Investigations into the behaviour of ants

An ASAB resource for those teaching GCSE, AS/A2, Higher and Advanced Higher students of Biology

Dr Liz Evesham (Mill Hill School)

ASAB

2011

Contents

This resource includes:

- Background information about British ants.
- A step-by-step approach to the collection and maintenance of some British ants with particular reference to the common red garden ant *Myrmica rubra L.*
- Ideas for both fieldwork and laboratory investigations, mainly using the ant *M. rubra.*
- Opportunities for students to follow instructions, design investigations, carry out, record and present data, use appropriate statistical tests, interpret secondary data, consider limitations to scientific evidence and ethical issues involved when using animals in investigations.

Suggestions presented here cover aspects of investigative work and behavioural ecology found in most UK National Curriculum syllabuses and examination board specifications. The background knowledge and sample investigations will provide sufficient materials for the 'How Science Works' component of the GCSE and A-level courses, as well as the assessment of practical skills. For example, the resource will help with designing investigations, sampling techniques, making measurements and the collection of quantitative data, presenting evidence, analysis, evaluation and its applications.

For a more detailed consideration of where the study of the behaviour of ants fits in with UK course syllabuses and specifications, see Appendix A.

British ants

Background

The information here will provide details of the British ant species you are most likely to find, see Figure 1, and their habitats.

You may have come across ant nests (made up of pine needles and decaying plant material) whilst walking through woods and forests. These belong to the group of ants called Formicinae. A common species encountered is called ***Formica rufa.*** These are large in size (approx.13 mm in length) compared to other British ants and squirt formic acid from the abdomen, if disturbed. Their ant hills may vary in size and can be up to 2 m high and 6 m wide. They are usually located at the edges of woodland where the light intensity and temperatures tend to be higher than elsewhere in woodland.

Other ants belonging to the Formicine group, and which use formic acid as a defence mechanism, are ***Lasius niger*** (a black-brown ant, approx. 6 - 7 mm in length) and ***Lasius flavus*** (a little, yellow ant, approx**.** 6 - 7 mm in length).

Lasius flavus ants build small mounds which can be seen in grassland. Both ***Lasius flavus*** and ***Lasius niger*** may build nests under paving stones. You may have come across piles of fine sandy soil in between the slabs of paving stones which mark the entrance to their nest. It is ***Lasius niger*** which gains access to buildings looking for food and can be a pest.

A group of ants that do not produce formic acid, but have a sting, are the Myrmicinae e.g. ***Myrmica rubra*** (a shiny, red ant, approx. 8 mm in length). This ant can be found in gardens, either under stones or amongst plant roots as well as in pasture.
(An indication of their relative size is evident in Figure 2.)

[NB: Queens of these species can be recognized by their larger abdomens, being about 3 times that of the workers.]

Figure 1 Four British species of ants

Formica rufa

© Richard Bartz

Myrmica rubra

© Gary Alpert

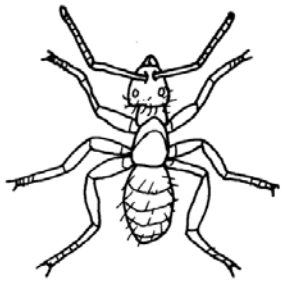
Lasius flavus

© Alex Wild - www.alexanderwild.com

Lasius niger

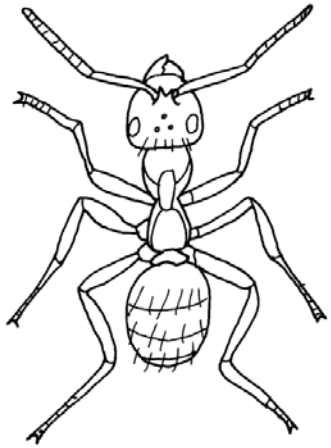
© Alex Wild - www.alexanderwild.com

Figure 2 Some British ants found in woods, pastureland and gardens

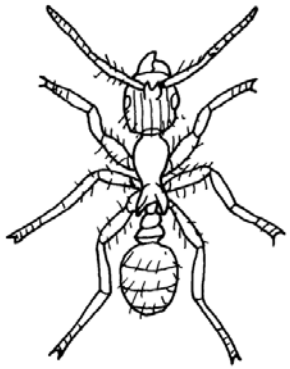


Lasius niger

Lasius flavus



Formica rufa



Myrmica rubra

Illustrations by Liz Evesham

Scale 10x life size

Both Formicinae and Myrmicinae are abundant in the British Isles, particularly in the south of England. ***Formica rufa*** can be found around and in areas of forest and woodland where there are pine trees, for example, the North York Moors, the Peak District and Lake District, Southern Uplands and Grampians in Scotland. ***Lasius*** and ***Myrmica*** species can be found in pastureland and grassland in areas such as the Chiltern Hills, North and South Downs, the Mendips, the Cotswolds, much of Wales, the North Dorset Downs, Blackdown Hills, Exmoor, Dartmoor, the Pennines and the mountains and vales of Ireland. Well known ***Myrmica*** sites are the limestone hills of Kimmeridge, Dorset. (Grid reference on a ***1: 25,000 map = SY 801918.*** For further information regarding suitable sites in Dorset contact the author: her e-mail address is ejme@millhill.org.uk)

It is the ant ***Myrmica rubra*** which is suggested for use with this guide, since their biology is well understood and they are easier than the Formicines to collect and maintain in the laboratory. However, there are some suggestions for fieldwork that can be carried out with Formicine ants, since these may be easier to locate in your area and can also be obtained from appropriate suppliers of animal specimens (such as Blades Biological Ltd. and other online sources, see Appendix B).

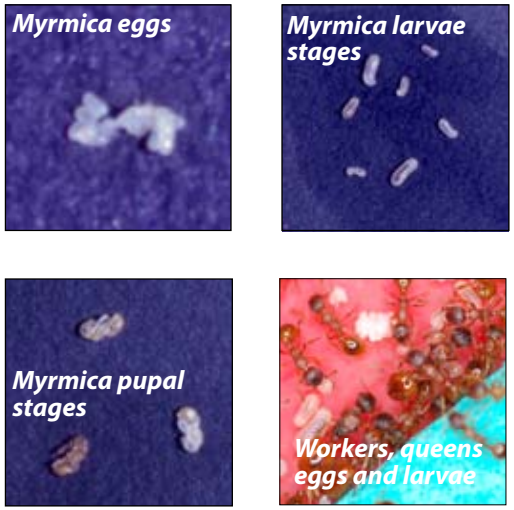
You may plan to carry out your investigations at a time of year that suits you. Therefore, an understanding of the ant life cycle would be of benefit since you will come across various life stages of ants when locating, collecting and observing them at different times of the year.

