Genital Preparations of Female Lepidoptera.

By
Jørgen Birket-Smith.
Nigerian College of Arts, Science
and Technology, Ibadan.

The present paper is in a way a continuation of an earlier paper on the preparation of the genitalia of male Lepidoptera and for instruments, consult this paper (Birket-Smith, 1959).

The female genitalia differ from the male ones in preparatory respects by being partly external, the so-called genital plate, partly internal, the bursa copulatrix.

A careful examination of the specimen to be prepared is most important prior to any action. The genital plate will sometimes only consist of the edges of the copulatory opening (lamella antevaginalis and lamella post vaginalis) which together may form a firm plate with an opening (the genital plate s. str.), but very often the previous ventral sclerite, sternum VII is modified as well and the corresponding dorsal sclerite, tergum VII may also be modified, possibly together with the lateral sclerites, pleura VII. While st. VII often is completely fused with the genital plate and pl. VII may be so, t. VII is usually free. At last, but not least important, the genital plate s. str. may have completely atrophied and only the more or less modified st. VII can be seen. It is very important to make out all the modified parts, since they all may be important for taxonomical purposes, and for that reason should be included in the final preparation.

Extirpation, dry specimens.

Fig. 1. The easiest way of extirpation is by first removing the abdomen. This is done by inserting from the side the needle scalpel in smaller (pygmy scalpel in bigger) specimens between meta-coxae and abdomen and rolling it along its own axis, the "ventralmost" edge of the scalpel towards the abdomen. In this way the abdomen will rather easily be disconnected ventrally, and it is then easy to disconnect it dorsally as well. It is sometimes seen recommended to press the abdomen from the dorsal side in a mounted specimen or just "to remove it with a pair of forceps". It is the writer's experience, that this method will far too often result in the specimen breaking between meso-

and meta-thorax, especially in smaller species with metathoracal tympanal organ, while in Geometridae and Pyralidae it will very often spoil the tympanal organ. The detached abdomen is moistened with 70 % alcohol, to ensure that it is uniformly moistened throughout, and then dumped in 5 % potassium or sodium hydroxide. The abdomen is left in the hydroxide from 5 minutes (small specimens) to 6 hours (big ones). The purpose of using hydroxide is not to clean off all soft parts, but only to soften the abdomen. The abdomen will swell and soften, and when it has changed to a consistency, which might best be equalled to that of a cooked saussage, (not softer), it is ready for extirpation.

Fig. 2. By means of the needle scalpel or pygmy scalpel the intersegmental membranes are cut where required under water in a watch glass. Begin at the ventral side and cut the intersternal membrane completely. Then cut dorsally and after having done the intertergal membrane, continue from there towards the ventral cut. The pleural membranes between the dorsal and the ventral sclerites are sometimes very tough and cannot very well be cut with the scalpels, but require the use of the iris scissors. Be careful and never cut too deep, that the bursa copulatrix should not be damaged; keep the scalpels "flat", pointing towards the head end. When the body wall is cut all the way round, grip the genital plate or st. VII as may be (never the anal papilla) with the fly-leg forceps and remove the hindmost free part of the abdomen by pulling backwards. The remainder of the abdomen is held with another pair of forceps by a firm grip in the foremost dorsal sclerites. In this way the bursa copulatrix will follow the genital plate and can be cut completely free as soon as it is visible. It is advisable to cut the gut as soon as it can be done. The abdomen withouth genitalia is now dumped into strong (90—95 %) alcohol, and dried after a few minutes; it will in this way retain its original shape nearly completely, and can, when dry, be replaced on the specimen with a small drop of thin cellulose glue.

Fresh specimens.

Naturally it is not necessary to soften a fresh specimen, but the abdomen is not expanded and the body wall is strengthened by the muscles. It is therefore slightly more difficult to extirpate the genitalia from a fresh specimen, and special care should be applied to the pleural areas. The specimen should be pinned

and placed upside down in the vice, and usually the abdomen should be supported on the sides by a pair of fine pins, to prevent the specimen from turning on the pin. It is necessary to have small pieces of blotting or filter paper ready to suck up any moisture which may come from the opened body.

