Core practical 7: Investigate the gas exchange system of a locust

Objectives

- Know how insects are adapted for gas exchange
- Consider the ethical issues of using organisms in the laboratory

Safety

- Take care to avoid cuts from sharp dissection tools. Keep these implements in an ordered fashion in a tray when not in use.
- Wash your hands with soap after handling insects. Students who suspect they may be allergic to locusts should inform you.
- Methylene blue is low hazard when diluted. Wear eye protection when using the stain and wash with water if there is any contact with skin.
- You should demonstrate safe dissection procedure to students.

Specification links

- Practical techniques 4, 5, 10
- CPAC 1a, 2a, 2b, 3a–3c, 4a

Procedure

The aim of dissection is to observe the internal organs of an organism in order to gain a better understanding of the function of the organs and the relationships between different structures.

There are ethical considerations that must be made when experimenting on animals and carrying out dissections. The benefits in terms of advancing knowledge must be balanced against any potential harm to a living thing. Dissection provides personal experience of the nature of fresh tissues and of the variation between individual animals. It also fosters an understanding of the organism as a whole and develops manual skills. These skills and knowledge cannot be fully provided by other means.

An ethical approach to the use of animals therefore involves responsibilities for you as a student. This includes a responsibility to derive the maximum possible learning benefit from any use of animals or animal tissues in your classes, as well as a duty of care for the welfare of any live animals that you use.

1. First observe a live locust inside a clear syringe or specimen jar. Take some time to locate the various external features and body parts shown in **fig A** in the Student sheet.
2. Insects have pores within the hard, impermeable, chitinous exoskeleton that allow gas exchange with the atmosphere. These pores are called spiracles. Find the spiracles that are located on the thoracic and abdominal segments and observe them using a hand lens. Use your observation of the live locust along with your observations of the dead specimen in step 4 to produce an outline sketch to show the position of these spiracles. Observe the larger spiracle on the thoracic segment just above and behind the second leg. Is there any evidence that this spiracle can be opened and closed? Add notes to your diagram.
3. Tilt the locust slightly to one side and look for rhythmic ventilation movements of the underside of the abdomen. These movements compress and expand the abdomen. Annotate your diagram to describe these movements.
4. Take a dead locust and view it from the side using a dissecting (stereoscopic) stereomicroscope. Again, look for the position and shape of spiracles and add to your sketch if necessary.
5. Place your dead locust in its normal upright position in the middle of a dissecting dish. Cut off the wings. Fix it to the wax or cork using dissection pins placed through the junctions of the second pair of legs with the thorax. Take hold of the tip of the abdomen with forceps, straighten it out gently and pin the tip down.

6. Using fine pointed dissection scissors, make cuts along each side of the locust exoskeleton, from the tip of the abdomen to a point just in front of the antennae, following the lines shown in **fig A** in the Student sheet. Keep the tip of the scissors raised as you do so to avoid cutting through body tissues. Cut across at the head and the tip of the abdomen to join these lines. Then carefully remove the strip of exoskeleton, using forceps and a seeker to separate tissues and leave organs intact within the body of the insect. Pin out the specimen further as necessary.
7. Flood the dissection with water. This will cause any air-filled tracheae and other structures to stand out as they will have a silvery appearance when under water. Locate the major organs. The gut (alimentary canal) will be obvious, running down the length of the specimen, and should have a brown colour (see **fig B** in the Student sheet).
8. Use a dissecting microscope and hand lens to examine the structure of the gas exchange system and make labelled diagrams to record your findings. Look particularly for the narrow silvery threads of tracheae that extend from the spiracles and examine any connections between them. Observe the position and structure of any air sacs that you can find (see **fig A** in the Student sheet). Look for tracheae in the head. Is there any evidence that air is supplied via the mouth?
9. Use the scissors to cut around a spiracle and remove it with a piece of trachea attached. Place it on a slide in a few drops of water, cover with a coverslip and observe under the microscope. Look for rings of thicker chitin along the length of the trachea. Add a drop of methylene blue at one side of the coverslip and draw it through using the corner of a paper towel on the other side. Observe again under the microscope. Record what you see.
10. Lift the mid gut using a pair of forceps and observe the attached tracheae. Using scissors, remove a small section of mid gut tissue with tracheae attached and place in a few drops of methylene blue in a watch glass for 5 minutes. Then rinse and mount a small section in water on a slide. Observe first under low power, then under high power using the microscope. Look for the narrowing of tracheae as they branch until they form blind-ending tracheoles, which do not have rings of thickening. It is likely that the smallest branches, which deliver oxygen to respiring cells, will be too small to see.
11. Use your observations and further research where necessary to answer the questions below.

