

Exercise 3: Internal Anatomy of the Lubber Grasshopper, *Romalea*

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Systematics

Arthropoda^P, Mandibulata, Hexapoda^{SC}, Insecta^C, Pterygota, Metapterygota, Neoptera, Orthopteroidea^{SO}, Orthoptera^O, Caelifera^{SO}, Acridoidea^{SF}, Romaleidae^F, *Romalea guttata*

Orthoptera

Orthoptera includes 20,000 species of grasshoppers, crickets, locusts, katydids, and their relatives. These are mostly large insects with an enlarged pronotum. The hind femora are large and adapted for jumping. Females have a large ovipositor but male genitalia are not visible externally. Most are herbivores. Romaleidae are the lubber grasshoppers.

Supplies

Dissecting microscope and dissection tools (forceps, scalpel, scissors, probes, needles)
Small dissecting pan or dish (a sardine tin with a wax bottom is ideal)
Living anesthetized, freshly sacrificed, or preserved *Romalea*
Carbon dioxide, chloroform, or ethyl acetate for living specimens
40% isopropanol

Laboratory Specimens

The eastern lubber grasshopper, *Romalea guttata*, is often used as the introduction to insect anatomy. *Romalea* is a large insect, reaching 50 to 75 mm in length, and is easily dissected. It is a good example of general, relatively unspecialized insect anatomy.

Romalea is best studied using anesthetized living, or freshly sacrificed specimens. Preserved animals are much less satisfactory. Unfortunately, the life cycle is such that living adults are not usually available during the academic year when they are needed. These instructions are based on unpreserved specimens and references to color usually do not apply to preserved material.

The lubber grasshoppers (Romaleidae), are mostly found in Central and South America but one species, *R. guttata* occurs in the eastern United States. This species ranges from Tennessee and the Carolinas south to Florida and Louisiana. Within its range its distribution is patchy but where present it may be very common in the summer. *Romalea guttata* is flightless and has reduced wings, so if the specimens provided have complete, functional wings, they are not the eastern lubber grasshopper.

Lubbers are herbivores with chewing mouthparts. Like other orthopterans they have hemimetabolous development. Eggs diapause over winter and hatch in April into miniature glossy black grasshoppers with orange, yellow, or red markings, with a mid-dorsal stripe. Juveniles undergo a series of molts during the summer and in July undergo the final molt and become a winged adult.

The adult is gray, black, and yellow, with pink on the forewings. The hind wings are hot pink with a black border but are usually hidden. Females (about 70 mm) are larger than males (about 55 mm) and, of course, have a short ovipositor which is lacking in males.

External Anatomy

Tagmata

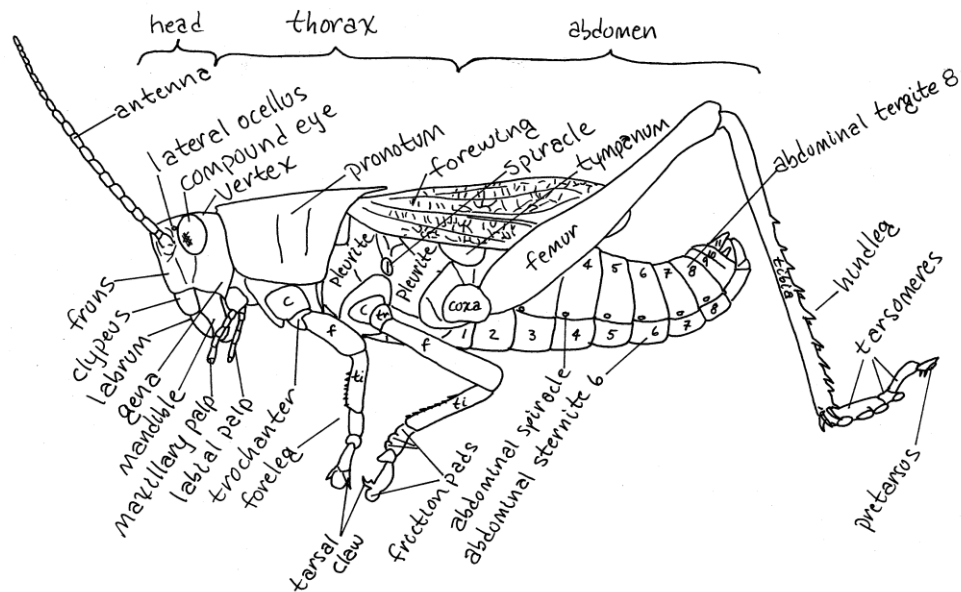
Examine a male or female *Romalea* under magnification. The three tagmata of the insect body are the head, thorax, and abdomen (Fig. 1). The head, which shows few external signs of segmentation,

bears the eyes, antennae, mouth, and mouthparts. Its major functions are sensory reception and feeding. The thorax is larger and bears three pairs of legs and two pairs of wings. The abdomen is the largest tagma and is conspicuously segmented. It houses most of the digestive, excretory, and reproductive viscera and appendages, when present, are specialized for copulation or oviposition. The external genitalia at the posterior end of the abdomen are highly modified segmental appendages.

Head

The head is enclosed in a hard, heavily sclerotized, unsegmented, exoskeletal head capsule, or epicranium. The mouthparts are attached ventrally to the capsule, a condition known as hypognathus. The mouth is located on the ventral surface of the head and is surrounded by the mouthparts. These mouthparts enclose a space, the preoral cavity, from which the mouth opens. The head is more or less ovoid in shape with a nearly vertical anterior surface.

Figure 1. Lateral view of an adult male lubber grasshopper from Greenwood, SC.



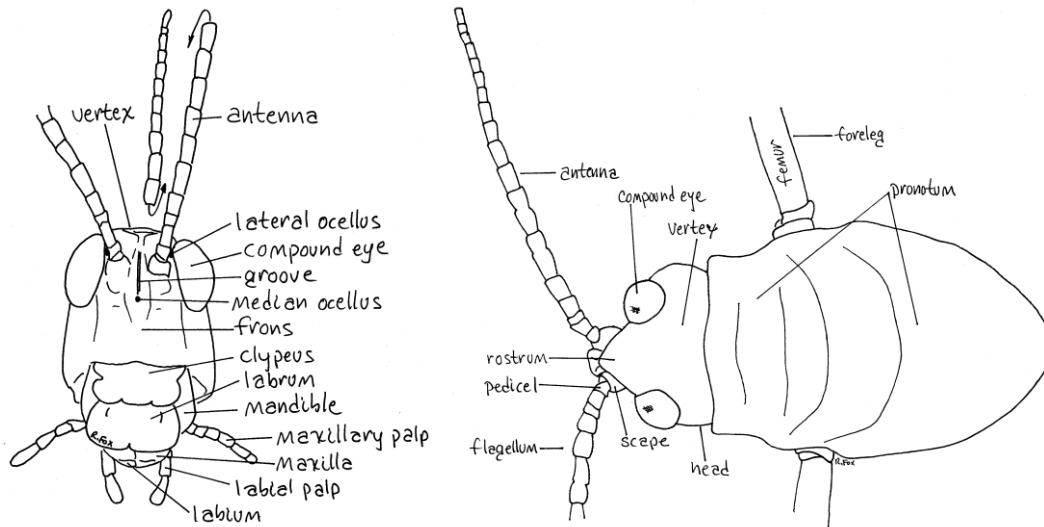
Most of the head is enclosed in a single hard piece of exoskeleton composed of the fused sclerites of the head segments. It covers the dorsal anterior and lateral aspects of the head and is divided into regions (Figs. 1 and 2). The regions are labeled in the Figures; the terms may be of use when identifying insects and these Figures can be used to locate the regions.