(See pages 5 to 8 and Figure 3)

Life cycle of ants

The queens and workers are females. The males appear in the colony only during the summer months, ready to mate with new queens, see Figure 3. Winged forms of *Myrmica* gather before their nuptial flights in late August to September, depending on the prevailing weather conditions. (During warm and humid evenings in late summer you may have seen winged ants flying, when they are vulnerable prey to birds, such as swallows.) The males collect in swarms over prominent land marks, such as trees and tall buildings. **Gynes** (the name given to unfertilized queens and queens that have not yet established a colony) fly in amongst them to be mated and then store enough sperm to last a lifetime, thought to be about ten years. Depending on the species, some ant queens may be mated with different males. Having mated, the males die and the gynes shed their wings and excavate a hole in the soil, which houses the gyne and is sealed off from the inside.

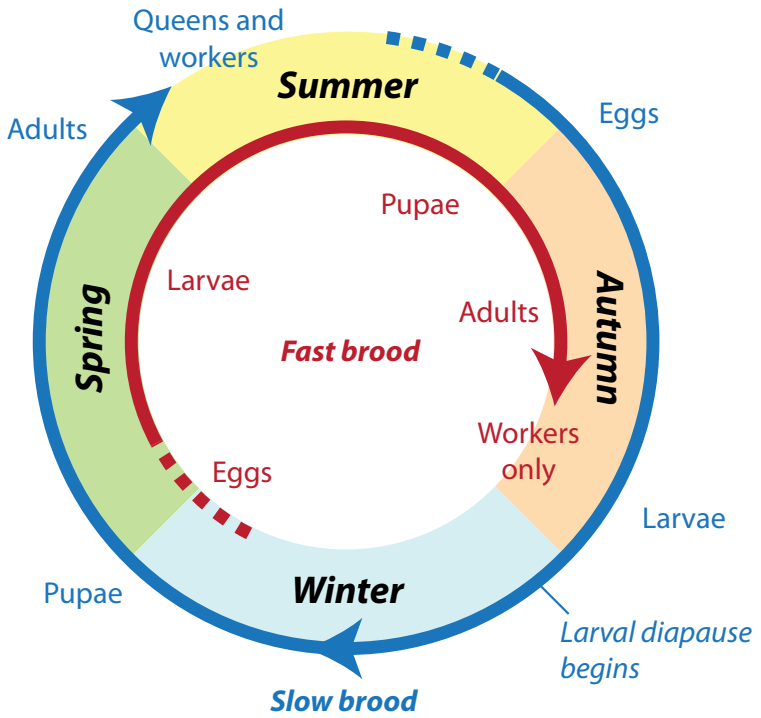
Figure 4 Stages in Myrmica development



The warmth of the following spring causes the queens to lay their first eggs, see Figure 4, some of which will develop into larvae. The larvae are white and transparent, covered in tiny hairs and have neither legs nor eyes. They do have mouthparts which enable them to take sugary liquids from their queen.

As the larvae grow, they become less transparent and will eventually lose the contents of their digestive system prior to pupation. This stage is referred to as the **pharate pupal** stage. A pupa then develops from this, which is a non-feeding stage and looks like a miniature ant. At first the pupae are white in colour, turning orange as time progresses and when the weather gets warmer. Male pupae can be recognised by their thinner shape and blackish colour, while queen pupae are much larger. If the pupae emerge as pale orange worker ants, they have the role of a **nurse** caring for the queens, their eggs and other larvae. Later, their coloration darkens and the worker duties within the colony changes from a nurse into a **forager**, moving outside the nest to find and collect food for other members of the colony. Larvae which are fed on a liquid diet of sugary fluids usually develop into **workers**.

Figure 3 Stages in the annual life cycle



If workers do not reach the adult stage by October, they hibernate, or **diapause** through the winter as larvae in the third instar. This happens when the larvae have been fed on sugary fluids and proteins and are larger in size. In the following spring, the hibernating larvae prepare to pupate. More queen eggs will also appear in the nest. Therefore, two brood cycles run together through the seasons, viz. a 'slow' brood and a 'fast' brood, see Figure 3. A well-established colony will have different ant stages in the nest at the same time.

In the Formicinae (e.g. ***Lasius*** and ***Formica***), the first batch of eggs laid by queens are likely to appear several weeks later compared to the Myrmicines, and sometimes not until the following spring. The larvae will, however, grow rapidly if given sufficient food.

Cocoons, not pupae, develop from the larval stage, see Figure 5. Queen cocoons tend to be larger than worker ones. The queens of ***Lasius***, will either disperse once the colony has matured or will be attacked by the other members of the colony, leaving one queen remaining, so it is a **monogynous** colony, like that of honeybees. ***Formica*** and ***Myrmica*** may have several queens in a mature colony, so have a **polygynous** system. A mature colony of ***Myrmica rubra*** can consist of about 10 - 80 queens and 1000 - 3000 workers, see Figure 6. The success of a colony will depend upon many factors, such as food availability, habitat and the prevailing weather conditions.

Figure 5 Adults and cocoons of Lasius flavus

Figure 6 Myrmica *ant colony*

Locating ant colonies in the field

During the cooler, winter months, ant colonies will be deeper in the soil, although you may still find a few foragers at the surface. You would probably need to dig to a depth of 1 - 3 metres to find a colony. In the spring, the colony will move nearer the soil surface and are easier to locate and collect. On very hot days, the majority of ants will be on the soil surface and location and collection is easier still.

Figure 7 Typical pasture with ant colonies

Figure 8 Stones on the hillside, beneath which ant colonies may be found

In order to attract ants into a garden or an unused area of a playing field (where mowing machines do not operate), scatter some flat stones or old roof tiles onto a spot that receives plenty of sunshine. If you do this early in the year, by springtime or early summer you may find you have some ants underneath the stones. If you already have a rockery or stones in the garden, start investigating them daily to see if you have an ant's nest. If you happen to live near a limestone area or pastureland, then while out for a walk, start turning over any stones or objects that you come across to see whether there are any ants underneath. Remember to return any stones to their original position afterwards.

