

5 B. Analysis of Pollen from Wild Bees

Paul Westrich

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Observations of individually marked bees in the wild are absolutely necessary, but are only really possible until the bees disappear from view. In difficult country or if flowering trees are visited, extended field observation is virtually impossible. The assessment of oligolecty and the range of pollen sources used by a species, can, therefore, only be clarified beyond doubt with the aid of pollen analysis.

I Field methods

I.1 Study areas & floral recording

The study area selected for consideration should not be too small. All possible pollen sources should be identified as well as their frequency, distribution, flower density and flowering condition. Individual flowering shrubs and trees in the vicinity should also be investigated.

I.2 Pollen removal in the field

A prerequisite for successful field work is a sound knowledge of bee taxonomy and a thorough literature study of the bees' behaviour (nesting sites, nesting behaviour, and known flower associations). Pollen can be removed at the collecting site (without anaesthetizing or killing the insects) either from foraging females of species that can be determined with certainty in the field, or from those which are required for further observation. If Megachilinae (e.g. *Anthidium*, *Osmia* & *Megachile*) are put into a small glass tube and are kept in the dark (for example in the trouser pocket) they remove the pollen in the course of a few minutes by cleaning the abdominal scopa. With the remaining bee species, pollen removal is often more difficult, since not all species clean off their pollen in the glass tube. Indeed, occasionally they will only shed their pollen load a long time later. Where necessary, such bees are dealt with by carefully brushing the pollen with a clean insect mounting pin into a watchglass. This method, however, can only be applied to specimens which have collected plenty of pollen. Carefully place and leave the bee (still in its tube) for a few minutes in a larger container which is packed with ice cubes.

I.3 Transporting collected bees

If the insects can not be identified with certainty in the field or if the pollen can not be removed, one will need to kill some of the captured specimens. To prevent contamination with foreign pollen the insect should not be placed in the same tube as any other bee, either living or dead, even of the same species and one must, of necessity, use thoroughly cleaned, dry containers. The bees should be killed directly after catching. This is especially important, if, for example, the entire pollen load needs to be photographed. In order to prevent the vestiture of the insect being stuck together with regurgitated nectar (which can make subsequent determination difficult), some strips of tissue paper should be placed in the killing jar.

2 Laboratory techniques

2.1 Light microscopy

2.1.1 *Preparation of the pollen load*

Specimens from collections and fresh specimens are both suitable for examination of their pollen loads, as the age of the pollen is not important for the quality of the preparation. Curators should, in principle, not remove the pollen loads from specimens, either in collections or from those being transported, as an important biological document is destroyed by cleaning.

Removal of the pollen from the Scopa

Before one takes the pollen, the colour and nature of the pollen load should be noted (e.g. solid or loose, moist or dry) as well as where it is carried. Very often one can readily recognize under the stereo microscope if the pollen load contains the pollen of more than one plant species by the size, the form, or the colour of the pollen grains. Furthermore the amount of pollen on the scopa should also be recorded as a measure of the amount of pollen being transported back to the cells of a nesting insect. An estimated value of, for example, approximately 3/4 of the complete pollen load, is written down. With specimens, that have been caught and transported separately, one must always assume that they are contaminated by foreign pollen from other specimens. The removal of the pollen is done under the stereo microscope with an insect pin of an appropriate size. Of course, all the equipment used (needles, forceps, microslides) must be very clean. To minimise the loss of pollen, the cleaned microslide, as well as the coverslip, should be placed on a piece of paper of approximately 12 x 12 cm. If pollen grains fall off the microslide during preparation, they can be returned to it with an insect mounting pin. It is of the utmost importance that no pollen is carried off to contaminate other slide preparations. Nevertheless the sequence by which the pollen is prepared should be adhered to as a precaution. The method may also give a clue as to the possible origin of any extraneous pollen. It will usually be sufficient to use only half the pollen load from the scopal hair. One then has some in reserve should the preparation fail or a more exact investigation be necessary (e.g. by scanning electron microscopy).