A pair of large multiarticulate, sensory antennae is attached in deep sockets on the antero-dorsal corners of the head (Figs. 1, 2, 3). The antenna consists of two basal articles, the proximal scape and distal pedicel, to which is attached the long multiarticulate filamentous flagellum (Fig. 3). The antennae are the first pair of segmental appendages of the insect head.

A large, bulging compound eye is located dorsolaterally on each side of the head just posterior to the base of the antenna (Figs. 1, 2). **Examine the surface of a compound eye** and note that it is composed of an uncountable number of small light-receiving ommatidia. The surface of the eye is a specialized, transparent part of the exoskeleton divided into countless tiny hexagonal cuticular corneas, one for each ommatidium.

In addition to the two compound eyes there are three tiny, simple eyes, or ocelli (Fig. 2). These are not composed of ommatidia. Two of them are lateral ocelli located between the compound eyes and the antennae (Fig. 2). The unpaired median ocellus is located on the anterior midline of the head in a pit on the vertical ridge between the two antennae (Fig. 2).

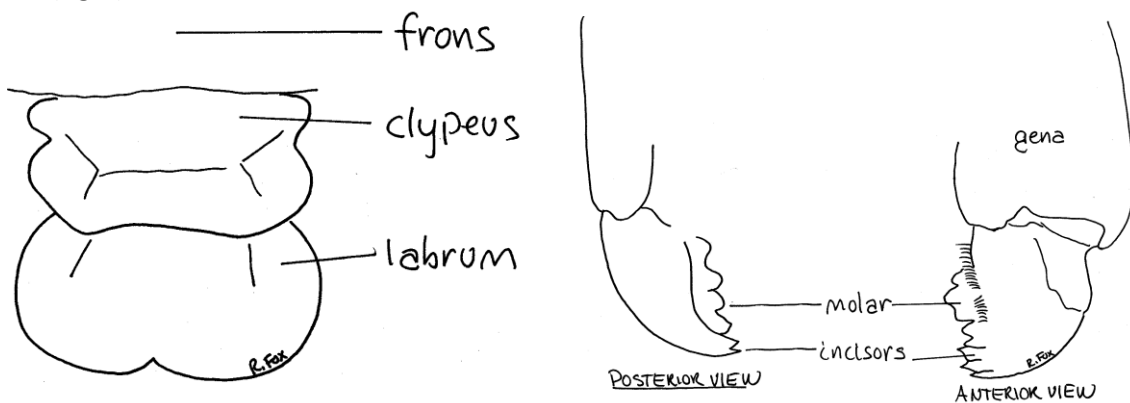
Figure 2 (left). Front view of *Romalea*. **Figure 3 (right).** Dorsal view of the head and prothorax of a female *Romalea*.



The labrum, or upper lip, is also located on the vertical anterior face of the head capsule (Figs. 1, 2, 4). It is a large movable plate, equipped with muscles, ventral to the clypeus. It bears a small median notch on its ventral border and a median groove on its anterior surface. The labrum covers the more posterior mouthparts. **Lift the labrum to demonstrate its mobility** and to reveal the preoral cavity; we'll return to this shortly.

Immediately posterior to the labrum is a pair of mandibles (Figs. 1, 2, 5). In grasshoppers these massive mouthparts are adapted for biting and chewing. Their heavily sclerotized and strongly toothed median surfaces are apparent when the labrum is moved aside.

Figure 4 (left). En face view of the clypeus and labrum of *Romalea*. **Figure 5 (right).** Anterior (left) and posterior (right) views of the left mandible of *Romalea*.

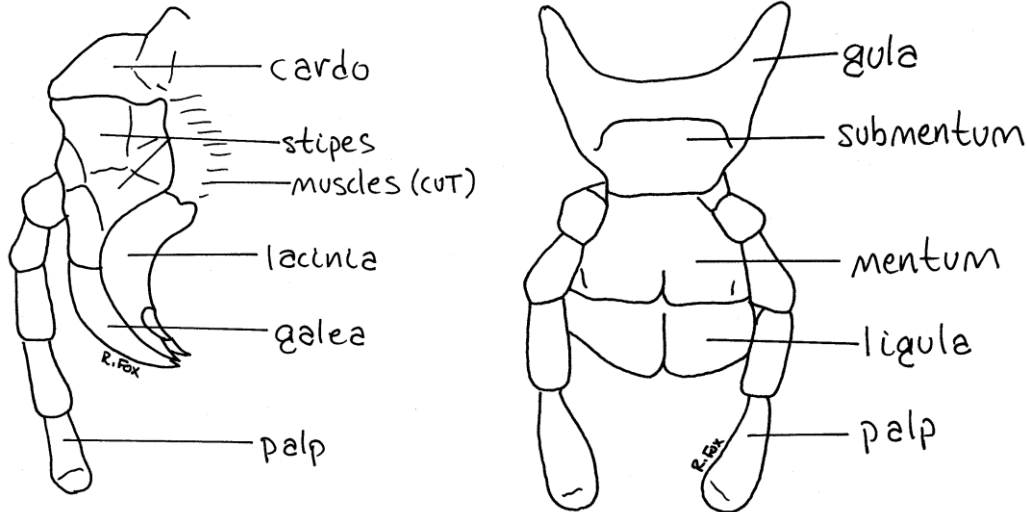


The toothed, median, cutting surface of the mandible includes a distal (ventral) incisor of sharp shearing teeth and a proximal molar of heavier grinding teeth (Fig. 5). **You can see these by lifting the labrum.**

The mandible articulates with the ventral edge of the posterior epicranium (gena). The mandibles lie on either side of the mouth and are the sides of the preoral cavity from which the mouth opens dorsally. Mandibles are operated by powerful muscles with the motion entirely in the transverse plane. Grasshopper mandibles are dicondylic, meaning they articulate at two hinges, thus limiting the

range of motion to a single plane. A monocondylic articulation, on the other hand, would be a ball and socket joint with a much greater range of motion. **Grasp a mandible with fine forceps and move it to demonstrate its motion.**

Figure 6 (left). The left maxilla of *Romalea*. **Figure 7 (right).** The labium of *Romalea*.