Figure 9 Looking under stones

Figure 10 Typical pasture with ant colonies

If you do find ants in an area, make a note of their general location. You could also try putting out bait. Find a small tin or shallow container and place something sweet into it, such as a sugar lump, and leave it overnight. If you find ants in the container, look carefully at the surrounding area and you will probably find a trail of ants moving to, or from, the sugar source. Trace the trail back and see if you can locate a probable ant nest. Place a flat stone where you think that might be.

Figure 11 Galleries of a nest created along plant roots

Once you have located a number of nests within the same area mark them in some way, either by placing a small plant label at the side of the nest or underneath the stone where the nest is situated. You are now ready to carry out some fieldwork with your students, if that is thought desirable or possible. Alternatively, investigations can be carried out in the laboratory, see page 15.

Figure 12 Stone removed to reveal a nest

Fieldwork investigations

Equipment needed for field investigations

- pooters (see Figure 14)
- spare boiling tubes
- cotton wool
- tape measures
- trowels
- plant labels
- marker pens
- washing-up bowls or shallow laboratory trays (with Fluon coating the rims, if at all possible)
- map of the field area (preferably large scale OS maps)
- ant key (see *The Oxford book of insects*, appendix B)
- note book and pen
- graph paper
- compass
- bin bags
- plastic bags
- quick-drying, non-toxic paint e.g. Humbrol, obtained from most art shops and online (see Appendix B) - (use colours which can be seen clearly, like white/yellow/orange/pale blue)
- tissue/paper towels
- cocktail sticks
- paintbrushes
- suitable bags/containers to carry equipment
- Fluon **(Ensure that you follow the relevant risk procedures for your institution.)**

Notes

The suggested investigations can be adjusted according to the ant species you find at your field site, how many colonies you intend to use and whether the work is intended for GCSE/Higher or A Level/Advanced Higher.

Depending on the investigation, you may only need some of the items mentioned here. Plan and prepare the equipment well in advance. Suitable clothing needs to be worn such as boots/wellies that cover the ankles, surgical gloves if prone to insect allergies or prone to severe allergic reactions from insects/vegetation etc., and a cagoule in case of wet weather.

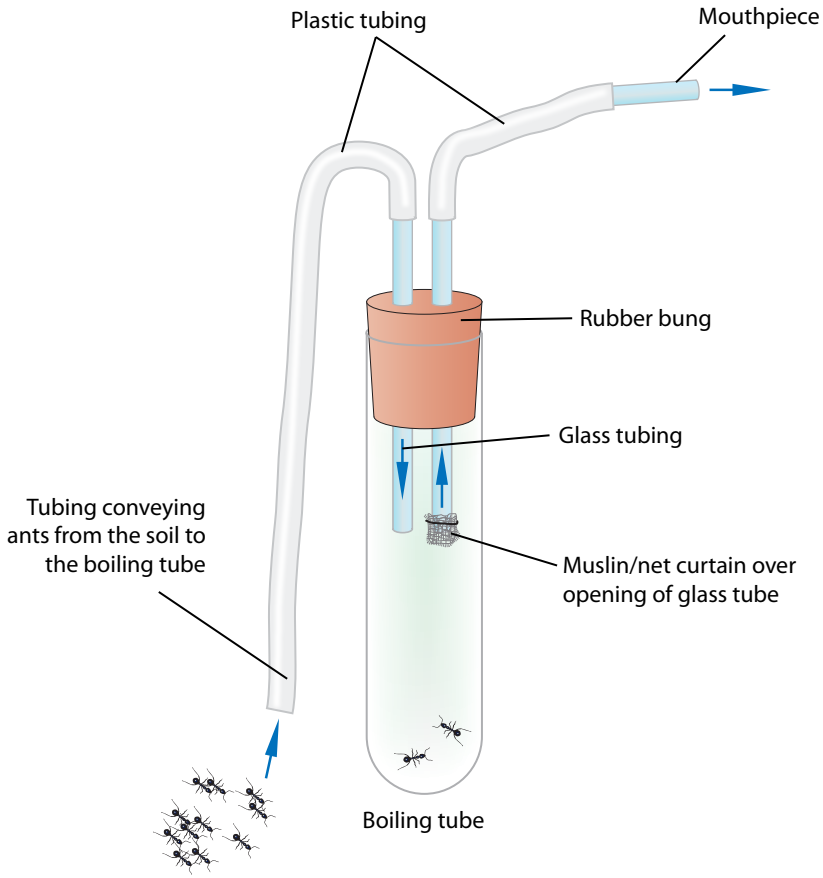
It would be useful to have some Fluon available. This is a white fluid which, when dry, provides a slippery surface on which the ants cannot climb, thus preventing their escape from a container. It can be obtained from Blades Biological (see Appendix B). Fluon needs to be applied, with cotton wool for example, (wearing suitable clothing such as a laboratory coat, goggles and surgical gloves), around the rim of washing-up bowls and/or small plastic containers and allowed to dry well before the fieldwork is carried out. To do this soak a pad of cotton wool with Fluon and give one complete wipe around the rim of each of the containers used. Alternatively, a piece of sponge can be used to apply the Fluon. The sponge can be rinsed out, immediately after application, in warm water and be re-used. Do not worry about drips of Fluon that run down the sides and into the container, because, once dry, it is harmless to the ants. [If needed, a hair-dryer can be used, by teachers or technicians, to accelerate the drying process.]

Pooters

Pooters are easy to make and use, see Figures 13 and 14. The commercial ones have shorter tubes and small containers and, therefore, can be difficult to use, but you can easily make your own. A home-made pooter requires a boiling tube which can be removed and replaced with another quite easily, should the first one become full of ants, see Figure 14. Two holes need to be made into the rubber bung that fits a short piece (65 mm in length) of glass tubing (6 mm diameter) which extends to the outside and inside of the boiling tube when the rubber bung is fitted. To the outside of the tube are placed two pieces of clear, flexible, plastic tubing (6 mm inside diameter and 8 mm outside diameter with a wall thickness of 1 mm and approximately 35 cm in length). Two short pieces (30 mm in length) of the clear, flexible plastic tubing are placed over the glass tubing to the inside of the boiling tube. Before placing the tubing over one of the pieces of glass tubing, place a small square of muslin or curtain netting over the end and secure with, say, a rubber band. This prevents students sucking up ants into the mouthpiece.

Figure 13 A pooter being used to collect ants in the field

Figure 14 A home-made pooter



Marking ants

[Below are instructions for marking ants and for carrying out an investigation into their foraging behaviour. The instructions are aimed at students, in case teachers wish to provide small groups of students with the required directions to successfully carry out observational studies in the field.]

