THE PREPARATION OF SLIDES OF LEPIDOPTERA GENITALIA WITH SPECIAL REFERENCE TO THE MICROLEPIDOPTERA

By GADEN S. ROBINSON

Dept. of Entomology, British Museum (Natural History), Cromwell Road, London SW7 5BD

INTRODUCTION

In modern taxonomic research on Lepidoptera the preparation of genitalia slides is a necessity and the preparations made by a worker, be he amateur or professional, will inevitably be referred to and used by others. The taxonomic value and information content of these preparations will often be greater than that of the specimens from which the preparations were made and this importance will not diminish with the passage of time. It is clear that genitalia preparations of rare or taxonomically important specimens must conform to the highest possible standards if these preparations are to form part of the legacy of knowledge of the Lepidoptera that past generations have amassed and upon which future generations will build. It follows that a genitalia preparation should be made in such a way that all features of the specimen, including those that are not of immediate interest to the preparator, should be preserved and displayed with as much clarity as possible; that the preparation should be suitable for drawing or photomicrography; that the preparation should be permanent and that it should be in a form which may be examined repetitively without damage.

Several recent publications (Cribb, [1974]; Kroon, 1973; Pierce, 1909 [repr. 1967]) have discussed the preparation of Lepidoptera genitalia but in these works the methods described are often too crude or unsuitable to be of use in the study of Microlepidoptera.

The morphology of Lepidoptera genitalia has been discussed in detail by Klots (1956) and this work is recommended to the reader.

In the following notes, certain of the methods used by the author and his colleagues are described: the results obtained using these methods are considered to go some way towards fulfilling the requirements outlined in the first paragraph.

INSTRUMENTS

The choice of dissecting instruments is, obviously, a personal one. The author and his colleagues find that two pairs of watchmaker's forceps, a very fine camel-hair brush, several very fine mounted needles (usually ·0076″ pins, some with the tip tapped into a hook), a snipe pin-feather, a pair of very fine spring scissors

and a hypodermic syringe with the needle ground square fulfil most requirements.

A good quality stereo microscope is essential for the dissection of Microlepidoptera. The instrument used by the author has a linear magnification range of $\times 6$ to $\times 100$. A monocular microscope of magnification to $\times 600$ may be used in subsequent examination of the preparation.

SUMMARY OF PREPARATION

The process of "dissection" may be divided into several distinct operations as follows:

1: Maceration in potash
2: Washing to remove potash; initial staining
3: Dissecting and cleaning
4: Staining
5: Dehydrating and hardening
6: Clearing and mounting

These are described below but it should be noted that staining (q.v.) may occur on several occasions: the worker exercises his own judgement in the matter.

INITIAL PROCEDURE

The complete abdomen is removed from the insect by upward pressure; the metathorax is gently pressed dorsally while the abdomen is removed to prevent severance of the metathorax and hindwings. Complete removal of the abdomen is recommended as cutting the abdomen damages it and leaves it incomplete and may also sever the saccus, aedeagus, apodemes or bursa copulatrix. The abdomen is placed in a test tube with about 5 ml of 10% potassium hydroxide (KOH—caustic potash) solution and the test tube placed in a beaker of boiling water for two to ten minutes, this time being dependent upon the size of the abdomen. The contents of the test tube are then poured into a small beaker, the abdomen removed and placed in 10% alcohol in a solid watchglass beneath the microscope.

DISSECTION

The author begins the dissection of a male abdomen by gently blowing a jet of 10% alcohol up the abdomen, using the hypodermic syringe, to clean out debris and extrude the genitalia. The tip of the abdomen is partly descaled by gently brushing with the tip of a snipe feather or fine brush and the genitalia then separated from the abdomen by cutting the attaching membranes. Extraneous debris and deciduous scales on the genitalia are removed by brushing or picking with forceps or needles. The aedeagus is removed, depending on species, by pulling posteriorly, anteriorly, or cutting through the anellus or manica with a needle. Superfluous membranes attached to the vinculum or tegumen are removed.

The abdomen is descaled by transverse brushing while holding the abdomen with forceps. With very small abdomens it is necessary to descale by picking off individual scales with a needle. The inside of the abdomen is thoroughly cleaned out with the syringe and forceps or a hooked needle: the interstices of the genitalia are likewise cleaned and the syringe may be used to evert the anal tube (otherwise accomplished with a hook) which is then trimmed. During this cleaning process the genitalia and abdomen are subjected to frequent changes of 10% alcohol. Any further dissection depends upon the species being dealt with; generally speaking, one assumes a method which exposes the taxonomically important characters of the group in question to the best advantage with the minimum of damage or distortion.

Dissection of females does not involve the initial spraying out of the abdomen as this is likely to cause damage. Preliminary descaling is followed by the separation of genitalia and abdomen: cutting of the abdominal wall anterior to the ostium may be necessary. The genitalia are cleaned, the anal tube is everted and the ovipositor extended: all dirt, debris, fungal hyphae, deciduous scales, receptaculum seminalis and remnants of the oviduct are removed. Great care is taken not to damage the apodemes of the 8th and 9th segments ("supporting rods" of Cribb, 1974). In clean 10% alcohol, the bursa copulatrix is perforated and the contents flushed out by gentle tapping with a needle: if present the spermatophore may require removal with a hooked needle. Debris lodged in the ductus bursae may often be removed at this stage by gently jetting 10% alcohol through the ostium using the syringe. The abdomen is thoroughly cleaned in the same way as in the dissection of the male.