Posterior to the mandible are paired maxillae (Figs. 1, 6). Each maxilla consists of two basal articles. A large, conspicuous, filamentary, and multiarticulate maxillary palp also arises from the stipes but on its lateral side (Figs. 1, 6). The last pair of head appendages is fused to form the labium, or lower lip (Figs. 1, 7). The labium is easily seen in its entirety by looking at the posterior surface of the ventral head. It forms the posterior boundary of the preoral cavity. The regions of the labium are shown in Fig. 7. The lateral borders of the mentum each bear a filamentous, multiarticulate labial palp.

Push the labium posteriorly and look into the preoral cavity. The labium forms its posterior wall. The anterior wall is the labrum. The mandibles and maxillae are the sides. Inside the cavity you will see the large hypopharynx extending from the ventral wall of the head immediately posterior to the mouth. It is an unpaired fold of the body wall and is not a segmental appendage. The mouth lies between the base of the hypopharynx and the labrum.

Thorax

The heavily sclerotized head and thorax are connected by a short narrow neck (Fig. 1), whose integument is lightly sclerotized and consequently flexible. The thorax consists of three fused segments, each of which bears a pair of legs. Two of the thoracic segments typically bear a pair of wings, which are complex folds of the body wall and are not segmental appendages. Wings, which are present only in adults, are characteristic of most insect orders.

The three thoracic segments are, from anterior to posterior, the prothorax, mesothorax, and metathorax (Fig. 1). The meso- and metathoraces are fused rigidly to form an inflexible box housing the flight muscles and bearing the wings. Together they are referred to as the pterothorax in reference to the wings they bear (ptero = wing).

A typical arthropod segment is enclosed in four exoskeletal plates, or sclerites, that form a ring around the segment. These are the dorsal tergite, ventral sternite, and two lateral pleurites. These sclerites are typically joined together by flexible articulating membranes but sometimes they are fused rigidly together. This primitive pattern often undergoes some modification in modern arthropods, including grasshoppers.

Prothorax

The grasshopper prothorax is covered by a large shieldlike tergal plate called the pronotum (Figs. 1, 3). (The tergites of the thorax are called nota, rather than tergites.) The pronotum covers the prothorax and partly overhangs the mesothorax. The prosternite, located ventrally, is much smaller and bears a conspicuous median prosternal spine.

The prothoracic legs (Fig. 1) articulate with the prothorax. The pleurites of the prothorax, the propleurites, are small and located between the prosternite and the pronotum. Together the prosternite and propleurites form sockets for the articulation of the forelegs. **Note the flexible, unsclerotized cuticle forming an articular membrane between the base of the leg and the socket. Rotate the leg around the articulation to demonstrate its mobility.**

Each body segment bears a pair of lateral openings, or spiracles, for gas exchange. One such spiracle is found on each side of the prothorax. It is located laterally near the border between the prothorax and mesothorax in the soft unsclerotized cuticle under the posterior edge of the pronotum. **Try to find it.** The prothoracic spiracles are difficult to see because they are hidden by the pronotum. The more posterior spiracles are easier to locate.

Pterothorax

The pterothorax comprises the meso- and metathorax. Each segment bears a pair of wings and a pair of legs. The wings of the mesothorax are the forewings (Fig. 1), and those of the metathorax are the hindwings. The mesothorax bears the middle legs (Fig. 1), or mesothoracic legs. The metathorax bears the hindlegs, or metathoracic legs.

Dorsally the two segments of the pterothorax are covered by two sclerotized tergites known as the mesonotum and metanotum. Similarly the ventral surfaces are protected by the mesosternite and metasternite. The pleurite on each side of both pterothoracic segments is divided into two smaller plates. The legs articulate between the ventral edge of the pleurites and the sternites. The wings arise between the dorsal edge of the pleurites and the nota (tergites).

A spiracle is situated laterally between the pleura of the metathorax and mesothorax (Fig. 1). **Note that its opening is guarded by two movable valves.** The opening is the slit between the two valves.

Legs

Study one of the middle legs. This appendage, typical of insect legs, is composed of a linear series of units, or articles. Each article consists of a sclerotized exoskeletal cylinder which contains muscles, tendons, blood, connective tissue, and nerves. **Note that while most of the limb exoskeleton is rigid and heavily sclerotized, some parts are flexible and unsclerotized.** The unsclerotized regions are the movable articulations between successive articles. **Look at some of the articulations under magnification and find these flexible articular membranes.**

The first (proximal) article of the leg is the **coxa** (Fig. 1). It is short, wide at the base, and narrows distally. Articulated with the distal end of the coxa is the short, cylindrical **trochanter**. The trochanter is fused immovably with the long **femur**. The femur is one of the two long articles of the leg. The **tibia**, which is the second of the long articles, articulates with the distal end of the femur.

Pay particular attention to the hard parts of the articulation between the femur and tibia. They form a dicondylic hinge joint similar to that in your elbow and knee (but exoskeletal). Such a joint restricts motion to a single plane. The tibia is long and slender with teeth on its margins.

Next in line along the leg is the **tarsus** composed of tarsomeres (Fig. 1). The number of tarsomeres varies among insects. The proximal tarsomere bears three soft, unsclerotized friction pads on its surface to prevent slipping of the tarsus on the substratum. The second tarsomere is much shorter and has a single friction pad. The final article of the leg is the **pretarsus**. It extends from the distal end of the tarsus and bears a pair of claws and a single soft friction pad.

The forelegs are nearly identical to the midlegs but the hindlegs are much larger. They make possible the characteristic jumping locomotion of grasshoppers. The large coxa is fused with the trochanter and the femur and tibia are much elongated. In addition, the femur is expanded to accommodate the jumping muscles, giving it a club or drumstick shape.

Wings

The two pairs of wings are evaginations or folds of the cuticle and body wall of the two segments of the pterothorax. Each wing is a double layer of body wall consisting largely of cuticle. The characteristic "veins" of the wings are thickened tubes of exoskeleton involved in support. Their primary function is structural but they contain epidermis, nerves, and blood. The hindwings of the flightless *Romalea guttata* are small and are not used for flight. The forewings of all grasshoppers, including *Romalea*, are heavy and form a pair of wing covers to protect the more delicate hindwings.

Lift and spread the wing covers (forewings) and examine them with magnification. Note the heavy longitudinal veins and the abundance of small, branching and anastomosing veins. Make a similar examination of the hindwings.

Abdomen

The grasshopper abdomen consists of 11 segments and extends posteriorly from the thorax (Fig. 1). It is the largest of the tagmata and most of its segments have no appendages. Its segmentation is obvious externally.

Numbered from anterior to posterior, abdominal segments 1-7 are similar to each other and are unspecialized insect segments. Segments 8-9 are reduced and modified and may bear appendages. These are the genital segments and may have appendages modified as external genitalia. Segments 10-11 are the postgenital segments.

