Genital Preparations of Male Lepidoptera.

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The following preparation method for genitalia of male Lepidoptera can actually be divided into two independent methods, the Extirpation and the Free Mount respectively. Compared to the usual methods they have the following advantages and disadvantages:

Extirpation,

Advantages:
1. It leaves the abdomen in situ.
2. Hardly any traces will be left on the specimen (except for the missing genitalia).

Disadvantages:
1. It requires some skill. (On the average 25 specimens will suffice for this)
2. It requires, apart from a stereomicroscope, some special dissection instruments.

Free Mount,

Advantages:
1. The entire genital apparatus will at any time be ready for examination in its natural perspective without distortion because of flattening.
2. The mounted genitalia can be kept on the same pin as the specimen itself.

Disadvantage: The mounted genitalia are not as well protected against injury, as when kept on a slide (but as well protected as the specimen itself).

The figures explaining the method are slightly diagrammatical. As an example a medium sized moth, *Eilema xantopa* Holl. is chosen, span 14 mm, length of abdomen 5 mm, diameter of abd. hardly 2 mm.

Fig. 1, The Special Instruments Used.
A. Fly-leg Forceps ($\times$ $^1/_2$). This instrument is readily made from an old hack-saw blade on an electric hone. The blade is broken into halves and these are solidly riveted together through the holes in the ends of the blade; the two halves should cover each other exactly. While

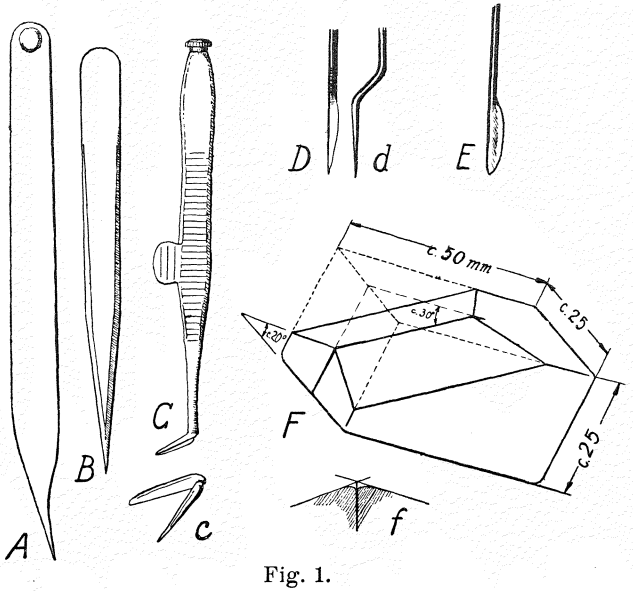

Fig. 1.

they are hold close together the two halves are shaped as shown on the figure and the teeth are removed on the electric hone. All sharp edges are removed with emery paper, and the final touch to the fine ends is given by hand on a fine hone ("Belgian hone"). The fine ends of the limbs shold be about 2 mm wide at base and 0.1 mm at the tips; their thickness at the base should be that of the blade, at the tips 0.05 mm, tapering down to this thickness mostly on the outermost 5 mm. Finally the tips should be carefully rounded. The internal surfaces of the fine ends of the two limbs should be nearly

parallel. Given this shape, these forceps will not crush even very delicate objects, as a too strong pressure on the upper part will cause the tips to open and release the object.

B. Watchmaker's Forceps ($\times$ 1/2). These can be bought at most watchmakers. The points must be needle sharp and of exactly the same length.

C. Iris Scissors or Weber's Ophthalmic Scissors ($\times$ 1/2). These can be bought at suppliers of surgical instruments. It is most useful to grind the tips of the blades as shown on the figure (c, $\times$ 1, where they are seen from the end).

D. Needle Scalpel ($\times$ 4). This can conveniently be made from a fine sewing needle or from a steel insect pin no. 3. The outermost 3 mm are hammered flat while red hot and then ground down on a fine hone until the "blade" is hardly wider than the diameter of the needle. The blade is tempered by dipping it into water while red hot and is then finally sharpened. It is mounted in a wooden handle. I have found it practical to give the needle a slight S-shape above the blade, as shown (d, $\times$4, where it is seen from the back).

E. Pygmy Scalpel ($\times$4). This is made in the same way as the needle scalpel, the blade, however, should be wider and it should be straight.

It is most important that all the points of the instruments are sharp and not bent or hooked and they must not be rusty. The points should be examined under the microscope before use, and they should allways be protected whilst not in use, for example by a small cork.

F. Rubber Vice ($\times$1/2). It is most important that the specimen is firmly supported during the preparation. The cheapest and easiest way of making a holder is from a piece of not too soft eraser gum, about 25$\times$25$\times$50 mm. A clean cut is made along the midline from one of the ends and half way to two-thirds through the piece with

a sharp, wet knife. The "vice" can be used in this state, but it is recommended to shape it as on the figure. This can be done with a sharp, wet knife, or still easier and better on the side of an electric hone. The cut should just divide the ridge between the oblique faces. Finally this ridge is rubbed on a piece of hard smooth paper which will give the shape shown (on f) to the transverse section of the ridge. This vice will readily hold any specimen from the size of a small pyralid to a medium noctuid, supporting the abdomen. Heavier specimens usually need not to have any support of the abdomen.

