population. The vertical axis is logarithmic. The photograph is a transmission electron micrograph of *Salmonella typhimurium* dividing ( 20 200).

competition (between species as well as within a species), disease, climate, predation, population density and parasitism. Their study is part of a branch of biology known as population ecology (*Biology 2*, chapter 3), but other areas of biology, such as animal behaviour, are also relevant. Observations of natural populations suggest that under a given set of environmental conditions, natural population sizes tend to stabilise and stay reasonably constant over long periods of time. In other words, they show typically sigmoid growth curves and do not enter a phase of decline. Each population comes into a dynamic equilibrium with its environment. This was first pointed out by the clergyman and mathematician Thomas Malthus at the end of the eighteenth century and was one of the observations which formed the basis of Darwin's theory of natural selection. Humans are an interesting example of an animal species which is not at present in equilibrium with its environment, and how we achieve such an equilibrium presents us with one of our greatest challenges as a species.

# Advantages and disadvantages of different methods

Having studied different ways of measuring growth in a range of living things, it is useful to summarise some of their advantages and disadvantages.

## Measuring growth by increase in length or height

### Advantages

- It is easier to measure growth in length or height of a whole organism or part of an organism than changes in mass, and for many purposes it is just as useful.
- Unlike dry mass measurements, the sample is not destroyed.

### Disadvantages

- There may be more variation between individuals in linear dimensions like length and height than in mass. For example, two leaves could have the same mass but one may be shorter and wider. Measuring length would give a misleading interpretation of growth. Mass is therefore

more representative of true growth than length in this case.
- Shoots may grow in length but not irreversibly increase mass. For example if growing in the dark, a shoot cannot add new dry mass by photosynthesis. It also continues to lose dry mass in the form of $CO_2$ during respiration. Similarly, roots may elongate during germination with no increase in mass.

## Measuring growth by increase in mass

### Advantages

The measurement of mass is generally regarded as a better guide to growth than the measurement of single dimensions such as height or length because:

- it is more representative of the whole organism or structure;
- it is a better guide to the eventual yield in agriculture.

Mass may be measured as fresh mass or dry mass.

### Advantage of measuring fresh mass

- Easier than measuring dry mass. Usually, relatively easy to weigh the whole organism. The problem of removing soil from the roots of plants before weighing can be avoided under laboratory conditions by growing the plants in soil-free nutrient solutions.

### Advantage of measuring dry mass

- More reliable than measuring fresh mass because living organisms, especially plants, can gain or lose water according to water availability, irrespective of growth.
- Dry mass contains the energy that can be traced back to photosynthesis.

### Disadvantages of measuring dry mass

- More difficult and time-consuming to obtain because samples have to be dried until they reach a constant weight.
- It destroys the sample, so that a different sample must be used each time and the total number of organisms required is much greater.
- Growth in plants may occur with only a little change, or even loss, in total dry mass. For example, towards the end of the growing season, the increase in dry mass slowly comes to a halt

at a time when the seeds are developing and increasing greatly in size with stored food. This is caused by a diversion of nutrients from leaves to seeds, the leaves dying in the process. By the rule of irreversible change in dry mass, plant growth has stopped. As already explained, dry mass may decline when a seed germinates until the seedling starts to photosynthesise.

## SUMMARY

◆ Growth can be defined in a number of ways. The best general definition is an irreversible increase in dry mass of living material.

◆ Growth is closely linked with development, a progressive series of changes which includes cell specialisation (differentiation) and results in greater complexity.

◆ Growth is complex and cannot easily be measured by a single variable. Different methods are used, each with its own particular advantages and disadvantages. For example, increase in dimensions, dry or fresh mass, or cell numbers can be measured. Absolute growth (actual growth), absolute growth rate (change in rate of growth with time) or relative growth rate (which takes into account size) can be plotted as graphs known as growth curves.

◆ Different patterns of growth occur. For example, insects show two different types of metamorphosis.

◆ Microorganisms provide a useful simple model of population growth.

## Questions

1 Explain what is meant by the terms growth and development.

2 Distinguish between
   a development and differentiation
   b individual growth and population growth.

3 Describe, giving full experimental details, how you could measure the absolute increase in dry mass of a plant from seed to maturity.

4 Name an insect showing complete metamorphosis. Outline four methods by which you could measure its growth and summarise the advantages and disadvantages of each method.

5 Growth may be measured as absolute growth, absolute growth rate or relative growth rate. Explain the advantages of each as a measure of growth.

6 Discuss the usefulness of unicellular organisms as models for population growth.