Method for marking ants should you wish to identify them, either in the field or in the laboratory, at any time during your investigation.

Make sure you have a pooter, paint, cocktail sticks and washing-up bowls, coated with Fluon around the rim, at the ready.

In the study area, carefully turn over the stones. As soon as you identify a *Myrmica* nest, pooter up a few workers/queens that you see underneath the stone and on the underside of the stone. Speed is of the essence when doing this!

Care must be taken to replace the stone immediately after you have collected the ants you want.

Transfer the ants to a washing-up bowl. Move the ants into the shade if the day is sunny and hot.

To get the ants out of the pooter, gentle tap the base of the boiling tube on to the palm of your hand and this will knock the ants to the bottom of the tube. Quickly remove the rubber bung and tip the ants into the washing-up bowl, see Figure 15.

Figure 15 Ants being placed in washing-up bowl

Gently pick an ant up between the thumb and forefinger of one hand, making sure you have its legs firmly secured and the top of the ant's body is facing you. Hold the ant very softly, see Figure 16 (a).

Hold a cocktail stick in the other hand (or get someone else to help you), dip the end of the stick into a small pot of non-toxic, quick-drying paint. Try to get only a small amount on the end of the cocktail stick. If you pick up too much paint, wipe some of it off with a tissue or paper towel.

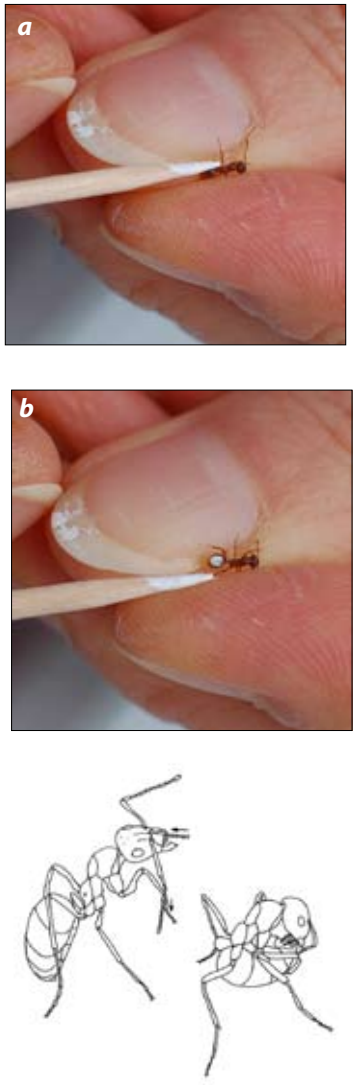
Place a small blob of the paint onto the abdomen or thorax of the ant, whichever you find easier, see Figure 16 (b).

Hold the ant for a few seconds before placing it back into the washing-up bowl.

You will probably notice that the painted ant will groom itself after having been picked up by you. It does this in order to remove your odour from its body.

Repeat with the other ants as necessary.

Figure 16 Holding (a) and marking (b) ants



An investigation into the foraging behaviour of ants

(Can be carried out with any species)

Having located your nests in your study area, observe the surrounding vegetation for ants. If there are nettles near by, look closely at the stems and underside of the leaves and you may see aphids (wingless green or blackfly).

M. rubra ants are known to 'milk' aphids for food (see Figure 17) and so there is likely to be movement of ants between the nettles and ant nests. The ants tap the rear end of the aphids with their antennae. The aphid responds by excreting a substance containing sugars called ***honeydew***. The aphid collects this itself from the sap of plants and trees.

Figure 17 Aphids and ants

1 Look closely at the trunk of any surrounding trees and you are likely to see movement of ants up and down it. The ants moving up the tree trunk are also 'milking' aphids, like those found on nettles, which are located on the underside of the leaves on the branches of the tree.

2 You will see that ants moving up the tree will probably meet those moving down the tree. When the two ants meet, they will examine each other with their antennae by tapping each other on the head. This acts as a way of communicating to the other ant that there is a food source along the route. The ants may exchange the food source collected with one another , see Figure 18. In this way, the one coming down the tree or stem will regurgitate food and pass it to the one moving up the tree. See if you can observe this behaviour.

Figure 18 Ants exchanging food

The ant also checks to see whether the ant they have met belongs to the same colony, see Figure 19. The ant uses odours that it has picked up using its antennae to recognise the other ant. Wood ants, being larger than ***Myrmica***, can be seen clearly moving up and down tree trunks. It is also easy to follow their trails between the trees and their nests.

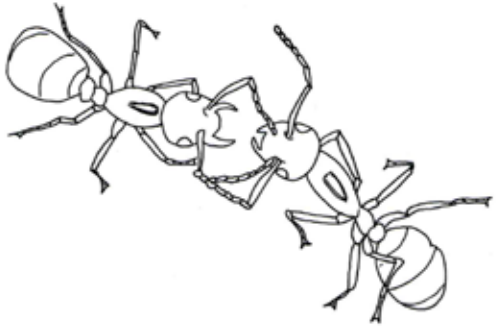
Figure 19
One ant attacking an ant from another colony

3 Working from the tree trunk or other food source where you have found ants, follow the ant route back to the nests. You may observe several trails of ants, moving up and down, on different sides of the tree trunk or plant stem that radiate out in different directions from the base.

4 Map out on the ground the trails from the food source to the nest. Is there any overlap of the trails i.e. do ants from different nests exploit the same food source or different food sources? Will ants that meet on a trail show aggressive behaviour? Will this be more or less intense if their nests are closer together or further apart?

5 One or other ant may have strayed from its own territorial boundary onto another, see Figure 20. You could try placing an ant from one trail onto another trail to observe their behaviour. What do you think might happen?

Figure 20
One ant threatening another ant



6 Now count the number of ants passing a particular point, either on the tree trunk/plant stem, the base of it or along the ground. For example, count how many pass that point, per minute for a 20 minute period.

7 Repeat on other trails that you have found. Are there more ants moving along one trail compared to another? If so, this could be because the nest is larger and more members of the colony need to be fed, particularly the larvae and queens. It could be that the food source is richer and more abundant.