A fine water colour brush (Sable hair no. 0) is also needed.

Extirpation, dry specimens.

Fig. 2. The specimen, which must be mounted on a pin, is placed upside down in the vice, the top end of the pin in the cut and the abdomen resting on the ridge, the anal end uppermost. To avoid hairs and scales being rubbed off the abdomen, a small piece of thin cellophane can be placed between rubber and abdomen. The end of the abdomen is moistened with a drop of 50 % alcohol (methylated is as good as any), big enough to moisten the anal half of the abdomen, but not more. This drop is immediately followed by a drop of distilled water; both drops are applied with the brush.

Fig. 3. After a lapse of about 30 minutes, the sternites will usually be soft and flexible. While holding the abdomen with the fly-leg forceps the needle scalpel is introduced ventro-laterally between the left valva and the 8th sternite, penetrating the intersegmental membrane between vinculum and the 8th sternite. Heavier specimens will usually need another drop of 50 % alcohol and one of water at this stage; the two last drops will readily penetrate into the interior of the abdomen through the hole in the membrane. (Especially difficult specimens can instead of the last drop of water be mois-

tened with a drop of 2 % ammonium hydroxide. This will not harm the specimen and disappears without trace. (This may not, however, be very pleasant for the preparator.) Smaller specimens will usually be sufficiently relaxed all through, for the immediate continuation of the preparation.

Fig. 4. The fine pointed blade of the Iris Scissors is inserted through the hole in the intersegmental membrane and a longitudinal ventro-lateral cut is made through the last 3-4 sternites. The edges of this cut are carefully separated by means of the fly-leg forceps.

Fig. 5. While holding, first in the edges of the longitudinal cut, later in the vinculum, the intersegmental membrane between the 8th sternite and the vinculum is cut by means of the needle scalpel (or, for heavier specimens, the pygmy scalpel as well), first ventrally, then along the left and the right side until the tegumen.

Fig. 6. The whole genital apparatus is now "broken" out with a backwards and upwards movement of the fly-leg forceps. The genitalia are still retained by the ductus ejaculatorius (which will often break), the gut and the muscles to vinculum. While holding the genitalia with the fly-leg forceps, these structures are cut with the iris scissors (the blunt blade lowermost). The remaining bit of the intersegmental membrane dorsal to the tegumen will rarely need cutting, but usually it will tear easily by removing the genitalia completely.

Fig. 7. While the abdomen is still moist and soft, the two edges of the longitudinal cut are carefully brought together and the specimen is allowed to dry completely. The abdomen is then moistened with a drop of strong alcohol, and immediately after with a large drop of xylol and dried quickly (under an electric bulb). After this treatment the hairs and scales will look perfectly fresh and undisturbed.

Fresh specimens.

