Making permanent fluid mounts of minute arthropods

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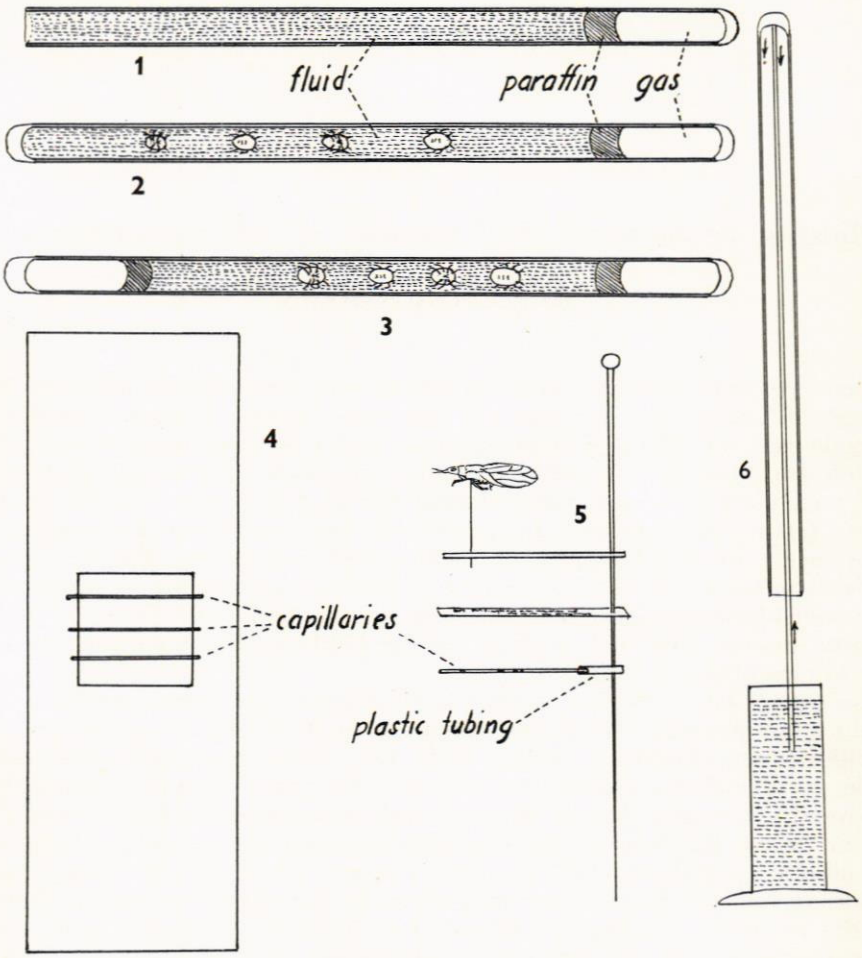
Good permanent microscopic mounts of very small insects like first-instar larvae of aphids and psyllids are not easily made. Mounting media like Canada balsam, Euparal, gum chloral, and polyvinyl alcohol compounds which are more or less useful for mounting adult insects and older larvae will nearly always cause much shrinking and deformation if used for first-instar larvae. Fluid mounts intended to be permanent are rarely quite reliable owing to the difficulty of sealing such mounts effectively. Nematologists use Gurr's Glyceel for sealing lactophenol or glycerine mounts which they consider to be permanent. Perhaps this method could be useful also for minute insects, but such mounts are probably easily damaged when one has to clean the coverslips.

Recently I have adopted another method based upon glass capillaries containing the mounting fluid into which the small insects are introduced. The capillaries are closed by melting both ends. Then they are placed across a slide, an adequate quantity of a suitable "external" mountant is added and a coverslip is superimposed. The procedure is described in detail below.

For broad, flat insects like Psyllid larvae, flat glass capillaries (i.e. with an elliptic or rod-shaped transverse section) are preferable. They can be made as follows. Heat thin-walled glass tubing about one cm in diameter in the flame of a fishtail or Bunsen burner until the heated part is quite soft. Compress the soft part moderately by aid of a pair of tongs with flat jaws. Heat the compressed part again and when it is soft enough take it out of the flame and pull. The resulting capillary will be more or less flat. Break it in pieces about 2 cm in length, inspect these under a magnifier or binocular microscope and discard those not having the desired shape of transverse section.

Filling the capillaries with mounting fluid and closing them can be done in several ways. In a laboratory where a vacuum equipment is available the following method is convenient because a great number of capillaries can be filled in one operation. Close the capillaries at one end by heating just the extreme end in the lower part of a spiritus flame. Avoid making a large knob of glass. Place the capillaries standing vertically with the open ends downwards in a cylindrical vessel containing the intended mounting fluid. Place the cylinder in an exsiccator which is then evacuated. When athmospheric pressure is restored, the capillaries will be filled with fluid but a small air-bubble will remain at the closed end. The better the vacuum obtained the smaller the bubble. This bubble is difficult to get rid of completely.



