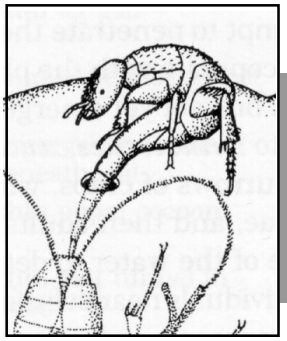


ENTOMOLOGY 322 LABS 22 & 23
External Male Genitalia

Sperm transfer in hexapods is primitively via indirect methods. Males of Collembola, Diplura, Protura, and Thysanura deposit spermatophores on the substrate and females later pick them up. While bizarre and interesting courtship dances occur in these groups (reviewed and illustrated in Schaller 1971), it was not until the evolution of the pterygotes that direct sperm transfer evolved. Direct sperm transfer has advantages in dry habitats where spermatophores are vulnerable to dessication and has advantages for males in terms of assured paternity.

In the majority of pterygote orders, the organs related to direct sperm transfer (intromittent organs) are formed from modifications of the 9th and 10th abdominal segments and/or their appendages (see below). However different systems have evolved in a variety of insect and arthropod groups and there is no single widely accepted terminology that can be applied across all insect orders. One reason for this is that male genitalia are extraordinarily diverse in form and function, implying extraordinarily rapid rates of morphological evolution. A number of alternative hypotheses have been proposed for the diversity in male genitalia. The most likely hypothesis is the internal courtship/sexual selection hypothesis (Eberhard, l985), which postulates rapid evolution of male genitalia via female choice during courtship.

Although a single terminology across all insect orders would be useful (especially for students of insect morphology!), attempts to establish a single terminology have proven difficult and most of the terminologies used are largely taxon-specific (e.g., hymenopterists, lepidopterists and coleopterists each have their own particular terminologies). Robert E. Snodgrass referred to this dilemma when he wrote: "in taxonomic descriptions of the genitalia there is such a lack of uniformity in concepts of homology and in the adopted terminology that one specialist hardly knows what the other is talking about" (Snodgrass, 1957).

With the exception of copulatory systems that are clearly of independent origin (e.g., the spider pedipalp and odonate accessory genitalia), there are two primary theories concerning the evolutionary origin of male genitalia: (1) the appendicular hypothesis and (2) the non-appendicular hypothesis.

Appendicular hypothesis — According to the proponents of this view (Snodgrass 1935, Michener 1944, Smith 1959, Scudder 1971, Matsuda 1976), male genitalia arose through modifications of the 9th abdominal appendages homologous to appendicular elements of the female ovipositor.

Evidence for this hypothesis comes from comparison of the male 9th abdominal segment in Thysanura (Fig. 22.2). The penis is located medially between two coxopodites (Fig. 22.2B, Cxpd) each of which bears a stylus (Fig. 22.2B, Sty) and paired, medial gonapophysis (Fig. 22.2B, 2Gon). This is very similar to the morphology of the female 9th segment, but the gonapophyses are shorter and more flexible.

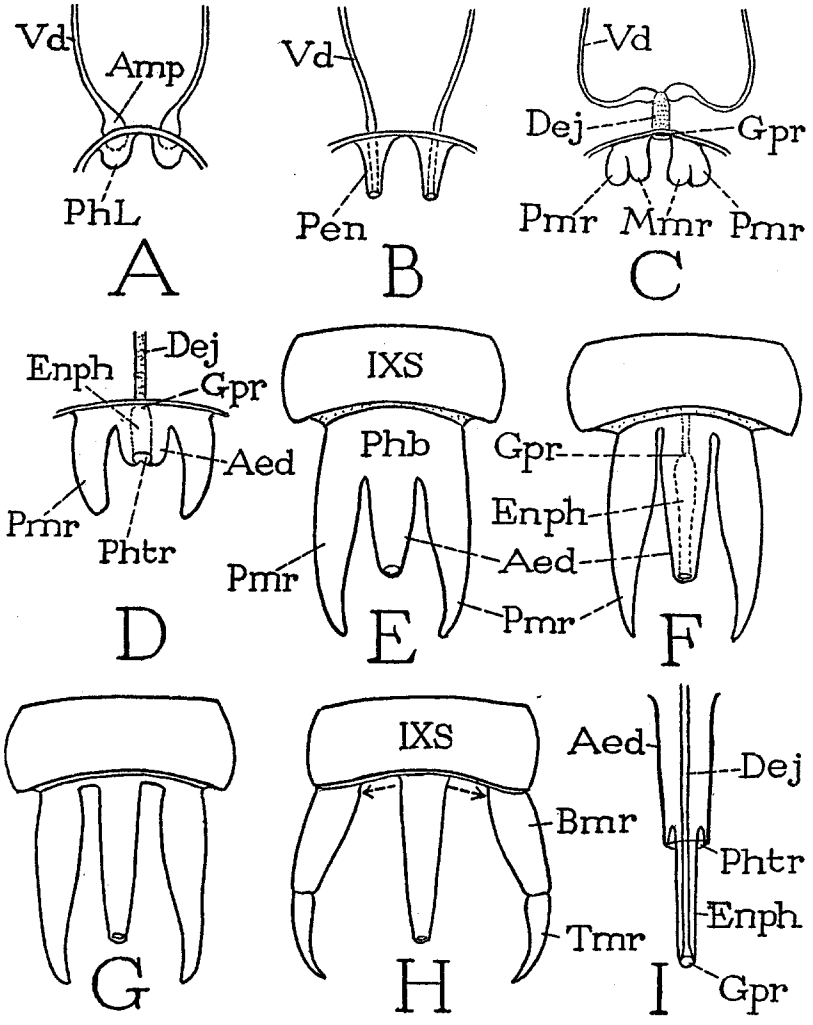


FIG. 1.—Diagrams illustrating the common origin and various types of development of the phallic organs.