Each segment is covered by a large dorsal tergite and a ventral sternite (Fig. 1). Eleven tergites are present but several of the posterior segments lack sternites and, as a consequence, there are only eight sternites in females and nine in males (Figs. 7, 8). The pleurites are reduced and fused to the ventral edges of the tergites.

The first abdominal segment is fused rigidly with the thorax. Its tergite is mostly hidden by the base of the hindwings. Each side of tergite 1 bears a large aperture covered by a thin cuticular tympanic membrane (= tympanum or eardrum). Find this structure. These are auditory organs for sound transduction. Some species of lubber grasshoppers have stridulating organs with which they produce sound. The spiracle of the first abdominal segment is located on the anterior edge of the tympanum. This spiracle opens into a large air sac attached to the inner surface of the tympanum.

The posterior abdominal segments are associated with the external genitalia and differ from other segments and between males and females. The large anus is on segment 11 ventral to tergite 11 (tergite 11 is the epiproct). It is covered by the single epiproct and flanked by two paraprocts. Exchange specimens with another student in order to study the opposite sex.

Female

Externally, female segment 8 is similar to the preceding segments except that its sternite is known as the genital plate (Fig. 8, 9). The egg guide is a triangular, median process of the genital plate. Segments posterior to 8 are reduced and lack sternites. The tergites of segments 9 and 10 are reduced and fused laterally although they remain separate dorsally (Fig. 8). The 11th tergite, or epiproct, forms a triangular dorsal shield rather than an arch over the body. Its pointed end projects posteriorly and covers the anus. Associated with it is a lateral paraproct on each side, ventral to the epiproct.

Between the 11th tergite and each of the paraprocts is a small cercus. The cerci are the appendages of segment 11. In some orthopterans, such as crickets, they are long antenniform sensory

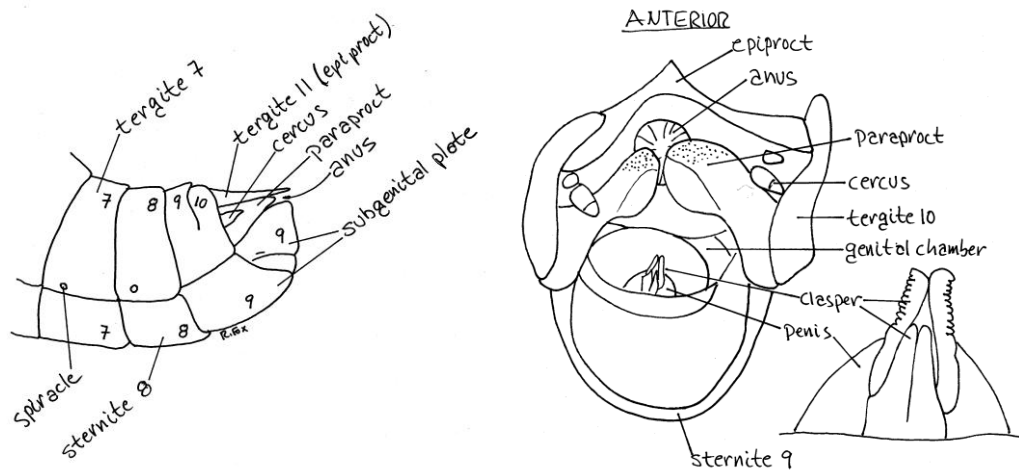
organs, but in *Romalea* they are very short. Female external genitalia are modified segmental appendages belonging to segments 8 and 9. Together they form the short robust ovipositor extending posteriorly from the abdomen. **Find as many of these structures as you can.**

Figure 8 (left). Lateral view of female abdomen. **Figure 9 (right).** Posterior view of the external genitalia of a female *Romalea*. The first and third valvulae are reflected ventrally and dorsally, respectively.



The ovipositor consists of three pairs of processes, called valvulae, which are used by the female to insert eggs into the ground. The first and third valvulae are conspicuous externally. The inconspicuous, membranous second valvulae (= inner valves) are hidden from view by the first and third and will not be seen. The gonopore lies between the first valvulae (Fig. 9). The first and third valvulae together form a hollow shaft that is used to penetrate the soil. The eggs pass from the gonopore, through the lumen of the shaft, assisted by the second valvulae, and are deposited in masses in the soil.

Figure 10 (left). Male abdomen in lateral view. **Figure 11 (right).** Posterior view of the external genitalia of a male *Romalea* with the epiproct and sternite 9 reflected to reveal the penis. A magnified view of the tip of the penis is shown on the right.



Male

The male posterior abdomen is simpler than that of the female (Figs. 10, 11). Segment 8 resembles 2-7 and has an unmodified sternite. Tergites 9-10 are fused ventrally and separate dorsally as

in females. Segment 9 possesses a large sternite, the subgenital plate, which extends ventrally to segments 10-11. It forms a cup enclosing the male external genitalia. Protected and hidden by the plate is the soft (largely unsclerotized) eversible penis (= aedeagus). Although referred to as "external" genitalia, the penis is not visible externally without moving the paraprocts and subgenital plate aside. Segment 11 is similar to that of females and has a dorsal tergite (= epiproct), lateral paraprocts, and short cerci. The anus is under the epiproct and between the paraprocts. **It can be seen by lifting the epiproct. Find the structures described here.**

The penis consists of a soft, eversible, bulbous base bearing claspers at its distal end (Fig. 10). It contains an extension of the ejaculatory duct through which spermatophores pass during copulation. **Pin the posterior tip of sternite 9 (subgenital plate) to the tray. Lift the epiproct and pin it.** The space revealed is the genital chamber. Associated with the penis are dark, hard, sclerites. The male gonopore opens on the midline at the tip of the penis and in *Romalea* it is flanked by two pairs of slender, dark, sclerotized valves that hide it from view.

Internal Anatomy

The study should be conducted with the specimen immersed in fluid (tapwater or 40% isopropanol) and with the aid of the dissecting microscope. **Place a specimen in a small dissecting pan with the dorsal surface up. Push the middle legs flat against the dissecting pan and anchor them in place with an insect pin through each femur. Insert a single pin through the posterior end of the abdomen.** This should be sufficient to secure the animal during the dissection. Use scissors to remove the wings.

Use scissors to make a transverse cut across the pronotum to remove its posterior overhang. Now, slip the point of fine scissors under the posterior edge of abdominal tergite 8, about 2 mm to one side of the dorsal midline, and cut anteriorly until you have a longitudinal dorsal incision beside the midline. Extend the cut anteriorly to the posterior edge of the truncated prothorax. Cut through the meso- and metanota were formerly covered by the pronotal overhang. The cut should be completely through the exoskeleton but no deeper. Avoid cutting the soft tissue under the tergites and the tympanum. Make a similar incision on the other side of the animal. Extend both incisions posteriorly by inserting the scissors under the anterior edge of tergite 9 and cutting posteriorly to the epiproct.