8 You could extend this investigation by counting the number of ants passing a particular point, either on the tree trunk/plant stem, base of it or along the ground for five minutes, once an hour, throughout the day. Is there more movement at certain times of day compared to others? If so, consider some reasons why. Try different days and different times of the year. With this kind of data, you could compare one trail with another, or a nest in one location compared with another, using a **chi-squared test (χ^2) (A level work).** There are also plenty of graphs that can be drawn **(GCSE and A-level work).** For example, plotting the frequency of ants passing (say every 5 minutes) against time.

You could also try putting out baits. Find a small tin or shallow container and place something sweet into it, such as a sugar lump, and leave it overnight. The following day, you will probably find ants in the container. Look carefully at the surrounding area and find the ant trail back to the nest. Record which nests the ants have come from? Is it the nearest nest? Do ants from several nests visit the same bait?

Try placing baits at different distances from a nest to see whether the ants visit the nearest one or not. You could try placing different concentrations of sugar into different baits by placing these at the same distance from a nest, or different distances, to see whether one type attracts more ants compared to another.

Collecting ants in the field in preparation for laboratory work

Once you have located your ant nests, see Figure 21, make sure you have plenty of pooters, boiling tubes, cotton wool and marker pens ready.

Carefully turn over the stone of the ant nest, see Figure 22, and immediately pooter up the ants underneath the stone and on the underside of the stone. Try to minimise the disturbance to the nest!!

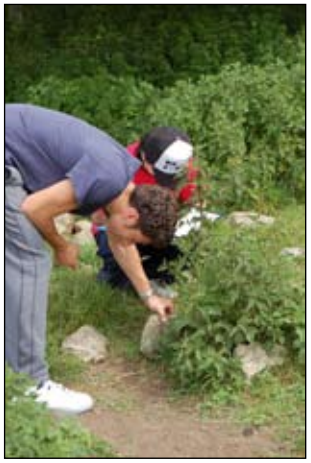


Figure 21 Locating an ant nest

Care must be taken to replace the stone immediately you have collected the ants you want, without crushing any animals.

Figure 22 Stone removed to reveal the nest

Figure 23 Checking another stone

Mark the boiling tube with a number/letter or other symbol so that you can identify the nest again once you are back in the laboratory. Make a note of location of the nest, label it, and make any other readings you require, see Figure 24.

Gently tap the base of the boiling tube on to the palm of your hand and this will knock the ants to the bottom of the tube. Quickly remove the rubber bung and replace it with a piece of cotton wool. In this way you can re-use the pooter by placing the bung into another boiling tube.

Once you have collected all the nests you want, lie the tubes down in a tray, interspersed with cotton wool to prevent them banging into each other. Cover the tray with a black bin liner and keep cool, out of direct sunlight. They are now ready to transport.

Figure 24 Close-up of a nest

Laboratory work

List of equipment for laboratory investigations with ants

- pooters
- spare boiling tubes
- cotton wool
- marker pens/labels
- washing-up bowls, with Fluon coating the rim, if possible
- small plastic containers, with Fluon coating the rim, if possible
- laboratory trays
- Petri dishes
- note book and pen
- paintbrushes
- quick-drying, non-toxic paint (different colours which can be seen clearly e.g. white/yellow/orange/pale blue) ***(Humbrol*** see Appendix B***)***
- cocktail sticks
- tweezers
- plastic plant pots which do not have holes at the base.
- sugar lumps
- ***Drosophila melanogaster*** larvae (Blades Biological) or mealworms (from garden centres) as protein sources
- distilled or deionised water
- time switches
- desk lamps with low-energy light bulbs (no more than 40W)
- Wetex pads (previously rinsed and left to dry). These are flat, moist cloths (about 5 per pack) that can be purchased from most supermarkets.
- Fluon **(Ensure that you follow the relevant risk procedures for your institution.)**

Maintenance of ant colonies in the laboratory

1 Place the covered tray, containing the boiling tubes of ants you have collected from the field, (or your specimen tube of ants that you have obtained from a laboratory supplier) in a cool and undisturbed place until you have set up your equipment.

If you are unable to set up the equipment for a few days, do not worry, your ants will survive if kept cool (i.e. at 10 °C – 15 °C).

Ensure that you check each boiling tube daily and if the ants appear dry, using a fine pipette, dribble some water onto the cotton wool at the mouth of the boiling tube. Push the cotton wool a little way down the tube and place a dry piece of cotton wool at the mouth.

Figure 25 Some of the equipment needed for laboratory studies

2 Prepare a washing-up bowl for each of the colonies collected. Wearing suitable clothing, such as a laboratory coat, goggles and rubber gloves, wipe Fluon (which provides a slippery surface that ants cannot climb) around the rim of the washing-up bowls and any other containers you might use. Do this by soaking a pad of cotton wool, or piece of sponge, with the Fluon and wipe it once around the rim of the container you intend to use. Use a fresh pad and Fluon once the Fluon on the pad has run out. If you are using a piece of sponge, rinse it out, in warm water, before each application.

3 With a scalpel (**students should not do this on their own**) cut two or three small triangular notches, around the rim of the plant pots.

Place one plant pot, upside-down, in each of the washing-up bowls you have prepared. The plant pots provide a dark enclosure where ants place and nurture their brood.

4 Cut small squares of the previously dried Wetex, approx 3 x 3 cm, and place in a beaker of distilled or deionised water. With tweezers, take them out and place one piece of Wetex under each of the plant pots and another piece in the foraging arena of each washing-up bowl.

Make sure the Wetex pads are kept moist daily. If dry, use a pipette to transfer some distilled water from a beaker onto the Wetex pad. If you cannot do this daily and may have to leave the ants for more than two days (over the weekend for example, or perhaps at a half term holiday), it is a good idea to fill a test tube with distilled water, plug it with cotton wool and leave, on its side, in the washing-up bowl. Ensure the water is in contact with the cotton wool. Sometimes, the ants will chew at the Wetex pad so that it gradually disintegrates and/or it deteriorates. If so, remove and replace with a fresh piece of Wetex.

5 Place one sugar lump and a couple of meal worms or some *Drosophila melanogaster* larvae into each washing-up bowl, or in a small Petri-dish or similar container. Keep an eye on the sugar lump. It should last a long time, but if it gets wet or begins to get smaller, remove and replace with a fresh one.

Top-up the protein source regularly, perhaps every other day. You could try out different foods on your colonies. Ants also like blackcurrant juice, oranges and apples as a source of sugar!

6 You need to find out, from a newspaper, when sunrise and sunset are and adjust a time switch, attached to a lamp, so that the lamp is on for the appropriate daylength. If you are setting up several ant nests with several lamps, use an extension lead or use more time switches.