A, the primary phallic lobes (*PhL*) invaded by the ampullae (*Amp*) of the vasa deferentia (*Vd*). B, penes of Ephemeroptera, giving exit to the genital ducts. C, the usual division of the phallic lobes into mesomeres (*Mmr*) and parameres (*Pmr*); ejaculatory duct (*Dej*) developed between the mesomeres, opening through the gonopore (*Gpr*). D, mesomeres united to form the aedeagus (*Aed*); aedeagal lumen, or endophallus (*Enph*), opening through the phallotreme (*Phtr*). E, aedeagus and parameres not completely separated from common phallobase (*Phb*). F, aedeagus and parameres with only narrow basal connections. G, parameres removed from aedeagus. H, parameres more widely displaced from aedeagus on margin of ninth abdominal sternum (*IXS*), divided into basimeres (*Bmr*) and telomeres (*Tmr*). I, aedeagus with endophallus everted, bringing the gonopore to its tip.

Figure 22.1 (Snodgrass 1957)

The gonopophyses are interpreted as forming the aedeagus (or the penis valves) in male insects and the gonocoxite and gonostylus are thought to form the genital capsule and clasping organ.

Non-appendicular hypothesis — An alternative hypothesis is that male genitalia are homologous to sternal evaginations associated with the gonopore (genital opening) itself (Heymons 1896, Matsuda 1958, Snodgrass 1957). This view is supported to some extent by embryonic studies as well as difficulties making homology statements across all insect orders. In embryonic development the male genitalia arise from paired lobe-like structures formed on the 9th segment immediately adjacent to the gonopore. Medially, two lobe like structures, called mesomeres (Mmr in Fig. 22.1C), give rise to the aedeagus (Aed in Fig. 22.1D, E, F). Laterally, two additional lobe-like structures, called parameres (Pmr in Fig. 22.1C), develop into the genital capsule, with a basal basimere (Bmr in Fig. 22.1H) and a distal telomere (Tmr in Fig. 22.1H). Proponents of the non-appendicular hypothesis prefer these latter terms (basimere, telomere) because they do not imply homology with either leg appendages or the female ovipositor. Keep in mind that for most insects we can use gonocoxite/basimere and gonostylus/telomere interchangably.

Because the diffulty of establishing homology across orders, we have chosen to use terminology specific to each order. Keep in mind that while similar structures may have similar names in (say) Hymenoptera and Diptera, this need not imply that these are necessarily homologous structures!

In this lab we will take an overview of the male genitalia in various orders of insects.

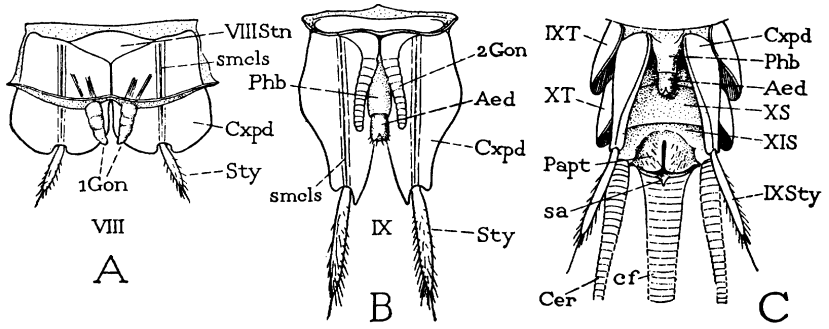


FIG. 298.—Male genitalia of Thysanura. A, *Machilis variabilis*, dorsal view of first gonopods, showing gonapophyses of eighth segment. B, same, dorsal view of second gonopods and median copulatory organ. C, *Nesomachilis maoricus*, ninth and terminal segments, ventral view.

Figure 22.2 (Snodgrass 1935)

1.

Archaeognatha: Examine the demonstration of the male genitalia of a bristletail (Archaeognatha: Machilidae). Locate the gonocoxae (Cxpd in Fig. 22.2)and gonostyli (Sty in Fig. 22.2) of segment 9 and the penis (Aed in Fig. 22.2), apparently formed from gonapophyses of segment 10, which is overlapped by the gonapophyses of segment 9 (2Gon in Fig. 22.2). This is the basis for the "appendicular" hypothesis promoted by Michener (1944) and others (see above).

2.

Ephemeroptera: Examine the demonstration of male genitalia of a mayfly (Fig. 22.3). The mayfly exhibits a primitive condition of paired genital systems that do not unite into a common ejaculatory duct, but rather open separately through a pair of gonopores behind the 9th sternum (perhaps on the 10th segment). A similar condition (a paired penis) is present (but not necessarily homologous) in the order Dermaptera.

Locate:

Sternum 9 (IX Stn)
"gonostylus" (Sty)
pair of penes (Pen)
cercus (Cer)
caudal filament (cf)

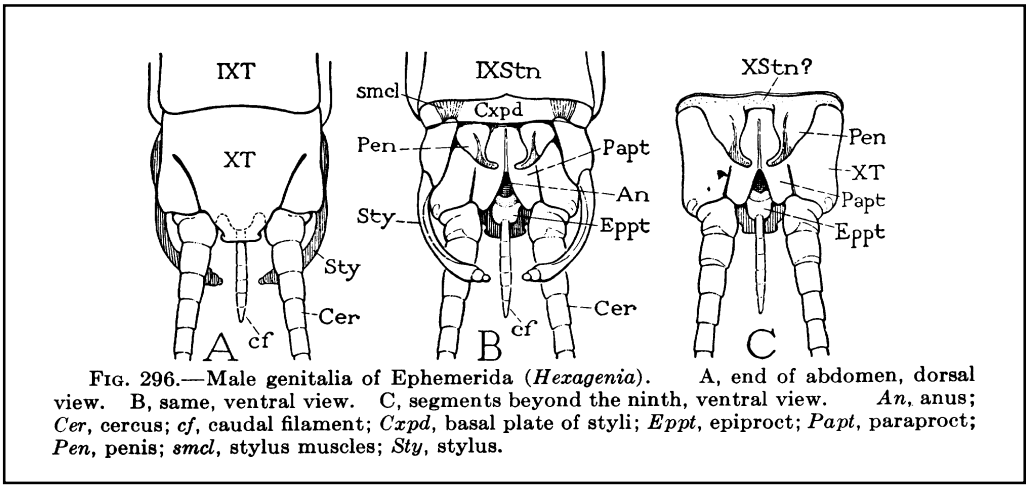
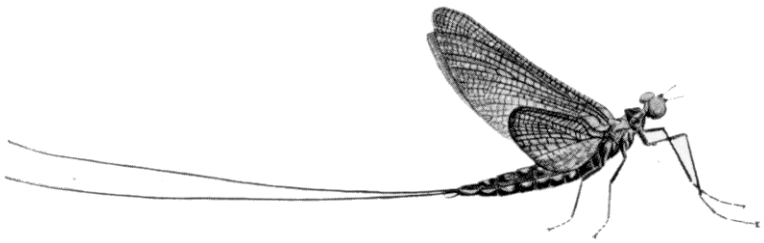


FIG. 296.—Male genitalia of Ephemerida (*Hexagenia*). A, end of abdomen, dorsal view. B, same, ventral view. C, segments beyond the ninth, ventral view. *An*, anus; *Cer*, cercus; *cf*, caudal filament; *Cxpd*, basal plate of styli; *Eppt*, epiproct; *Papt*, paraproct; *Pen*, penis; *smcl*, stylus muscles; *Sty*, stylus.