Use fine forceps and fine scissors to separate the middorsal strip of exoskeleton from the underlying soft tissues and remove it. Try to remove the separated pieces of tergite while leaving as much soft tissue as possible behind and intact. If you are careful, the heart will remain with the body. The large perivisceral sinus with the gut should not be visible yet. Instead you will see only the much smaller cavity, the pericardial sinus enclosing the heart.

During the dissection particles and debris will accumulate in the fluid of the dissecting pan and obscure your view of the specimen. **When this occurs make sure everything is securely pinned to the wax of the dissecting pan and then gently pour the fluid and particles into the sink. Replace the fluid and continue.**

Hemal System

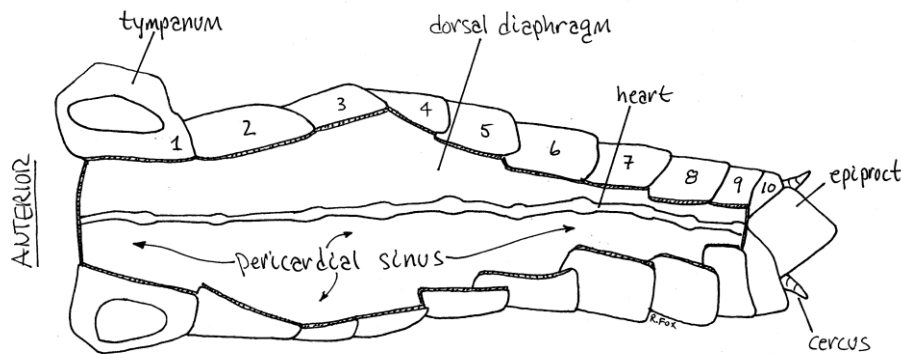
A successful dorsal dissection first reveals the heart in the pericardial hemocoel. These features must be destroyed after study to reveal the remainder of the hemocoel and its organs.

The arthropod body cavity is the hemocoel, divided into three longitudinal sinuses by two horizontal diaphragms. The largest of the three is the perivisceral hemocoel, which contains most of the viscera. The smaller pericardial hemocoel lies dorsal to the perivisceral and surrounds the heart. Another small hemocoel, the perineural, lies ventral to the perivisceral sinus. The heart is a transparent, slender, longitudinal tube extending the entire length of the perivisceral hemocoel just ventral to the terga. It bears paired segmental ostia and is equipped with alary muscles to expand its lumen in diastole. Its walls contain circular muscles whose function is to constrict its lumen during systole.

You should see a transverse membrane, the dorsal diaphragm, which separates the relatively small, dorsal pericardial hemocoel from the much larger, more ventral perivisceral hemocoel. Both are filled with colorless blood. The diaphragm will have been destroyed if the initial scissors cuts were too deep.

The heart is an inconspicuous transparent tube on the dorsal midline of the abdomen and thorax (Fig. 12). It is closely attached to the dorsal diaphragm but is just ventral to the dorsal tergites and thus is easily lost when the exoskeletal strip is removed. Look for a narrow longitudinal tube adhering to the dorsal surface of the diaphragm exactly on the midline. Carefully tease away adhering tissue as necessary to improve your view of it. Also present are tubular, branching, silvery tracheae, which should not be confused with the heart. Only the heart lies on the midline and the longitudinal tracheae are lateral to it and the occasional transverse tracheae cross it. Paired, segmental alary muscles extend from the heart to the body wall and diaphragm. The muscles fan out to form a thin layer over the upper surface of the dorsal diaphragm. The heart has inconspicuous segmental swellings equipped with paired, segmental ostia. The swellings are apparent but the ostia will not be seen.

Figure 12. Dorsal dissection of the abdomen of a male *Romalea* showing the pericardial sinus and heart. Abdominal tergites are numbered.



Perivisceral Hemocoel and Viscera

When you have finished your study of the abdominal hemal system, perivisceral hemocoel, and external genitalia, remove the legs from both sides by cutting across their trochanters or coxae with heavy scissors. Use fine scissors to make a middorsal longitudinal incision through the dorsal diaphragm of the abdomen. This incision should be just deep enough to cut through the thin diaphragm and should not damage the organs in the underlying perivisceral sinus. Pin the abdominal walls aside as you go. Note the large white air sacs of the tracheal system under the tympana in the anterior abdomen and thorax. Note also the yellow, amorphous fat body lying beneath the diaphragm throughout the hemocoel.

Upon reaching the thorax, use the medium scissors to remove the heavy dorsal and lateral thoracic sclerites in pieces. Use the medium scissors to cut around a piece, then use fine scissors to cut the muscles holding the piece in place. Use heavier scissors to make the thoracic incision. As you cut through the thoracic sclerites, note and then cut as necessary, the massive flight muscles contained within it. Many of them originate on thoracic nota and must be cut or removed before the sclerites can be removed. Pin the walls of the thorax aside.

Without further dissection, make a preliminary inspection of the interior of the animal. The cavity occupying most of the interior is the perivisceral hemocoel. It is not true coelom. You will see numerous silvery, branching tubes of the tracheal system. Some of them are expanded to form air sacs. The yellow fat bodies may obscure your view of the viscera. A slender, transparent, longitudinal,

middorsal muscle extends the length of the abdomen just dorsal to the chief fat body. In reproductive individuals gonads will occupy much of the space in the abdomen. The regionally specialized gut extends lengthwise from the anterior mouth to the posterior anus but is presently covered by fat body and gonad so that little of it is visible. The ventral diaphragm, usually covered by yellow fat body, forms the floor of the perivisceral hemocoel and separates it from the perineural hemocoel.

Fat Body

The fat bodies extend throughout the hemocoel but are most prevalent in the abdomen. These are sites of intermediary metabolism with a function similar to that of vertebrate liver. Usually they are arranged in two sheets or amorphous masses, one near the body wall and the other near the gut, and are supported by connective tissue. In *Romalea* the fat bodies are thin connective tissue sheets supporting small yellow lobes. The lobes are masses of trophocytes which are bathed in the blood of the hemocoel. The most conspicuous fat body is a sheet covering the surface of the gut in the anterior abdomen. **Locate the fat bodies.**

Respiratory System

The respiratory system, consisting of 10 pairs of spiracles (two thoracic, eight abdominal), a network of branching tubular tracheae, and several air sacs, will be gradually destroyed as you study the perivisceral hemocoel. **As you study these systems note the profusion of silvery-white tracheae extending to the organs.** Each spiracle on the body surface opens into the system of interconnected tubular trachea.

The tracheal system includes several large, silvery, bladder-like expansions called air sacs. Their walls are not reinforced and consequently are expansible. They function in ventilation. In addition to its respiratory role, the tracheal system is important structurally and functions like connective tissue to support the tissues it pervades. The gut, as you will soon see, is a good example of an organ system supported by tracheae.