If in doubt about the length of day needed, use 14 hours and gradually increase to 16 hours if carrying out these investigations in late spring/summer. The idea is to create as near to natural conditions as possible for your ant colonies. The room temperature needs to be between 20 °C and 23 °C (or as near to this range as possible).

7 Place each one of your boiling tubes of ants horizontally, in turn, into each prepared washing-up bowl and remove the cotton wool.

Leave the ants to explore their new home, and to settle out from any soil that was pootered up with them, for at least 24 hours before counting them and beginning your investigations. If you cannot begin your investigations after 24 hours, provided the ants have plenty of water, they can be left for longer.

It is advisable to leave the ants in an undisturbed and quite, dark place. If you are unable to do this, try to create an area of the classroom/laboratory specifically for the ants. Place notices up and around the area to inform the students of the investigations going on and that they should not disturb them.

If the washing-up bowl begins to smell or is looking dirty due to ant faeces (seen as black marks around the bowl), moisten a piece of cotton wool and clean it up. Do not worry if you find dead ants in your bowl. It is natural for there to be some mortality in an ant colony since their life cycle is very short.

8 You will find it useful to label each washing-up bowl and to make a note of the following:-

DATE:- When the ants were collected from the field.

NUMBER:- Numbers of each caste (type of ant/queen/larvae), see Figure 26.

COLONY:- If you have collected more than one colony, distinguish between them. For example:- M 1, M, 2, etc. or A, B, etc..

OBSERVATIONS:- Make notes of what you see daily. For example, what happens when two ants meet, where the queens are, whether the ants are carrying brood or food or other ants, etc..

Counting the numbers of ants in a colony

Make sure you are in a comfortable position with the nest you want to assess in front of you, see Figure 26. You need a note book and pen next to you and a pooter.

1 Unfortunately you will need to disturb the ants for a while by removing the upside-down plant pot from the washing-up bowl. Make sure you tap the base of the plant pot to remove any ants attached to the inside of it.

2 Pooter up the queens first and count them as you do so. You can identify the queens quite easily, since they are about three times the size of a worker ant.

3 Make a note of the numbers and the numbers, if any, of gynes (queens with wings).

Figure 26 Some ant stages

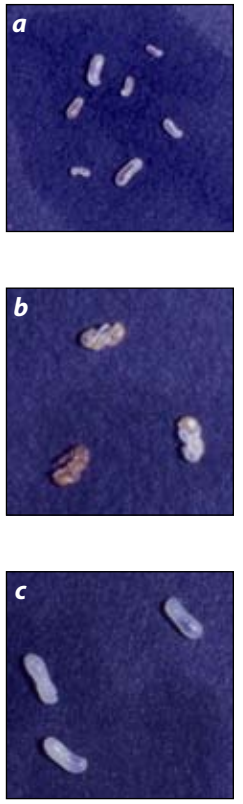
4 Now count the males, if any, seen as almost black with slender bodies and wings. Make a note of the numbers.

5 Now count the workers. You will need to tally these. For example, make a note of every 5 or 10 you pooter up and then add them up.

6 If you need to count the larval stages, it should be easier without the workers and queens in the bowl. Don't worry if you accidentally pootered up some of the brood along with the workers. This is easily done.

7 If you need to make a count, see Figure 27 a to c, of each larval (a) and pupal (b) stage, then first separate them out, using a fine paintbrush, into the different types, for example, small, medium and large larvae. Pharate pupae (c) (a stage prior to the pupal stage where the larvae appears very white so that you cannot see any internal tissues), white pupae and then orange pupae (the stage before the pale, orange worker ant emerges). Male pupae will be narrow and grey in colour. Queen pupae will be larger than worker pupae. As you count the individual larvae and pupae, move each one to the side of the cluster. Make a note of the numbers.

Figure 27 Larval and pupal stages



8 If you are intending to use the larvae for experimentation immediately, remove them now, with a paintbrush, and transfer them to a Petri-dish. If you find them difficult to pick up, moisten the tip of the paintbrush with distilled water.

9 Return the colony to the washing-up bowl by either removing the rubber bung of the pooter and tapping the end of the boiling tube gently or place the boiling tube into the washing-up bowl to allow the ants to come out by themselves.

10 Repeat with the other colonies, if any.

11 Always allow your colonies to settle for at least 24 hours before using them again.

12 If you have more than one colony, you can see whether the population density and caste composition within each nest is as expected or whether there is some other factor which has contributed to the size of each colony: for this analysis you can use a chi-squared test.

Investigations using a horizontal glass nest of soil

[NB: You will require a laboratory technician or teacher to help build the nest.]

Figure 28 Horizontal glass nest of soil

List of equipment for building a horizontal glass nest of soil

- glass sheets (25 x 25 cm), 2 per nest ***(obtained from any glass merchant)***
- black paper
- Fluon
- cotton wool
- desk lamps with low energy light bulbs (no more than 40W)
- time switches
- Wetex pads (previously rinsed and left to dry)
- protein and sugar source
- distilled water
- soil (loamy type (i.e. with a balance of sand, silt and clay particles), left to dry and sieved)
- draught excluder
- double-sided tape
- scalpel
- plastic food boxes or similar (approx. 10 x 10 x 7.5 cm)
- rubber tubing (3 mm diameter)
- bunsen burner
- old metal rod or other (3 mm diameter)
- bulldog clips, 3 - 4 per nest.

Method for building and using a horizontal glass nest of soil

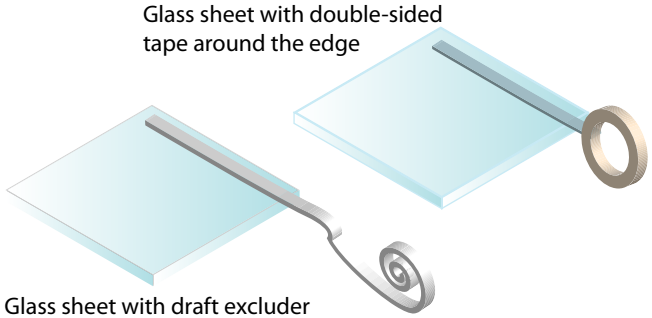
1 Wearing a lab coat throughout, collect all the materials you need and clean the glass sheets thoroughly and any containers you intend to use.

2 Wearing rubber gloves, use a pad of cotton wool or a piece of sponge to wipe Fluon around the rim of the plastic container(s) and allow it to dry.