Figure 22.3 (Snodgrass 1935. p. 584)



3.

Odonata: Examine the demonstrations of the damselfly (Odonata: Zygoptera: *Calopteryx*). Odonates show a remarkably modified mode of sperm transfer whereby males have evolved a new structure located on abdominal segments 2 and 3. Nevertheless, the intromittent organ shows the same degree of genitalic diversity as the genitalia of other insects.

The male gonopore is on the venter of abdominal segment 9, opening between two "penis valves" (e in 22.4C) (=gonocoxae?). A pair of strong cerci (Cer in Fig. 22.4C), at the end of the abdomen (segment 11), are used to grasp the female during courtship (Fig. 22.5A). Before encountering a female, the male curls the tip of his abdomen anteriorly and transfers sperm to accessory genitalia (Fig. 22.4A) on the venters of abdominal segments 2 and 3. The copulatory apparatus consists of several plates (laminae and hamuli) derived from these abdominal sterna (Fig. 22.5B). The sperm is stored in a sperm vesicle, which in ventral view meets the "head" of the penis. In lateral view (overlapping terga and sterna removed), the penis is evident as a long curved rod with two curved "horns" at the tip. During copulation the penis head enters the female's bursa copulatrix and the horns enter the paired spermathecae and remove up to 100% of sperm previously stored there; the "old" sperm is then visible on the penis head, held in place by backwards-pointing spines (Waage, 1979). After this displacement, the sperm vesicle opens and "new" sperm is transferred along a sperm canal on the penis to the female's bursa.

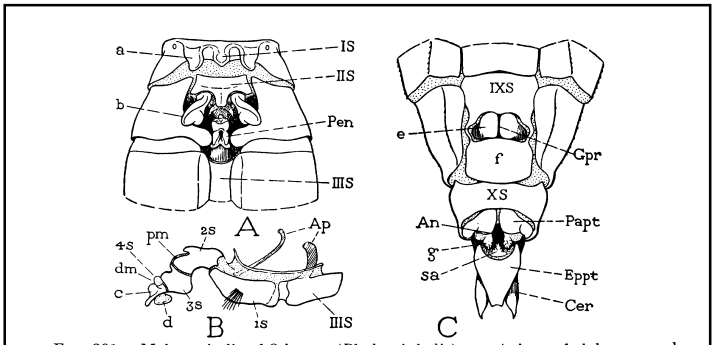


FIG. 301.—Male genitalia of Odonata (*Plathemis lydia*). A, base of abdomen, under surface, showing secondary copulatory organs. B, secondary penis of second abdominal segment, lateral view. C, posterior end of abdomen, under surface, showing true gonopore (*Gpr*) on ninth segment between two valve-like plates.

Figure 22.4 (Snodgrass. 1935. p. 593)

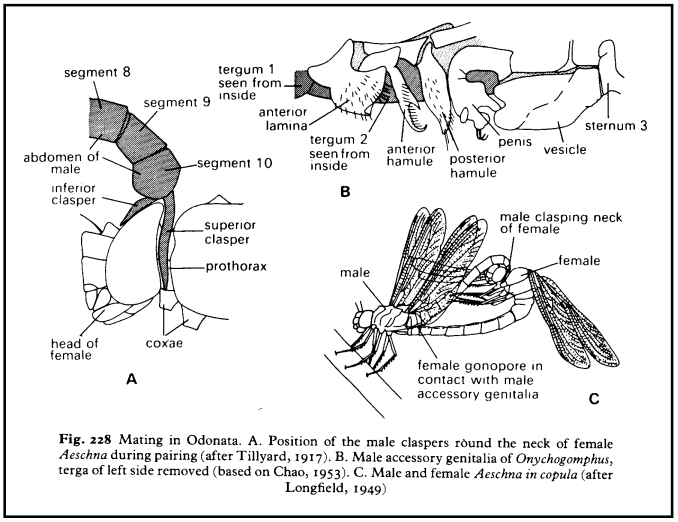


Fig. 228 Mating in Odonata. A. Position of the male claspers round the neck of female *Aeschna* during pairing (after Tillyard, 1917). B. Male accessory genitalia of *Onychogomphus*, terga of left side removed (based on Chao, 1953). C. Male and female *Aeschna in copula* (after Longfield, 1949)

Figure 22.5 (Chapman. 1982. p. 360)

4.

Hemiptera/Homoptera: Examine the demonstrations of the milkweed bug, *Oncopeltus*, whose genitalic structure and mechanics of copulation have been examined by Bonhag & Wick (1953; J. Morph. 93:177-255). In copulation, the 8th abdominal segment of the male rotates 90^{o} to the right and the genital capsule rotates 180^{o}. The final position of copulation, end to end facing in opposite directions (Fig. 22.6), may last up to 5 hours. The male "gonostyli" (=claspers) grasp the gonocoxites (=valvulae) of the female ovipositor while the aedeagus is being inserted into the female. The aedeagus is erected by hydraulic pressure with fluid that was stored in a special reservoir and forced into the phallus by a pump (Fig. 22.7). The terminal portion of the aedeagus, the endophallus, acts as an intromittent organ and is extended directly in the spermatheca of the female during copulation.