Digestive System

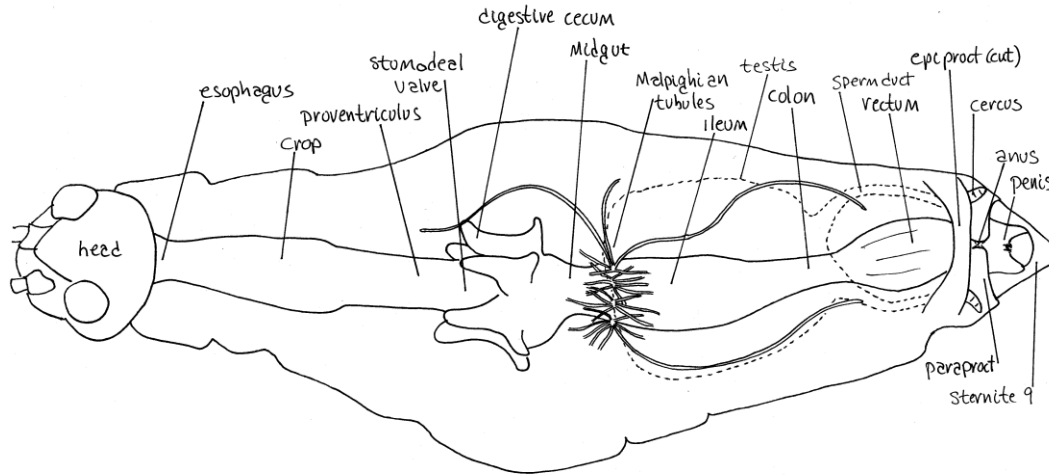
Remove the fat body from the dorsal hemocoel to reveal the gut under it. Find the large gonad covering the posterior regions of the gut in the posterior hemocoel (Fig. 15). Remove the fat body covering the gonad then use fine scissors to free the gonad from its anterior connections with the remaining viscera. Note that most of these connections are tracheae. Locate the gonoducts (oviduct or sperm duct) exiting the gonad laterally. Cut the duct on the left so the gonad can be reflected to the right, thereby revealing the posterior gut but keeping the gonad intact for later study.

Like that of other arthropods, the gut consists of anterior ectodermal foregut, middle endodermal midgut, and posterior ectodermal hindgut. The foregut consists of mouth, pharynx, esophagus, crop, and proventriculus and is lined by a cuticle secreted by its epidermal epithelium. The gut begins, of course, with the mouth which opens from the preoral cavity but it cannot be seen from your present vantage point inside the hemocoel. The mouth opens into the short, narrow pharynx, which is entirely contained within the head capsule and is also not visible at present.

The pharynx leads to the esophagus, which is largely confined to the head but extends a short distance into the prothorax (Fig. 15). Posterior to the esophagus the gut widens to become the crop. The separation between esophagus and crop is not distinct on the exterior but will be obvious later when you open the gut. Next, the gut narrows again as it becomes the proventriculus.

The proventriculus, the last region of the foregut, ends where it joins the short midgut. An elaborate valve, visible only from inside the gut, marks the separation of the fore- and midguts. Protruding conspicuously from the periphery of the midgut are six hollow diverticula, the digestive caecae. **Find these structures.**

Figure 15. Dorsal dissection of a male *Romalea* showing the digestive system. The dorsal diaphragm and heart have been removed, most of the Malpighian tubules and most of the epiproct has been removed. The few Malpighian tubules in the drawing are shown much shorter and much less numerous than in life. The testis has been removed but its position is indicated by a dashed line. The ovary occupies a similar position in females.



The remainder of the gut is hindgut, which accounts for about half the length of the gut. Internally, a valve marks the midgut-hindgut junction. The junction of midgut and hindgut is marked externally by clusters of long slender Malpighian tubules (Fig. 15). These form a tight mass in the vicinity of the junction but also extend anteriorly and posteriorly. The hindgut consists of three regions. First is the large ileum followed by the short, narrow colon. The longitudinally ridged rectum is the final region and it opens via the anus to the exterior. Like the foregut, the hindgut is lined by epidermis, which secretes a cuticle. Use fine scissors to open the entire length of the gut from esophagus to anus with a longitudinal incision.

Excretory System

Locate the chief insect excretory organs, the Malpighian tubules, which arise at the midgut-hindgut junction (Fig. 15). These are long, slender, hollow diverticula of the gut lumen. In *Romalea* the tubules are very numerous and very long. Nitrogenous breakdown products of protein metabolism, mostly ammonia, are released into the hemocoel, converted to uric acid in the fat body. The uric acid is then absorbed by the tubules, precipitated, and released into the hindgut where it becomes part of the feces.

Reproductive System

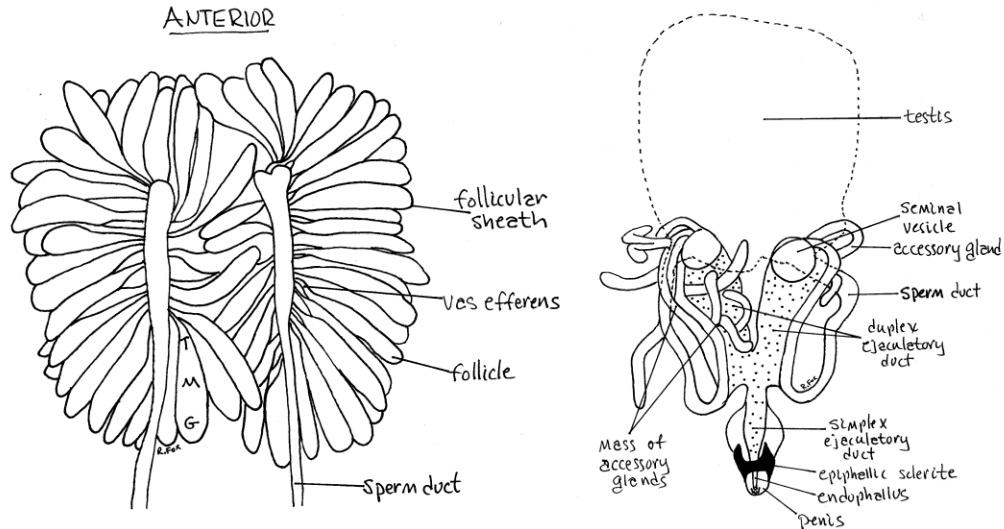
Study both male and female reproductive systems by sharing specimens. If you have followed instructions, the gonad will be displaced to the right but will be intact and available for study.

Male

The male system begins with a pair of dorsal testes (Fig. 15, 16). The two testes are joined together over the dorsal midline and appear to be a single testis. A sperm duct exits the posterior lateral corner of each testis. The ducts are wrapped in fat body and are easy to miss. Each duct extends laterally and posterior to disappear under the gut.

From the ventral surface each testis can be seen to consist of numerous elongate sperm tubes draining into a central white sperm duct (Fig. 16). Each follicle is a blind ending sac lined with epithelium and connecting with the sperm duct.