Notes on procedure

- Start the lesson with a consideration of the educational benefits of dissection and a discussion of the related ethical issues. If a student who strongly objects to dissection on ethical grounds cannot be persuaded of the benefit of participation, then those views should be respected. If the student is not comfortable with observing the dissection, an alternative activity should be offered. Numerous presentations and videos of insect dissections are available online that will allow students to complete the drawings and questions on this sheet.
- Live locusts should preferably be placed in tubes or syringes for the students to reduce the risk of escapes and harm to the organisms.
- Magnification will be needed to see spiracles and ventilation movements. A magnifying glass or hand lens should be sufficient. A digital video camera with a macro function or a video microscope can also produce very good images, which can be projected. This would allow steps 1–4 to be carried out as a class demonstration, reducing the number of live animals used.
- When the locust is active, spiracles in the thorax may open during abdominal expansion and close on compression, while the opposite is true for spiracles on the abdomen. Hence a one-way air flow may be set up. The advantages of this to respiring cells in the muscles of the thorax could be discussed.
- Ventilation movements are more frequent in air which has a greater concentration of CO_2. This could be shown by gently blowing towards the intake of the syringe from a distance of at least 10 cm, drawing air in and out slightly as you do so with the plunger. Take care not to disturb the locust. Compare the frequency of ventilation movements in exhaled and normal air. Supervise any students who carry out this step.
- Step 4 can be carried out using a monocular microscope. Students should place the locust on the stage under low power and light it obliquely from above.

- Students unfamiliar with dissection techniques may need some guidance during steps 5 and 6.
- Tracheae in the head can be traced back to the first thoracic spiracle; locusts do not receive their air supply from the mouth. Detailed diagrams of the anatomy of the locust tracheal system can be found in Harrison, J. F. et al., 2013. How locusts breathe. *Physiology* 28:18–27. The article is available at http://physiologyonline.physiology.org/content/28/1/18.
- Sections of gut wall can be opened up and flattened out and may need to be rinsed to remove contents.

Answers to questions

1.
   (a) The chitin spirals support the tracheae. They hold the tracheae open if they are squashed as the insect moves.
   (b) Ventilation movements actively pump air into the tracheal system and increase the supply of oxygen to very active tissues. Expansion of the abdomen increases the volume and therefore decreases the pressure inside the body so that pressure in the tracheae becomes lower than atmospheric pressure, drawing air in through open spiracles. Compression of the abdomen decreases the volume and increases the pressure inside the body so that air moves out of the tracheae through open spiracles.
   (c) Air sacs act as air reservoirs or bellows. They increase the volume of air moved through the respiratory system. They have flexible walls so that changes in pressure caused by ventilating movements of the abdomen (or the thorax when in flight) inflate and deflate them.
2. Opening and closing of the spiracles allow the rate of gas exchange to be controlled. But water vapour also diffuses out of spiracles, so sphincters allow spiracles to be kept closed as much as possible to minimise the amount of water lost.
3. Suitable approaches that students might describe include reducing the number of animals used by working in groups or observing a teacher demonstration, any steps taken to reduce suffering of animals or prevent them experiencing stress (e.g. humane killing). This may be based around the three 'R's: Replacement, Reduction, and Refinement. Students may also describe steps they took to derive maximum benefit from the use of animals.

Core practical 7: Investigate the gas exchange system of a locust

Objectives

- Know how insects are adapted for gas exchange
- Consider the ethical issues of using organisms in the laboratory

Safety

- Take care to avoid cuts from sharp dissection tools. Keep these implements in an ordered fashion in a tray when not in use.
- Wash your hands with soap after handling insects. Inform the teacher if you suspect you may be allergic to locusts.
- Methylene blue is low hazard when diluted. Wear eye protection when using the stain and wash with water if there is any contact with skin.

Equipment

- live large adult locust
- freshly killed large adult locust
- fine forceps
- fine dissecting scissors
- seeker
- small dissection dish with a layer of hardened wax, or cork board with raised edges
- six dissecting pins
- large clear syringe or specimen jar with ventilated lid
- 2 slides and 2 coverslips
- methylene blue 0.5% aqueous
- compound microscope
- dissecting (stereoscopic) microscope
- strong hand lens
- plastic dropping pipette
- watch glass