Dry specimens, abdomen in situ.

It is possible to soften dried specimens and then extirpate the genitalia as in fresh ones, but the process is a wearisome one and requires much skill. First the tip of abdomen is softened by frequent applications of small amounts of 70 % alcohol. When soft enough, the intersegmental membrane is cut ventrally and the softening continued, first with 70 % alcohol, then with distilled water and finally with 2 % ammonia hydroxide. Only the abdomen should be moist, not the thorax. Since it is important that the entire surroundings of bursa copulatrix are softened, this is especially difficult in species in which the bursa reaches into the first abdominal segments. By extracting the bursa, great care must be taken not to break the abdomen off. The method is not recommended.

There are several ways of mounting the genitalia.

Mounting on Slides.

The most common mounting method is mounting on slides. The genitalia are left in 5 % potassium (or sodium) hydroxide for a day or two until all soft material, muscles etc. is dissolved. (They may be boiled for a few minutes in the hydroxide, but it is not recommended, since the bursa may burst.) The genitalia are then rinsed in distilled water, if necessary cleaned with a fine brush. Heavily sclerotized genitalia may not need staining, but usually, in order to examine the bursa, it is advisable to stain the genitalia before mounting. There is no specific stain for chitin. Erythrosin in watery solution will stain faintly red, and is especially useful for photographic objects. For common use, Mallory's Double Stain (Methylene Blue-Orange G) seems more suitable. The cleaned genitalia are kept in the stain for a day or two and should appear nearly black when finished. The genitalia are then differentiated first in distilled water, in a watch glass, while watching them in the microscope, to wash out most of the Orange G, and then tranferred into 70 % alcohol to wash out surplus of Methylene Blue. When adequately stained, the genitalia will now have a strong blue colour, only the tubu-

lar part of the spermatophores being yellow (the globular part having been dissolved). The genitalia are now transferred to absolute alcohol, which will dissolve a little more of the Methylene Blue, for which reason they should not be left there for