STAINING

It is usual to stain genitalia preparations in order to improve both visual and photographic resolution of the resultant preparation. The author prefers Chlorazol Black E (1% solution in 70% alcohol) as a stain although aqueous solutions of mercurochrome are widely used. Preparations are lightly stained several times during the dissection process to aid recognition of fine membraneous structures. It is all too easy to overstain a preparation and with Chlorazol Black it is extremely difficult to reduce the degree of colouration: an overstained preparation is difficult to examine and well-nigh impossible to photograph.

MOUNTING

The practice of storing genitalia preparations with glycerine or clove oil in a glass tube is abhorrent. A preparation in this form needs to be handled to be examined, is difficult to store and is likely to suffer damage. Preparations in which the genitalia are glued to a piece of card pinned beneath the specimen are similarly

obnoxious. If a specimen is worth dissecting it is surely important enough to warrant the preparation being well and sensibly mounted. All Microlepidoptera genitalia preparations in the British Museum are mounted on glass microscope slides.

The author and his colleagues exclusively use Euparal as a mounting medium. The reasons for this are twofold: it is our object to standardise the media used in genitalia preparations in the collections under our care; the high refractive index of Euparal gives better definition of fine chitinous structures than, say, Canada Balsam.

Dissections are passed through 50% alcohol and thence to absolute alcohol. Before the preparation reaches absolute alcohol (which hardens it) the genitalia may be arranged so as to give maximal exposure of the relevant features: for example the genitalia may be placed on a slide, the valves spread and the gnathos pulled ventrally before absolute alcohol is dripped on to the preparation.

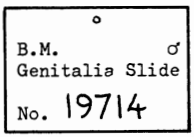
From absolute alcohol the preparation is passed to "Euparal Essence" (the Euparal solvent), which clears it and then the genitalia and abdomen are added to a small drop of Euparal on a slide. The parts of the preparation are manipulated, the convention of "ventral side uppermost" being observed. The coverslip is gently lowered on to the preparation, care being taken not to allow the genitalia to roll, bubbles to lodge in the abdomen or the aedeagus to float out from under the coverslip. The slide is kept flat and allowed to dry, either on a covered tray at room temperature or in an oven at 45° C. for 48 hours.

ARRANGEMENT AND LABELLING OF THE SLIDE

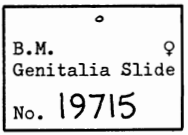
A standard form of labelling is in use in the Microlepidoptera Section of the British Museum (Natural History) for both slides and the specimens from which the preparations are made. The specimen carries a three line label (figs. 1, 1a) which is placed on the pin so as to protrude below the data label, thus obviating the need to remove a specimen from a drawer to read the preparation number. Slides are labelled in a standard manner (fig. 2) and the abdomen and genitalia arranged beneath the coverslip in a consistent pattern (fig.2, 2a). The form of labelling of the slide with the complete label data of the specimen permits easy reference where work is being done with slides (rather than with specimens), where slides are loaned and where the specimen label may have been lost or the specimen cannot be found.

INTANGIBLES

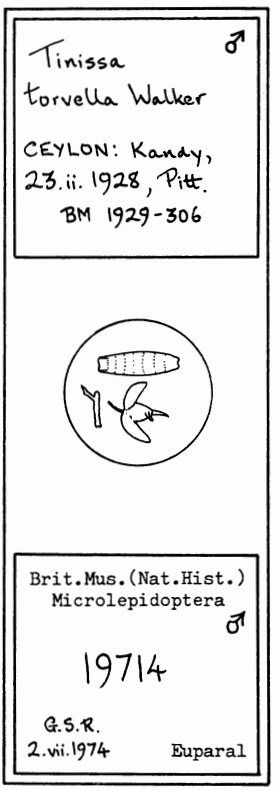
The quality of genitalia preparations depend very much upon the attitude of the worker who makes them. They are not easy to make; instant or one hundred per cent. success is not assured yet high standards are possible if one remembers that the funda-



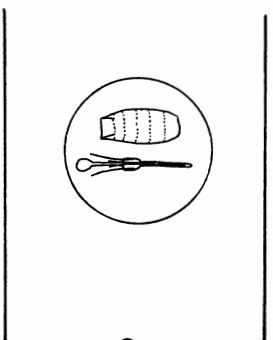
1



1a



2



2a

Labelling and slide arrangement used for Microlepidoptera by British Museum (Natural History).

1, 1a. Specimen labels for ♂ (1) and ♀ (1a) genitalia preparations.

2, 2a. Labelled slide with standard arrangement of ♂ (2) and ♀ (2a) genitalia.

mental requirements for making successful preparation are cleanliness and patience. Preparations cannot be completed in fifteen minutes and the author and his colleagues believe that six slides a day is a high level of output. It takes many hours of delicate work to re-dissect and re-mount a poor preparation and salvage work of this type should not be necessary. Sadly, it frequently *is* necessary and even for preparations made by well-known professional entomologists. Quantities of preparations (and publications) are no substitute for quality. The legacy of substandard genitalia preparations (often of type specimens) of Microlepidoptera left to posterity by workers unable or unwilling to produce clean and carefully prepared slides is now a serious problem. This has necessitated the recent introduction of stringent limitations on the dissection of British Museum Microlepidoptera material and the requirement being imposed that slides produced conform to the standards outlined in the introduction to this paper.

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