3 Make a small hole on one side of each plastic container, near the base. Do this by heating the metal rod in a Bunsen burner and melt the plastic slowly. (**An adult needs to do this. Student must not carry out this part**). Make sure the hole has not been made too large, otherwise you will find that your ants will be able to squeeze through the gap between the plastic container and the inserted plastic tubing.

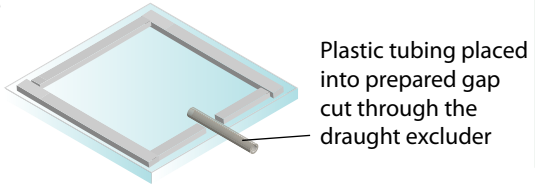
4 Leaving no gaps, place double-sided tape around the edge of one sheet of glass and draught excluder around the other sheet, see Figure 29 (a) and (b). Make sure the draught excluder is a continuous piece, do not be tempted to cut it for the corners of the glass sheet.

Figure 29 Applying tape (a) and draught excluder (b)



5 With a scalpel (make sure an adult is present if a student is doing this), cut away a small section from the draught excluder (2.5 - 3 mm wide), in the middle of one side of the draught excluder. Again, make sure the cut has not been made too large. The tubing should fit the gap snugly, see Figure 30.

Figure 30 Plastic tubing in place



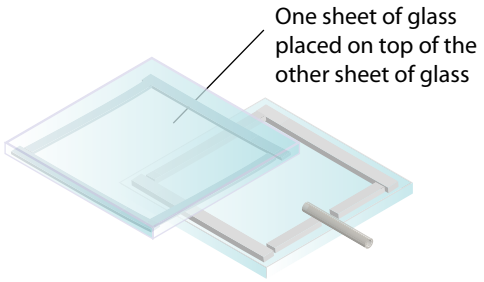
6 Place some dry, sieved soil on to the glass sheet which has the draught excluder on it. Make the soil nice and compact, but make sure the soil has **not** been sprinkled on top of the draught excluder. This would prevent a proper seal when the two sheets of glass are placed together. Moisten the soil with distilled water, but **do not over-water.**

7 Place, approximately, a 30 cm length of the 3 mm tubing (enough to fit between the glass nest and the plastic container) from the gap cut into the draught excluder on the glass to and through the hole cut out of the plastic container. Make sure there is enough of each end inside the nest and inside the plastic container.

8 Place the second sheet of glass, with the double-sided tape on it, on top of the glass sheet which now has soil on it. Press firmly in place so that the double-sided tape on one sheet of glass sticks to the draught excluder on the other, see Figure 31.

9 Cover each outer side of the nest with black paper and secure each corner of the nest with a bulldog clip.

Figure 31 Aligning the two glass sheets



10 Place the nest in a darkened room at approx. 20 °C. Attach a desk lamp to a time switch which has been set for natural daylength and night time. Place the lamp about 60 cm away from the centre of the nest.

11 Place food and water inside the plastic container.

12 Pooter up the ants and brood you wish to investigate, from the washing-up bowl, and place inside the plastic container. Remember to gently tap the bottom of the boiling tube to gather all the ants together before tipping them out.

13 Leave the ants to settle into their new environment for at least 24 hours before making your observations and measurements.

14 Repeat for other nests you wish to investigate.

Suggestions for studying ants in the horizontal glass nest of soil

1 You will need to plan your experiments carefully, prior to making your observations. You will need to decide what you are recording, how you will make your measurements, when and for how long. You may decide to paint particular ants with non-toxic, quick-drying paint beforehand so that you can identify them throughout the duration of your experiment. [See earlier for method of painting, page 11.]

2 When you make your observations, try to be quiet. Carefully remove the black paper and make a note of what you see. You could use a dimmer light or place a red bulb in the desk lamp while you make your observations. This will ensure the colony inside the glass nest is not adversely affected by the light. Record what you see at the same time, and perhaps every hour or every four hours, for a day (and night if you are up to it!).

3 It is useful to obtain acetate sheets and permanent, waterproof or non-permanent, water-soluble pens suitable for drawing onto the acetate. Each time you make an observation, lay a sheet of acetate over the top of the nest and mark out the tunnels (long, thin areas which act as travel routes for the ants connecting different parts of the nest) and chambers that the ants have excavated (larger areas which normally house the brood, queens and workers which care for the queens and brood), indicating the position of particular ants and brood. You can then lay your acetate sheet over some graph paper and calculate the area of the nest, chambers and tunnel widths. Acetate sheets can also be placed on a photocopier so that you can build up a record of the shape of the nests, scan them and transfer them to a computer, see Figure 32.

4 You could experiment with different soil types in your glass nests. For example, one with mostly clay and one with mostly sand, and see whether the ants build their nests in different ways.

5 You could also try dividing your nest into quarters (using the draught excluder and tape). Each quarter needs to be connected to a plastic container. You may find this an easier option so that you can make comparisons by viewing one nest instead of several, separate glass nests. Within each quarter, you could place different types of soil to see whether the ants build different shaped nests.

6 If you have decided to set up a number of nests, then you can subject them to different conditions, for example, using a different soil type or food source. You can then compare the total amount of soil excavated between different nests, perhaps tunnel width and chamber size as well as see the effect of time (day/week/month) upon the development of the nest.

7 The data could be represented graphically and/or analysed with a suitable statistical test. If you record the area of soil excavated each day or week, you could see whether there is a significant correlation between the total amount of soil excavated and time.

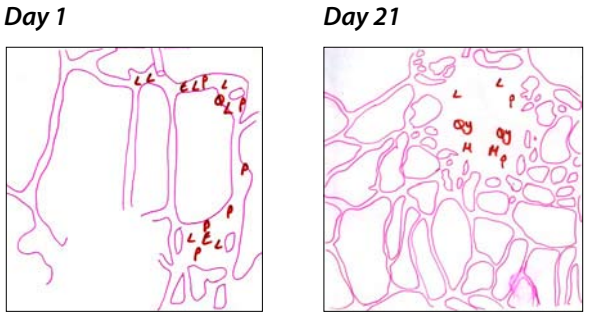


Figure 32 Comparing acetate sheets on day 1 and day 21

Dismantling the nest

1 The design of the nest is such that it can be dismantled should you wish to reset your experiment or when you have finished the investigation. Make sure the entire apparatus is placed in a suitable container before you start, such as a large washing-up bowl, or laboratory tray, with Fluon round the rim so the ants do not escape. (An adult needs to be present if the students are doing this.)