In demonstration dissections of *Oncopeltus* male genitalia, locate the following:

phallobase
aedeagus
"gonocoxae" (genital capsule)
vesica
endophallus
"gonostylus" (=clasper)

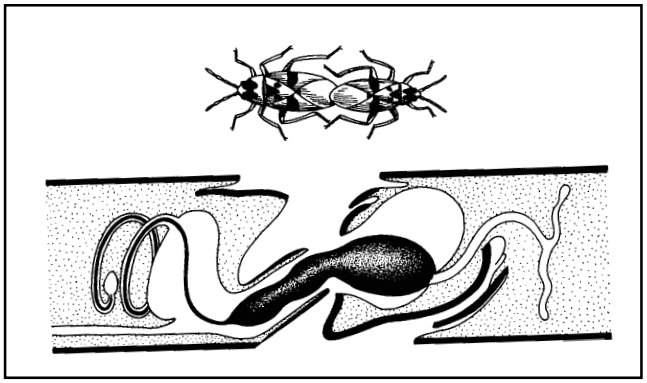


Figure 22.6 (Thornhill & Alcock. 1983. p.324)

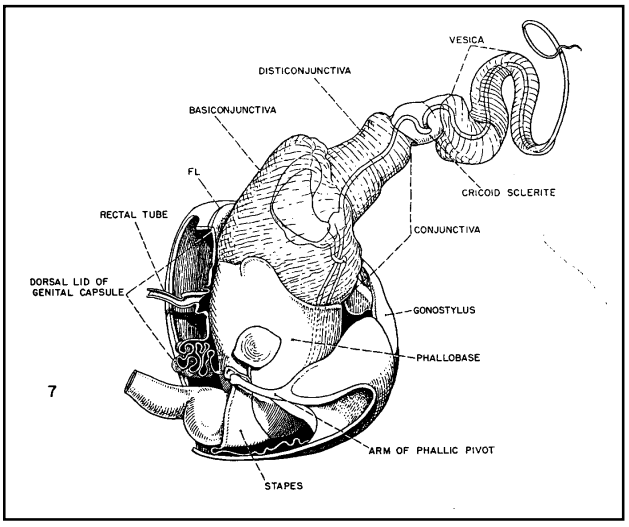


Figure 22.7 (Bonhag & Wick. 1953)

5.

Coleoptera: The male genitalia of Coleoptera shows considerable variation, but the demonstration of *Trox* (Polyphaga: Trogidae) illustrates a relatively unmodified example (Fig. 22.8). There is some ambiguity even among Coleopterists as to the correct terminology for male genitalia, but we will adopt that of Lawrence and Britton (1991) which is largely based on the comprehensive work of Sharp and Muir (1969 (1921)). Under this interpretation, the aedeagus is comprised of three parts: (1) the median lobe (= penis) which often has two anterior support rods (median struts; visible in *Trox*), (2) phallobase and (3) the parameres, which as in Hymenoptera can bear distal styli (not present in *Trox*). The phallobase and the parameres together form the tegmen, which in some beetles forms a complete ring around the median lobe. Common variations to this generalized "trilobed" form include reduction (absence) of the phallobase and/or asymmetrical development of the parameres.

The median lobe has an eversible sac (internal sac) which inflates during copulation and is often associated with complex setation, microtrichia, spines, lobes and sclerotized patches. The gonopore is located at the apex of the internal sac. In a number of beetle groups a long, thin flagellum has developed independently as a sclerotized extension of the gonopore.

The aedeagus typically rests inside a genital capsule. The coleopteran genital capsule represents invaginated 9th and 10th abdominal segments. When complete, the genital capsule consists of: (1) a dorsal proctiger (T10), (2) two lateral paraprocts (divided T9), and (3) the spiculum gastrale (S9). All of these comonents are present in the demonstration of *Trox*. In some beetles of the superfamily Cucujoidea, the genital capsule is itself encapsulated inside an invaginated 8th abdominal segment, which is also (confusingly enough) called the genital capsule.

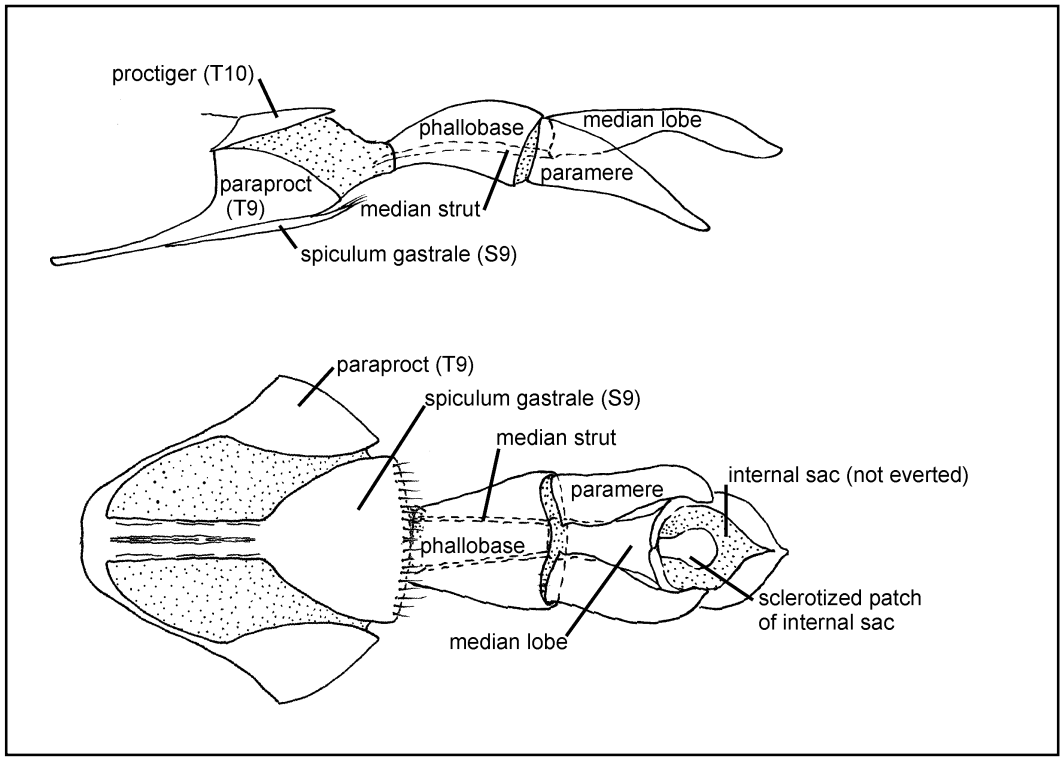


Figure 22.8 (Chris Marshall, 2001)

6.

