MOUNTING AND CLEARING

Notes on some useful arachnological techniques

by J.A.L.COOKE.

In the course of identifying spiders it is becoming increasingly necessary to examine the internal genitalia (vulva) of female spiders in addition to the epigynum. Although there are quite a number of techniques that may be employed, all have certain disadvantages and there is no agreement on which one is best. This paper summarises methods that I have found satisfactory for my own particular purposes.

Basically, the choice is between submitting the whole specimen to treatment, thereby risking damage to non-genital structures such as spines and hairs, or cutting out the genital region and treating only this small portion of the specimen. In this latter case there is a very real danger that the prepared genitalia will become separated from the rest of the specimen and lost in the course of time.

If the whole specimen is to be treated there are two main alternatives. Either the entire spider is macerated in hot potassium hydroxide (5-10 percent), or it is allowed to clear slowly in a solution of suitable refractive index such as clove oil, beechwood creosote or phenol. After maceration in potassium hydroxide, which removes all the soft parts and leaves only cuticular structures, the remains may either be mounted entire on a slide as a permanent microscopical preparation or examined and stored in alcohol. In either case, little of value remains apart from the genitalia and the rest of the specimen is virtually useless for taxonomic purposes. Total immersion in a clearing agent is usually a rather protracted business and liable to be time-consuming. Although the most informative picture is usually obtained well before the whole abdomen is cleared, nevertheless this may still take several hours and need continual surveillance. Moreover, the specimen may well sustain permanent damage either through irreversible clearing or by the abdomen swelling and bursting. Levi (1965) has proposed a particularly useful modification of this technique. The genital region is cut round on three sides and the resulting flap folded up at right-angles to the abdomen. The specimen is then immersed in clearing agent which clears the small flap, together with the genitalia, very rapidly and effectively. The structures can be examined and the spider returned to alcohol long before any damage is done to the rest of the animal.

If the genitalia are excised from the spider there are a variety of treatments available to reveal details of structure, but ultimately the excised portion must either be mounted as a permanent slide preparation or stored separately in a microvial, which should if possible be kept with the rest of the animal in a larger tube. When a permanent slide mount is required, consideration must be given to the best mounting medium to employ. Traditionally, this has usually been canada balsam or euparal, both of which require meticulous dehydration through several changes of alcohol. Several water-soluble mountants based on gum arabic and chloral hydrate (e.g. Hoyer's medium) have been quite widely used in recent years, but slides prepared in this way cannot be regarded as permanent, even though ringing with gold size can prolong their life considerably. Quite the best mountant for arachnological purposes is the new synthetic resin Dimethyl Hydantoin Formaldehyde (D.M.H.F.). It is col-