Diagram

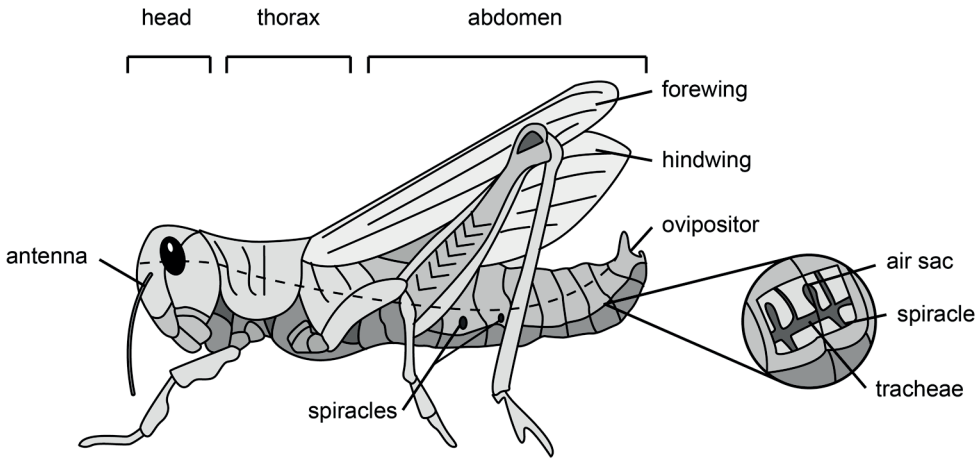

fig A The external features of a locust with inset to show some parts of the respiratory system. The dashed line shows the position of cuts to be made on each side in the dissection.

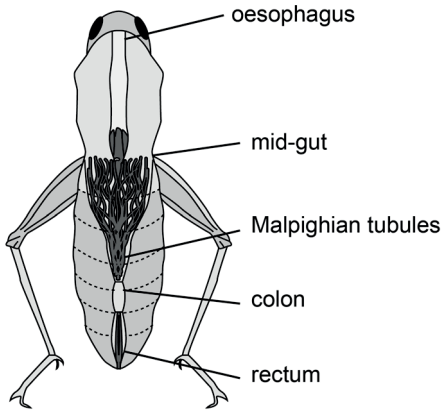

fig B The location of the gut of the dissected locust (dorsal view).

Procedure

The aim of dissection is to observe the internal organs of an organism in order to gain a better understanding of the function of the organs and the relationships between different structures.

There are ethical considerations that must be made when experimenting on animals and carrying out dissections. The benefits in terms of advancing knowledge must be balanced against any potential harm to a living thing. Dissection provides personal experience of the nature of fresh tissues and of the variation between individual animals. It also fosters an understanding of the organism as a whole and develops manual skills. These skills and knowledge cannot be fully provided by other means.

An ethical approach to the use of animals therefore involves responsibilities for you as a student. This includes a responsibility to derive the maximum possible learning benefit from any use of animals or animal tissues in your classes, as well as a duty of care for the welfare of any live animals that you use.

Insects such as the locust are too large to rely on diffusion over the external body surface to supply oxygen to the cells of internal organs and so must possess a respiratory system. Dissection can help us to answer questions about this system.

1. First observe a live locust inside a clear syringe or specimen jar. Take some time to locate the various external features and body parts shown in **fig A**.
2. Insects have pores within the hard, impermeable, chitinous exoskeleton, which allow gas exchange with the atmosphere. These pores are called spiracles. Find the spiracles that are located on the thoracic and abdominal segments and observe them using a hand lens. Use your observation of the live locust together with your observations of the dead specimen in step 4 to produce an outline sketch to show the position of these spiracles. Observe the larger spiracle on the thoracic segment just above and behind the second leg. Is there any evidence that this spiracle can be opened and closed? Add notes to your diagram.
3. Tilt the locust slightly to one side and look for rhythmic ventilation movements of the underside of the abdomen. These movements compress and expand the abdomen. Annotate your diagram to describe these movements.
4. Take a dead locust and view it from the side using a dissecting (stereoscopic) microscope. Again, look for the position and shape of spiracles and add to your sketch if necessary.
5. Place your dead locust in its normal upright position in the middle of a dissecting dish or cork board. Cut off the wings. Fix it to the wax or cork using dissecting pins placed through the junctions of the second pair of legs with the thorax. Take hold of the tip of the abdomen with forceps, straighten it out gently and pin the tip down.
6. Using fine-pointed dissection scissors, make cuts along each side of the locust exoskeleton, from the tip of the abdomen to a point just in front of the antennae, following the lines shown in **fig A**. Keep the tip of the scissors raised as you do so to avoid cutting through body tissues. Cut across at the head and the tip of the abdomen to join these lines. Then carefully remove the strip of exoskeleton, using forceps and a seeker to separate tissues and leave organs intact within the body of the insect. Pin out the specimen further as necessary.