Degreasing and mounting the pollen

It is only really necessary to degrease pollens which are oily (e.g. Asteraceae and Fabaceae) and it is difficult to do without losing at least some of the pollen but, however, during the preparation stage examples of each pollen should be degreased so that comparative examination is possible. When being degreased the pollen loads are brought on a clean watchglass and a few drops of Diethyl Ether (inflammable) are added. After a short stir, the Ether is drained into a waste container for subsequent safe disposal. The watchglass remains in a well ventilated place until the Ether has evaporated completely. The degreased pollen is placed on a microslide with an insect mounting pin. For mounting the pollen preparation, use a glycerine jelly mixture warmed up to 40 degrees Celsius. The glycerine jelly is warmed up in a double boiler (this can be done inexpensively by using a baby bottle warmer for example) or in an airing cupboard. The pollen needs to be spread in a thin layer, as identification becomes difficult if pollen grains are lying on or over one another. If one wants to examine the entire pollen load of larger bee species, a single microslide will no longer suffice. The microslide is put on a hotplate to allow the pollen grains to absorb moisture and to achieve their maximum size. One can make a home made hotplate by using a ceramic infra-red emitter, a switch and an aluminium plate. One or two drops of glycerine jelly are placed on the pollen by using a glass rod. Pollen grains that adhere to one another can easily be separated by stirring with an insect mounting pin. When older collections are being used, the pollen load will occasionally dry into a lump (e.g. with Fabaceae or Asteraceae pollen). In such cases, the carefully retrieved pollen lump is squashed in somewhat more glycerine jelly than one would normally use. For avoiding air bubbles when mounting, Zander (1935) recommends placing a tiny drop of glycerine jelly on the underside of

the preheated coverslip but it is usually sufficient just to preheat the coverslip on the hotplate for a short while. Use enough glycerine jelly to prevent air bubbles becoming trapped under the edge of the coverslip. After putting on of the coverslip (this can be from 18x18 mm up to 21x26 mm in size depending on the quantity of pollen) the slide should be left on the hotplate, until the glycerine jelly has distributed itself evenly under the coverslip. If one makes pollen preparations from material that has been in a collection for more than fifteen years, pollen degreasing is no longer necessary, the pollen oils apparently becoming denatured (?through bacterial action) in the course of the time.

2.1.2 Pollen preparation from the brood cell contents and from larval frass

It is also very revealing to examine pollen from brood-cells. If one colour-marks the bees during nest construction and regularly, for example, over a one or two day period, keeps the nest under observation, one can make an exact statement, on the basis of the pollen analyses, which pollen sources were visited and if and when a change of the forage plant(s) has taken place. (For marking the bees one can use a quick drying non-toxic model construction paint or a lacquer-pen. The colour marks can be placed on thorax or the abdomen with a fine brush or a wooden toothpick). Most bee larvae void their frass for the first time only when they reach the last larval stage, after the food reserve is consumed and prior to entering the pre-pupal stage. However, with many species of Megachilinae, frass is produced in small "frassballs" during the earlier stages of larval development. In each case, the frass remains behind after the adults have emerged from the cells and subsequent analytical examination of the pollen can reveal interesting clues as to the composition of the larval food. When preparing this material, single "frassballs" are placed in a drop of distilled water on a microslide and distributed using an insect mounting pin. After the water has evaporated, the preparation is mounted with glycerine jelly as described above.

2.1.3 Reference collections

Accurate determination of the pollens collected by wild bees is often only possible with help of a reference collection. A "Pollen Herbarium", (the refence collection of microslides) is a valuable and necessary supplement to the specialist literature. For a start, reference material of pollens of all the possible wild bee forage plants that are in flower in the study area should be made. With long-term studies, it is advisable to maintain a more extensive Pollen Herbarium. Newly opened flowers and inflorescences are separated by species into unused plastic bags. The plastic bag is sealed with a rubber band so that the air trapped inside protects the flowers during transportation. Zander recommends that the collected plants should first be left flowering in the laboratory, to minimise the risk of cross-contamination from other flowers in the home, either by wind or through insect visits. Under the stereo microscope, some anthers are pulled off with fine forceps and are put in a watchglass. If the anthers are still closed and the pollen still within, they can be dried in a dust-free environment and this will cause the anthers to dehisce. By using this technique the pollen will not germinate or a pollen tube start to grow. For degreasing, the stamens can be treated with a few drops of Diethyl Ether. After degreasing, the pollen can be prepared in exactly the same way as the bee pollen loads (see above). The beginner should first make degreased and undegreased slide preparations from all the flowers. One learns most quickly from comparison with reference material which plant families do and which do not not require treatment of their pollen with Ether.

2.1.4 Sealing the slide preparations

After several days, the pollen reference material can be sealed around the edge of the coverslip with nail varnish. This will protect the preparation from moulds. If the slides are stored in a

dry environment there is no need to seal the coverslip. This is based on only short term experience, but applies equally to pollen loads or to brood cell contents.

