On mounting Aphids and other soft-skinned insects

by
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In 1948 E. O. ESSIG published an article on the mounting of Aphids and other small insects. In this paper on p. 13 a method is discussed which is said to have originated in the Netherlands, but which in the form in which ESSIG describes it has certainly never been used in this country. Therefore a correction of ESSIG's paper as to this subject seems necessary. For there really exists a method for mounting Aphids which was developed in the Netherlands and which so far has given most satisfactory results.

The first paper on this method was published by FRANSSEN in 1927. He boils his fresh material for some time in 96% alcohol and then in lactic acid of 70—75% in tubes in a waterbath and finally washes out the lactic acid in destilled water. The aphids are mounted in a gum-arabic mixture. The results were seldom satisfactory, because the body is not cleared completely and crystals are usually after some time obscuring parts of the aphids. ROEPKE in 1928 and 1929 considerably improved FRANSSEN's method of clearing. Instead of washing out the lactic acid, the aphids were transferred from lactic acid into chloralphenol, a mixture of chloralhydrate and pure phenol in equal weights, in which they were heated for some time on a waterbath, after which they were mounted in a gum-mixture. Aphids treated this way are generally excellently cleared and the mounts show more details than aphids prepared in any other way of which I so far saw the results. The slides keep well and slides made in the year of publication of ROEPKE's method show no trace of destruction of the tissues to which ESSIG refers. The mounting medium is not less permanent than balsam, euparal or similar substances as far as we know after 20 years of constant use of a gum-mixture as mounting medium.

The newer methods of Aphid-classification as developed by BÖRNER often make use of the chaetotaxy of first instar larvae. As these forms are often absent in samples, and as there is no way by which to decide with certainty that a certain larva really is a first instar larva (even BÖRNER in his 1930 paper seems to have described second instar larvae in a key as first instar larvae) fullgrown embryones inside the mother usually are an excellent substitute for first instar larvae. Besides, the study of the morphology of the embryones inside the mother often has given valuable information about the biology of the species. And in some cases embryones or eggs inside the mother are the only ready character by which one can find out what morph one is examing : oviparous female or viviparous female. Therefore one certainly should avoid the removal of embryones as much as possible, and where embryones make examination of some parts of the mother more or less difficult, one should improve one's methods of clearing and mounting, rather than remove the embryones as prescribed by ESSIG. Certainly none of the younger European workers on Aphids would remove embryones from the mother's body, since they are not in the way and in correctly cleared specimens form no hindrance for the study of any morphological detail.

The method of clearing as described by FRANSSEN and ROEPKE has some disadvantages. The whole process of clearing usually takes at least

one hour. And certain species, notably members of the Phyllaphidina ("Myzocallina") in the alate form, explode when heated in lactic acid, like long pickled specimens of any species of aphid ; the thorax bursts and after mounting, the specimens show shrinking to an often intolerable degree. The formula of the mounting medium as used by ROEPKE is not quite satisfactory ; the fluid dries slowly or remains soft in an environment of not excessively low relative humidity.

The following method is a combination of long known and described methods. As far as I know it is the quickest and most satisfactory of the described methods of mounting of which I saw the results.

I. Collecting and Pickling. Aphids are removed from their substratum with a small soft brush, which often damages the insects, or with forceps. The latter method is done by compressing the forceps with thumb and middle finger while the index is pressed on the edge of the closing forceps, so that also part of the skin of the fingertip is seized. Thus the forceps can be closed very slowly and carefully without any danger of damaging the soft insects. The aphids are submerged in 96% ethyl alcohol. In this they should preferably not stay longer than 1½ year, as they harden slowly, and are more difficulty cleared after a longer period of storage. They soon become very brittle, and as most species have long legs, antennae and siphunculi, special care must be taken to avoid broken appendages. Therefore I use small and very narrow tubes, 3—4 mm wide and 30—50 mm long. These are filled to the brim with alcohol, and after the aphids have been put in, a number referring to extensive notes on colour, wax-excretion, hostplant, etc., is added. They are closed with a thoroughly wet cottonplug, so that no air is left in the tube, and the filled tubes are put in a small, solid, wide-mouthed bottle which also contains some alcohol and which is closed with a stopper. Full bottles can be transported and mailed without any danger to the aphids, because there are no moving air-bubbles inside the tubes.

II. Clearing. The clearing process is done in tubes of 6—7 by 120 mm, small test-tubes, in a waterbath. As such any vessel in which the tubes can stand may serve. As aphids are often damaged in handling them with a needle or other instrument, they should not be touched more than strictly necessary.

Fresh material is first heated in the tubes to or just below the boiling point in 96% ethyl-alcohol for 5—10 minutes. Before placing the tubes with alcohol and aphids in the waterbath, explosive boiling of the alcohol should be prevented. This is done in my lab by adding a small piece of sinter of a burnt matchhead. Material which has been in alcohol for more than a month needs not be boiled in alcohol.

The alcohol is decanted and some 10% KOH is added to the aphids. In this they are heated in the waterbath for 1—5 minutes, so that the KOH does not or hardly boil. Small specimens are heated for 1 minute only, very large specimens for 5 minutes, dark, coccidiform species like *Ceratapis*, etc., till they are pale. The effect of the heating in KOH often is hardly visible, though some species show distinct clearing even after so short time.

