Anatomy and development of Orthoptera (Locusts):
Locusta migratoria

Overview:
- Introductory lecture
- Study of adults from outside; drawing of a complete animal from outside (about 2 hours)
- Study of juvenile locust (about 1 hour)
- Study the internal anatomy of the adult animal, drawing of gut (about 2 hours).
- Study of the development of locust embryos inside the egg (2 hours)
- Bringing it all together/conclusions

Please read the whole script for this part before you start with your study of the locusts.

Part 1: Anatomy of the locust

Aim: You should become familiar with the functional anatomy of the locust.

You will receive an Imago (=adult insect) and later some juveniles. First the external anatomy and morphology should be examined. Use the stereo microscope as an aid.

Outer anatomy:
Clarify the composition of the locust body. Differentiation of head, thorax and abdomen. Look for the sterna (abdominal shield) and terga (dorsal shield). The pleura on the sides are hardly visible. Which sense organs are recognizable? Where are they? What is their function? Locate the tympanum (Tympanalorgan). Find the respective structures in juvenile animals (if available).

Examine the legs. How do they differ? (e.g. amount or length of the individual limbs). Assign the parts: Coxa (hip), Trochanter, Femur (thigh), Tibia, Tarsus (foot), praetarsus and claws.

Find out whether you have a male or female. How do you recognize that? Compare your animals with your neighbours’ animals until you have seen both, males and females.

Find out how the sound (stridulation) is created by L. migratoria! Where are the spiracle (=outer openings of the tracheae).

Outer anatomy of juvenile:
How do the juveniles distinguish themselves from the adults relating to the outer anatomy? Qualitative: Which organs do you find in the juvenile stadium, which in the adults? Look for the wings, the tympanum, sexual characters, mouthparts, and other traits. Quantitative: Do adult and juvenile vary in their amount or size of organs and/or body segments? Order the various stages according to their advancing development. What is different after each shedding of the skin? Measure the animal so you can describe the growth (absolute and relative).

Inner anatomy of adult locust:
Dissect the adult locust. For that, you first cut off the wings and legs near their basis. With fine dissecting scissors cut open the dorsal cuticula from the last abdominal segment to the pronotum (the saddle shaped top of the thorax) about 1 mm lateral to the middle line. Take care not to insert the scissors too deep, so you don’t injure the inner organs. Fix the animal in a bowl with needles, peel open the bodywalls sideways and immerse in water. Get a general idea about the position and relationship of the organs you see.
Get to know the trachea-system! Follow the course of the tracheal tubes from the spiracles (=openings to the outside) to the organs. The tracheal tubes are characterized by firmness and elasticity.

Now examine the sexual organs! Do you have a male or a female? The female sexual organs consist of pairs of ovaries, pairs of oviducts, the spermatheca (to store sperms), the spermathecal gland and glands, whose secretion form the embryonic membrane. The male sexual organs consist of a pair of testicles (=testes, composed of seminal tubes, a pair of sperm ducts, 2 seminal vesicles for storage of sperm), glands that provide secretions for the buildup of spermatophores and a ductus ejaculatoris (ends in copulation apparatus). Dissect the sexual organs and allocate these structures!

Identify the digestive tract (Verdauungstrakt). It consists of a pharynx (swallowing, sucking), the oesophagus (Schlund), the crop (Kropf; to store the coarsely crushed food), the proventriculus (= gizzard; Kaumagen), which serves the crushing of food, the caecae (Blindsäcke; for the ion-water-exchange and resorption of food) and the ventriculus (= midgut, place of digestion), the pylorus (=colon, opening of the malpighian tubules), the ileum (the collecting point of excrement) and the rectum (Enddarm; water resorption from the excrement). Identify the individual segments of the digestive tract! You need to free parts of the digestive tract, since the gonads are above the rectum. To get access to the intestine, cut through the gonads, so you can push them to one side. The intestine can then be cut through and dissected with scissors at the anus, by removing (cutting off) the tracheae and if necessary the fat-body (Fettkörper; yellow tissue). Put the intestine to the side and fix with a needle.