Figs. 1—6. (1) filled capillary closed at one end by the paraffin method. (2) capillary mount filled in vacuo or by a thinner capillary and finally closed by the paraffin method. (3) capillary mount closed at both ends by the paraffin method. (4) slide with capillaries mounted under a coverslip. (5) pinned insect with genitalia mounted in capillary. (6) method of filling a capillary by a thinner one.

This is different with different viscosity and other properties of the fluid. With lactophenol, the remaining bubble will often disappear if the capillaries are kept standing with the open end upwards. Glycerine is far less favourable regarding this detail. But in most cases a small air bubble at one end of the capillary will probably do little harm in the mounts.

If no vacuum equipment is available, capillaries closed at one end can be filled individually by the following procedure (see Fig. 6). Make capillaries thin enough to be conveyed into your flat capillaries. Break them to pieces somewhat longer than the latter. Push a thin capillary into the one

which is to be filled so that the inner end of the former reaches the closed end of the latter. Dip the outer end of the thin capillary only in your mounting fluid which will then enter and fill both tubes by the capillary force. Let the mounting capillary be submerged in mounting fluid when you are drawing the fine capillary out of it. This technique works well if your fluid is not too viscous, and the capillaries will often be completely filled.

Alternatively the capillaries can be filled and closed by what is here referred to as the paraffin method which is also used for the final closing of capillary mounts. Start with a capillary open at both ends. Fill it by dipping one end in the mounting fluid. Heat one end slightly by holding it near the periphery of the lower part of a spiritus flame. Some fluid will then be removed by a small explosion. Dip the hot end of the capillary immediately in melted paraffin wax. Some paraffin will then enter the capillary. Close the end of the capillary containing the paraffin by heating in the lower part of a spiritus flame. Make sure that the closure is complete by inspection under a binocular microscope. If there is a hole or open duct heat again until a complete closure has been obtained but avoid making a bulb. The perfect result of these operations will look as in Fig. 1. A paraffin plug will now isolate the gas bubble always present at the closed end of the capillary from the mounting fluid.

Store filled capillaries in a vessel containing some mounting fluid. Make glass rods fine enough to be introduced into the capillaries.

Mounting procedure: Specimens cleared and ready for mounting are transferred into a Petri dish or other flat-bottomed vessel containing some mounting fluid and a few filled capillaries. Choose a capillary with dimensions suitable for your material and introduce a specimen into its opening with a fine needle (micro-pin). Use a fine glass rod to push the specimen into the desired position within the capillary. A few specimens can be mounted in one capillary, but leave room at the open end for the inevitable gas bubble. Take the capillary out of the fluid, clean it on the outside and close it by the paraffin method. The resulting mount should look as in Figs. 2 or 3.

Place your capillaries across a slide in a large drop of a suitable "external" mountant. Mounting media which do not lose too much of their volume while drying should be preferred, and it is an advantage if they are easily soluble in water or in some other solvent. I use coverglasses with a diameter slightly smaller than the length of the capillaries (see Fig. 4). Then the position of the capillaries can be adjusted after application of the coverslip, until the external mountant is dry. If only one capillary is to be mounted on one slide, pieces of glass rod should be used as supports for the coverslip to obtain a horizontal position of the latter. Let the slides dry at room temperature.

With this method you have a liberal choice of possible fluid mounting media and a practically free choice of refractive indices from that of pure water ($n=1.336$) upwards. Chemicals dissolving paraffin should not be used. Avoid using concentrated salt solutions which may crystallize. With concentrated lactic acid there is a risk of anhydrid crystals appearing in the mounts as a result of the heating for closing. Mixtures of water with glycerine, lactic acid or acetic acid as well as lactophenol and pure glycerine are available. If distilled water or very diluted aqueous solutions of lactic or acetic acid are used you should probably add a small amount of some

sterilizing agent, but this is perhaps unnecessary if the specimens have been treated with phenol in the clearing procedure.

So far I have used this mounting method for first-instar Psyllid larvae only, but I have also tried it for nematodes. The method has its obvious limitations, but I think that it can be useful for many purposes where the material is very small and delicate, like early larval instars of aphids, coccids, mites, &c.

Permanent mounts of the genitalia of pinned insects can also be made in the same way. For this purpose cylindrical capillaries should be used making it possible to study the mount in more than one direction. Such a mount can be fixed to the needle by inserting one end of it in a small piece of plastic (polyethylene) tubing which is then pinned under the insect's locality label. (See Fig. 5.) When one wants to examine these mounts they should be placed in a drop of glycerine on an excavated slide and covered with a coverslip.