2 Pooter up any ants in the plastic container first, and then prise the two sheets of glass away from each other very carefully. Pooter up the ants from the soil and place them into a washing-up bowl which has Fluon around the rim.

3 Clean all the materials thoroughly so they are ready to be re-used.

4 If the ants have been finished with, place them in a garden or similar environment, under a stone or similar object.

Acknowledgements

I am grateful to the Association for the Study of Animal Behaviour (ASAB) for the Teacher Researcher Grant to enable me to produce this resource. I would also like to thank Richard Bartz, Gary Alpert, Alex Wild and Mick Hoult for giving permission to use their images and Mick Hoult for designing the teacher notes: all other images were provided by the author.

Particular thanks must also go to Dr Michael Dockery who supervised the project and Dr Lottie Hosie (University of Chester) for her constructive comments when reviewing the teacher notes.

APPENDIX A – Examination board specifications

AQA GCSE Science A
Biology 1b: Evolution and Environment - especially 11.5, 11.8 and practical skills.
AQA GCSE Science B
Biology 1- especially 11.5, 11.8 and practical skills.

AQA GCSE Biology
Biology 1b - especially 11.5 and 11.8
Biology 2 - especially 12.4 and practical skills.

AQA GCSE Additional Science
Biology 2 - especially 11.4 and practical skills.

AQA AS and A2 Biology
AS 3.2 Unit 2. Biology 2: The Variety of Living Organisms.
3.2.1., 3.2.8., 3.2.11. Investigative and practical skills.
A2 3.4 Unit 4. Biology 4: Populations and Environment.
3.4.1., 3.4.5., 3.4.7. Investigative and practical skills.

Edexcel GCSE 360 Core Science and Biology
B1.a.1: Environment - especially 1.1 - 1.4 and 1.10. Practical skills.

Edexcel iGCSE Biology and Science Double Award
Section 1: The nature and variety of living organisms.
Section 4: Ecology and the Environment.
Optional Coursework: To carry out experimental work and investigations.

Edexcel GCSE 360 Additional Science and Biology
B2.4: Interdependence - especially 4.2 - 4.4, 4.9 and 4.10. Practical skills.

[N.B. Changes will be made to GCSE specifications in Science for first teaching in September 2011.]

Edexcel GCSE 360 Biology Extension Topics
B3.2. Topic 2: Behaviour in Humans and other Animals.
Practical skills.

Edexcel AS and A2 Biology
AS Unit 2: Development, Plants and the Environment.
2.4. Topic 4: Biodiversity and natural resources.
AS Unit 3: Practical Biology and Research skills.
A2 Unit 4: The Natural Environment and Species Survival.
3.3. Topic 5: On the wild side.
A2 Unit 6: Practical Biology and Investigative Skills.

OCR GCSE Gateway Science Suite - Biology B and Science
B2: Understanding our Environment.
B2 a: Ecology in the School grounds.
B2 b: Grouping organisms.
B4: It's a Green World.
B4 e: Energy Flow.
B4 f: Farming.
Assessment Unit 4 (Optional): Research Study, data task and practical skills.

OCR GCSE Environment and Land-based Science
Unit 3: Management of the Natural Environment.

OCR AS and A2 Biology
AS Unit F212: Molecules, Biodiversity, Food and Health.
Module 3: Biodiversity and Evolution.
Practical Skills.
A2 Unit F215: Control, Genomes and Environment.
Module 3: Ecosystems and Sustainability.

Practical Skills

Module 4: Responding to the Environment.

SQA Science Standard Grade
Topic 4: A study of Environments.

SQA Science Access 1
Unit Science (Access 1): Carrying out experiments.

SQA Science Access 2
Unit Biology (Access 2): Carry out practical experiments - The Animal kingdom.

SQA Biology Standard Grade
Topic 1: The Biosphere.
Investigative work.

SQA Biology Intermediate 2
Unit 2: Environmental biology and genetics.
Practical work.

SQA Biology Advanced Higher
Unit 2: Environmental Biology.
Unit 3: Investigative Report.
Unit 5: (optional): Animal Behaviour.

APPENDIX B – References

Maps of ant locations
http://data.nbn.org.uk
http://www.bwars.com/maps_ants.htm

Insect specimens
http://www.blades-bio.co.uk/
Address: Blades Biological Ltd, Cowden, Edenbridge, Kent, TN8 7DX, Tel: 01342 850242
E-mail: sales@blades-bio.co.uk

Non-toxic enamel paint
http://www.humbrol.com/paints/enamel-paints/

Books

Pia Korsholm 1982 ***Social Insects.*** London: Addison-Wesley Publishers Ltd. ISBN: 0 201 14350X

Pia Korsholm 1982 ***Ants.*** London: Addison-Wesley Publishers Ltd. ISBN: 0 201 143518

Brian M.V. 1977. ***Ants. The New Naturalist: A Survey of British Natural History.*** London: Collins. ISBN: 0 00 219378 7 (good for identification of species)

Brian M.V. 1979. ***The World of an Ant Hill.*** London, Boston: Faber. ISBN: 0 571 11320 0

Brian M.V. 1983. ***Social Insects: Ecology and Behavioural Biology.*** London, New York: Chapman and Hall. ISBN: 0 412 2290 X Hardcovers ISBN: 0 412 22930 7 Paperback

Burton J. 1974. ***The Oxford Book of Insects.*** London: Oxford University Press. ISBN: 0192177257 or 9780192177254 (good for identification of species)

Evesham E. J. M. 1982. Regulation of the production of female sexual morphs in the ant *Myrmica rubra* L. *Ph.D Thesis, University of Southampton*

Hölldobler B. and Wilson E. O. 1990. ***The Ants.*** Berlin, London, Paris, Tokyo: Springer-Verlag. ISBN: 3 540 52092 9

Pontin J. 2005. ***Ants of Surrey.*** Surrey: Surrey Wildlife Trust. ISBN: 0 9526065 93

Skinner. G. J. 1987. ***Ants of the British Isles.*** Aylesbury, Bucks: Shire Natural History; . ISBN: 0 85263 896 5

Skinner G. J. and Allen G. W. 1966. ***Ants. Naturalists' Handbooks 24.*** Great Britain: The Richmond Publishing Co. Ltd.
ISBN: 0 85546 306 6 Hardcovers ISBN: 0 85546 305 8 Paperback