Remove the KOH by decanting, after cooling. Usually the aphids sink but sometimes they do not, and then it is advisable to add a larger

quantity of alcohol. In the resulting liquid the aphids, filled with the heavier KOH sink rapidly and the liquid is decanted. Clearly the quality of the alcohol is of no interest, only its specific gravity, so that methylated spirits can be used as well. The alcohol removes nearly all the KOH, which is useful because KOH reacts in an undesirable way with the next step of the process.

Add a few cc of chloralphenol, a saturated solution of chloralhydrate in phenolum liquefactum. Heat in this for 5—10 minutes at the boiling point of water in the waterbath. The aphids clear rapidly in this liquid.

This process of clearing has the advantage over ROEPKE's method, that it works faster, and excludes the explosion and shrinking of certain species. The short period of clearing in KOH does not affect the pigmentation of the sclerotic areas in the skin to any extent. Transfer of the aphids from one step to the next is done without touching them, thus reducing the danger of broken appendages to a minimum.

The cleared aphids can be kept for a long time in the cold chloralphenol, provided that they are stored in the dark. They can be mounted at any time after being cleared.

III. M o u n t i n g. The aphids are poured with the chloralphenol in a watchglass or other suitable container. From there they are transferred with a dissecting needle, or with forceps in a drop of choralphenol to the mounting medium which is spread on the slide. Wings, legs and antennae are placed in the desired position, strongly swollen specimens are slightly depressed with the needle and alatae can be prevented to roll over by depressing their thorax. Some species possess very thin-skinned apterae with wax-glands and the study of the latter becomes difficult when the abdominal skin shows wrinkles or folds. To prevent any shrinking in such exceptional species a ring of mounting medium is painted on the slide and the center filled with chloralphenol ; the aphids are then arranged in the chloralphenol. After this the coverslip is brought into position. The chloralphenol later mixes with the mounting fluid.

I use a mounting medium of the following formula : gum arabic 12 g., concentrated glycerine 6½ cc, chloralhydrate 20 g., destilled water 20 cc. Select pale lumps of gum arabic, dissolve them in the cold (room temperature) with the other substances, but use 40 instead of 20 cc water. Filter without heating through glasswool, eventually twice. Then place the filtrate in a dustfree thermostate of 30—40° C. in a flattish dish and let the water evaporate till the fluid has the desired viscosity, when it will have the first mentioned formula. If one does not add a surplus of water, one has to filter the medium on a heated funnel ; the losses in material and time are considerable and besides one may loose a considerable portion of the chloralhydrate in the process. If the medium is too thick, the aphids may shrink, and if it is too liquid, the aphids may be depressed when the slides are dry, because too much of the mouting medium has evaporated. After the slides have been drying slowly for about a month, they can be sealed with Murrayite, a sealing fluid made by FLATTERS & GARNET, Manchester, Sealing, however, seems not to be necessary in normal circumstances. Besides, sealing with Murrayite may be the cause of a sub-microscopic granulation of the mounting medium.

The technique described above takes a little time, but it is ideal for

rather large samples such as are wanted for descriptive purposes.

Sometimes it is very desirable to examine immediately a few specimens in order to know what they are. For such purposes the following method is advised.

a. Paint a ring of the described gum-mixture on a slide, heat and harden it with a lighted match or lighter. Fill the center with chloralphenol.

b. Kill some specimens in 96% alcohol.

c. Transfer them to the chloralphenol in the gum-ring, put on the coverslip and heat with a match till the chloralphenol just begins to boil. The aphids clear in a few seconds and if not sufficiently, they will do so if the heating is repeated once.

This method gives very good slides, and is highly useful for mounting young larvae which tend to get lost in the normal technique. The slides, however, dry slower and since the technique is only 2 years old, it is not yet known how long the slides will keep.

IV. Storing and labelling slides. Instead of normal labels I use labels glued or printed on soft cardboard of 1 mm thick, which are glued on the slide. On the left side of the slide such a label, bearing the name of the genus, the species, the author who described it, the morphs included and the person who identified them is fixed, on the right side a label on which is written the country, the hostplant, the date, the locality, the collector and reference numbers. Because of the labels the slides can be piled one on top of the other without danger of the coverglasses touching. Slides labelled this way are used as index cards. I store them in vertical boxes with a hinged lid, each box holding about 60 slides in a horizontal position, the slides arranged in alphabetical sequence. Ten such boxes, each with the name of the genus written on the top-end are stored in alphabetical sequence in a dustfree drawer, the front of which bears a label indicating the first letters of the genera in the drawer, and a handle. The drawers are also arranged alphabetically. In this way very large numbers of slides can be stored in a minimum of space, arranged alphabetically, so that no special card-index is required.

If no special attention is given to the glue with which the labels are stuck to the slide they will come off sooner or later. One should use a glue which becomes never wholly hard and brittle. As such the described mounting medium without chloralhydrate will do, but also other water-soluble glues, provided a little glycerine is added, and besides Miracle, a cement described in the Readers Digest of May 1947.

It will be noticed that staining is not mentioned in the method. So far I have not seen stained aphids which could compete with unstained ones mounted with the technique here described for the first time. Except to those who are in love with the bright colours produced by staining I would advise against staining, because it obscures the contrasts between pigmented and unpigmented areas of the tergite, contrasts which are essential to modern classification of aphids.

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