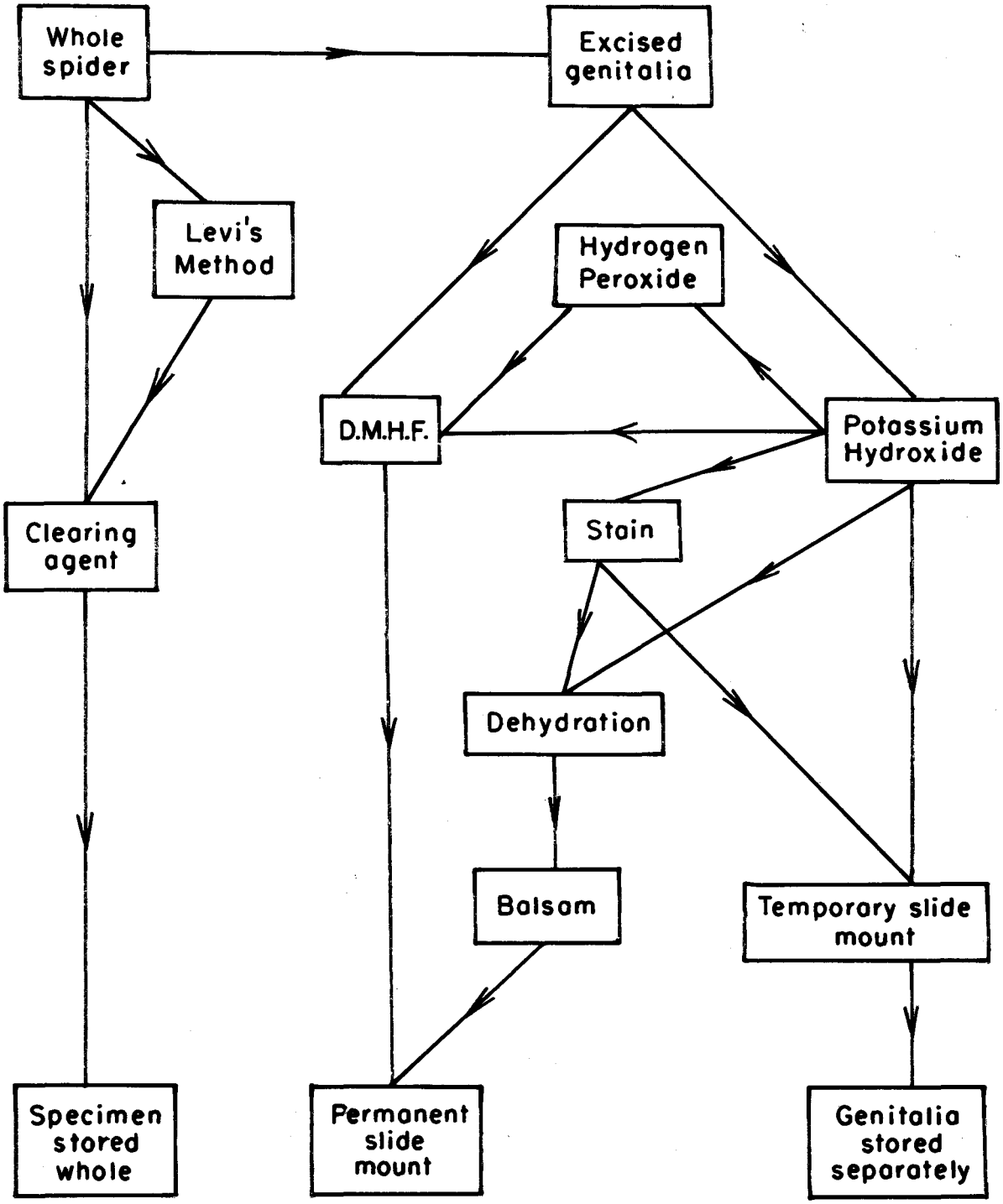

Fig.1. Synopsis of the main techniques for examining spider genitalia.

ourless, miscible with both water and alcohol and does not shrink. Its refractive index (1.46 approx.) is perfect for clearing spider cuticle. Although 'instantaneous' mounts may usefully be made by transferring the genitalia (or even the whole spider if small) to D.M.H.F. on a slide and putting on a coverslip, it is sometimes helpful to remove adhering soft parts by gentle warming in dilute potassium hydroxide first.

It not infrequently happens that even after treatment with potassium hydroxide the genitalia are too deeply pigmented to be clearly seen. Komatsu & Yaginuma (1968) have published details of a technique for depigmenting spider genitalia. This involves heating the specimen for 5-15 minutes in 3 percent hydrogen peroxide, either with or without prior maceration in potassium hydroxide.

If the genitalia are to be stored in a microvial, then it is often useful to prepare temporary slide mounts. Experience has shown that lacto-phenol is a particularly good and fast-acting clearing agent that can readily be used as a temporary mountant. This is especially so when parts of the genitalia are unpigmented, as in many of the haplogyne spiders, and the lacto-phenol is used in conjunction with alkaline aniline blue as a stain to make them visible.

Details of Techniques and Reagents

1. Removal of genitalia. This is best done under the dissecting binocular microscope, holding the spider's abdomen with fine (No.5) forceps. The cuticle surrounding the genital region may either be cut with a fine blade (e.g. surgical eye-scalpel) or with sharpened tungsten needles. These are made by dipping tungsten wire into molten sodium nitrate. Tungsten wire may be obtained from: Johnson, Matthey & Co. Ltd., 73-83 Hatton Garden, London, E.C.1. Fine forceps (No.5) may be bought for about £1 a pair from: Ferrier & Alston Ltd., 49-50 George IV Bridge, Edinburgh I.

2. Potassium hydroxide treatment. The generally recommended technique of boiling in 10 percent potassium hydroxide is best avoided as the specimen is very likely to be distorted. There is also a grave risk of the highly corrosive fluid 'bumping', which is not only dangerous but almost certain to result in the loss of the specimen. The use of more dilute (5 percent or less) potassium hydroxide warmed in a test tube in a beaker of boiling water is much to be preferred.

3. Hoyer's medium. Details of the preparation of Hoyer's and other water-soluble mountants are given by Evans, Sheals and Macfarlane (1961).

4. Dimethyl Hydantoin Formaldehyde (D.M.H.F.). This excellent mountant was first introduced by Steedman (1958). It is obtainable from: Rex Campbell Ltd., 7 Idol Lane, London, E.C.3. Grind the resin and dissolve 80 gm. in 30 ml. of distilled water, which takes about three days. 70 percent alcohol may be used instead of water. Filter at least once (rather tedious). If an aqueous solution is used, add phenol to 5 percent to suppress the growth of mould. When drying slides made with D.M.H.F. take care not to heat above 40°C or bubbling may occur.

5. Lacto-phenol. Commercial lacto-phenol contains too much water. The best solution is made by dissolving phenol in concentrated lactic acid until it begins to crystallize out. It should be noted that extraordinarily large amounts of phenol will dissolve in quite small quantities of lactic acid, particularly if heated.

6. Aniline Blue. Usually stocked as a 1 percent aqueous solution. This should be diluted considerably and made alkaline by the addition of concentrated (.880) ammonia, when the blue colour will disappear. The specimen should be immersed in a drop of this solution for a few minutes and then transferred to lacto-phenol, when the blueness will reappear. If over-stained, rinse briefly in dilute ammonia and return to lacto-phenol. Aniline blue is obtainable from any of the usual biological supply houses.

Further information on staining and other useful techniques can be found in Peacock (1966), Pantin (1948) and Eltringham (1930). The latter is long out of print, but is well worth consulting in libraries.

References.

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FIELD MEETING AT WHITEDOWNS, SURREY (TQ 1249), 1st JUNE 1969.

Five members, their families and friends attended the field meeting at Whitedowns, where a total of 40 species of spiders were taken, mostly by grubbing among the short turf. Among these 40 species, the following are additional to those listed for Whitedown by Nellist (Bull.No.4 Vol. 1, pp. 55-60). Nomenclature to L.& M. Vol. II, 1953.

Atypus affinis, Dysdera crocata, Clubiona neglecta, C.compta, Agroeca brunnea, Xysticus erraticus, X. bifasciatus, Neon reticulatus, Lycosa monticola, L.tarsalis, L.amentata, L.lugubris, L. hortensis, Episinus angulatus, Asagena phalerata, Enoplognatha thoracica, Pachygnatha clerki, Singa sanguinea, Wideria antica, Metopobactrus prominulus, Erigone dentipalpis, Microneta viaria, Bathyphantes concolor, Lepthyphantes obscurus.