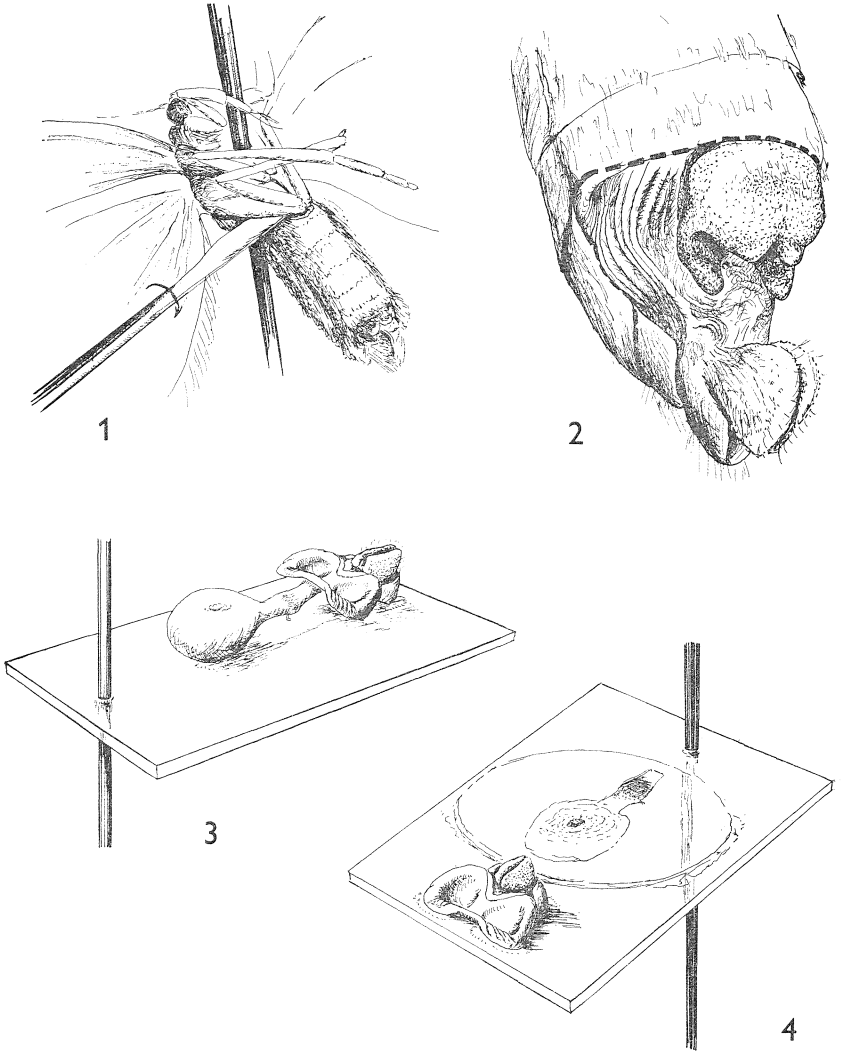

Fig. 1—4: 1. Removal of abdomen of *Eilema uelleburgensis* Strd. — 2. Extirpation of the genitalia of same. The broken line indicates where to cut the integument. The hairs are removed in the figure. — 3. Free mount of female genitalia of *Phryganopsis flavifrontella* Strd. — 4. Combined mount of the same.

than 5 minutes. Finally the genitalia are cleared in xylol and mounted on a slide in dammar dissolved in xylene, and covered with a coverslip. Another method, especially for persons who cannot obtain absolute alcohol without duty, is to transfer the genitalia to as strong methylated alcohol as obtainable (usually "Industrial 74", but even "Denaturated" will do), then into clove oil, which will remove the remaining traces of water, and finally to xylol and to dammar. It should be mentioned that mounting in Canada balsam is not recommended, since even the best neutralized Canada balsam will turn acid after some time, and cause the stains to fade, while dammar will stay neutral. Both the stains mentioned (and others as well) will fade in light. A slightly different method, particularly suitable for unstained genitalia, is mounting directly from strong alcohol into Venetian turpentine on the slide; the refraktion index of chitin differs more from that of Venetian turpentine than from that of dammar (or Canada balsam) and this method will therefore render the chitineous structures more readily distinguishable.

In the slide mount all internal structures such as signa in bursa and the apophyses will be clearly visible, but the surface structures of the genital plate will at best be very indistinct, if visible at all.

Free mount (Fig. 3).

When a free mount is wanted, the genitalia after extraction are examined under the microscope. If the bursa is well extended ("blown up") the genitalia are dropped into 4 % formaline and left there for about a day; if the bursa is more or less collapsed, a short stay in the hydroxide will usually expand it fully. From the formaline the genitalia are rinsed shortly in distilled water and then taken through 30, 50, 70, and 90 % alcohol to absolute alcohol (or to the strongest available) and then to xylene, from where it is removed and dried slowly in a corked tube.

By careful preparation the bursa will now have its natural shape and so will the genital plate. The walls of the bursa will be opaque and even if a large signum is visible, no finer structures can be distinguished. The walls of bursa can be made transparent by carefully "painting" them with a thin solution of dammar in xylene under microscope (be careful that the dammar does not reach the genital plate), but even this will not al-

low the finest structures in the wall of the bursa to be seen clearly.

The dry genitalia can be kept in a small gelatine capsule on the same pin as the insect, or glued on a small piece of celluloid mounted on the pin.

Since the genital plate is dry and exposed, the method allows direct comparison to undissected specimens, and thus in many cases a certain identification of these. In species, where it has been desirable to remove st. VII and even t. VII, the external parts of bursa will be hidden.

Combined Mount (Fig. 4).

This method is more elaborate than the previously mentioned ones, but it allows a more detailed study as well.