Hymenoptera: Examine the demonstration of cleared genitalia in a non-*Apis* bee (we recommend using a colletid, andrenid, or halictid bee). In the bee, the genitalia are completely retracted into the abdomen when not in use. Sterna 8 and 9 are small, highly modified sclerites that show huge species to species variation in shape and form. Each consists of basal apodemes for muscle attachment and distal lobe-like extensions that come into contact with the female during copulation. The diversity of morphology in the bee S8 and S9 indicates that, like the genital capsule, these structures are under sexual selection via female choice.

The genital capsule of the bee illustrates the basic elements in the genital capsule of most Hymenoptera. The genital capsule is surrounded basally by the gonobase (present in most bees, Fig. 22.9). Laterally are the paired gonocoxites, which can be divided apically into gonostyli. Mesally are the paired clasping organs (called volsellae) and the aedeagus (supported laterally by sclerotized rods called the penis valves).

Locate the following structures in the demo (using Fig. 22.9):

gonobase
gonocoxite
gonostylus
aedeagus
penis valves
volsellae

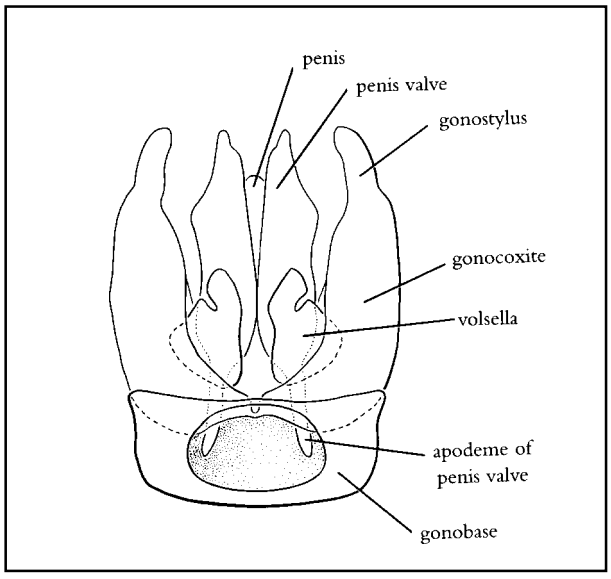


Figure 22.9 (Michener, McGinley & Danforth, 1994)

7.

Lepidoptera: The genitalia in the Lepidoptera are highly complex and composed of modified elements of the 9th and 10th abdominal segments plus a complex clasping structure derived from the parameres of the groundplan insect genital capsule (Fig. 22.10). Structures derived from the ninth segment include the tegumen (modified T9, located dorsally) and the vinculum (lateral arms of S9, located ventrally). Together the tegumen and the vinculum form a divided or undivided ring around the base of the genitalia in Lepidoptera (in Trichoptera they form an undivided ring, suggesting that this is the primitive condition for the Lepidoptera). Structures derived from the tenth segment include the uncus, a triangular sclerite located dorsally with either a simple or a divided apex, and the gnathos, a prominent modification of S10. Socii (singular, socius) are soft, hairy lobes located at the base of the uncus. The homologies of the socii are uncertain. They have been interpreted as modified parts of T10, extensions of the intersegmental membrane between segments 9 and 10, or as homologous to the cerci (appendages of abdominal segment 11)!

The genitalia proper consist of lateral valvae (singular, valva), which function to grasp the female during copulation and the aedeagus. Valvae are considered homologous to parameres in the groundplan insect genitalia. (See Scoble, 1992 for a more complete discussion of genitalic diversity in Lepidoptera).

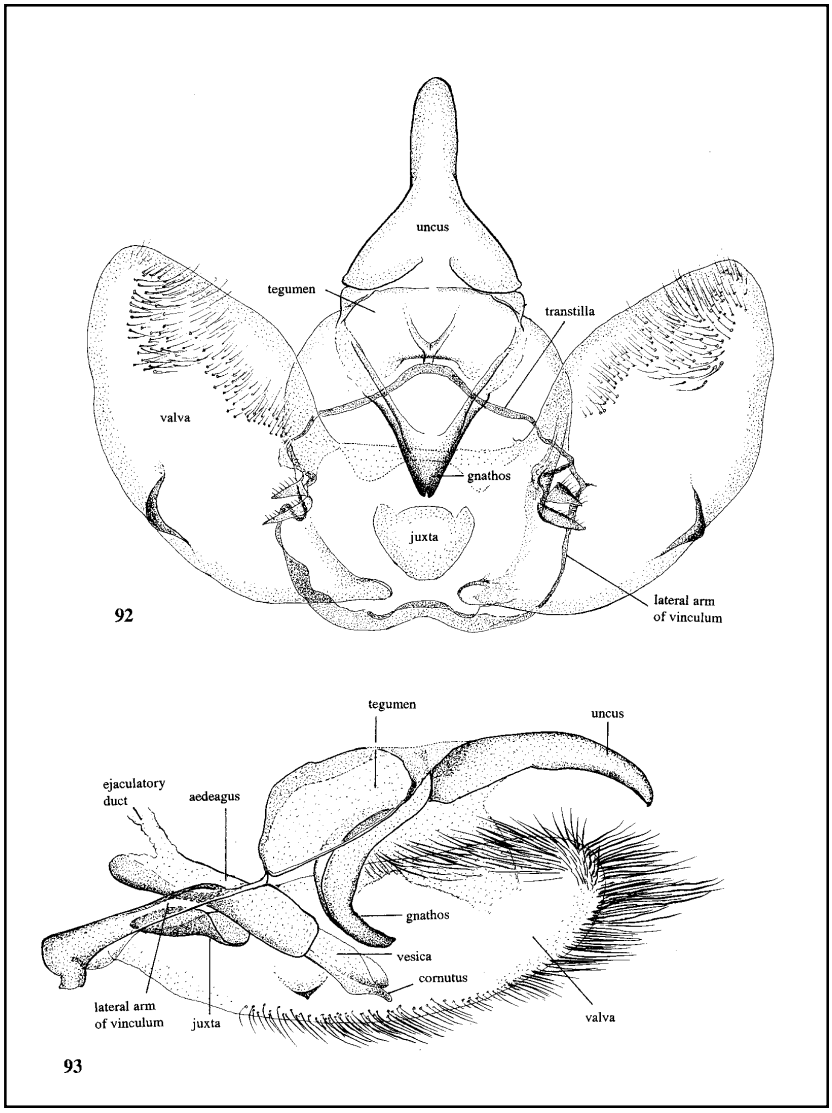


Figure 22.10 (Scoble 1992)

8.