A fresh specimen should first be pinned and then

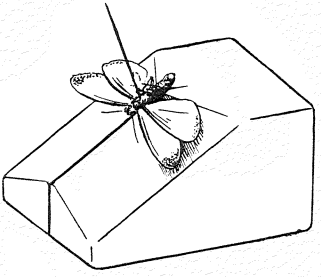

Fig. 2

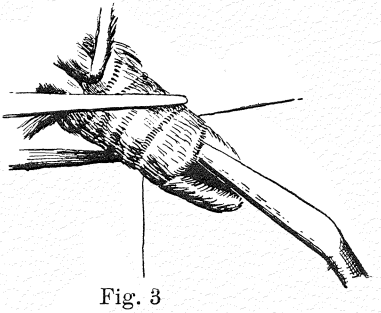

Fig. 3

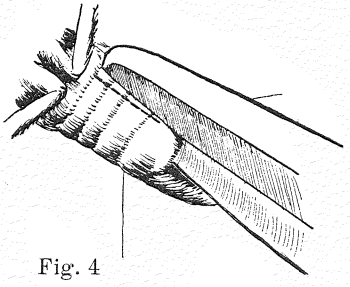

Fig. 4

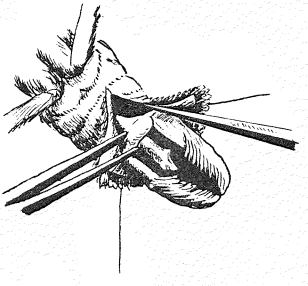

Fig. 5

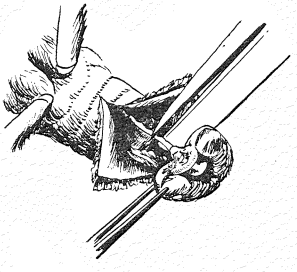

Fig. 6

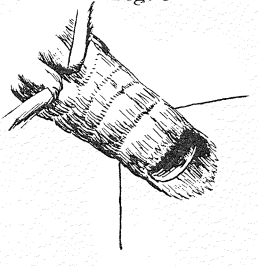

Fig. 7

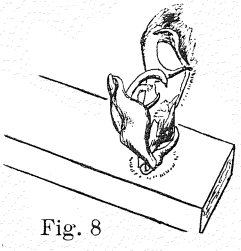

Fig. 8

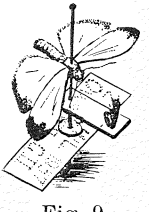

Fig. 9

placed in the vice (Fig. 2). If the specimen is completely relaxed, a slight pressure on the sides of the abdomen with a broad pair of forceps just in front of the genitalia will cause these to protrude. Whilst still the pressure is exerted on the sides of the abdomen, the intersegmental membrane between vinculum and the 8th sternite can easily be cut with the needle scalpel; this allows a grip with the fly-leg forceps in the vinculum, and the genital apparatus can then be removed (Fig. 6). It should be noted that a longitudinal section through the sternites is n o t necessary in this case. Care should be taken however, to suck up (with filter paper) all moisture from the abdomen, since this will soil the hairs and scales.

Damaged specimens.

If the interior of the abdomen of an old specimen has been eaten away, great care should be taken, not to apply too much moisture to soften the specimen, as this may cause the entire abdomen to collapse. If only a minute amount of 50 % alcohol is applied, this type of specimen is often the easiest one to deal with.

The genitalia are dumped into cold 5 % potassium (or sodium) hydroxide and left until next day (smaller specimens) or for a couple of days (heavier specimens). They s h o u l d n o t b e h e a t e d, as this makes the membranes unnecessary fragile and may cause ruptures. From the hydroxide the genitalia are transferred into distilled water in a watch glass under the microscope and cleaned with the brush. At this stage it is fairly easy to turn the vesica inside out by means of the watchmaker's forceps, while holding the aedeagus with the fly-leg forceps.

The cleaned genitalia can either be mounted in Canada balsam or Venitian terpentine on a slide in the usual way, or they can be mounted as a Free Mount.

Free Mount.

In cases where the genitalia are devoid of hairs and other fine structures they can be dried directly from the water, while kept in the desired shape by means of insect pins. If it is desirable to keep any hairs, scales or other fine structures, the following procedure is recommended. The genitalia are kept in the desired shape while still soft on a piece of filter paper under the microscope, and a drop of strong alcohol is applied to them. This will harden them in the desired shape, and they are now passed through 2 lots of strong alcohol and one of xylol (5 minutes in each). From the xylol they are placed on a piece of filter paper and dried quickly (under an electric bulb). While the genitalia are wet from xylol, minor adjustments of the shape are still possible.

It is practical to keep the different liquids needed in small (approx. 10×50 mm) flat-bottomed glass tubes, closed with corks. There should never be more than one set of genitalia in the same tube. A pin with a numbered label, corresponding to a similar label on the specimen, can be stuck in the cork and follow the set of genitalia from tube to tube. The tubes may be conveniently placed in holes in a piece of board, having for example 4 times 10 holes.

Fig. 8. A tiny drop of a mixture of 4 parts of amyl acetate and 1 part of a good cellulose glue ("Pandetikon", "Durofix" etc.) is placed towards the end of a small sheet of celluloid (approx. 2×10 mm) and the completely dry genitalia are placed in the drop and kept in the required position until the glue has stiffened (a few seconds). The thinned cellulose glue will by capillar retraction enter the hollow sclerotized parts of the genitalia, where it on drying will leave a thin invisible internal membrane of cellulose. At the same time it will dissolve the surface of the celluloid and thus the attachment of the genitalia

to the celluloid will be a very firm one. To remove the genitalia again, it is necessary to moisten them with amyl acetate and after having detached them, to rinse them in amyl acetate.

Fig. 9. Finally the small sheet with the genitalia is stuck above the labels on the same pin as the specimen.

The method described above may be applied to most Lepidoptera (and to some other orders as well); in certain groups of Lepidoptera are, however, modifications of the genitalia and the dissection must be modified accordingly. In cases where it is desirable to study the intersegmental membrane between the 8th segment and the vinculum, this membrane should not be cut; in cases of doubt as to the desirability of such a study, the whole 8th segment should be removed and cleaned together with the genital apparatus, before a decission about the mounting is taken. Sometimes structures on the 8th or 7th or earlier segments are to be studied as well. It is then recommended to remove and clean such segments together with the genitalia before detaching the genitalia proper.

The entire preparation as described will take 5—10 minutes for each specimen, when more preparations are made at the same time and it is not necessary to wait for each specimen to soften.

The description of the method is made for right-handed persons. For left-handed preparators right and left should be changed.

For informations on the anatomical names, consult: S. L. Tuxen (ed.): Taxonomist's Glossary of Genitalia in Insects, Copenhagen, 1956.