Figure 16 (left). The testes of a male *Romalea* in ventral view. G = germarium, M = zone of maturation, T = zone of transformation. **Figure 17 (right).** Dorsal view of the reproductive system of a male *Romalea*. For clarity, the testis is shown as a dashed line. Most of the accessory glands have been omitted. The ejaculatory duct is stippled.



Each sperm duct curves ventrally around the gut to join the ejaculatory duct which leads to the penis and gonopore (Fig. 17). **Cut across the gut in the middle of the ileum, free the posterior gut from the body wall, and pin it aside to reveal the ventral regions of the reproductive system. Trace the sperm ducts around the rectum to the ejaculatory duct.** Upstream the ejaculatory duct is double but they join each other distally to form a single duct that enters the penis. Associated with each ejaculatory duct is a bright white seminal vesicle for sperm storage. Numerous tubular accessory glands extend from the duplex ejaculatory ducts and form a loose mass around the duct. Some of the accessory glands are bright white and some are transparent. Some are long and some short. Some extend away from the mass.

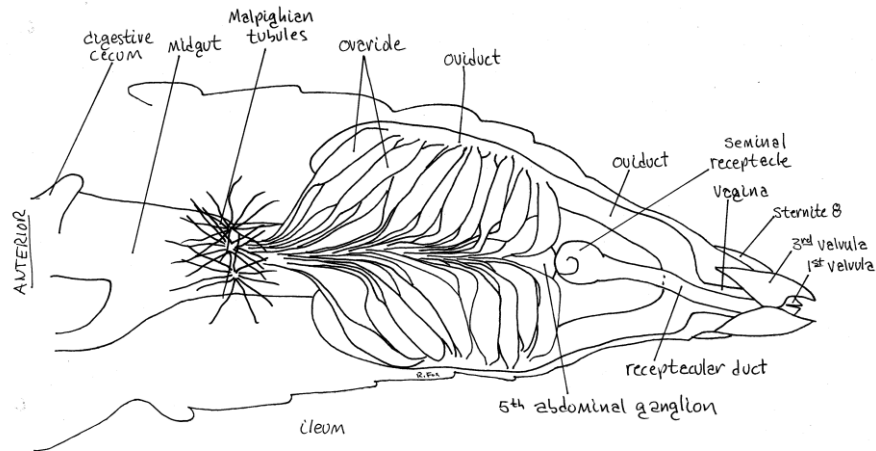
Female

The female reproductive system consists of two ovaries, two oviducts and a common vagina opening by the gonopore between the two first valvulae (Figs. 9, 18, 22). In addition, an independent seminal receptacle opens to the exterior via a separate duct. Sperm stored in the seminal receptacle are used to fertilize the eggs during oviposition.

The two ovaries are the most conspicuous feature of the female system because they are large, bright yellow, and most dorsal. They are located in the posterior abdomen, dorsal to the gut and ventral to the dorsal diaphragm. **You need remove only the investing layer of fat body and tracheae to reveal them.** Each is a cluster of tubular ovarioles, similar to the testicular follicles, emptying into a lateral gonoduct. The two ovaries of reproductively mature females are large and occupy, along with the fat body, most of the space in the dorsal posterior abdomen, between the dorsal diaphragm and the gut (Figs. 15, 22).

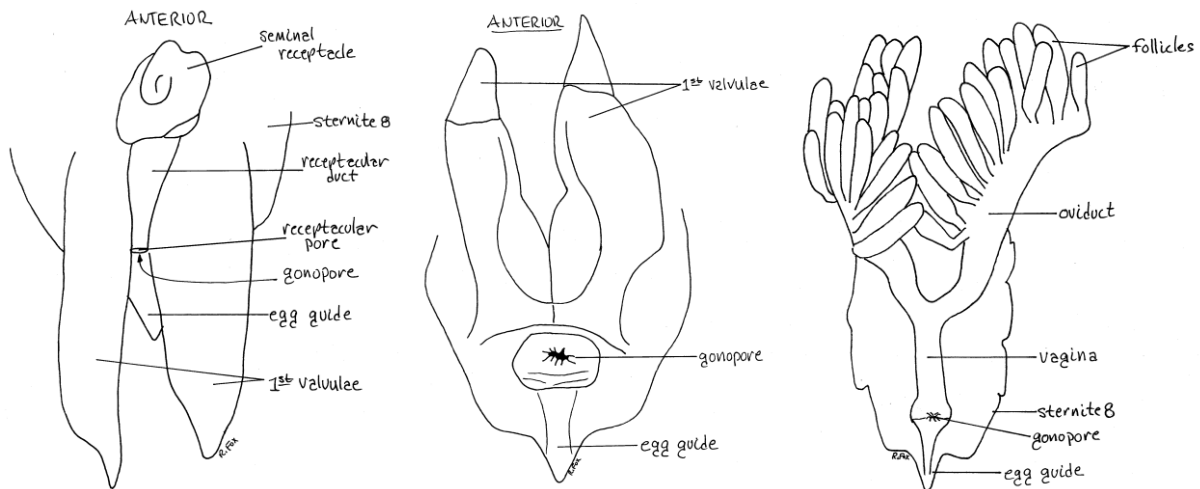
Mature eggs in the ovaries are bright yellow and, since they account for most of the volume of the ovary, impart their color to these organs. Immature eggs are white. The fat body around the ovaries is also yellow and care must be taken that the two are not confused. **Remove as much of the fat body as possible from the ovary.** Each ovary is composed of numerous tubes, known as ovarioles (Figs. 18, 19), homologous to the sperm tubes. Ovarioles drain into a lateral oviduct, which extends along the outside border of the ovary.

Figure 18. Dorsal view of the reproductive system of a female *Romalea*. Malpighian tubules are drawn much shorter and less numerous than in life.



The two oviducts depart from the posterior lateral corner of their respective ovaries and curve ventrally around the gut to join on the midline ventral to the gut and form the single vagina. Observation of the vagina must be postponed, however, in order to find and study the seminal receptacle which is dorsal to it.

Figure 20 (left). Dorsal view of the posterior end of a female *Romalea*. The epiproct, paraprocts, and third valvulae have been removed and the first valvulae are in their normal position, revealing their dorsal surface. The gonopore is ventral to the receptacular pore, between it and the egg guide. **Figure 21 (center).** Dorsal view of the posterior end of a female *Romalea*. The epiproct, paraprocts, and third valvulae have been removed and the first valvulae reflected dorsally to reveal their ventral surface and the gonopore. **Figure 22 (right).** Dorsal view of the female reproductive system. The gut, seminal receptacle, epiproct, paraprocts, and valvulae have been removed to reveal the vagina.



Cut across the posterior rectum where it joins the anus and pull the intestine forward, between the two ovaries and out of the field of view. This will reveal the last ganglion of the ventral nerve cord and the seminal receptacle. The receptacle is nestled against the curved posterior border of this ganglion (Figs. 9, 18). Try to avoid damaging the ganglion as the nervous system has not yet been studied. Remove the third valvulae (Fig. 20). The gonopore is at the base of a fingerlike, sclerotized process on sternite 8, the egg guide, located ventrally in the genital chamber (Fig. 9). The spermathecal pore is a little dorsal to it.