The intestinal tract can now be carefully removed. Now you can recognize the ventral nerve cord (Strickleiternervensystem). It is partially covered by fatty tissue and muscle, which have to be removed to see the ganglia. The exposure of those parts of the nervous system that are in the cranium (Kopfkapsel) is best achieved by removing the skullcap (Kopfdecke) with superficial slices (oberflächlich geführte Flächenschnitte). If you achieved the opening of the cranium and the central nervous system, try to understand the structure. Allocate the section of the central nervous system: protocerebrum, deutocerebrum and tritocerebrum! Alternatively you can examine the separated head by opening it with a sagittal cut and examining the mouthparts. (You may collaborate with your neighbour such that each of you opens the head in a different direction).

Examine now at the separated head the mouthparts (labrum = „upper lip“, mandibles (jaw), maxillae (jaw), labium = „lower lip“) in situ, and try to understand their function! What kind of diet do those mouthparts indicate? Dissect the mouthparts and examine them more closely.

**Part 2: Development of the Locust embryo**

**Aim:** Study the embryonic development of a locust.

**GENERAL INTRODUCTIONS**

Female locusts lay their egg in packets called pods? They are deposited in damp sand (in captive breeding of locusts „Vermiculit“ is used as an oviposition substrate). Depending on species, humidity and temperature, the eggs develop in 10 to 24 days. In *Locusta migratoria*, the embryonic development, that is the time from the fertilization of the egg to hatching, takes 10 days in a temperature of 38° C. We get the animals from the Zoological Garden in Basel.

At the egg deposition, the eggs have an average length of 5mm and a diameter of almost 1mm. Water absorption during the development causes a major enlargement. This happens through so-called micropyles, which can be recognized as small, dark lines around one pole of the eggs.
The eggs and embryos used in the course can be studied very well. Only the embryonic membranes of the eggs are not transparent. They can be made transparent easily by immersing them in a 2-3% sodium hypochlorite solution (Javelle water, = bleach). (We use household bleach (2.5%).)

You will use eggs in the course of which the date of deposition is known. (Check the date on the ovipositing cups (Legebecher). Since the eggs are fertilized when they are deposited, the time of the depositing can be considered as the starting point of the development.

At the beginning of the course you should use eggs with relatively well developed embryos to begin with (older than 6 days). When you learned to handle these advanced embryos, try with younger embryos, which are smaller and more fragile and which are more difficult to locate in the egg. For each embryo note the location/orientation in the egg (indicate the anterior/posterior axis and the ventral and dorsal side).

**Studies on egg and embryo**
Eggs of various ages are immersed in 3% sodium hypochlorite solution. Observe under the stereo microscope how the non-transparent embryonic membranes become gradually transparent (remove gas bubbles carefully!). Clearing times of more than 5 minutes damage the embryos, so move the egg before to a small bowl with water. Not all stages are equally easy to study. Careful! The embryonic membrane is very delicate. It should not be damaged during the manipulation. Don’t use pointed tweezers and don’t squeeze the eggs! Try to recognize the embryo in the now transparent egg: this works best on a dark background and with lighting from the side. How does the embryo lie relative to front-end and back-end of the egg (rounded pole with micropyles, brownish pigment)? How do embryo and yolk lie relative to one another?

Can you recognize the bodysegments and appendages? Try to understand which parts will be antennae, mouthparts, legs and cerci. Are there other appendages? Observe the movements of the embryo from various directions. Record your observations; make sketches.

Dissection of an embryo: Open carefully the embryonic membrane (chorion) of the transparent egg prepared previously and remove embryo and yolk. Free the embryo as far as possible from the yolk. Examine carefully everything you didn’t see clearly previously.

**OVERVIEW OF EMBRYONIC DEVELOPMENT**
Order the specimen according to age or developmental stage respectively. Compare the material. Your protocol should contain a short but precise descriptions of your observations of the developmental stages. Those descriptions should be complemented with sketches. Record what changes in the course of the development and how those changes look. **Make sure that you indicate the anterior/posterior axis and the ventral and dorsal side.**

The protocol should contain the following aspects:

1. Where are the embryos inside the egg located? Are they always in the same place or do they change their position during the embryonic formation. Where is the yolk?
2. Size increase during development (in relation to egg length or absolutely)?
3. How do antennae, mouth parts, legs and cerci develop?