Mecoptera: The commmon name of this order, scorpionflies refers to the enlarged genital capsule of the male. In Mecoptera the genital capsule is supported at the apex of the abdomen by S9 (ventrally) and T9 (dorsally). The genital capsule consists of a basal gonocoxite (or basimere, Bmr in Fig. 22.11) and a distal gonostylus (or telomere, Tmr in Fig. 22.11). Examine a demonstration of mecopteran genitalia, if available.

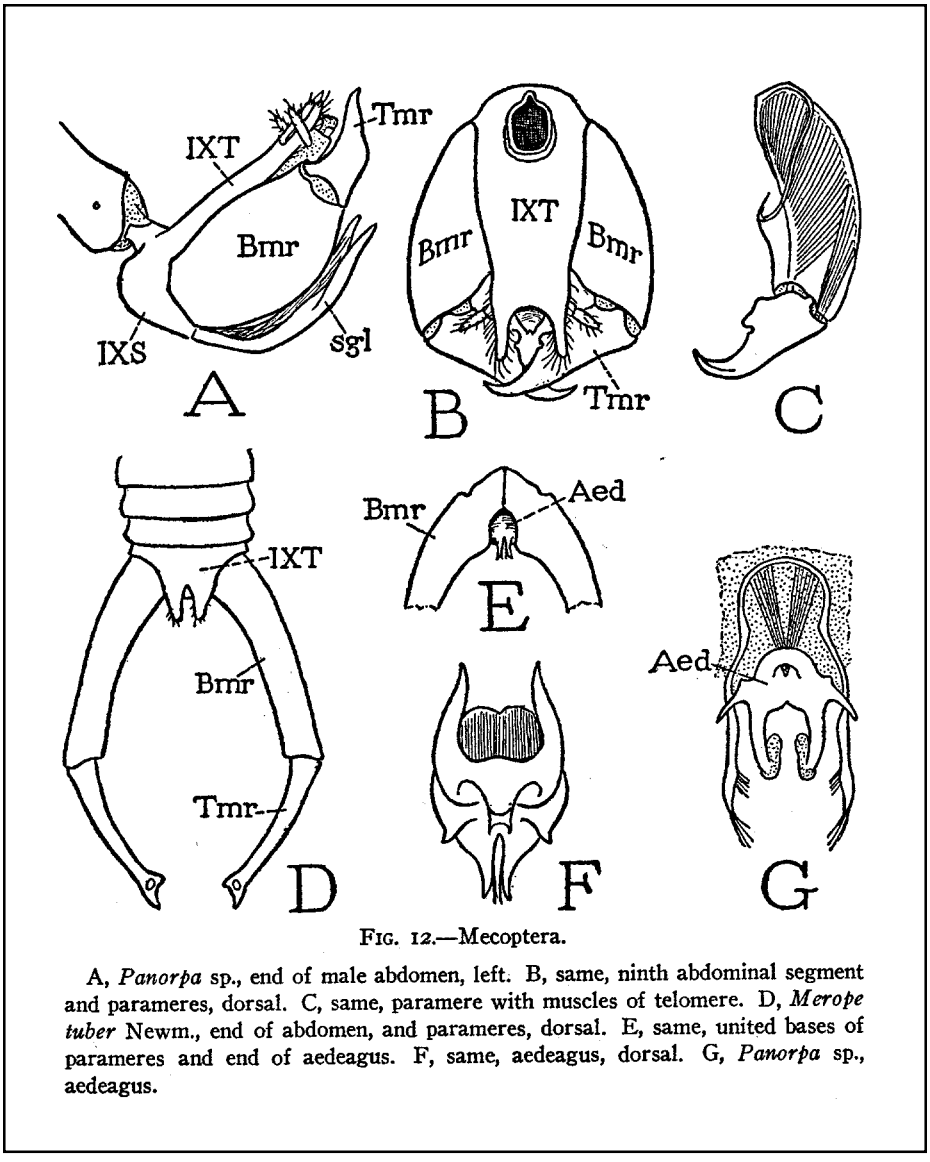


FIG. 12.—Mecoptera.

A, *Panorpa* sp., end of male abdomen, left. B, same, ninth abdominal segment and parameres, dorsal. C, same, paramere with muscles of telomere. D, *Merope tuber* Newm., end of abdomen, and parameres, dorsal. E, same, united bases of parameres and end of aedeagus. F, same, aedeagus, dorsal. G, *Panorpa* sp., aedeagus.

Figure 22.11 (Snodgrass 1957)

9.

Diptera: Diptera show a bizarre and highly modified genitalic morphology, especially within the "higher flies" (the suborder Brachycera, and the two Divisions, Orthorrhapha and Cyclorrhapha; see Insects of Australia for more on Dipteran classification). Homologizing the parts of the male genitalia among higher fly families is extraordinarily difficult.

Examine the demonstration dissection of a member of the suborder Nematocera (a crane fly [Tipulidae] or mosquito [Culicidae] will be ideal for this). Basally, the genitalia are formed from modifications of the 9th abdominal segment. T9 is referred to as the epandrium and S9 is referred to as the hypandrium (Fig. 22.12 A, C). The surstyli (jointed, clasper-like structures) are modifications of T9. The genital capsule of flies consists of a gonocoxite (Fig. 22.13, goncx) and a distal gonostylus (Fig. 22.13, gonst). The gonocoxite may bear modified appendages of lobes called claspettes (Fig. 22.13, clasp). Between the gonocoxites is the variously modified aedeagus (Fig. 22.13, aed). The apex of the male abdomen is formed from the fusion of segments 10 and 11 to form the proctiger (which bears the cerci and the anus; Fig. 22.12, proctiger).

A bizarre modification of the male genitalia in the Diptera involves a process called "torsion." In some Nematocera and Orthorrhapha the apex of the abdomen (from segment 9 onwards) is rotated 180 degrees so that what was dorsal is now ventral. In the Dolichopodidae and the Cyclorrhapha this is carried one step further and the original orientation of the genitalia is restored through an additional rotation of 180 degrees, giving these flies a 360 degree rotation to their genitalia! (Fig. 146 in McAlpine et al. 1981 shows how this rotation is related to copulator positions). Examine a demonstration of a calliphorid, sarcophagid, or muscid fly to see this.