Remove the seminal receptacle, its duct, and the first valvulae. Trace the lateral oviducts from the ovaries posteriorly toward the midline where they join to form the vagina (Figs. 18, 22). The vagina is ventral to all other organs in the posterior hemocoel.

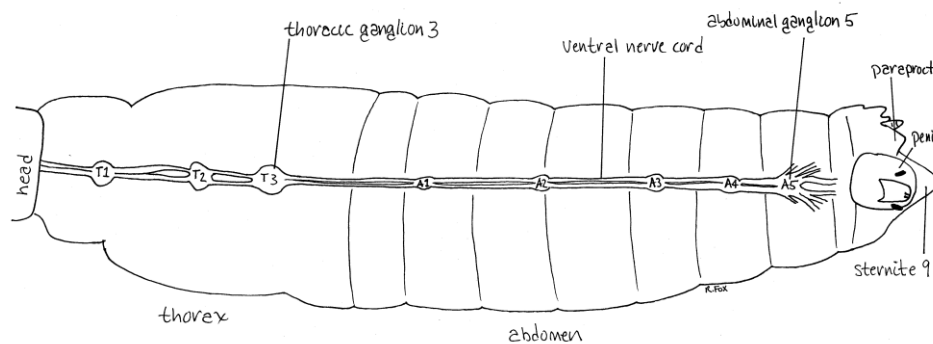
Nervous System

The nervous system is divisible into the central nervous system (CNS) consisting of the brain and ventral nerve cord, and the peripheral nervous system (PNS) consisting of sensory and motor neurons connecting the CNS with the tissues. The PNS has somatic and visceral components but will not be studied in this exercise. The CNS consists of a ventral nerve cord in the thorax and abdomen and a dorsal brain in the head.

Ventral Nerve Cord

Remove the reproductive system and discard it. Cut across the esophagus and remove the gut so you can see the floor of the perivisceral sinus. The floor is the ventral diaphragm. Through it you can see the perineural hemocoel and the double ventral nerve cord. The nerve cord is a white band immediately inside the diaphragm and adheres to it. The fat body covers its dorsal surface. Carefully remove the ventral diaphragm and fat body from the nerve cord over the entire length of the abdomen and thorax. In the thorax the massive flight muscles must also be removed. The abdominal region of the cord is easily revealed but considerably more digging is required to find the thoracic region.

Figure 24. Dorsal dissection of the postcranial nervous system of a male *Romalea*. Heart, fat bodies, dorsal and ventral diaphragms, and gut have been removed.



In the thorax removal of the fat body and ventral diaphragm will reveal the salivary glands, each of which consists of numerous white spherical follicles arranged in clusters, rather like clusters of grapes. Where the salivary glands obscure the ventral nerve cord they must be removed.

The ventral nerve cord is conspicuously double and connects a chain of ganglia on the floor of the perineural sinus (Fig. 24). The ganglia consist of three large thoracic ganglia, T1-T3 and five smaller abdominal ganglia, A1-A5. Notice the abundant nerves entering and leaving the thoracic ganglia in contrast with the relatively few of the abdominal ganglia. Consider why this is so.

Brain

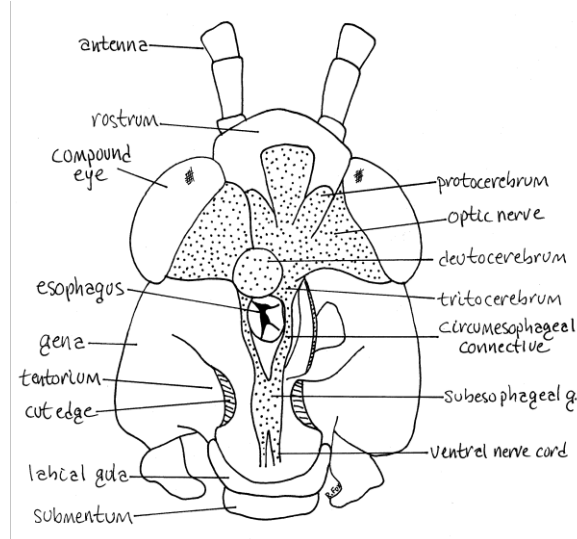
Dissection of the head capsule to reveal the brain is optional. The brain is well protected in the heavily sclerotized cranium and exposing can be difficult. Use medium scissors and forceps to open the cranium. Do not attempt to cut or manipulate the hard cranium with delicate scissors. It will ruin them.

Cut across the esophagus and remove it. Cut across the posterior cervix to separate the head from the body. Look into the severed cervix and find the foramen magnum. Find the severed ventral nerve cord where it enters the foramen magnum. Find the severed esophagus. Notice the abundant muscles filling the head capsule. These are responsible for operating the head appendages, especially

the mandibles. The brain is dorsal to the esophagus.

With medium scissors make two longitudinal cuts anteriorly through the cranium from the foramen magnum, one on each side of the vertex. Make the cuts as shallow as possible to avoid damage to the brain. Extend these incisions anteriorly dorsal to the eyes to the frons. Remove the strip of cuticle thus freed. The space opened is filled with muscles and the esophagus. Insert an insect pin through the labium into the wax to hold the head in place. Cut away the posterior portion of the genae. Use fine forceps to remove the muscles in the posterior head. Do not remove the esophagus, brain, or nerve cord. The muscle has a fibrous texture lacking in nerve tissue. Nerve tissue is yellowish, muscle is dull white.

Figure 25. Dorsal dissection of the head capsule of *Romalea* with the vertex and mandibular muscles removed to reveal the brain. The right deutocerebrum is omitted for clarity. The esophagus is cut transversely. The nervous system is stippled.



As the muscles are removed the brain will gradually come into view (Fig. 25). It consists of the three major paired ganglia of the mandibulate brain which make up the three brain regions. These are the protocerebrum, deutocerebrum, and tritocerebrum, of which the deutocerebrum is most dorsal. A large optic nerve extends laterally from each protocerebrum to the nearby compound eyes. A pair of circumesophageal connectives extend from the tritocerebrum around the esophagus to unite at the subesophageal ganglion ventral to the esophagus. The paired ventral nerve cord extends posteriorly from the subesophageal ganglion. Remove the soft tissue of the cervix, including its cuticle. Remove all non-nervous tissue surrounding the esophagus, including the medial mandibular apodeme. This will expose a heavy transverse ventral bar of cuticle just ventral to the esophagus. This is the central body of the tentorium, an internal structural brace of the head capsule. The ventral nerve cord enters the head capsule ventral to the tentorium. The subesophageal ganglion is immediately dorsal to the central body of the tentorium. The latter must be removed to reveal the ganglion and the circumesophageal connectives. The subesophageal ganglion represents the combined ganglia of the mandibles, maxillae, and labium.