7. Flood the dissection with water. This will cause any air-filled tracheae and other structures to stand out as they will have a silvery appearance when under water. Locate the major organs. The gut (alimentary canal) will be obvious, running down the length of the specimen. It should have a brown colour (see **fig B**).
8. Use a dissecting microscope and hand lens to examine the structure of the gas exchange system and make labelled diagrams to record your findings. Look particularly for the narrow silvery threads of tracheae that extend from the spiracles and examine any connections between them. Observe the position and structure of any air sacs that you can find (see **fig A**). Look for tracheae in the head. Is there any evidence that air is supplied via the mouth?
9. Use the scissors to cut around a spiracle and remove it with a piece of trachea attached. Place it on a slide in a few drops of water, cover with a coverslip and observe under the microscope. Look for rings of thicker chitin along the length of the trachea. Add a drop of methylene blue at one side of the coverslip and draw it through using the corner of a paper towel on the other side. Observe again under the microscope. Record what you see.
10. Lift the mid gut using a pair of forceps and observe the attached tracheae. Using scissors, remove a small section of mid-gut tissue with tracheae attached and place in a few drops of methylene blue in a watch glass for 5 minutes. Then rinse and mount a small section in water on a slide. Observe first under low power, then under high power, using the microscope. Look for the narrowing of tracheae as they branch until they form blind-ending tracheoles, which do not have rings of thickening. It is likely that the smallest branches, which deliver oxygen to respiring cells, will be too small to see.
11. Use your observations and further research where necessary to answer the questions below.

Analysis of results

1. Look back over your diagrams and annotations and add any further detail that you can.
2. Write a paragraph to explain how gas exchange is achieved in insects such as the locust, based on your observations.

Learning tips

- Note that while many features of the gas exchange system, such as spiracles, tracheae and tracheoles, are typical of all insect respiratory systems, others, such as valves on spiracles and ventilation movements, may only be found in more active species.

Questions

1. Explain the role of the following features of the locust gas exchange system.
   (a) Spirals of thickened chitin in tracheae
   (b) Ventilation movements of the abdomen
   (c) Air sacs
2. Some insects have spiracles that have sphincters, allowing them to be opened or closed. Suggest two advantages of the ability to close and open spiracles.
3. Describe the approaches that were taken to ensure the ethically responsible use of animals in this practical activity.

Core practical 7: Investigate the gas exchange system of a locust

Objectives

- Know how insects are adapted for gas exchange
- Consider the ethical issues of using organisms in the laboratory

Safety

- Ethyl acetate (ethyl ethanoate) is highly flammable and irritant. Vapours may cause drowsiness and dizziness. The sweet smell can be addictive. This chemical should be used and stored with care. Use in a fume cupboard and wear eye protection. Dispose of it carefully, adding no more than 50 cm^3 to 1 litre of water before disposing of it down a foul-water drain.
- Wash hands with soap after handling insects.
- Locusts can be kept temporarily in a suitable cage and fed on grass. Do not keep locusts in long-term culture because allergies can be triggered with repeated exposure. Take appropriate care when cleaning out the cage to avoid inhaling dust.
- Take care with sharps.
- Methylene blue solid is harmful. Avoid ingestion, skin contact and inhalation of the powder when making up solutions. Wear eye protection and disposable nitrile gloves. Carry out the procedure in a fume cupboard.
- Insects can be well-wrapped and then disposed of in the normal refuse on the day refuse is removed from the premises. Dissected insects should be kept frozen or refrigerated until then.

Equipment per student/group	Notes on equipment
large live adult locust	One per group. Place in a syringe or specimen tube (see below) before giving to students. For ethical reasons, teachers may alternatively wish to use a single live specimen for a class demonstration.
freshly killed large adult locust	Locusts can be obtained from biological suppliers or from larger pet stores (as food for reptiles). Kill using a few drops of ethyl acetate (ethyl ethanoate) on a pad of cotton wool placed at the bottom of a jar with a tightly fitting lid. Alternatively, insects could be killed by placing them in a freezer for 48 hours then defrosting, although this may damage some fine tissues. You could also perform an Internet search for 'L227 Stick insects' to find a CLEAPSS protocol for killing insects using crushed laurel leaves.
fine forceps	One pair per group
fine dissecting scissors	One pair per group
seeker	One per group
small dissection dish with a layer of hardened wax, or cork board with raised edges	This must be deep enough to allow the dissected locust to be covered in water.
dissecting pins	Six per group
large clear syringe or specimen jar with ventilated lid	To contain the live locust for observations of spiracles and ventilation movements. Gas syringes can be used for very large individuals.
slides and coverslips	Two of each per group
methylene blue 0.5% aqueous	0.5 g in 100 cm^3 water

compound microscope	One per group if stereoscopic microscopes are not available
dissecting (stereoscopic) microscope	Teachers may wish to use a video microscope for a class demonstration. See the Teacher sheet for details.
strong hand lens	One per group
plastic dropping pipette	One per group
watch glass	One per group

Notes