McAlpine et al. 1981 (Vol. 1) provide a detailed discussion of fly genitalic diversity.

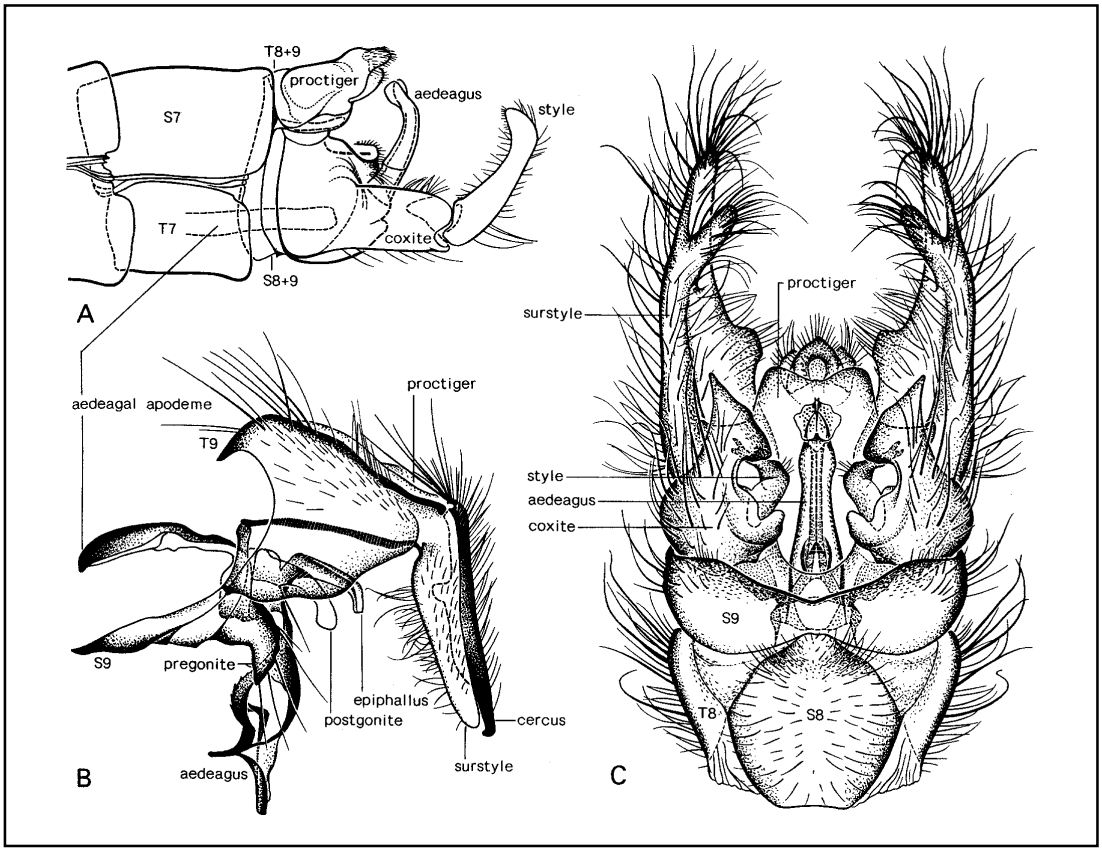


Figure 22.12 (Insects of Australia, Vol. II)

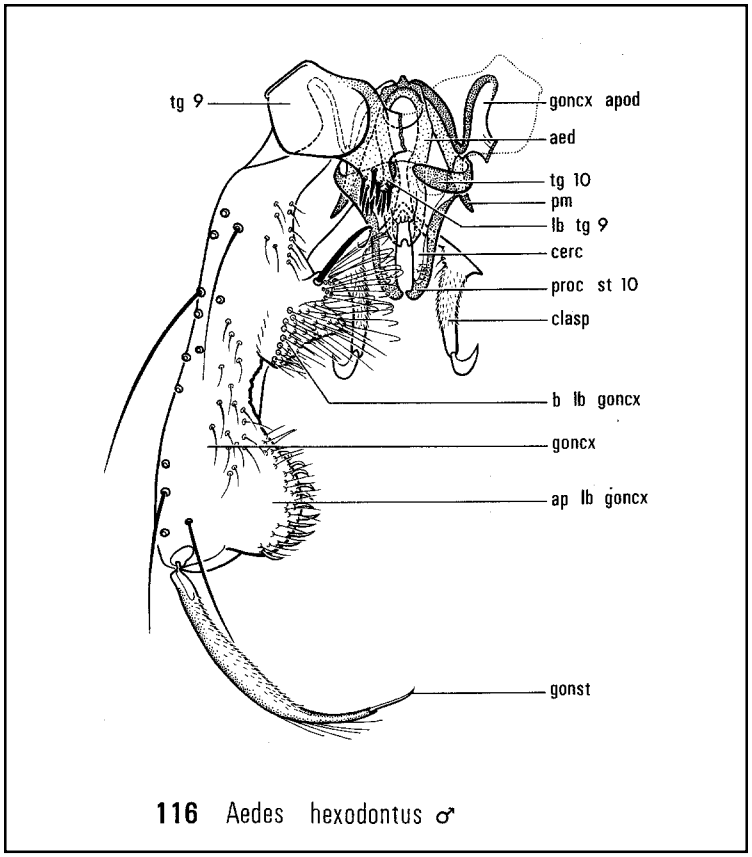


Figure 22.13 (McAlpine, et al. 1981)

10.

Obtain a male specimen of your insect (it need not be well preserved). Clip off the terminal portion of the abdomen and clear in KOH or NaOH, following usual procedures. Do not overclear. Rinse in distilled water and separate the genitalia from associated sterna and terga. Examine in a drop of glycerine held in a depression dish or slide with transmitted light. The genitalia may be stained with Chlorazol Black E, Eosin-Y, or other stain if desired. A glycerin-jelly mount in a depression slide may also be prepared (see adjoining sheet), which keeps the genitalia in a stable position for drawing.