2.2 Scanning Electron Microscopy (SEM)

The preference for using light microscopes in pollen analysis lies in the fact that larger quantities can be processed in a relatively short time. For verifying results Scanning Electron Microscopes are eminently suitable, and can also be used to examine the packing density of the pollen loads in species which have different pollen collecting strategies.

2.2.1 Scanning Electron Microscopy of the pollen loads of bees

This method can be used to examine pollen from freshly killed or from different aged cabinet specimens. Parts of the body with pollen loads (for example the hind leg, or abdomen) can be carefully removed using pointed fine forceps and under a stereo microscope. Care should be taken to avoid touching the pollen load itself. In most cases it will not be necessary to use the Ether degreasing technique mentioned above. Degreasing can move the pollen grains and alter the pollen grain density in the scopa. If the existence of oils in the pollen load impairs the quality of the micrographs, then a second, degreased preparation of, say, the other hind-leg can be attempted. The specimens are mounted on the preparation plates using carbon or silver. When attaching, it is important to watch carefully to ensure that the preparations are embedded with their entire underside in the adhesive, and that they have contact with the plate surface through it. Both the legs and the abdomen should remain free of the adhesive to prevent the hairs becoming rigid. Care should be taken, as the solvents present in the adhesive may be drawn up into the vestiture of the bee and thus spoil the preparation. Before starting, the adhesive should be thoroughly stirred with, for example, a wooden toothpick. After placing a drop of adhesive on to the preparation-plate, keep the material under observation using the stereo microscope, until some of the solvent has evaporated and the adhesive has become a thick paste. The object is then pressed into the adhesive, with a pair of fine pointed forceps. Affixing the specimens is a matter of experience, and one should first practise on a less precious specimen (e.g. a honey bee leg). If the object is stuck in the desired position, look at the pollen load under high magnification with the stereo microscope. If you have a steady hand, remove pieces of dust and detritus using a fine, clean, insect mounting needle. The specimen is now coated with gold so it is electrically conductive and can be viewed under the electron microscope. A polystyrene-lined plastic box serves well for the dust free storage of the prepared plates. When the bee's pollen load is loosely packed and dry (as in many Megachilinae and some *Andrena* species) pollen grains are frequently lost during preparation of the material for viewing under the electron microscope. Pollen loss is reduced or avoided if the specimen is examined directly after coating or if the intensity of the electron beam is decreased. If specimens are from collections and should not be damaged, or if pollen loads are likely to be shed whilst preparing the material for the electron microscope, pollen grains can be removed from the bee and prepared with a mounting needle using the method described in the following section.

2.2.2 Electron microscopy of the reference collection

For the determination of electron micrographs of pollen, it is essential to make compare them with preparations of pollen from possible forage plants. The procedure is as follows: a coverslip is covered with aluminium foil and is broken into pieces of approximately 5 x 7 mm by pressing it with a knife blade. The coverslip shards are cleaned in alcohol or Propanone (= Acetone) and are put into separate, clean, watchglasses. Under the stereo microscope, a dehisced anther is pulled off with fine pointed forceps. A drop of Ether is now placed on the glass shard, and the anther is dipped into the Ether. Some pollen grains float in the Ether, and, when the Ether has evaporated remain stuck to the surface of the glass coverslip shard. With carbon or silver the glass shards are stuck on a stub

with carbon or silver, ensuring that the entire underside of the shard is in contact with the adhesive. Further preparation proceeds as described in the previous section.

3 Determination and evaluation

For determination, the work of Zander (1935) is still indispensable. Also Moore & Webb (1978) can be recommended. Sawyer (1981) is particularly useful for the beginner. Among the newer standard works, that of Punt (1976) is highly regarded as is the pollen-flora of Punt and Clarke (1980, 1981, 1984). Critical determination of pollen to species level frequently only becomes possible with help of a reference collection. The precision of the qualitative analysis sits alongside pollen morphology and the knowledge of the vegetation of the study site. Often the plant species can be identified, purely on the basis of the pollen structure, but one may just be able to recognise the genus and sometimes only the plant family. With the regulation of honeys by origin, a percentage evaluation of the pollen content is possible using statistical methods (Louveaux et al., 1970). This is not transferable to the examination of wild bee pollen loads, since no homogeneous pollen mixtures are available. Percentage statements can therefore only be founded on quantitative analyses or on estimations. With mixed pollen loads, extraneous pollen may be present at values of about 5%. Provided contamination is excluded, these can usually be interpreted as pollen picked up accidentally when the insect is nectar foraging. For details on further techniques of pollination biology see Kearns & Inouye (1993).

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