After extraction the genitalia are cleaned; it may be that they are not yet softened enough for this, but another half hour in the hydroxide will usually be enough for a successful cleaning by carefully applied brush and small scalpels. In the clean genitalia the bursa and the genital plate are separated just behind the latter by means of the iris scissors or the fine scalpels (this requires some skill). The genital plate and other parts of the body wall are then dumped into strong alcohol to harden. Formaline is not necessary except where the tip of the abdomen is strongly extensible as an ovipositor. The bursa is stained (see above under slide mount). After one or two days the genital plate can be removed from the alcohol and dried directly. The bursa is cleared and mounted in the usual way, not on a glass slide, but on a small piece of celluloid and covered with a coverslip. The celluloid slide should be allowed to dry for some days, preferably a week at room temperature, slightly less at about 40° C. The superfluous dammar is then removed, which is easily done by careful scraping. The genital plate is glued on the celluloid with a small amount of thin cellulose glue, and the whole preparation can then be kept on the same pin as the insect.

This method allows a study of the finest structures in the bursa, and at the same time of the surface of the genital plate, which is equally well exposed for direct comparison to undissected specimens.

The last method, combined with the first extirpation method, has been successfully applied to dried moth with abdominal lengths and diameters of 3—45 mm and .5—12 mm respectively.

References.

Tuxen, S. L. (edd.), 1956: Taxonomist's Glossary of Genitalia in Insects. (Munksgaard) Copenhagen.

Birket-Smith, J., 1959: Genital Preparations of Male Lepidoptera. — Ent. Medd. 29 p. 170.

Anmeldelse.

Hermann Wiehle: **Spinnentiere oder Arachnoidea (Araneae) XI: Micryphantidae — Zwergspinnen.** Die Tierwelt Deutschlands, 47. Teil. Jena: Gustav Fischer, 1960. XI + 620 pp. Pris: 107,65 DM.

Med dette bind om *Micryphantidae* — eller *Erigonidae*, om man vil — har araneologen faaet et godt hjælpemiddel i hænde. Bogen rummer bestemmelsesnøgler til og tegninger og beskrivelser af 144 arter med oplysninger om deres biologi og forekomst i og uden for Tyskland. Den bliver nok den nyttigste bog for danske samlere af familien, idet de ca. 80 arter, som inden længe vil være kendt fra Danmark, næsten alle er behandlet i den.

Anmelderen har kun i et beskedent omfang benyttet bestemmelsesnøglerne og har kun i et enkelt tilfælde fundet anledning til at betvivle deres anvendelighed: Man ledes paa vildspor, hvis man vil bestemme et svensk eksemplar af *Lasiargus hirsutus,* der kun har een dorsal børste paa tibia I og II; de tyske har ifølge saavel nøgle som beskrivelse aabenbart to. Figurer og beskrivelser er derimod blevet udnyttet meget intenst, og de er gode: Man føler sig i høj grad paa fast grund, naar man bruger denne bog under bestemmelsesarbejde.

Der er 1147 tekstfigurer, altsaa gennemsnitlig otte for hver art. Foruden figurer af epigyn og palpen set fra ydersiden, hvilket er det minimale behov, er tillige afbildet vulva, palpens inderside, tibialapofyse, cephalothorax fra oven og fra siden, øjnenes placering, chelicerer, tarsalkløer m. m., afhængigt af hvad der er brug for. Man kunne have klaret sig med færre figurer, men som bogen er nu, savner man næsten aldrig yderligere en figur. Deres kvalitet er upaaklagelig, langt fra at være smukke portrætter er deres nøjagtighed alligevel fortrinlig.

Man kan indvende mod bogen, at oversigten over arternes udbredelse uden for Tyskland næppe er ganske paalidelig, men baseret paa andre, ikke helt troværdige, kompilationer. F. eks. foruddiskonteres fundet af *Trichopterna thorelli* i Danmark, fordi Bonnet ved en misforstaaelse angiver dens forekomst her. Men havde forfatteren ikke givet denne summariske oversigt, maatte man selv til en første orientering have slaaet op — i Bonnet!

Literaturfortegnelsen er mangelfuld og uden angivelse af sidetal for tidsskriftartikler. Der er bemærkelsesværdigt faa trykfejl, og de faa der er synes for flertallets vedkommende at have eksisteret allerede i manuskriptet; f. eks. forekommer *Savignya* ogsaa stavet *Savignia*, den hidtil gængse form.

Ole Bøggild.