Prepare a second dissection by clipping off the abdomen of a well-preserved male and removing the genitalia from associated terga and sterna under alcohol. This uncleared, alcohol-preserved specimen should be anchored in a dissecting dish and examined under surface lighting.

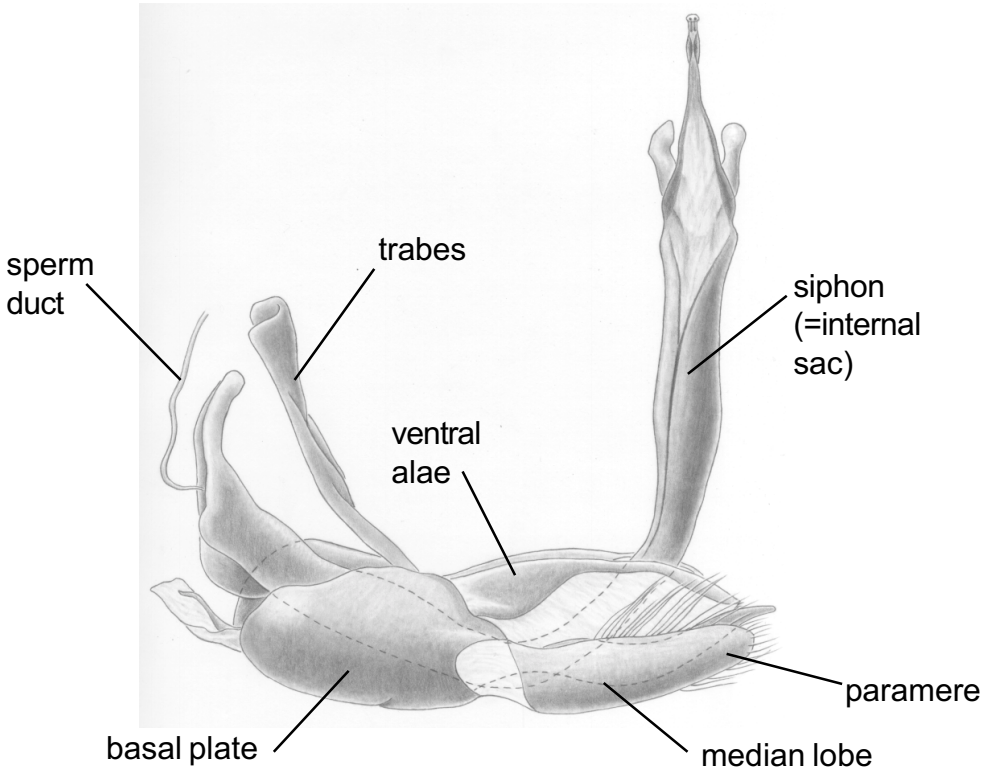
Name the principal components of the genitalia, using Tuxen, 1978 (Taxonomist's Glossary of Genitalia in Insects, 2nd Edition), or other references. Cite this reference in the legend of the following sketch (last of the semester!).

11.

Observe the genitalic preparations of other students in the lab, according to procedures given by the TA. These should be briefly observed to gain an appreciation of the diversity of male external genitalia in insects.

Sketch #6: External genitalia of male

Sketch either dorsal, lateral, or ventral view of the external male genitalia, using either cleared or alcoholic preparation. Muscles should not be shown. Identify the prominent morphological structures and use terminology specific for your insect order. This drawing should be drawn as a shaded pencil drawing, as shown below.



Curtis Ewing (Spring, 1996)
Hippodamia convergens
(Coccinelidae). Male genitalia, lateral view, siphon extended.

Slide Mounts of Male Genitalia
in Glycerin Jelly

1. Remove the tip of the abdomen or dissect out the genital capsule, and clear using standard procedures.

2. Remove from the clearing agent and rinse in distilled water. If heavily melanized the specimen may be bleached with hydrogen peroxide.

3. The genitalia may be stained if desired, 20-24 hours in Eosin-U (1% aqueous).

4. Remove from the stain and rinse in distilled water. Dissect under distilled water.

5. Remove from water and place in glycerin until glycerin has replaced water in specimen (at least 10-15 minutes).

6. Heat a small amount of glycerin jelly until melted (a few minutes in a stendor dish or spot slide on a hot plate set at 250°).

7. Place a small drop of glycerine jelly on a microscope slide.

8. Transfer genitalia. The specimen may be positioned as the jelly cools and solidifies. If necessary, the genitalia may be repositioned by warming the slide on the hot plate until the jelly begins to liquify.

9. The specimen may be permanently preserved by cutting the solidified jelly around the specimen on the slide with a scalpel or razor blade, carefully lifting it off the slide, and placing the jelly-enclosed genitalia into a small, corked genitalia vial which is then attached to the pin holding the specimen.

Slide Mounts of Male Genitalia
(adapted from procedures used by J.G. Franclemont)

1. Remove tip of abdomen and clear in 10% KOH, using standard procedures.

2. Remove from KOH, wash in distilled water, and examine in water. Carefully remove terga and sterna to expose genitalia. In some insects, the aedeagus may be everted by carefully pushing it out with a minuten probe inserted into the ejaculatory duct, or by pumping it with a hypodermic needle, and syringe.

3. If the genitalia are not heavily melanized, they may be stained. Place the genitalia in a 2% aqueous solution or mercurochrome. The time of staining will vary according to your specimen usually 5 to 10 minutes is ample.

4. Remove from stain and wash thoroughly in distilled water.

5. Remove from water and place in 95% ethanol. The parts may be positioned by spreading them and holding them in place with small pieces of glass. Leave in ethanol at least 15 to 30 minutes).

6. Remove from ethanol and place in terpinol, cedarwood oil, or clove oil for a minimum of 15 to 30 minutes, to clear.

7. Remove from clearing oil and place in xylene for 10 to 15 minutes.

8. Mount on microscope slide in Canada balsam. Most genitalia are mounted in ventral or dorsal view, and should be carefully positioned so they are level. The cover slip may be propped up with 4 small pieces of celluloid or broken cover slip pieces to prevent the genitalia from being crushed or distorted.

9. Label the slide temporarily with your initials and specimen number with wax pencil and place to dry on a level slide warming tray or slide oven. The finished slide should bear a permanent label with the species name, your name, and code number. The specimen from which the genitalia were removed should also bear a pin label giving your name and the code number.